Segmentation of magnetic resonance images of brain and abdomen

Annamalai Sarayu Parimal

2010

Annamalai Sarayu Parimal. (2010). Segmentation of magnetic resonance images of brain and abdomen. Master's thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/47466>

<https://doi.org/10.32657/10356/47466>

Nanyang Technological University

Downloaded on 26 Apr 2023 21:02:45 SGT

SEGMENTATION OF MAGNETIC RESONANCE IMAGES OF BRAIN AND ABDOMEN

ANNAMALAI SARAYU PARIMAL

School of Computer Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Master of Engineering

2010

Acknowledgements

I would like to acknowledge the effort from all the people who have helped towards the work in this dissertation.

I am in the deepest gratitude to my project supervisor Dr. Vitali Zagorodnov, who provided me with this project. His valuable guidance and encouragement through out the course of study is the most important factor to the completion of this work.

I would also extend a heartfelt thanks to Prof. Michael Chee of Cognitive Neuroscience Lab, Duke-NUS Graduate Medical School for providing me with the datasets and insights into the problem and its applications. I would like to thank Mr. Suresh Anand Sadananthan for his help with courses and projects during the entire course of my study. I also thank Prof. Nick Ivanov who gave me insightful suggestions about the project during the initial phase. I would also like to extend my thanks to Mr. Zheng Hui for assisting me in Matlab programming, Ms. Karen Chen for patiently providing me with the manual tracing, Mr. Vivian and Ms.Cindy Goh for their discussions about the methodologies.

Last but certainly not the least, I am most indebted to my parents and sister who have always supported me with their love and affection.

Table of contents

<i>Summary</i>	<i>iv</i>
<i>1 Introduction to automated segmentation</i>	<i>1</i>
1.1 Motivation	1
1.2 Aim of the thesis	2
1.3 Organization of the thesis	3
<i>2 Magnetic Resonance Imaging</i>	<i>4</i>
2.1 Basics of MRI	4
2.2 MRI for Brain and WMH	8
2.3 Imaging for Abdominal Fat Tissues	15
<i>3 Segmentation Techniques for MRI</i>	<i>19</i>
3.1 Thresholding	19
3.2 Region growing	23
3.3 Edge-based segmentation techniques	25
3.4 Active contour modeling	26
3.5 Graph based segmentation approach	29
<i>4 Segmentation of WMH tissues</i>	<i>35</i>
4.1 Motivation	35
4.2 Review of previous literature	36
4.3 Proposed approach	49
4.4 Performance evaluation	61
4.5 A note about performance metrics	68
4.6 Conclusion	69
<i>5 Segmentation of MR abdomen images</i>	<i>71</i>
5.1 Motivation	71
5.2 Review of previous techniques	72
5.3 Proposed approach	82
5.4 Performance evaluation	91
5.5 Conclusion	97
<i>Publications</i>	<i>98</i>
<i>Appendix A – WMH segmentation analysis</i>	<i>99</i>
<i>Appendix B – Fat segmentation analysis</i>	<i>102</i>
<i>References</i>	<i>104</i>

Summary

Segmentation of MRI data is required for many applications, such as the comparison of different structures or time points, and for annotation purposes. Manual segmentation requires high-level expertise and is subject to inter- and intra- observer variability, time and labor consuming. All these urge the need for automatic segmentation algorithms. In this work we try to address two segmentation problems in MR imaging and design automatic algorithms to solve them.

The first problem is segmentation of White Matter Hyperintensities (WMH) from brain images. WMH occurs in healthy, elderly and in patients with abnormalities. It is important to accurately quantify WMH volumes in longitudinal and epidemiological studies in order to understand the distribution of these pathological tissues over time. We propose an algorithm to segment WMH. This algorithm suggests an improvised way to estimate global threshold followed by cluster threshold locally. The false positives are well accounted for in this approach. Validation of the method is done using two datasets.

The Second problem is the quantification of adipose tissues of the abdomen images. This work proposes an algorithm to segment the adipose tissues and further classify it into visceral abdominal fat tissue (VAT) and surface abdominal fat tissue (SAT). Accurate segmentation of VAT and SAT is necessary since they provide a better indicator of cardiovascular risk factors. The proposed method quantified VAT and SAT and also overcame many problems of the other existing works in literature.

List of Figures

Figure 1: Example of MR Images	6
Figure 2: Anatomical Brain Images. MRA is from http://radi.med.hokudai.ac.jp . Ultrasound is from http://www.medphys.ucl.ac.uk	9
Figure 3: Functional Brain Images. SPECT is from http://webnt.physics.ox.ac.uk . EGG is from www.chasque.net .	10
Figure 4: Example of WMH	11
Figure 5: Example of MRI brain scan with WMH lesions - (a) T1-weighted, (b) T2-weighted and (c) PD-weighted images	12
Figure 6: Sample slices from FLAIR MRI studies of patients with WMH	14
Figure 7: Sample Image of CT	15
Figure 8: Abdomen Imaging. Left: T1 image; Right: T2-weighted image	17
Figure 9: The 4 contrast images from the DIXON sequence. (a) In-Phase; (b) Out-Phase; (c) Fat Image and (d) Water Image.	18
Figure 10: An example of bimodal histogram with selected threshold T	20
Figure 11: An example of global thresholding. (A) Original Image, (B) histogram of image A, (C) result of thresholding with $T=-127$, (D) outlines of the white cells after applying 3x3 Laplacian to the image shown in C.	21
Figure 12: Steps of segmentation with the hill climbing algorithm	24
Figure 13: Edge detection using sobel operator. (A) Original angiography image showing blood vessels, (B) edge magnitude image obtained with a 3x3 Sobel mask, (c) edge image thresholded with a low threshold (300), (D) Edge image thresholded with a high threshold (600).	26
Figure 14: A graphical framework for a 5x5 image - region based approach.	30
Figure 15: Graphical framework for a 5x5 image - boundary based approach	32
Figure 16: Boundary based graph cuts (a) foreground and background seeds (b) Optimal Segmentation	33
Figure 17: Classification of a patient with moderate lesion load. (A) FLAIR image, (B) manual segmentation, (C) probability map, (D) segmentations derived from probability map with different thresholds: black: probability (P) = 0, blue: $0 < P \leq 0.3$, green: $0.3 < P \leq 0.5$, yellow: $0.5 < P \leq 0.8$, red: $0.8 < P \leq 1$.	37
Figure 18: Histogram based segmentation of FLAIR image	40
Figure 19: FLAIR-histoseg. The top left panel represents a FLAIR image from a moderate synthetic phantom. The top right panel demonstrates the results of the FLAIR-histoseg method. The bottom panel represents the actual histogram of the FLAIR image after the skull and scalp have been removed. Pixels classified as normal brain are assigned green; leukoaraiosis lesion, red; CSF, blue.	41
Figure 20: WMH Segmentation Flowchart. The processing steps used to automatically segment the WMHs on FLAIR MR Brain images.	43
Figure 21: An overview of the WMH localization procedure.	44
Figure 22: Need to use other modalities to perform segmentation	45
Figure 23: General work flow of automatic segmentation algorithm	46
Figure 24: Segmentation outcome (GS - Gold Standard; Auto - Automatic segmentation result)	46
Figure 25: Flowchart showing the algorithm procedure	50
Figure 26: Skull stripping of the FLAIR images	52
Figure 27: Interslice intensity correction	53
Figure 28: The two distributions within the histogram	54
Figure 29: Ground truth and the false positives and negatives	56
Figure 30: Iterative approach for enhancing threshold estimation	57
Figure 31: Result of segmentation - a) Flair raw; b) Groundtruth; c) Thresholding+BlockTrimming d) Cluster thresholding	62
Figure 32: Result of segmentation - Top left: FLAIR raw; Top Right: Groudtruth; Bottom Left: Thresholding (and block trimmed); Bottom Right: Cluster thresholding.	63
Figure 33: Problem of growing the GM tissues during cluster growing	65
Figure 34: Reliability of the ground truth - overestimation of manual segmentation	66
Figure 35: Result of segmentation on Phase II data	67

Figure 36: Result of segmentation on Phase II data	67
Figure 37: a: Water-saturation MRI image (L3). b: Same level screenshot computer segmentation technique showing VAT (white) and SAT (green)	74
Figure 38: a: Non-water-saturation MRI image (L3). b: Same level screenshot computer segmentation technique showing underestimation of VAT (white) and overestimation of SAT (green).	74
Figure 39: Single slice illustration of the pelvis model in use. (a) Adipose Tissue (AT) pixels within the mask (VAT), (b) highlighted area illustrates the region anterior to the pelvis model and (c) the result (VAT) after the AT threshold is raised posterior to the pelvis model.	75
Figure 40: Illustration of step (a) shows the pixels in VAT and the striped region shows one slice of the vertebra model used to include geometrical a priori knowledge.	75
Figure 41: An example of the curve deformation to achieve a rough estimate of the abdomen boundary in an image slice blurred with a Gaussian kernel.	78
Figure 42: Automatic segmentation performed by the standard FCM algorithm (a) and by the AFCM algorithm (b). Green and yellow contours define external and internal boundaries of SAT, respectively. Histogram for VAT evaluation is calculated inside the blue contour. Histogram extracted from the VAT area before (c) and after (d) signal correction performed by the gain field calculated by AFCM algorithm. Red curves represent automatic fitting of the VAT peak.	80
Figure 43: Fat initial images. Left: Fat image, right: In-Phase image	83
Figure 44: Mask of Abdomen Image excluding arms of the patient	84
Figure 45: The Adipose tissue mask is shown. Left: Initial Fat Slice; Right: Adipose tissue mask from the thresholded image	85
Figure 46: Adipose tissue mask. The place where boundary between SAT and VAT needs to be cut is indicated as blue. A cut at this place will separate the SAT and VAT	85
Figure 47: Left: Binary image of adipose tissues; Right: Grayscale distance transform of the image	88
Figure 48: Figure showing different FG seeds. (a) Adipose tissue mask, (b) FG based on outer layers of binary mask, (c) based on convex hull approach, (d) based on multi-modal information	90
Figure 49: Top: Thresholded Image. Bottom left: Incorrect foreground layer due to image boundaries with holes (leaks). Bottom right: Segmentation output. Leaking of SAT into VAT	92
Figure 50: Convex hull of boundary solves the leaking problem. Thresholded image is shown in left; Foreground seed is shown in middle; segmentation result is shown in the right.	93
Figure 51: A case of failure of convex hull boundary approach by not detecting SAT accurately	93
Figure 52: (a) The initial thresholded image; (b) the boundary from the convex hull of image; (c) Better estimate of foreground layers using boundary information from In-Phase modality image; (d) Segmentation output from the better estimate of foreground seed layers.	94
Figure 53: Segmentation output for images with discontinuous surface fat	96

List of Tables

Table 1: Jaccard similarity between groundtruth and automatic segmentation for 16 subjects. The JS 2008 is the column of interests. The JS2008_HC column gives the JS with high confidence WMH. _____ 64

Table 2: Failure rates of the segmentation output based on different methods of seed selection _____ 95

Table 3: False positives and missed detection _____ 99

Table 4: Correlation of automatic volumes with manual volumes and other health parameters. _____ 101

Table 5: Association of VAT and SAT with cardiovascular risk factors. The VAT showed good associations with risk factors than the SAT _____ 102

1 Introduction to automated segmentation

1.1 Motivation

Different modalities of images are available in medical imaging for non-invasive viewing of the internal organs of the human body, such as the brain and abdomen. Among all the medical image techniques, Magnetic Resonance Imaging (MRI) has recently been most popular in many of the research areas. The analysis of medical images acquired from the MRI is a complex task. They usually involve more data, compounded with extraneous artifacts and noise. As advancements of this type of computerized imaging systems become widespread, it is increasingly important that the large amounts of digital information, thus obtained, be automatically processed. This causes an arising need for quantitative analysis of MRI. Examples of some MRI image analysis works include precise delineation of tumors, reliable and reproducible segmentation of images and so on. Segmentation of MR image is very important and can be used for various applications like computer-guided surgery, treatment planning, studying the anatomical structures and measuring tissue volumes, for registration across patient, time, or other image modalities. Formerly manual segmentation was the most popular segmentation technique. But it has several disadvantages: generally, (i) it requires high-level expertise, (ii) it consumes time and labor, and (iii) it is subjective and therefore not reproducible. A typical MR analysis of a patient involves vast amount of data. Hence manual segmentation is but too time-consuming. Automatic segmentation of medical images wards off this problem and speeds up imaging-based diagnosis. However, an automated algorithm for segmentation is inevitably required.

1.2 Aim of the thesis

In this thesis methods for automatic segmentation of MR images with reference to human brain and abdomen are presented. Two prevalent problems of medical imaging segmentation are addressed. The first problem is to design an automatic segmentation method for quantifying the MR brain White Matter Hyperintensities (WMH). The WMH occurs in healthy, elderly as well as patients with abnormalities. Hence it becomes an important problem in medical imaging to accurately quantify WMH volumes in longitudinal and epidemiological studies in order to understand the distribution of these pathological tissues over time.

The second problem addressed is the automatic segmentation of MR abdominal images. Only a few studies have been done previously on the segmentation of MR abdominal images. This study aims to develop an algorithm that can segment the fat tissues from the non-fat tissues and further classify the fat tissues into Visceral Adipose Tissues (VAT) and Subcutaneous Adipose Tissues (SAT). The need for such segmentation is that the quantification of abdominal fat volumes may provide a better indicator of cardiovascular risk factors.

1.3 Organization of the thesis

This thesis is organized into five chapters. The first chapter is the introduction, giving the motivation and aim of the work and an outline of the thesis. Chapter 2 introduces the basics of magnetic resonance imaging. It elucidates the various modalities of MRI and the types of techniques used for imaging WMH. Also, in this chapter the imaging modality used for abdomen tissues has been discussed. The next chapter highlights some of the common segmentation approach applied on MR images. Chapter 4 addresses the problem of quantification of WMH. It gives an overview of the previous methods in literature followed by a proposed novel algorithm for the segmentation. Chapter 5 deals with the problem of segmentation of abdomen fat tissues and the further classification of fat tissues into VAT and SAT.

2 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) technique uses strong magnetic fields and radio frequency waves to produce high quality two- or three-dimensional images of biological tissues [1]. The important advantage of MRI is that it allows for non-invasive high quality imaging of patient anatomy. For designing a successful automated algorithm to do image morphometry on MR images, knowledge of different types of MRI sequences, their advantages and disadvantages are necessary. Sources of noise and artifacts and their characteristics should also be known in order to design an automated algorithm that is not highly influenced by quality of image. This chapter presents the basics of MRI, different types of acquisitions and also briefly explains about noise and artifacts in MR images. This chapter also explains why we use the current modality that we work on, in order to perform image analysis. The MR sequences for brain and the abdomen are highlighted in subsections.

2.1 Basics of MRI

Isidor I Rabi first discovered the physical phenomenon of Nuclear Magnetic Resonance (NMR) in 1938 [2]. He was awarded the 1944 Nobel Prize in Physics for his method for recording the magnetic properties of atomic nuclei. Felix Bloch and Edward Purcell are considered to be the inventors of Magnetic Resonance Imaging (MRI), for which they were

awarded the Nobel Prize in Physics in 1952. In 1973, Paul Lauterbur, a chemist at the State University of New York, Stony Brook, produced the first NMR image [3]. Peter Mansfield, a physicist working in Nottingham, England, further developed the utilization of gradients in the magnetic field. Mansfield's work showed how fast MR imaging could be achieved [4]. In 2003 Mansfield and Lauterbur were awarded the Nobel Prize in Medicine for their pioneering contributions, which later led to the application of magnetic resonance in medical imaging.

An MRI system is constructed of at least three basic subsystems: a main magnet to produce a strong, homogenous, static field denoted as the B_0 field; a subsystem for generation of a gradient magnetic field, for signal localization; and a radio-frequency (RF) subsystem, for generation and transmission of a rotating magnetic field, denoted as the B_1 field, and measurement of NMR signals [5]. In order to create MRI images, an oscillating magnetic field is used. When this field is turned off, the atomic nuclei that had absorbed electromagnetic energy in a specific frequency, release that energy [6]. Sensors read these emissions and the target's images are created. Using MRI it is possible to create images of both surface and subsurface structures, with a high degree of anatomical detail.

An MRI system evokes NMR signals from tissue to be imaged. By controlling the acquisition parameters of the scan, different image weightings may be obtained, allowing for different and/or improved image contrast between different types of tissue. Image contrast in MRI studies is fundamentally based on the measurement of spin-lattice relaxation (T_1) time, spin-spin relaxation (T_2) time, and nuclear spin density (PD). An example of MRI image is shown in Figure 1.

Figure 1: Example of MR Images

2.1.1 T1 contrast

Usually the protons align to the external magnetic field. The ability of the proton to align to the external magnetic field differs for different tissue types. In lipids protons will relax faster than protons in water, or in molecules that are much larger. In the time period of returning to lower energy state, the NMR signal will gradually decay and disappear. The T1 relaxation time is the time it takes for 63% of the protons to align with external field of magnetization. A sampling of the NMR signals in a T1 relaxation gives T1 weighted images. T1 time is tissue class dependent and one of the reasons why MR images have good tissue contrast. In the brain, T1-weighting causes the nerve connections of white matter to appear white, and the congregations of neurons of grey matter to appear grey, while cerebrospinal fluid appears dark [7].

2.1.2 T2 contrast

The T2 weighted images are obtained after a refocusing of the spins in the x-y direction following a 180° RF pulse. T2 relaxation is caused by reduction in magnetization in the X - Y plane, also called transverse relaxation. This is caused by in-homogeneity of the magnetic field on a molecular level, leading to a dephasing of the protons with decay of the transverse

magnetization. T2 relaxation time describes how fast the decay of the NMR is because of T2 relaxation. T2 time is longer in pure water than tissues or liquids containing protein. The contrast of "white matter," "grey matter" and "cerebrospinal fluid" is reversed using T2 imaging compared to T1 imaging. In comparison with other techniques, MRI has the advantage of being almost completely harmless to the subject's health and allowing distinguishing between soft tissues [8]. All these properties make MRI comparable with the ones obtained through CT scans. One disadvantage of this technique is its inability to be used in subjects with some kind of electronic implants, like pacemakers [9].

2.1.3 Noise and artifacts in MRI

There are various sources of noises in the MR images. The main source of the noise originates in the patient or the object to be imaged and is added during the processing of the signal in the receiver chain. In the receiver chain, noise may be generated in the preamplifier and at the connection between the preamplifier and the RF receive coil. In the RF coil, which is a conductor, the stochastic motion of free electrons produces thermal noise. This motion is caused by ohmic losses in the RF coil itself, and by eddy current losses in the patient, which are inductively coupled to the RF coil. (High conductivity of receiver coils avoids noise, whereas conduction in the patient causes noise.)

The resistance induced in the receiving circuit by eddy currents in the patient (called loading) is much more significant in a modern high-field MRI system than a receiver coil's own resistance. A larger mass in vivo causes greater coil loading—and more noise.

The signal available in a magnetic resonance experiment is dependent on many factors, including tissue specific parameters, such as T1, T2 and proton density, and the choice of pulse sequence parameters, such as the echo time (TE), repetition time (TR), flip angle, preparation-pulse delay time (if applicable), number of measurements and so on.

Apart from the noises the signal may also be affected by the inhomogeneities, which are perceived as smooth variation of intensities across the image. The reason for these inhomogeneities may be induced by a number of factors, such as poor radio frequency coil uniformity, static field inhomogeneity, radio frequency penetration, gradient-driven eddy currents and overall patient anatomy and position.

2.2 MRI for Brain and WMH

2.2.1 Brain Imaging

There exists a wide range of 3D medical image modalities that allow neuroscientists to see inside a living human brain. This 3D brain imaging allows, for instance, to better localize specific areas inside the brain and to understand the relationships between them. Brain imaging can be divided into two main groups: *structural* and *functional* imaging. The former deals with imaging brain structures and diagnosis of intracranial pathologies. Structural imaging allows the study of the anatomical structures of the head such as the bones or the different brain tissues. Some of the structural brain imaging includes techniques like *Computed Tomography (CT) scan* which uses a series of X-ray beams passing through the head, followed by a tomographic reconstruction, to build a 3D image of the head where bones and soft tissues are clearly identified; *Magnetic Resonance (MR)*

imaging (as explained before) which provides also an anatomical view of the tissue and deep structures of the brain using the magnetic properties of the tissues; Diffusion tensor MR brain imaging which is a relatively new image modality that permits in vivo measures of the self-diffusion properties of water in living tissues [10]. This measure becomes highly anisotropic and oriented in areas of compact nerve fiber organization of the brain providing an indirect way of white matter mapping and fiber tract identification [11], [12]. Other anatomical images can be obtained, for instance, using MR or CT principles for *Angiography* (MRA or CTA respectively), that provide a 3D image of blood vessels (Figure 2)

Figure 2: Anatomical Brain Images. MRA is from <http://radi.med.hokudai.ac.jp>. Ultrasound is from <http://www.medphys.ucl.ac.uk>

Functional magnetic resonance imaging, or fMRI, is a technique for measuring brain activity. It works by detecting the changes in blood oxygenation and flow that occur in response to neural activity. When neuronal activity increases there is an increased demand for oxygen and the local response is an increase in blood flow to regions of increased neural activity. Hemoglobin is diamagnetic when oxygenated but paramagnetic when deoxygenated. This difference in magnetic properties leads to small differences in the MR

signal of blood depending on the degree of oxygenation. Since blood oxygenation varies according to the levels of neural activity these differences can be used to detect brain activity.

fMRI can be used to produce activation maps showing which parts of the brain are involved in a particular mental process [8]. This allows the study of the brain activity by picturing the motor, sensor or cognitive tasks of the brain. A few types of functional images are shown in Figure 3.

Figure 3: Functional Brain Images. SPECT is from <http://webnt.physics.ox.ac.uk>. EEG is from www.chasque.net.

2.2.2 White Matter Hyperintensities of the brain

White matter hyperintensities occur in healthy brain and also in affected brains. The extent of distribution of the WMH may be an indicator of the pathology. The occurrence of WMH (Figure 4) is due to the disruption of the nervous system by demyelination of the white matter (myelin is an important substance of white matter). Although it is not directly lethal, it still has many side effects, which lead to noticeable deterioration in the quality of life [13]. Increased amount of WMH tissues are termed as multiple sclerosis (MS). MS has a wide range of symptoms including fatigue, visual problems, balance problems and altered

sensations. The cause of death is not known, but genetic changes are suspected to be a partial cause [14]. This disease is not easy to diagnose, because the symptoms vary heavily from one patient to another. Often the patient has to live in an uncertainty for a long period of time until proper diagnosis is made and treatment can be started. With MRI it is possible to confirm the presence and extent of the lesion and, by processing the images with computers, valuable information can be obtained [15]. The progressive growth of WMH is often referred to as brain lesions, WMH lesions or multiple sclerosis.

Figure 4: Example of WMH

2.2.3 Imaging for White matter hyperintensities of brain

MRI is known to be by far the best para-clinical test in detecting White matter hyperintensities, depicting abnormalities in 95 percent of patients [16]. Today, diagnosis of WMH is virtually always accompanied by a confirmatory MR study [16]. Until recently the

three modalities namely T1, T2 and PD were used to detect hyperintensities of the brain. WMH lesions appear as regions with increased signal intensity on the T2- and PD-weighted images, and an iso-intense or hypointense appearance in the T1-weighted channel. An example of these three modalities with occurrence of WMH lesions (circled) is shown in Figure 5 below.

As explained earlier, a study is said to be a T1-weighted study when the dominant tissue characteristic generating image contrast is the T1 time of a tissue [5]; a study is said to be T2-weighted when the dominant tissue characteristic generating image contrast is the T2 time of a tissue [5]; a study is said to be PD weighted when the dominant tissue characteristic generating image contrast is the nuclear spin density of a tissue [5].

Figure 5: Example of MRI brain scan with WMH lesions - (a) T1-weighted, (b) T2-weighted and (c) PD-weighted images

Recently a new imaging modality, Fluid Attenuation Inversion Recovery (FLAIR), using the Inversion-recovery sequences are used to utilize T1 contrast, while allowing for differentiation of tissues with approximately equal T2 times or nuclear spin densities [5]. FLAIR studies are implemented with inversion-recovery sequences designed to suppress the

intensity of cerebro-spinal fluid (CSF). To simplify, with a T1 or FLAIR weighted images the CSF is darker relative to the surrounding brain tissue, while in T2 weighted study, CSF is brighter relative to surrounding brain tissue (See Figure 5).

Comparing the appearance of the lesions in the FLAIR image to the T2 image, it is observed that the T2 image shows limited contrast of lesions against brain material for some lesions. Furthermore, the diseased tissue imaged in the FLAIR image is not always contrasted as abnormal tissue in the T2 image, and at other times, the diseased material found in the T2 image appears quite differently depending on the lesion. Since the FLAIR image gives the best indications of disease, and because the T1 and T2 images are either not useful (T1), or not consistent with the FLAIR imagery (T2), the FLAIR imagery alone is widely being used for classification. The features that are generally used in classification will be the intensity observed in the FLAIR imagery. Figure 6 below shows several slices of different FLAIR MR Images of patients with WMH.

Figure 6: Sample slices from FLAIR MRI studies of patients with WMH

The use of MR imagery in the evaluation of WMH involves the identification of abnormal brain tissue (WMH lesions), and normal, non-diseased brain tissue (gray and white matter). MRI has been found to be a sensitive marker to changes in disease progression, and evaluation of MRI studies of WMH/MS patients can be useful as an outcome measurement in MS studies [17].

Despite these statements, evaluation of the disease cannot be completely based upon MRI findings. WMH lesion activity observed in MRI studies of the brain does not always correspond to clinically observed deficits. As well, quantification of MRI studies of WMH has yet to be perfected.

2.3 Imaging for Abdominal Fat Tissues

Abdominal fat has been well known to be a cardiovascular risk factor and the distribution of body fat tissues needs to be evaluated to investigate the complications of obesity [18]. Anthropometric parameters such as body mass index (BMI), waist-to-hip ratio, or waist circumference are used in clinical settings as a rough estimation of abdominal fat volume. A precise method of quantification the fat volume is through imaging the abdomen and segmenting out the fat tissues.

Up-to-date CT has been offering an economical analysis of the body fat tissue with better contrast in clinical studies. Conventional studies implemented manual 2D contouring analysis of slice-by-slice CT scan images taken at the umbilical level [19] and 3D volume analysis of helical CT images [21]. Automated image analysis differentiated 2D abdominal fat into subcutaneous and visceral fat components and removed equipment-related artifacts in CT images [22]. An example of CT scan is shown in Figure 7.

Figure 7: Sample Image of CT

Since CT may over-expose subjects to ionizing radiation of X-rays, MRI has found its use in imaging abdomen nowadays. MRI produces high image quality and good conspicuity of

disease with several sequences that are robust and reliable avoiding artifacts. Respiration, bowel peristalsis, and vascular pulsations result in artifacts that have lessened the reproducibility of MR imaging (MRI). Breathing-independent sequences and breath-hold sequences form the foundation of high-quality MRI studies of the abdomen. General MRI of the abdomen can consist of T1 or T2 weighted spin echo, fast spin echo (FSE, TSE) or gradient echo sequences with fat suppression and contrast enhanced MRI techniques. Respiratory compensation and breath hold imaging [23] is mandatory for a good image quality. Gradient echo in phase T1 breath hold can be performed as a dynamic series with the ability to visualize the blood distribution. Phases of contrast enhancement include the capillary or arterial dominant phase for demonstrating hyper-vascular lesions, in liver imaging the portal venous phase demonstrates the maximum difference between the liver and hypo-vascular lesions, while the equilibrium phase demonstrates interstitial disbursement for edematous and malignant tissues. Since the T1 relaxation time of adipose tissue is shorter than that of most other types of tissue this was more commonly used. However, due to partial volume effects, this technique has been shown to consistently underestimate the fat volume. Another modality that was used for abdomen imaging was the T2 weighted images. Although these images are insensitive to inhomogeneity of the images, the overall signal intensity of the image is reduced in single shot sequences and hence making it not suitable for fat quantifications. A sample slice of T1 and T2 acquisition is shown in Figure 8.

Figure 8: Abdomen Imaging. Left: T1 image; Right: T2-weighted image

Dixon imaging sequence [24] which has been around for long time now, have gained more popularity in the recent years due to the improvements of the sequences for better acquisition of abdomen images. The Dixon imaging provides an image showing fat content in each voxel, an image showing water content in each voxel, an in-phase and an out-phase image. The images are show in Figure 9. Because of availability of separate water and fat images, partial volume effects do not adversely affect Dixon images. Also the separate images make it very useful for the segmentation process. The Dixon sequence images are also not affected by inhomogeneities. Because of these significant advantages, Dixon images are beginning to be used lately. In short, Dixon imaging is performed by acquiring two separate images: one where the signals from fat and water are 180° out of phase ($I_1 = w - f$) and one where they are in phase ($I_2 = w + f$). Ideally, water and fat can then be obtained as the sum and difference of these images, respectively, and the total fat content in any region of interest can then easily be calculated. However, in practice, magnetic field inhomogeneities cause the complex phase of I_1 and I_2 to vary over the images and a robust phase correction is needed before w and f can be calculated. Several different methods for

correcting Dixon images have been proposed in i.e., [25][26] which has been shown to perform well even in the presence of severe phase artifacts.

Figure 9: The 4 contrast images from the DIXON sequence. (a) In-Phase; (b) Out-Phase; (c) Fat Image and (d) Water Image.

3 Segmentation Techniques for MRI

The principal goal of the segmentation process is to partition an image into regions (also called classes, or subsets) that are homogeneous with respect to one or more characteristics or features. There is not any unique segmentation technique that can produce satisfactory results for all imaging applications. The segmentation technique varies according to the goal of the study and the type of the image data. This chapter describes the commonly used segmentation techniques for MR images and their limitations. The techniques presented in this chapter will often be referenced in chapter 4 and 5. Familiarizing these techniques is important to understand the procedures of the proposed algorithm in later chapters. Some of the MR segmentation techniques are explained below.

3.1 Thresholding

Thresholding is a common region segmentation method [33]. In this technique a threshold is selected and an image is divided into groups of pixels having values less than the threshold and groups of pixels with values greater or equal to the threshold. There are several thresholding methods: global methods based on gray-level histograms, global methods based on local properties, local threshold selection, and dynamic thresholding.

3.1.1 Global thresholding

Global thresholding is based on the assumption that the image has a bimodal histogram and therefore the object can be extracted from the background by a simple operation that compares image values with a threshold value T [34]. Suppose that we have an image $f(x,y)$ with histogram as shown in figure below showing two dominant modes for object

Figure 10: An example of bimodal histogram with selected threshold T

and background then the obvious way to extract the object from the background is to select a threshold T that separates these modes. The thresholded image $g(x,y)$ is defined as

$$g(x,y) = \begin{cases} 1 & f(x,y) > T \\ 0 & f(x,y) \leq T \end{cases} \quad (1)$$

The result of thresholding is a binary image, where pixels with intensity value of 1 correspond to objects, while pixels with value 0 correspond to the background.

An example of global thresholding is shown in Figure 11.

Figure 11: An example of global thresholding. (A) Original Image, (B) histogram of image A, (C) result of thresholding with $T=127$, (D) outlines of the white cells after applying 3x3 Laplacian to the image shown in C.

The threshold T is called the global threshold and if it is defined within a local neighborhood, it is called the local threshold. The main problem in thresholding is the determination of the correct value of threshold for segmentation. The simplest way to determine that is from the intensity histogram. However, statistical and probabilistic measures can be used to determine the threshold to achieve better results. One common way to estimate the threshold is using the Otsu's method [35]. This method assumes that histogram of image is bimodal. It minimized the within-class variance of foreground and

background pixels to find the optimum threshold. This method gives satisfactory results when sizes of the two classes are close to each other. It fails to provide a good threshold when one class is large and the other class is small.

3.1.2 Local adaptive thresholding

In many applications, a global threshold cannot be found from a histogram or a single threshold cannot give good segmentation results over an entire image. If the background variations can be described by some known function of position in the image, one could attempt to correct it by using gray level correction techniques, after which a single threshold should work for the entire image. Another solution is to apply local (adaptive) thresholding. [36] Local thresholds can be determined by (1) splitting an image into subimages and calculating thresholds for each subimage, or by (2) examining the image intensities in the neighborhood of each pixel. If a subimage has a bimodal histogram, then the minimum between the histogram peaks should determine a local threshold. If a histogram is unimodal, the threshold can be assigned by interpolation from the local thresholds found for nearby subimages. In the final step, a second interpolation is necessary to find the correct thresholds at each pixel. In the latter method, a threshold can be selected using the mean value of the local intensity distribution. Sometimes other statistics can be used, such as mean plus standard deviation, mean of maximum and minimum values or statistics based on local intensity gradient magnitude.

3.2 Region growing

Region growing is another class of region segmentation algorithm that assigns adjacent pixels or regions to same segment if the image values are close enough, according to some preselected criterion of closeness [36]. Whereas thresholding focuses on the difference of pixel intensities, the region growing method looks for groups of pixels with similar intensities. Region growing, also called region-merging starts with a pixel or a group of pixels (called seeds) that belong to the structure of interest. Seeds can be chosen by an operator, or provided by an automatic seed finding procedure. In the next step neighboring pixels are examined one at a time and added to the growing region, if they are sufficiently similar based on a uniformity test, (also called a homogeneity criterion). The procedure continues until no more pixels can be added. The object is then represented by all pixels that have been accepted during the growing procedure [37][38].

One example of the uniformity test is comparing the difference between the pixel intensity value and the mean intensity value over a region. If the difference is less than a predefined value, for example, two standard deviations of the intensity across the region, the pixel is included in the region; otherwise, it is defined as an edge pixel. The results of region growing depend strongly on the selection of the homogeneity criterion. If it is not properly chosen, the regions leak out into adjoining areas or merge with regions that do not belong to the object of interest. Another problem of region growing is that different starting points may not grow into identical regions.

The advantage of region growing is that it is capable of correctly segmenting regions that

have the same properties and are spatially separated. Another advantage is that it generates connected regions.

Instead of region merging, it is possible to start with some initial segmentation and subdivide the regions that do not satisfy a given uniformity test. This technique is called splitting [34]. A combination of splitting and merging adds together the advantages of both approaches. A type of region growing is the hill-climbing algorithm [39]. A self-explanatory figure is shown in Figure 12 that explains the algorithm and elucidating the concept of region growing algorithm.

Figure 12: Steps of segmentation with the hill climbing algorithm

Various other approaches to region growing segmentation is highlighted in [40]. An excellent review of region growing techniques can be found in Fu and Mui [41].

3.3 Edge-based segmentation techniques

An edge or boundary on an image is defined by the local pixel intensity gradient. A gradient is an approximation of the first- order derivative of the image function. For a given image $f(x,y)$, we can calculate the magnitude of the gradient as

$$|G| = \sqrt{G_x^2 + G_y^2} = \sqrt{\left[\left(\frac{\partial f}{\partial x}\right)^2 + \left(\frac{\partial f}{\partial y}\right)^2} \quad (2)$$

and the direction of the gradient as

$$D = \tan^{-1}\left(\frac{G_y}{G_x}\right) \quad (3)$$

where G_x and G_y are gradients in directions x and y , respectively. Since the discrete nature of digital image does not allow the direct application of continuous differentiation, differencing does calculation of the gradient. Both magnitude and direction of the gradient can be displayed as images. The magnitude image will have gray levels that are proportional to the magnitude of the local intensity changes, while the direction image will have gray levels representing the direction of maximum local gradient in the original image. Most gradient operators in digital images involve calculation of convolutions, e.g., weighted summations of the pixel intensities in local neighborhoods. The weights can be listed as a numerical array in a form corresponding to the local image neighborhood (also known as a mask, window or kernel). For example, in case of a 3x3 Sobel edge operator, there are two 3x3 masks:

$$\begin{array}{ccc} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{array} \quad \begin{array}{ccc} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{array}$$

The first mask is used to compute G_x while the second is used to compute G_y . Combining

G_x and G_y generates the gradient magnitude image. Figure 7B shows an edge magnitude image obtained with the 3x3 Sobel operators applied to the magnetic resonance angiography (MRA) image of figure below. The results of edge detection depend on the gradient mask. Some of the other edge operators are Roberts, Prewitt, Robinson, [42].

Figure 13: Edge detection using sobel operator. (A) Original angiography image showing blood vessels, (B) edge magnitude image obtained with a 3x3 Sobel mask, (c) edge image thresholded with a low threshold (300), (D) Edge image thresholded with a high threshold (600).

The common problem of edge-based segmentation is that often the edges do not enclose the object completely. To form closed boundaries surrounding regions, a post-processing step of linking or grouping edges that correspond to a single boundary is required.

3.4 Active contour modeling

Boundary based techniques use the edge information to locate the object boundaries. The active contour models have been an intensely researched area ever since proposed by Kass et al [43]. These active contours, popularly called snakes, are computer-generated

curves that undergo deformation to fit the region of interest. The deformation is controlled by a set of internal forces that are defined within the curve of surface, and a set of external forces that are computed from the image data and the optimization function. The internal forces typically comprise the elasticity forces and the bending forces that are designed to keep the curve smooth during deformation and prevent it from stretching and bending too much.

These forces are in general designed using the prior shape information/smoothness. The external forces move the snake towards the desired boundary and are usually represented by edge information. The main advantage of these deformable models is their mechanism to allow expert knowledge in the form of prior information to be incorporated in the model-based image segmentation.

The active contour models can be divided into the parametric active contours and geometric active contours. Parametric deformable models represent curves explicitly in their parametric forms during deformation [44], [45]. This parametric representation allows direct interaction with the model. However the main drawback with this method is that it cannot adapt to topological changes, such as splitting or merging during the deformation. Geometric deformable models, on the other hand, can handle topological changes naturally [46]. These models are based on the theory of curve evolution and the level set method [47], [48] which represents the curves and surfaces implicitly as a level set of a higher-dimensional scalar function. Their parameterizations are computed only after complete deformation, because of which it has the capability to adapt to topological changes. Despite this fundamental difference, the underlying principles of both methods are very similar.

Level sets are able to handle topological changes. These methods give poor results in the presence of more noise. A good survey of the deformable models for medical image analysis can be found in [49].

3.4.1 Parametric active contours – snakes

The parametric deformable models use an energy minimizing formulation of deformable contours that minimizes the weighted sum of the internal energy and external energy. The snake is a parametric curve $x(s) = (x(s); y(s))$, where $s \in [0; 1]$ and the energy function to be minimized is of the form

$$E(x) = E_{\text{int}}(x) + E_{\text{ext}}(I, x) \quad (4)$$

where I represent the image. The internal energy is given as

$$E_{\text{int}}(x) = \int_0^1 \alpha |x'(s)|^2 + \beta |x''(s)|^2 ds \quad (5)$$

where α and β are the coefficients associated with the elastic energy and the bending energy respectively.

The external energy is a function of the image gradient and is given by

$$E_{\text{ext}}(x) = \int_0^1 -|\nabla I(x, y)|^2 \quad (6)$$

The snake that minimizes $E(x)$ has to satisfy the Euler equation

$$\alpha x''(s) - \beta x'''(s) - \nabla E_{\text{ext}} = 0 \quad (7)$$

The optimal position of the contour is obtained when the external forces and the internal forces balance each other. Snakes suffer from two main problems, close initialization

requirement and inability to progress towards boundary concavities. The close initialization requirement can be overcome by the use of multiresolution methods [50], pressure forces [51], and distance potentials [52]. The basic idea is to extend the capture range of the external force fields to guide the contour towards the desired boundary. The use of gradient vector flow (GVF) fields [53] solves the problem by capturing the snake at long range and forcing it into concave regions. Further extensions of GVF to improve the results can be found in [54], [55].

3.5 Graph based segmentation approach

Similar to active contour models, graph theoretic approaches solve energy minimization problems; the difference between them is that discrete energy minimization function is defined in the case of the latter and a continuous function in the case of the former. Graph theoretic approaches represent the image in the form of a weighted undirected graph $G = (V; W; E)$, where each vertex $v_i \in V$ corresponds to a pixel in the image, edge $e_{ij} \in E$ connects neighboring vertices v_i and v_j and $w_{ij} \in W$ is the weight assigned to the edge e_{ij} . Weighted graphs are generalized form of unweighted graphs, where $w_{ij} = 1$ for all $e_{ij} \in E$. Each vertex is also connected to two additional vertices (source and sink). Figure 14 shows an example of a graph constructed for a 5x5 image. Every edge is assigned a weight, usually based on the intensities of the pixels it connects. The method requires the selection of foreground seeds and background seeds. The background seeds are connected to the source and the foreground seeds to the sink. The weights of edges connecting foreground seeds with the source are denoted by W_0 and the weights of edges connecting background seeds with the sink are denoted by W_1 .

Figure 14: A graphical framework for a 5x5 image – region based approach.

A graph cut is a set of removed edges that partitions the vertices in the graph into two disjoint sets. The cost of the cut is the sum of the weights of all the removed edges. The minimum cut on the graph produces the optimal segmentation and it can be determined by computing the maximum flow on the graph. The maximum flow in the graph is equal to the minimum cut in the graph and it can be found using the Ford-Fulkerson maximum flow algorithm in polynomial time [56]. The graph theoretic approaches can be classified into two categories: boundary-based techniques and region-based techniques.

3.5.1 Region-based graph theoretic approaches

Region-based graph theoretic approaches tend to find regions with uniform properties. The distribution of the foreground as well as the background pixels is given as prior information into the segmentation process. This avoids the need for selecting the foreground and background seeds explicitly. The weights of the edges connecting the vertices to the source/sink are assigned based on the distribution. Let $W_0(s)$ represent the weight of the

edge connecting the vertex s with the source and $W_1(s)$ represent the weight of the edge connecting the s with the sink. All other edge weights are equal to β . The value of β is chosen empirically. One example for the weight assumption is as follows:

$$W_0(s) = -\log p_0(I(s)) \quad (8)$$

$$W_1(s) = -\log p_1(I(s)) \quad (9)$$

where p_0 is the probability distribution of intensity of foreground and p_1 is the probability distribution of intensity of background. Figure 14 above shows an example of a region-based graph framework. The graph cut then determines the minimum cut that separates the source and the sink, thus yielding the segmentation.

3.5.2 Boundary-based graph theoretic approaches

Boundary-based graph theoretic approaches are employed to determine the boundary between regions. They work by cutting along the boundaries of the region. This approach requires the selection of foreground seeds and background seeds. These seeds act as hard constraints in the segmentation process, i.e., pixels selected as foreground seeds always appear as foreground and pixels selected as background seeds always appear as background in the output. The background seeds and foreground seeds are connected to the source and sink respectively. The weights of the edges connecting these seeds with the source/sink are assigned as follows:

$$W_0(s) = W_1(s) \geq \sum_{k \in N_s} w_{sk} \quad (10)$$

where N_s is the neighborhood of s consisting of all its adjacent vertices. The weights of edges connecting the vertices are assigned based on a function that is chosen depending on

the application. Finding the minimum cut that separates the two seeds yields the optimal segmentation. Figure 15 shows an example of the graph framework for the boundary-based approach. The shaded region corresponds to the foreground and the rest of the pixels correspond to the background region.

Figure 15: Graphical framework for a 5x5 image – boundary based approach

Given below is an example of a function to determine the weights of the edges connecting the vertices of the graph.

$$w_{ij} = \exp(-(I(i) - I(j))^2) \quad (11)$$

where w_{ij} is the weight of the edge e_{ij} connecting the vertices v_i and v_j and $I(i)$ is the intensity value at vertex v_i . This edge weight assignment gives smaller weights to the edges connecting less similar pixels and vice versa. The minimum cut on this graph separates the regions by cutting along the region boundaries producing optimal segmentation. The result of boundary based graph cuts is shown in Figure 16. This approach [57] penalizes longer boundaries and is biased towards finding smaller regions.

Figure 16: Boundary based graph cuts (a) foreground and background seeds (b) Optimal Segmentation

In order to overcome the problem of graph cuts showing bias towards smaller regions, Wang and Siskind [58] proposed an approach that minimizes the mean value of a cut by dividing the total weight by the number of removed edges, thus favoring regions with the highest contrast boundary. But this will produce cuts around spurious regions if the image is noisy. Shi and Malik [59] addressed this by the normalized cut criterion, which is defined as a ratio of the cost of the removed edges to the total cost of the edges inside the region. This ratio is large for smaller regions and hence the cut will prefer larger regions. Eigen vector approximation is used for solving the NP-hard problem of minimization of the normalized cut criterion. Interactive graph cuts approach [60] uses the minimum cut as an optimization method for user interactive selection of object and background regions. Xu et. al. [61] assume that the cut corresponding to the desired object boundary is a global minimum of the cuts inside some contour neighborhood specified by the user and aim to find the closest contour that is a global minimum within its neighborhood, given an initial contour.

4 Segmentation of WMH tissues

4.1 Motivation

Manual WMH or WMH lesion segmentation is a fastidious task and depends on intra and inter-expert variations. For this reason, a lot of automatic lesion segmentation algorithms have been developed in the past 20 years. Automatic detection of abnormal brain structures, and particularly MS lesions, is difficult. Abnormal structures exhibit extreme variability. Their shapes are deformable, their location across patients may differ significantly, and their intensity and texture characteristics may vary. White matter hyperintensities are such common pathological occurrences in elderly subjects. The white matter hyperintensities or lesions are caused by blood vessel diseases, which are usually caused due to hypertension, diabetes etc., and are, related to cognitive decline - making it a need for accurate segmentation of these tissues

It is desirable to apply computer vision techniques to the study of WMH using MR imaging. Allowing a computer to automatically identify normal and abnormal brain tissue would free an expert from the arduous task of manually examining each slice of a study, while generally increasing the reproducibility of the identification by removing the subjectivity of the human observer. Computer vision tools would also be useful for automatic, retrospective alignment of patient studies, taken at different points in time, to allow for qualitative comparison of different studies of a patient of the course of his or her treatment. This type

of analysis would aid the expert in deciding if the disease is responding well to the present treatment, or if a change in the treatment is warranted.

4.2 Review of previous literature

Several methods have been explored to automatically or semi-automatically segment the WMHs. Each of these methods uses different approach to deal with the segmentation problem. In this section we discuss some of the common approaches used for WMH segmentation and then propose a novel method to do the same.

Anbeek et al [62] validated a fully automated method using probabilistic k-nearest neighbors voxel-based scheme of classification. This method of tissue classification technique integrates information from different MRI sequences. The algorithm used information from T1-weighted, inversion recovery and proton density-weighted and FLAIR scans. Its feature space is derived from the voxel intensities and spatial information. The technique generates images representing the probability per voxel being part of WMH. By applying thresholds on these probability maps, binary segmentation can be obtained. The results are shown in Figure 17. The learning set for segmentation of one patient was built from the voxels of the other 19 patients. All voxels in the learning set were labeled with value of 0 (non-WMH class) or 1 (WMH class) derived from manual segmentations. Because of the innumerous cases, 20% of the voxels were included in the learning set.

Figure 17: Classification of a patient with moderate lesion load. (A) FLAIR image, (B) manual segmentation, (C) probability map, (D) segmentations derived from probability map with different thresholds: black: probability (P) = 0, blue: $0 < P \leq 0.3$, green: $0.3 < P \leq 0.5$, yellow: $0.5 < P \leq 0.8$, red: $0.8 < P \leq 1$.

The features used in this study can be divided into two categories: voxel intensities and spatial information. The first group is defined by the signal intensities of a voxel in the acquired images: T1-weighted, IR, PD, T2-weighted and FLAIR, which provide a five-dimensional feature space. The second group of features incorporates the spatial location of a voxel in the brain. These were added because in some regions of the brain, lesions are more likely to occur than in others. The spatial features were defined in-plane by two coordinates and through-plane by the z-coordinate. In-plane, the voxel coordinates were

measured from the center of gravity in the FLAIR image, which was the reference image for registration, by two different methods. Two types of in-plane coordinates were used separately: Euclidean coordinates as well as the polar coordinates. Since different features have different ranges, a rescaling of the feature space was necessary to define a proper metric to compare distances in the feature space, which is essential to justify classification based on KNN. This was achieved by variance scaling: subtraction of the mean of feature values and division of the outcome by standard deviation. The choice of K in KNN classification depends on the number of features and the number of cases. The WMH probability of every voxel was determined by inspection of the K-nearest neighbors of the examined voxel in the feature space. It was defined as the fraction of WMH voxels among those K neighbors. The voxel probabilities were presented in a so-called probability map, which is an image where each voxel intensity value is defined by the WMH probability of that voxel.

This study validated the segmentation approach on 20 subjects and a variety of performance metrics were given. The similarity index was reported as high as 0.80. The classification of each image voxel from a new patient relies on the voxel intensities and spatial information of previously manually classified training set. Since the MR image of different subjects at the same centre and across centers may have different intensity distribution ranges, this method may encounter difficulties for some subjects.

Earlier Pachai et al [63] had proposed a machine-learning algorithm including artificial neural networks, which face similar problem of dependencies on a training set as for the previous approach. An automated method from Stamatakis [64] is used to delineate large

brain lesions on T1-weighted structural images, which involves comparing the smoothed individual T1-weighted image to a control group using general linear model (GLM). The accuracy of this method depends on the performance of the spatial normalization technique. The normal anatomical variations in brain structure between the individual subject and the control group may present a problem for the registration accuracy and GLM, so a Gaussian smoothing filter is used to smooth out the anatomical differences which may also affect the reliability of the volumetric quantification of the lesions.

Another important pioneering work on segmentation of FLAIR images was by Jack et al [65] in 2001, which used histogram information to segment the WMH. The histogram of the FLAIR image was used in regression model to describe a cut-off intensity threshold, with the pixels above the threshold classified as WMHs. As a part of analysis contrast synthetic image phantoms were used with deliberately leukoaraiosis lesions were added to the MR images acquired in volunteers. The volume of leukoaraiosis lesions was precisely known and this was used as a gold standard for developing and testing their method. In order to do the segmentation the histogram was plotted (see Figure 18). The mode value of the histogram was identified (labeled M in Figure 18). Prior to determining the mode value of each slice, the histogram was smoothed using a moving average of three adjacent bins in order to improve the stability of mode determination. Then a horizontal cut point (labeled P) is established at $1/3$ of the height of the mode value.

Figure 18: Histogram based segmentation of FLAIR image

The intensities at which the horizontal cutoff value (P) intersects the histogram are denoted as X_L and X_U . The pixels in the intensity histogram that lie in the interval X_L and X_U define a central region of the histogram that will always represent normal brain tissue. The statistical properties of this central region of the histogram were characterized by five standard parameters: mean skewness, SD, kurtosis, and the total number of pixels in this region of the histogram. The statistical parameters above and the values X_L and X_U were used as independent variables in regression equations that are used to determine the threshold values T_L and T_U which are used to segment the histogram into three domains. Pixels to the left of T_L are assigned to the CSF; pixels between T_L and T_U are assigned to brain; and pixels to the right of T_U are assigned to the lesion. The segmentation output is shown in Figure 19 below.

Figure 19: FLAIR-histoseg. The top left panel represents a FLAIR image from a moderate synthetic phantom. The top right panel demonstrates the results of the FLAIR-histoseg method. The bottom panel represents the actual histogram of the FLAIR image after the skull and scalp have been removed. Pixels classified as normal brain are assigned green; leukoaraiosis lesion, red; CSF, blue.

The main disadvantage of this approach is that the algorithm was trained in advance using phantom image data sets in which correct tissue classification was known. Also the algorithm is solely based on synthetic WMH lesions and simulated phantom dataset, which may not possess the real biological properties of WMH. This segmentation works only for images with prominent differences in contrasts. This method also uses a single intensity threshold to segment the WMHs for whole brain for each slice of the brain images, which may misclassify some non-WMHs as WMHs, since some gray matter demonstrates signal intensity above the threshold in Hirono et al 2000 [65] and also image intensity inhomogeneities may be problematic.

Fuzzy connected algorithm is also a common approach that has been used in segmentation of WMH, lesions and Multiple sclerosis volumes. The use of fuzzy connected algorithm for segmentation can be found way back in 1997 by Mike et al [66] and Udupa et al [68] which needed user interaction and did not give spatial information on the WMHs. Wu et al [69] used an iterative fuzzy connected region growing approach starting from seeds that were 3.5 standard deviations above the mean. This approach used a fuzzy connected algorithm to segment the WMHs, and the Automated Labeling Pathway (ALP) to localize the WMHs into anatomical space. This method uses histogram to generate the WMH seeds, and then the fuzzy connected algorithm uses specific parameters to form a WMH cluster (containing the respective seed). The system updates the seeds iteratively and combines the scattered WMH clusters into the final WMH segmentation. Since the fuzzy connected algorithm uses different parameters for each seed, this method enables different threshold for each WMH cluster and avoids a single cut-off threshold for the whole brain or brain slice. Localization of WMHs was done by transferring

the John Hopkins University WM Atlas to subject's 3D image and further carried into subject FLAIR image space. The atlas regions in the subjects FLAIR image spaces were there used as ROI masks to localize the WMHs. The localized WMH volumes were quantified by multiplying voxel size by the number of WMH voxels inside the ROIs including anterior thalamic radiations, cortico-spinal tracts. The WMH volume estimates from WMH localization describe the spatial distribution of the WMH burden. The flow chart in Figure 20 explains the methodology of this approach. Figure 21 shows the procedure of WMH localization.

Figure 20: WMH Segmentation Flowchart. The processing steps used to automatically segment the WMHs on FLAIR MR Brain images.

Figure 21: An overview of the WMH localization procedure.

This method achieved correlations as high as $R^2 = 0.91$ against visual rating in 19 subjects. Although this method is accurate, the initial seed selection is still a challenge.

Another algorithm based on fuzzy interferencing along with the MRI brightness and region information was discussed in Admiraal Behloul, Van den Heuvel et al 2005 [70]. The segmentation method combines information from 3 different MR images: proton density (PD), T2-weighted and FLAIR image (Figure 22). In this type of approach the Montreal Neurological Institute (MNI) atlas template was used to define white matter regions. This way of template usage may risk missing peripheral lesions and the intensity-based approach is likely to miss faint lesions. Also like any method using multispectral information, the registration must be very accurate.

Figure 22: Need to use other modalities to perform segmentation

Figure 23 and Figure 24 shows the general algorithm workflow and the segmentation outcome respectively. Classification is based on prior knowledge about tissue properties for e.g. If voxel is inside brain and intensity in T2 is bright and in Flair is dark - CSF; Voxel is WM and Intensity in T2 is bright and Intensity in Flair is bright – WMH. This method validates against 100 subjects achieving the intra-class coefficient (ICC) score of 0.98 for WMH volume and similarity index (SI) or 0.75. This method has disadvantages that are related to the notions of redundancy and complementarities. PD, T2 and FLAIR images provide redundant information concerning WMH: they are hyperintense in all three scans. However, the extent of the lesions may not look the same on the different images leading to underestimation or overestimation problems always. Accuracy also depends on the registration between the three modalities.

Figure 23: General work flow of automatic segmentation algorithm

Figure 24: Segmentation outcome (GS – Gold Standard; Auto – Automatic segmentation result)

In another study involving multicenter datasets Dyrby et al [71] used a neural network segmentation approach. This study compared the automatic segmentation with a semi-automated segmentation approach and reported a mean SI of 0.45 (SD = 0.15) for WMH volume < 10 ml and 0.65 (SD = 0.15) for WMH volume > 30 ml.

Another option is to merge automated and manual methods (semi-automated) segmentation in order to achieve a balance between accuracy and efficiency. Previous work on semi-automated approaches described by DeCarli [72] and by Wen and Sachdev [73]. DeCarli used an operator-assisted method for removing the non-brain tissues and then proceeded by selection of sample tissue areas followed by threshold-based segmentation of WMH at 3.5 standard deviations (SD). Significant correlation ($r = 0.83$, $p < 0.001$) was reported between the WMH volume computed using intensity thresholding alone and operator guided tracing. No performance metrics were reported. Wen and Sachdev used MNI white matter probability map to indicate likely white matter and then used threshold-based segmentation at 3 SD above mean white matter intensity followed by manual editing to remove false positives. The ICC was reported to be 0.43 for whole brain WMH volume against the Fazekas visual rating scores by Fazekas, chawluk et al [74]. Payne, Fetzer et al [75] uses manual selection of tissue types to perform a tissue classification procedure in 16 scans. They reported a moderate correlation of r between 0.37 and 0.62 with previous rating scales.

A recent approach of segmentation uses the textural properties of the WMHs [76]. The intensity properties of textures are described using a grey-level co-occurrence matrix (COM). An element in two-dimensional matrix is represented as the probability of occurrence of a pair of intensity levels i_1 , i_2 of neighboring voxels v_1 , v_2 . The COM

describes the joint intensity distribution of neighboring voxels. Other useful characteristics like the gradient magnitude and the angle between gradients are also used as texture features and lesions are segmented from normal tissue by discriminating their texture features. It has been reported that false detection of WMH is estimated along the boundary of WM/GM interface and also at the border of thalamus and third ventricle, which are distinguished using a post processing approach, based on locations. This way of post processing based on locations may not be accurate. Another concern of this approach is the output of the segmentation if there are no WMH in the image. The clustering will still assume a predefined number of classes and may still try to keep searching for the WMH tissues.

4.3 Proposed approach

Most of the existing approaches use the global modeling of WMH intensities. Since the properties of WMH may vary across the image, global modeling is always not appropriate. Hence we introduce a local adaptive thresholding based on the contrast information of the WMH and the background tissue.

Our segmentation approach consists of four main steps.

1. Image Pre-processing.
2. Estimation of global threshold.
3. Adaptive local cluster based thresholding.
4. Post processing.

Image preprocessing: This is the first step. Linear alignment of the FLAIR onto the T1 image is done here. Interslice normalization is also performed here.

Estimation of global thresholding: A global threshold is estimated in this step. Different ways of estimating global threshold is explained in detail in the next section.

Adaptive local cluster based thresholding: This third step is to adaptively do a local thresholding on each cluster obtained after global thresholding. The initial clusters as obtained from the global thresholding, when subjected to this module would either grow or shrink or may even be removed completely depending on their tissue contrast and that of their background.

Post-processing: The final step is the post-processing step, which removes some of the spuriously detected WMH. A modification was tried in the global threshold estimation step by accounting for false positives beforehand. We also tried out another possibility of

enhancing threshold by an iterative approach in which the initial segmentation output can be used to re-estimate the global threshold based on the load estimation from the initial output. The flowchart in Figure 25 shows the processing workflow of the algorithm. We describe each step in detail as follows.

Figure 25: Flowchart showing the algorithm procedure

4.3.1 Image pre-processing

Prior to WMH quantification, tissue composition and selective brain image pre-processing are required for better analysis of the data. The preprocessing step includes skull stripping the FLAIR image followed by interslice intensity normalization.

Skull stripping of FLAIR using T1 information

The FLAIR image has to be skull-stripped before being analyzed for white matter hyperintensity estimation. Direct skull stripping algorithm may not be applicable on 2D FLAIR due to their very bright gray matter. So information from T1 image acquired at the same time as FLAIR images were used to solve the skull-stripping problem. The T1 images are skull stripped using a Brain Extraction Tool (BET) [77]. The BET algorithm uses spatial position and possibly voxel neighborhood information, in addition to the MRI intensity to perform the skull stripping. The output of skull stripping is a binary image with all the non-brain tissues masked out. Since the acquisition of T1 and FLAIR are almost at the same time, the brain mask obtained from the BET algorithm was applied onto the FLAIR image after a simple alignment to orient and map the FLAIR image with T1 images was done. By this way the skull stripped image of FLAIR is acquired. After skull stripping we use the simple histogram based GM removal in order to get the white matter mask of the FLAIR images, which will be later, used for segmentation procedure. The mask acts as a rough estimate of the WM mask. Our algorithm is based on this WM mask, any software or programs that can yield a more precise WM mask can be fit in this step and will aid in better segmentation output. A schematic representation of the process of extracting the intracranial component of FLAIR image and also the WM of the FLAIR is shown in the figure below.

Figure 26: Skull stripping of the FLAIR images

Inter-slice intensity normalization

The next pre-processing step is the inter-slice normalization. The intensity between and within the slices may not be uniform throughout all the slices during a scan. (See chapter 2 for sources of the non-uniformity). The top and bottom slices are usually not in the same intensities as the middle slices. To account for such variation it becomes indispensable to have this step of normalization. Histogram of each slice is plotted and the mode value is estimated. Each slice is then normalized by shifting the mode value, of the histogram, to a pre-assigned value equal to 0.3 (This value is arbitrarily chosen and can be changed to any value within which the white matter of the normalized image lies). An example of such intensity normalization is shown in the figure below

Figure 27: Interslice intensity correction

4.3.2 Global threshold estimation

The next step is the threshold determination. General procedure to determine the threshold is by the minimum mean-square error approach [78]. Suppose it is known a priori that an image contains only two principal brightness regions. The histogram of such a picture may be considered as an estimate of the brightness probability density function, $p(x)$. This overall density function would be the sum or mixture of two uni-modal densities, one for the light and one for the dark regions in the image. Furthermore, the mixture parameters would be proportional to the areas of the picture of brightness. If the form of the densities is known or assumed, then it is possible to determine an optimal threshold (in terms of minimum error) for segmenting the image into the two brightness regions. Suppose that an image contains two values combined with additive Gaussian noise, the mixture probability density function is given by

$P(x) = P_1 p_1(x) + P_2 p_2(x)$, where $p_1(x)$ and $p_2(x)$ are the probability density functions of two brightness levels. For the Gaussian case, it is

$$p(x) = \frac{P_1}{\sqrt{2\pi}\sigma_1} \exp\left[-\frac{(x-\mu_1)^2}{2\sigma_1^2}\right] + \frac{P_2}{\sqrt{2\pi}\sigma_2} \exp\left[-\frac{(x-\mu_2)^2}{2\sigma_2^2}\right] \quad (12)$$

where μ_1 and μ_2 are the mean values of the two brightness levels, σ_1 and σ_2 are the standard deviations about the means, and P_1 and P_2 are the a priori probabilities of the two levels. If the variances are equal then the threshold can be derived [78] easily to be

$$T = \frac{\mu_1 + \mu_2}{2} + \frac{\sigma^2}{\mu_1 - \mu_2} \ln\left(\frac{P_2}{P_1}\right) \quad (13)$$

Our approach of threshold estimation is based on maximizing the similarity index. For estimating the threshold we may need the two distributions, which we have to separate. For this we need to estimate the initial load of WMH to some approximation. Sixteen ground truth data were taken and their mean and standard deviation of the WMH was computed. From the knowledge about distribution of WM and WMH from ground truth, the WMH peak was found to be 0.4. (In reality it can be chosen any value greater than WM peak (0.3) and can be iteratively changed as we discuss later).

Figure 28: The two distributions within the histogram

The volume of the WM and WMH are also computed from the ground truth. The WMH distribution is given by

$$\pi_1 = \frac{V_{wmh}}{V_{wm}} \quad (14)$$

and the distribution of WM (only) was given by

$$\pi_1 = \frac{V_{wm} - V_{wmh}}{V_{wm}} \quad (15)$$

The ratio of thresholded pixels to the total volume of white matter can be estimated as the sum of pixels that are have intensity over 0.4 in distribution of only WM and the sum of pixels that have intensity over 0.4 in the distribution of WMH only. This is given by

$$\frac{\# \text{ thresholded pixels}}{V_{wm}} = \pi_1 p(I_1 > 0.4) + \pi_0 p(I_0 > 0.4) \quad (16)$$

The distribution of WMH can be derived as

$$\pi_1 = \left[\frac{\# \text{ thresholded pixels}}{V_{wm}} - p(I_0 > 0.4) \right] / \left[p(I_1 > 0.4) - p(I_0 > 0.4) \right] \quad (17)$$

After estimation of the WMH distribution, the similarity index is calculated. The similarity index is given as a ratio of correctly detected WMH to the total number of volume of WM (excluding the WMH). The Venn diagram and following equation may help understand this better.

Figure 29: Ground truth and the false positives and negatives

$$SI = \max \left(\frac{V_o - FN}{V_o + FP} \right) \quad (18)$$

The False negatives (FN) and the false positives (FP) can be computed as

$$FP = V_{wm} \cdot f p \quad f p = \int_{-\infty}^{\infty} P_0(x) dx \quad (19)$$

$$FN = V_o \cdot f n \quad f n = \int_{-\infty}^T P_1(x) dx \quad (20)$$

Substituting in the SI formula will give

$$SI = \left(\frac{V_o - V_o f n}{V_o + V_{wm} f p} \right) = \frac{1 - f n}{1 + (V_{wm}/V_o) \cdot f p} = \frac{1 - f n}{1 + (\pi_0/\pi_1) \cdot f p} \approx \frac{1 - f n}{1 + (f p/\pi_1)} \quad (21)$$

The threshold that maximizes this ratio is determined to be our global threshold.

Threshold estimation by accounting for false positives

$$FP^* = \frac{FP}{K}; SI \approx \frac{1 - fn}{1 + (fp/k * \pi_1)} \tag{22}$$

In the threshold estimation formula discussed above a small alteration is done in order to account for the false positives. We try to reduce the false positive by a factor K. The false positives are now designed to be reduced by this factor and hence estimating the threshold better by accounting for the false positives.

Threshold estimation by an iterative approach of segmentation

An iterative approach of segmentation was suggested as an enhancement of global threshold estimation. As described earlier the initial load estimated was based on certain assumptions. The algorithm assigns the initial load of WMH is based on the available groundtruth data as explained earlier. This initial load of WMH may not be accurate. Hence it is desirable to try out the segmentation process with an updated value of load estimates that are derived from the initial segmentation output.

Figure 30: Iterative approach for enhancing threshold estimation

The new WMH load can be computed from the initial segmentation. The new load values obtained from the initial segmentation along their means was updated and fed back to the algorithm. The entire process is re-run again to obtain a refined global threshold. A maximum of two iterations were tried out. Beyond two iterations the threshold is more likely to converge. We found that in some subjects this way of iteration enhanced the results and in some subjects this reduced the SI.

4.3.3 Segmentation and post processing

The estimated global threshold is applied on the FLAIR image to segment the WMH tissues. The segmented output needs to undergo the post-processing steps to remove the spuriously detected WMH tissues and hence to reduce the false positives of the segmentation. The underlying assumption is that white matter lesions occur either in the deep white matter or along the edge of the lateral ventricles. The WMH in the deep white matter is termed as deep-ventricular WMH and those along the edge of ventricles are termed as peri-ventricular WMH tissues. The goal of the post-processing is to incorporate the “high-level” information about the location of the detected lesions. The information about the shape and size of the lesion may also be used to do the post-processing. Our post-processing analysis removed the voxels within a distance from the edge of the brain. This is achieved by using a parameter, which accounts for the erosion of the brain surface pixels. The next post-processing procedure removed those WMH that are smaller than a specific size. These small blobs are likely to be caused by noise and falsely detected GM and hence have to be removed. If the blob size is less than a few pixels (the number of pixels can be decided either from the quality of data or from the optimization over a range of blob sizes),

then it is removed from the output. Also spurious WMH in between the lateral ventricles were also removed, as they do not form a part of the WMH volumetric. The three parameters for the outer erosion, size of the blob, filter size to remove pixels in between the ventricles were all iterated over a range of values to get the best optimal parameters. The outer erosion parameter ranged from 5 pixels to 50 pixels if the brain mask is used directly. These numbers were calculated based on the If the WM mask is used then this parameter ranged from 2 pixels to 20 pixels. The size of the blob ranged from 5 pixel to 50 pixel counts. The middle filter size was tried from 5 pixels to 30 pixels. These parameters were optimized for all these ranges of values. It was found that the best optimal parameter for outer erosion of brain is 15 pixels. Trying a range of many blob sizes, the best value was found to be 20 pixels meaning that blob sizes of less than 20 pixels can be treated as noise and can be removed. The mid-filter size gave the best results for 17 pixels. The results are measured by using the similarity index metrics.

4.3.4 Local adaptive thresholding based on the clusters

This step is based on the local adaptive thresholding method, which was discussed in section 3.1.2. After the global thresholding there are many clusters of different size and location in the image. The local thresholding treats the clusters individually and analyze the background of these tissues. Four layers of pixels around the clusters were taken as the background of the clusters. Mean and standard deviation of the cluster and the background tissues were calculated. If the component mean and standard deviation are notable different from that of the background then the cluster is thresholded (either grown or shrunk). Direct mean and standard deviation may not be appropriate because usually the mean of the

component is higher than background in most of the case and hence this always grows the cluster. We tried a few statistical descriptors like mean, median inter-quartile range to use as the mean of the component (foreground). We found that taking the mean of the inter-quartile range gave a very good indication of the foreground tissue contrasts. For the background, the mean and standard deviation are taken without any alteration. We use a simple formula to determine if there is a considerable difference between the contrast properties between the foreground and the background.

$$C_{FG} - C_{BG} / \sigma_{FG} > T_{FB} \quad (23)$$

where the C_{FG} is the contrast estimate of the foreground and it is the mean of the first inter-quartile range of the component and the C_{BG} is the mean of the background. σ_{FG} is the standard deviation of the component. T_{FB} is the cut-off threshold for the difference between the foreground and background. This value was optimized over a range of values and was found to be best at $T_{FB} = 2.9$. If the cluster satisfies the criteria in the above equation then a new threshold is determined for the cluster. The new threshold is based on the mean of the background and the standard deviation only.

$$T_{new} = C_{BG} + k_{BG} * \sigma_{BG} \quad (24)$$

We varied the parameter K_{BG} , which will determine how many standard deviations of the background, should be accounted for in order to estimate the new threshold. The value of K_{BG} , was found to be 2. The cluster is segmented with the new threshold. Preliminary results with these parameters are shown in the results section. Further fine-tuning of the algorithm is underway.

All the above described steps and processes are implemented in such a way that any step can be turned on or off during the algorithm run time. This important way of

implementation can help to use the algorithm even as a semi-automatic approach to deal with other medical image segmentation problem in future if needed.

4.4 Performance evaluation

4.4.1 Dataset

The algorithm was tested on the data obtained from the Cognitive Neuroscience Laboratory, Duke-NUS Graduate Medical School. Two sets of data were used. Both the data set were part of the Singapore Longitudinal Aging and Brain Study (SLABS). The first data set was a part of SLABS Phase I data acquired in 2005-2006, which was acquired from the 3T Siemens Allegra Scanner ($n = 248$ subjects). The FLAIR image was very low resolution and had a slice thickness of 5mm. The second data set is from the Phase II SLABS data, acquired in 2007-2008 ($n = 300$ subjects). The Phase II data was obtained from a 3T Siemens Trio Scanner. This has a high resolution and slice gap is 1mm.

4.4.2 Results of SLABS Phase I data

First we show the application of the algorithm on the Phase I data and then followed by the Phase II data. The Phase I data is of low resolution and segmentation on these low-resolution image was tedious. Manual segmentation by a single rater was available for this data set and was used as a ground truth. The segmentation output for the FLAIR Phase I data is shown in Figure 31 below.

Figure 31: Result of segmentation - a) Flair raw; b) Groundtruth; c) Thresholding+BlockTrimming d) Cluster thresholding

Figure 31 shows a slice of FLAIR original image and the ground truth from manual segmentation, a thresholded image and the cluster thresholded image. The cluster growing and also removal of cluster can be seen here. A slice from another subject after segmentation is shown in Figure 32.

Figure 32: Result of segmentation – Top left: FLAIR raw; Top Right: Groudtruth; Bottom Left: Thresholding (and block trimmed); Bottom Right: Cluster thresholding.

The initial shape of blob after thresholding can be seen to change after cluster thresholding. The final shape is closer to the shape of the ground truth than before.

Various parameters have been used for the segmentation as explained earlier. Some of them are the K value to determine the ratio of false positive that can be pre-accounted for, the block size threshold which is a threshold for pixel count. Any blob's pixel count is less than this threshold then it will be removed. Other parameters include the mid-filter size that will

remove the spurious WMH between the ventricles. The threshold parameter that was used for the cluster growing was $T_{BG} = 2.9$ and $K_{BG} = 2$. See section 4.3.4 for details of the parameters. The Jaccard similarity (JS) index for Phase I data was computed for different combinations of all this parameters. The best JS was found to be 0.47 with the manual segmentation. The manual segmentation was further divided into high confidence and low confidence WMH tissues. The JS for this high confidence WMH was found to be 0.55. Another classification was made as low loads and high loads based on the volume of WMH. It was found that for low loads our JS was found to be 0.37 and for high loads it was about 0.60. A note about performance metrics as how the JS was computed is given in the next section, which also highlights some of the draw back of this way of performance evaluation. The JS is given in Table 1. Missed classification and false positives are given in Appendix.

subject	AutoVol	GT2007Vol	GT2008Vol	GT2008_HC	JS2007	JS2008	JS2008_HC		
SS056a	55944	68832	78312	55500	0.532272	0.538081	0.685022		
SS068a	58764	99960	82308	49740	0.466815	0.54177	0.616808		
SS075a	8058	28620	14016	10392	0.218457	0.38935	0.450472		
SS076a	57396	86058	72030	54078	0.607112	0.599392	0.75224		
SS077a	50664	72918	68010	51006	0.626807	0.637199	0.775461		
SS116a	60006	73482	68088	46566	0.521647	0.567129	0.632387		
SS117a	70278	112926	83622	63402	0.507108	0.586467	0.704928		
SS120a	15648	13854	23004	17610	0.337959	0.569688	0.691486		
SS132a	85344	155088	129456	89172	0.484313	0.525157	0.705824		
SS136a	40578	34548	28314	19086	0.401846	0.425981	0.421993		
SS138a	9222	22470	5832	1638	0.116938	0.112639	0.163239		
SS139a	10482	19980	14136	9840	0.321791	0.391791	0.517473		
SS140a	71544	67446	82650	67248	0.649811	0.582938	0.654058		
SS142a	37260	60720	48258	30036	0.486708	0.518538	0.601371		
SS143a	38580	58458	50016	34560	0.447767	0.400816	0.503639		
SS148a	8376	7068	8154	0	0.258065	0.186988	0	lowloads	highloads
		0.910799	0.964977	0.968319	0.436589	0.47337	0.554775	0.374111	0.602885

Table 1: Jaccard similarity between groundtruth and automatic segmentation for 16 subjects. The JS 2008 is the column of interests. The JS2008_HC column gives the JS with high confidence WMH.

The Correlation analysis of manual volumes with automatic volumes and other health parameters were performed and are discussed in Appendix.

In Phase I data we encountered certain problems on applying the cluster thresholding. One of the problems is shown in Figure 33.

Figure 33: Problem of growing the GM tissues during cluster growing

The problem of GM inclusion can be resolved by using a more accurate WM mask that does not include the GM pixels. Generating an accurate WM mask is beyond the scope of this algorithm. Here in this algorithm we use a rough estimate of WM mask but if a more accurate WM mask can be provided then the algorithm may perform much better.

Another potential problem we faced by not matching with the groundtruth could be due to the inaccuracy of our ground truth estimation. The groundtruth was measured only by a single rater and hence it may not be accurate.

Figure 34: Reliability of the ground truth - overestimation of manual segmentation

There are instances when it was found that the manual segmentation was not estimated accurately. An instance in the figure above shows that the rater has many false inclusions of WMH voxels. Also, inconsistency was found around the ventricle areas. Hence, the comparison with the current ground truth (manually traced by single user) may not yield very good performance metrics for evaluation of our algorithm.

4.4.3 Results of SLABS Phase II data

As a second measure of validation, we tried our algorithm on the Phase II data set, which was from a different scanner (Siemens Tim Trio). In the segmentation of Phase II data, the FLAIR skull stripping can be directly done by using the BET algorithm. The problem of alignment with T1 is also overcome by this way. More importantly, only FLAIR modality is now sufficient to do the segmentation of WMH. The algorithm was applied for all the

subjects in the SLABS Phase II study ($N = 300$). Since there is no manual traced groundtruth for this dataset only visual inspection was done using manual raters. A sample of segmentation output from the Phase II data is shown in Figure 35 and Figure 36.

Figure 35: Result of segmentation on Phase II data

Figure 36: Result of segmentation on Phase II data

The algorithm can now be applied on other datasets and across different scanners too by just fine-tuning the input parameters detailed in the previous section.

4.5 A note about performance metrics

Results are typically reported in terms of similarity indices or volume correlations, compared with the manual ground truth (for Phase I data) and by visual inspection (for Phase II data as manual ground truth is not available). The similarity index values should be used with caution, as they depend on the quality of the ground truth as well as the total lesion load. Higher loads will lead to better performance. Jaccard similarity was used to estimate the similarity index between two samples. It is a standard metric for comparing the similarity and diversity between two datasets. Jaccard similarity between two sets A and B is defined as the size of intersection of the sets divided by the size of their union. It is given as

$$J = \frac{|A \cap B|}{|A \cup B|} \quad (25)$$

This can also be expressed in terms of true positive (TP), false positive (FP) and false negative (FN) as $TP / (FP + TP + FN)$. The Jaccard index is zero if the two sets are disjoint, i.e., they have no common members, and is one if they are identical. Higher numbers indicate better agreement in the sets, so when we apply this index to evaluate the agreement of brain segmentation results, the goal is to get as close to 1 as possible. In estimation of the performance metrics we do face some problems in the reliability of the performance evaluation because in general performance analyses based on similarity indices or correlations are inherently flawed, as there appears to be a great mismatch at how experts define WMH. The manual measurements, performance metrics and the comparison

of the automatic segmentation have not been consistent throughout in the literature. The difference of measurement (in volume) can reach a factor of 2, which means some experts are much more conservative than the others. In other words, some of them will include a lot of WMH, while others, would only choose the most prominent one. The conservativeness of the ground truth also depends on which images were used.

4.6 Conclusion

The proposed algorithm consisted of a few novel steps like accounting for the number of false positives beforehand; iteratively changing the global threshold; cluster based local thresholding. The algorithm was tested and validated for the SLABS Phase I and Phase II datasets and worked well for both the datasets. Quantitative validation was done for Phase I data and qualitative validation was performed for Phase II. The algorithm was able to pick most of high confidence WMH. The Jaccard similarity index for high confidence WMH for Phase I data (16 randomly chosen subjects with available groundtruth information) was found to be 0.55. The JS for subjects with high loads was 0.60. The Pearson's correlation analysis of the manual volumes and the volumes generated from the automatic algorithm (all subjects) was found to be 0.76. The computed automatic volumes followed a similar trend as manual volumes when correlated with the health risk factors and cognition scores of the subjects. As shown in examples before, the groundtruth used for this study was not very accurate. With a more standard groundtruth, the automatic output would match the manual output more closely and accurately. The algorithm was also applied on the Phase II dataset (no manual groundtruth data available). Qualitative validation was done with the

help of experts. The algorithm was hence tested and validated with images across different scanners. Further validation of the algorithm can be done upon availability of more datasets.

5 Segmentation of MR abdomen images

5.1 Motivation

Human adipose tissues are highly active metabolic, endocrine and are integrally involved in coordinating a variety of biological process including energy metabolism, neuro-endocrine function, and immune-function. Excessive human body fat distribution in humans is closely correlated with increased risk of cardiovascular and metabolic diseases such as atherosclerosis, hypertension, and non-insulin-dependent diabetes mellitus [79],[80] and [81]. In particular, the amount of intra-abdominal fat (or visceral fat) has direct bearing on the risk factor of these diseases. Therefore, traditional simple approaches to quantify obesity based on anthropometric parameters (e.g., body mass index [BMI], and waist-to-hip ratio, etc) do not give the best picture of the correlated risk factors of certain diseases [82]. Instead, human body fat imaging can be an effective diagnostic tool since both the absolute amount of fat and the anatomical fat distribution can be derived from images. MRI is preferred over CT due to non-ionizing radiations and also due to the feasibility of longitudinal studies. Manual segmentation of all the slices of the acquired MRI image is more time consuming and may lead to intra- and inter- observer variability. This arises for the need to design an automatic segmentation algorithm to segment out abdomen tissues. Also prime importance lies in the quantification of fat tissues as visceral adipose tissues (VAT) and the subcutaneous adipose tissue (SAT). VAT can be measured accurately only by imaging techniques, since waist circumference, often used as index of abdominal adiposity in the clinical setting, is associated, but not as well as VAT, with the risk of

cardiovascular disease (CVD) [83]. Another important index is the ratio between VAT and SAT that is associated with the development of all features of the metabolic syndrome, accompanying insulin resistance and CVD [83]. Therefore, detection and quantification of VAT and SAT is a crucial issue for identifying subjects with abdominal obesity-related risks.

5.2 Review of previous techniques

The segmentation problem of abdomen tissues separates the fat tissues from the non-fat background. Furthermore it aims to separate the fat tissues into intra-abdominal (also known as VAT) and extra-abdominal fat tissues (also known as SAT). Most of the segmentation technique that is available as of date is applicable mainly to the CT data [84][85]. Recently with the advent of new MRI sequences, MRI abdomen imaging is getting more popular than CT and the segmentation techniques applicable on CT images are usually modified to suit for MRI Parameters.

Measuring the fat quantity and distribution in the abdomen by MRI is generally laborious. A simple approach is to quantify one slice and then extrapolating this single slice to the entire volume [86]. This analysis with single abdominal slice is advantages in case of acquisition and further analysis. However single abdominal slice does not give an accurate measure of entire fat volume as it relies only on the location of the single slice chosen for quantification. If this slice is not chosen appropriately then the amount of SAT and VAT measured may not be accurate also affecting the correlations of total volumes of SAT and VAT [87]. Thus, accurate determination of SAT and VAT requires multislice imaging and

analysis. Starting from the seminal work by Lancaster et al [88], several image analysis techniques were proposed to address this issue.

Generally two steps are required to identify the volume of internal and external adipose tissues. The first step is to separate fat from nonfat tissue. Thresholding on a histogram [89][90] is frequently used for this task and could be accomplished automatically with good agreement between automatically and manually set thresholds. The second step is to locate VAT and SAT on MR image slices, location of either one can give the other one by just subtracting from the total fat tissues. It is often time consuming to delineate the region of interest (ROI) of VAT because of the complex structure of the viscera. [91]. Almost all the previous studies of this step have reported requiring more or less human interventions, which are either laborious or subject to technical problems [92][93].

Elbers et al. [94] measured visceral fat areas using an algorithmic seed-growing procedure. The neighboring pixels of this seed and their adjacent pixels that lay between a selected upper and lower signal intensity value were automatically included in the delineation of the fat area. However, both the seed points and the thresholds had to be defined manually. In Gronemeyer et al.'s study [95] in 2000, although the thresholds were automatically set, VAT had to be manually removed for the calculation of the SAT area. Poll et al.'s study [96] in 2002 took a similar approach, but the VAT was separated by ROIs drawn manually.

Diane et al [97] proposed a method for quantifying VAT and SAT from water-saturation MRIs. The water saturation and non-water saturation sequences were intensity thresholded to get the adipose tissues. The SAT was extracted using NLM Insight Toolkit's

implementation of confidence-connected algorithm, which uses the region growing approach. This approach is robust if there is only a thin connection between the VAT and SAT. If the connections are more prominent then this approach fails by underestimating VAT and overestimating VAT.

Figure 37: a: Water-saturation MRI image (L3). b: Same level screenshot computer segmentation technique showing VAT (white) and SAT (green)

Figure 38: a: Non-water-saturation MRI image (L3). b: Same level screenshot computer segmentation technique showing underestimation of VAT (white) and overestimation of SAT (green).

Kullberg et al 2007 [98] used threshold information to segment the adipose tissues and a prior geometric model to separate VAT from SAT. This approach is highly dependent on the pelvis model being incorporated into the algorithm. This may not be the same for all

populations and may also fail if they dimensions of images are a variant. The pelvic model is shown in the figure below

Figure 39: Single slice illustration of the pelvis model in use. (a) Adipose Tissue (AT) pixels within the mask (VAT), (b) highlighted area illustrates the region anterior to the pelvis model and (c) the result (VAT) after the AT threshold is raised posterior to the pelvis model.

Figure 40: Illustration of step (a) shows the pixels in VAT and the striped region shows one slice of the vertebra model used to include geometrical a priori knowledge.

Fuzzy clustering for thresholding has also found its application widespread for the fat segmentation. A study that used fuzzy clustering followed by the active contour algorithm was found in [99]. The adipose tissue is labeled across the abdomen by unsupervised

classification using fuzzy c-means clustering (FCM) and locally determined thresholds. The abdomen boundary is segmented, and the visceral adipose tissue is separated from the subcutaneous tissue by means of active contours;

The FCM algorithm differs from other clustering algorithms (such as k-means clustering) as each voxel is associated with more than one cluster. This is done by means of continuous, fuzzy, membership values reflecting a certain degree of membership to all clusters – instead of assigning voxels to only a single cluster each. These membership values form a common reference frame of the connection between intensity and tissue types comparable between images, since they are derived to resemble the individual structure of the data. The algorithm is formulated around the minimization of a criterion function, J_{FCM} , expressing the quality of the clusters identified by a weighted sum of squared distances:

$$J_{FCM} = \sum_{j \in \Omega} \sum_{k=1}^C u_{jk}^q |y_j - v_k|^2 \quad (26)$$

The subscript j denotes a voxel location in the image domain, k is the class index, C is the total number of classes. The parameter u_{jk} is the fuzzy membership value of class k for voxel j , and q is a weighting exponent greater than 1 defining the fuzziness of the classifications. y_j is the intensity of voxel j and v_k is the centroid of class k . The membership values must

conform to the following constraints: $0 \leq u_{jk} \leq 1$ and $\sum_{k=1}^C u_{jk} = 1$

The criterion function J_{FCM} is minimized with respect to u_{jk} and v_k . This is done through iteratively updating them by evaluating the following two equations:

$$v_k = \frac{\sum_{j \in \Omega} u_{jk}^q y_j}{\sum_{j \in \Omega} u_{jk}^q} \quad \text{and} \quad u_{jk} = \frac{|y_j - v_k|^{\frac{-2}{q-1}}}{\sum_{k=1}^C |y_j - v_k|^{\frac{-2}{q-1}}} \quad (27)$$

The threshold is initially determined using this fuzzy c-means clustering. After extracting out the adipose tissue using the threshold active contour algorithm is applied on the adipose tissue to determine the boundaries between SAT and VAT. The subcutaneous adipose tissue layer is subdivided into deep and superficial part by dynamic programming and a polar transformation of image data. After the unsupervised FCM approach to determine a threshold, active contour algorithm is applied to get the rough estimate of the abdomen boundary. The curve deformation of the active contour algorithm is shown in Figure 41.

Figure 41: An example of the curve deformation to achieve a rough estimate of the abdomen boundary in an image slice blurred with a Gaussian kernel.

Another unsupervised methodology based on a adaptive fuzzy clustering approach was demonstrated by Positano et al [100] to allow processing of 3D MRI datasets in a short time without any user interaction. This method is quite popular approach for segmentation of MR abdomen tissues. This method is fairly complex and involves multi-step process. The first step involves a adaptive fuzzy clustering algorithm (AFCM). Positano extended the idea of

fuzzy clustering to adaptive fuzzy clustering algorithm (AFCM) where intensity homogeneities $g(x,y)$ was modeled as a gain field that caused image intensities to vary smoothly and slowly through the image space. The gain field iteratively adapted to the intensity inhomogeneities at each iteration step following the process of the fuzzy clustering algorithm.

$$J_{AFCM} = \sum_{x,y \in I} \sum_{k=1}^C u_{jk}^q D_k(x,y) + \beta \sum_{k=1}^C \eta g_k(x,y) \quad (28)$$

where $D_k(x,y)$ was a term that imposes a spatial constraint on grouping similar pixels extending the distance defined in above equation to the pixel neighbors. The gain $g_k(x,y)$ represented a spatially smooth and slowly varying gain field, obtained as a weighted sum of 4th order cubic spline functions. The correction gain $g_k(x,y)$ calculated during the AFCM process could be exploited to reconstruct the corrected image by the equation:

$$\bar{I}(x,y) = I(x,y) \sum_{k=1}^C \frac{u_k(x,y)}{g_k(x,y)} \quad (29)$$

where the corrected image $\bar{I}(x,y)$ was calculated weighting the original image by the tissue masks and dividing the results for the relevant compensation gain. The mathematics is quite complex and implementation of this algorithm is tedious.

The histogram is refitted after correcting for the signal inhomogeneities. Then active contour algorithm is applied to estimate the SAT and VAT boundaries. With better signal inhomogeneity correction the active contour model accurately segments out SAT from VAT. The final output is shown in Figure 42.

Figure 42: Automatic segmentation performed by the standard FCM algorithm (a) and by the AFCM algorithm (b). Green and yellow contours define external and internal boundaries of SAT, respectively. Histogram for VAT evaluation is calculated inside the blue contour. Histogram extracted from the VAT area before (c) and after (d) signal correction performed by the gain field calculated by AFCM algorithm. Red curves represent automatic fitting of the VAT peak.

This methodology has been successfully used in abdominal fat distribution studies [100],[102] and [103]. Independent validation of this approach can be found in [104]. However, the results of the unsupervised method strongly depend on image quality and in particular on the presence of image intensity inhomogeneities [104]. In this study an automatic MR signal correction procedure was integrated in the fuzzy clustering algorithm to obtain a good quality of the unsupervised segmentation procedure also in presence of relevant image intensity distortions. The performance of the new approach was compared with the standard methodology by the volumetric analysis of adipose tissue distribution in

20 subjects. However this algorithm will fail for thick boundaries between internal and external fat. Suppose the VAT and SAT does not have narrow connections between them the active contour approach may form a loop within the internal adipose tissues.

Liou in 2006 [105] proposed a fully automatic algorithm for separation of SAT and VAT that allowed abdominal fat distribution to be analyzed in a short time without any user intervention. The method was reported to be accurate to within a few percent errors as compared to manual partition of SAT and VAT distributions. In this method firstly a Total Abdominal Adipose Tissue mask is generated using initial threshold that was empirically set at 20% higher than the average pixel value of an image slice. An approximate body mask was obtained by binarizing the image at that threshold. The next step involved the formation of visceral mask. Two initial visceral masks were generated by different approaches on the basis of anatomical knowledge of the abdomen. The results were visually examined by a physician and compared to a manual segmentation approach. This study assumes that the anterior boundaries that ran along the inner and outer sides of the SAT on an axial MR slice were simple smooth curves convex ventrally except for the umbilical discontinuity on some slices. This assumption may not always be valid. Another drawback of the approach is that they have a predefined threshold for deciding the classification of the visceral adipose tissue. Only those tissues that fall within the Euclidean distance of the pre-determined threshold is classified as visceral fat. This approach may not be suitable when the connections between VAT and SAT are not narrower because filling up of the internal area may not be closed if there are broader connections between the two tissues.

5.3 Proposed approach

As highlighted earlier, the entire process of segmentation of MR abdomen tissues consists of two major steps. The first step, segments out the fat tissues from the non-fat background of the image. The second step further classifies the fat tissues into VAT and SAT. Of all the different existing methods available for MR data, only two approaches, [100] and [105] were considered to be completely automatic. The drawbacks of these methods were discussed earlier in the chapter.

We hence propose a novel algorithm, which is completely automatic, and also overcoming most of the problems of the existing methods. For the proposed approach we make use of the In-Phase and the Fat modality of the acquired images. Water and Out-Phase modality has not been used. Our segmentation approach consists of the usual two steps of any segmentation algorithm used for fat quantification. In the first step we threshold the fat images of the DIXON sequence and hence segment out the fat tissues from the non-fat tissue background. The next step uses a modified graph-cut algorithm [105] to classify SAT and VAT volumes from the fat tissues. For this step we use the information from the In-Phase modality images as well for better classification. A sample slice of In-Phase and Fat modality can be seen in image below.

Figure 43: Fat initial images. Left: Fat image, right: In-Phase image

5.3.1 Segmentation of fat tissues:

This step separates the fat tissues from non-fat tissues. This step comprises of a few image pre-processing steps followed by thresholding of the image. As an initial preprocessing step, the arms of the patient, which appears as a part of the scanned image, needs to be segmented out from the abdomen image. In order to remove the hands from the image, the image is treated as a 3D volume and all the connected components of the image are estimated. The problem upon inclusion of arm was resolved by retaining the largest connected component (which is the abdomen 3D volumes) and removing the component that is not connected to the largest connected component and also which lies close to the outer bound of the image. This will eventually remove the arms in the side of the image. A masked image of the abdomen data excluding hands is shown in Figure 44.

Figure 44: Mask of Abdomen Image excluding arms of the patient

As a next preprocessing step the intensity of the image is normalized. This step is needed to account for the image intensity inhomogeneities. We apply a non-parametric non-uniformity correction algorithm [107] to correct the image for inhomogeneities.

Simple thresholding using Otsu's method [35], described in section 3.1 was applied on to the inhomogeneity corrected images. We also tried an entropy-based thresholding [107] but since the results were identical to the more widely used Otsu's method, we used the Otsu's approach for thresholding. The entropy is a statistical measure of randomness that can be used to characterize the texture of the input image. Hence an entropy-based thresholding can be used if the image is noisier. The entropy-based thresholding can also be applied if the image will be from across different scanners. More exploration can be done in this direction as a part of designing an algorithm that can be applied across different scanners and sequences (This is beyond the scope of the current work). A serious problem in using only fat image will be that the bones may have similar intensities, as the adipose tissues also will be segmented along with the adipose tissues. For enhancement purpose the water images can be used to assists in removing the bones to a certain extent. Trying to completely segment out adipose tissues excluding all the bone tissues is a something that can be done as

an extension to the current work. The scope of the current work is only segmentation of adipose tissues and separating them into SAT and VAT.

Figure 45: The Adipose tissue mask is shown. Left: Initial Fat Slice; Right: Adipose tissue mask from the thresholded image

5.3.2 Segmentation of VAT and SAT

Once we extract out the mask of the adipose tissue as shown in the previous step the next problem is to classify the adipose tissues further into VAT and SAT with the help of a cut at the boundary joining VAT and SAT. An example of a boundary where a cut is needed, in order to segment the VAT and the SAT, is shown in the image below.

Figure 46: Adipose tissue mask. The place where boundary between SAT and VAT needs to be cut is indicated as blue. A cut at this place will separate the SAT and VAT

Usually when the connections between the VAT and the SAT are narrower then the active contour algorithm provides a good segmentation output as explained in the previous section. This may not be assumed always. The active contour algorithm may also fail if the image gradient is not well defined. So we propose a Graph-cut based algorithm that can solve this problem.

5.3.2.1 Graph theoretical framework and min-cut segmentation

A connected graph $G = (V; E)$ is constructed from the image as detailed in Section 3.5. Each vertex is connected by a weighted edge to the source and the sink. Consider a cut on this graph separating the source and the sink into two disjoint sets. The cost of this cut is defined as the sum of the weights of the edges severed during the process. The minimum-cut problem on a graph is to find a cut of the minimum cost over all possible cuts in the graph. This minimum-cut can be determined by finding the maximum flow from the source to the sink. Using graph cuts, the global optimum can be found with polynomial time complexity.

5.3.2.2 Edge weight assignment using distance transform

In grayscale images, the edge weights are usually assigned as a function of the intensity values of the pixels, with edges connecting similar pixels possessing larger weights and edges connecting dissimilar pixels possessing smaller weights. The cut therefore depends on the gradient information. Ours is binary image segmentation and we need to find an alternative way other than the use of intensity information to assign weights and make sure

that the cut still happens at the desired position. The minimum cut should happen along the weak boundary between the VAT and the SAT regions thus separating them both. It should also be able to handle certain strong connections, which are false and may exist between the two classes of adipose tissues.

In order to comply with these conditions, the weights of the edges connecting the foreground (π_0) and the background (π_1) should be of a lower value and the edges connecting the foreground pixels should have larger weights, but not large enough to prevent breaking the strong connection between the VAT and SAT. We make use of the distance transform for weight assignment. We determine the distance transform of the binary image. The distance transform produces a grayscale image with the boundary pixels having value equal to one, which increases as we proceed to the center of the region. The distance transform applied on the binary image results in a grayscale image where the intensity of the foreground pixels denotes the distance of the pixel to the closest boundary from that pixel. It can be defined mathematically as

$$D(v_i) = \min_{v_0 \in \pi_0} (d(v_i, v_0)) \quad (30)$$

where v_i is the foreground vertice, v_0 is a vertex corresponding to the background and d is any distance measure. An example of the distance transform is shown in Figure 47. Weights are assigned based on the distance transform. The weight of an edge (w_{ij}) can be mathematically expressed as

$$w_{ij} = \max_{v_i, v_j \in \pi_1} (D(v_i), D(v_j)) \quad (31)$$

where $D(v_i)$ and $D(v_j)$ are the distance transform values of the foreground vertices v_i and v_j respectively.

Figure 47: Left: Binary image of adipose tissues; Right: Grayscale distance transform of the image

Assigning weights based on the distance transform will therefore ensure cutting along the boundary and along the weak connections between the two tissues, VAT and SAT. Further, this type of edge weight assignment provides the option of using any higher power p of the distance transform values as shown below:

$$D(v_i) = \left[\min_{v_0 \in \pi_0} (d(v_i, v_0)) \right]^p \quad (32)$$

This helps to prevent cutting of stronger connections. Currently, we shall use $p = 1$. Higher value of p is yet to be explored. The distance transform thus provides an efficient way to assign weights for the edges connecting foreground vertices. The edges connecting background vertices are assigned a common weight equal to a constant β . (See 3.5 for details). The value of β has to be appropriately chosen for proper segmentation.

5.3.2.3 Seed point selection

The foreground pixels and certain background pixels are selected automatically from the image. The background seeds are connected to the source and the foreground seeds to the sink. The edges connecting the seeds with the source/sink are assigned larger weights. With

edges connecting the neighboring pixel vertices equal to β , weights of the edges connecting the seed points with the source or sink should be equal to at least 4β . This prevents the min-cut algorithm from cutting through the seed pixels. The foreground seeds will always be part of the object segmented by the min-cut algorithm and the background seeds will always be part of the background obtained by minimum-cut. We selected the perimeter of the abdomen as a seed belonging to foreground seed. A few pixels on the centre of the image and also those that are a part of the abdomen tissue mask are taken as the background seeds. Although this is a crude approach of seed selection a little retrospection showed that this way of selecting the seed will always assign the foreground and background seeds to the SAT and VAT. The background seeds can be chosen to be the dark pixels (image background) as well. We use a region inside the abdomen tissue as we are sure that this regions lies in the background of the image. Once these seeds are selected, they act as hard constraints for segmentation. Since the min-cut algorithm cuts along edges such that the sum of the edge weights being severed is the smallest, the severed edges will thus provide the best fit to the actual region of interest.

5.3.2.4 Foreground seeds

The foreground seed acts as a hard constraint and is very important for the segmentation to run appropriately. Hence a small note of the different types of foreground seeds that was used may be useful. The adipose tissue mask for which the separation of SAT and VAT is to be done is shown in Figure 48 (a). Initially foreground seeds were chosen to be the few layers of pixels from the boundary of the image (Figure 48 (b)). This type of foreground seed does not account for the leaks and gaps in the surface fat of the image. Hence a convex hull was estimated. The convex hull for a set of points X in a real vector space is the

minimal convex set containing X . In other words let us suppose we have set of points X in vector space. The convex hull is the boundary that engulfs all points in X such that line joining any two points in X will be engulfed by the boundary completely. A FG boundary was modified using the convex hull algorithm. The FG seed based on convex hull approach is shown in Figure 48 (c).

Figure 48: Figure showing different FG seeds. (a) Adipose tissue mask, (b) FG based on outer layers of binary mask, (c) based on convex hull approach, (d) based on multi-modal information

Furthermore an enhanced estimate of FG seed was calculated based on the mutual information from In-Phase modality images. Image boundary is extracted from the In-Phase modality images. Since the tissues are brighter than the fat modality the In-Phase images may help in better foreground seed selection (Figure 48 (d)). The output of each approach and the drawbacks are shown in the next section.

5.4 Performance evaluation

The proposed approach was implemented using Matlab. Manual segmentation is very tedious and is not available. Only qualitative validation was done with the help of experts. Any segmentation output that did not correctly segment out the SAT and the VAT was considered to be a failure. Depending on the failure rates the seed selection was refined such that the approach has the least failure rate. The process of seed selections and their refinement along with the failure rates has been mentioned below.

5.4.1 Dataset

We used the abdominal data of Singapore Longitudinal Aging and Brain Study (SLABS) Phase II obtained from the Cognitive Neuroscience Laboratory, Duke-NUS Graduate Medical School. Phase II SLABS data, acquired was acquired in 2007-2008 ($n = 300$ subjects) using the DIXON sequences (See Chapter 2 for more details). The Phase II data was obtained from a 3T Siemens Trio Scanner.

5.4.2 Results based on Initial Foreground seeds

In order to get the foreground seed we use the boundary information from the fat image. A few layers of the outer boundary of the image are taken as the foreground seed. The background seed is taken as a small 10×10 voxel rectangular region in the central portion of the image (which is always a part of the internal fat) or just dark pixels (background) of the image. Then the min-cut algorithm is applied on the thresholded image with the respective foreground and background seeds. Although this approach gave reasonably good results, there was a problem of SAT leaking into the VAT areas. Visual inspection was done

on the inputs. The algorithm was applied on a sample dataset of 50 randomly chosen subjects from the SLABS Phase II study. The failure rate was found to be approximately 23%. The wrong segmentations were due to the inaccurate image boundaries. An example of one such failure is shown below.

Figure 49: Top: Thresholded Image. Bottom left: Incorrect foreground layer due to image boundaries with holes (leaks). Bottom right: Segmentation output. Leaking of SAT into VAT

In order to correct for the leaking problem we used an image boundary formed from the convex hull of the fat image. The convex hull approach for the image boundary solved the above-mentioned problem. The same slice with a convex hull boundary approach is shown in the Figure 50.

Figure 50: Convex hull of boundary solves the leaking problem. Thresholded image is shown in left; Foreground seed is shown in middle; segmentation result is shown in the right.

However the boundary based on convex hull gave rise to a new set of problem of missing portions of surface fat. This is because the minimum path is chosen along the image boundary, which is a result of convex hull and hence may not be a part of the real boundary. This approach was tested on the same sample of 50 random subjects as mentioned earlier and the failure rate was found to be about 12%. One of the failure slices using the convex hull boundary approach is shown in figure below.

Figure 51: A case of failure of convex hull boundary approach by not detecting SAT accurately

5.4.3 Results based on a better estimate of foreground seed

To overcome the previous problem we used mutual information from In-Phase modality images. Image boundary is extracted from the In-Phase modality images. Since the tissues are brighter than the fat modality the In-Phase images may help in better foreground seed

selection. Information from the In-Phase image and the Fat image is used for this step. Initially the convex hull boundary of the fat image is computed. Next the boundary of the In-Phase image is computed by simple thresholding. The absolute difference of the two boundaries is estimated and subtracted from the boundary of In-Phase image. This way the problems causing the leaks as mentioned above can be resolved because of complete filling of the image and the problems due to the convex-hull boundary approach can be corrected as the gap between the convex-hull and the actual boundary of image is removed thereby the foreground seed passing through the real boundary of the image. This method of estimating the foreground seed gives the best segmentation output. The algorithm was then applied on all the 300 subjects of the SLABS Phase II study and the failure rate was found to be only less than 3%. The output (single slice) is shown in figure below.

Figure 52: (a) The initial thresholded image; (b) the boundary from the convex hull of image; (c) Better estimate of foreground layers using boundary information from In-Phase modality image; (d) Segmentation output from the better estimate of foreground seed layers.

The table below shows the different seed selection approaches and their failure rates.

Method of seed selection for segmentation	Number of subjects tested	Number of subjects failed	Failure rate
Using boundary information from Fat image	150	35	23.3%
Using Convex hull approach on Fat image	150	18	12.0%
Using mutual information from In-Phase modality	300	8	2.6%

Table 2: Failure rates of the segmentation output based on different methods of seed selection

The algorithm performed well for almost all the subjects. The algorithm worked well for subjects with very thin and thick surface and visceral fat also. The approach did not fail for subjects with umbilical discontinuities. Example of one such image is shown in figure below.

Figure 53: Segmentation output for images with discontinuous surface fat

Correlation of segmentation output volumes with the various health risk factors (data available as a part of SLABS Phase II study) was done as a measure of external validity. This analysis is reported in Appendix.

The algorithm takes approximately 5-8 minutes to complete the 3D volumes estimation of the adipose tissues depending on the inclusion of inhomogeneity correction step, which is an optional step. The proposed method has successfully overcome many difficulties encountered in automatically identifying SAT and VAT using MR images as explained in the previous section. Since no manual segmentation is available for this dataset and hence only qualitative validation was done. Currently SIEMENS MEDICAL SOLUTIONS is testing the algorithm for various pathological and non-pathological images.

5.5 Conclusion

The algorithm performed good segmentation of the abdominal fat images in spite of the different brightness of adipose tissues, different thickness and shapes of SAT and VAT. The algorithm performed well even in case of subjects with umbilical discontinuities that disrupted SAT. This approach accounted for heterogeneous magnetic field by intensity inhomogeneity correction before thresholding. Although the pulse sequences used in the previous studies and our studies were different, our proposed method seems to be more accurate and less biased. Our approach overcomes these problems and can be applicable to images, which are non-homogenous. Since our approach of classification of SAT and VAT is from a binary mask of adipose tissue, we can apply our algorithm to images from other sequences provided the adipose tissue is segmented using their respective approach that has been used for that specific sequences. In conclusion, we have shown that the proposed automated analysis of visceral and subcutaneous abdominal fat volumes using axial MR images is sufficiently rapid, reliable, and accurate to be a surrogate measure to replace laborious manual measurement. This technique may serve as a methodological basis for many clinical and scientific applications, particularly those dealing with a large amount of data such as epidemiological studies.

Publications

1. Parimal SA, Zagorodnov V, Chuah YML, Chee MWL. Establishment of an automated method of intra and extra abdominal fat quantification: Correlates with BMI, Fasting glucose and blood pressure. Singapore General Hospital – 17th Annual Scientific Meeting (SGH-ASM), 2008.
2. KHM Chen, Parimal SA, Sam KY Sim, Hui Zheng, Vitali Z, Sadananthan SA, Michael WL Chee. Abdominal adiposity, vascular risk factors and cognitive decline in healthy elderly Singaporeans. 4th Congress of the International Society for Vascular Behavioural and Cognitive Disorders (VASCOG), 2009.
3. SA Parimal, Karren HM Chen, V Zagarodnov, SA Sadananthan, Michael WL Chee. Establishment of a novel automated approach for quantification of adipose tissues: Correlates with BMI, Fasting glucose and Blood Pressure. International Society for Magnetic Resonance in Medicine (ISMRM), 2009.

Appendix A – WMH segmentation analysis

The segmentation output is shown in 4.4. The false positives and the missed detections for segmentation output with respect to the groundtruth data is shown in table below. All the values are in percentages.

Subject	FalseAlarm (%)	MissedDetection	Prob of Detect	Miss classification Rate
SS056a	8.917364	47.968968	52.031032	56.886332
SS068a	13.799317	58.24169	41.75831	72.041007
SS075a	141.658031	44.248705	55.751295	185.906736
SS076a	23.259037	27.428109	72.571891	50.687146
SS077a	10.452962	27.093596	72.906404	37.546558
SS116a	55.691531	37.791375	62.208625	93.482906
SS117a	13.494301	50.170368	49.829632	63.664669
SS120a	61.931572	50.151581	49.848419	112.083153
SS132a	14.042946	58.521056	41.478944	72.564001
SS136a	56.547412	46.509205	53.490795	103.056617
SS138a	282.613333	100	0	382.613333
SS139a	33.608815	75.068871	24.931129	108.677686
SS140a	41.704475	49.924384	50.075616	91.628859
SS142a	28.00048	58.569371	41.430629	86.569851
SS143a	19.735072	58.832661	41.167339	78.567733
SS148a	54.702495	93.28215	6.71785	147.984645

Table 3: False positives and missed detection

In Phase I data, 234 subjects had all the manual segmentation and other health parameter indicators. Hence for the total of n=234 subjects the correlation analysis of automatic volumes with manual volume and other health parameters were performed. The correlation with manual volume was 0.76. The automatic volumes also followed similar trend to manual volumes when correlated with the health parameters. The subjects with low contrasts were visually identified. There were three kinds of classification based on the confidence of the rater – highly confident about low contrast subjects, less confident about low contrast subjects.

Correlation with health parameters	WMH – Manual (N = 248)	WMH- Auto (N = 234)	WMH – Auto; Low Contrast (N = 183)	WMH – Auto 116
Description	Manual	Automatic	Low Contrast	Low & Medium Contrast
WMH - Manual		0.76**	0.80**	0.84**
Age	0.38**	0.22**	0.23**	-
Gender	-	-	-	-
Education	-	-	-	-
BMI	-	-	-	-
LDL	-	-	-	-
HDL	0.18**	-	-	-
Systolic	0.21**	0.14*	0.24**	0.29**
Diastolic	-	-	-	-
Glucose	-	0.13*	-	-
Homocysteine	-	0.15*	-	-
Folate	-	-	-	-
B12	-	-	-	-0.21*
Cognition				
Attention	-	-	-	-
Verbal Memory	-	-	-	-
NV Memory	-	-	-	-
Speed	-0.26**	-0.17**	-0.14*	-
Executive	-0.13*	-	-	-
Language	-	-	-	-

Table 4: Correlation of automatic volumes with manual volumes and other health parameters. (* $P < 0.05$; ** $P < 0.01$)

Less confident means the WMH is of both low and medium contrast images. The correlation with manual volumes and other health parameters for these subjects were performed. The correlation for low contrast WMH subjects was 0.80 ($n = 183$). The correlation of subjects with combined low and medium contrast was about 0.84 ($n = 116$). The results are shown in Table 4.

Appendix B – Fat segmentation analysis

Correlation of segmentation output volumes with the various health risk factors (data available as a part of SLABS Phase II study) was done as a measure of external validity. As highlighted in previous literatures, the VAT was found to be more associated with the cardiovascular risk factors than SAT.

Variables	VAT	SAT
BMI	0.37*	0.54*
Systolic BP	.22*	.11
Diastolic BP	-.01	.04
Fasting blood glucose	.17	-.07
Serum triglycerides	.26**	-.04
Homocysteine	.26**	.00
Total cholesterol	.13	-.11
LDL-C	.20*	-.07
HDL-C	-.35**	-.10
Folate	-.17	-.04
Vitamin B-12	-.13	.06

Table 5: Association of VAT and SAT with cardiovascular risk factors. The VAT showed good associations with risk factors than the SAT

The abdominal fat volumes followed similar or better indication of risk factors. The results are shown in table below (BMI – Body mass index; BP – Blood Pressure; LDL – low; HDL – High). The results show that internal fat (VAT) is more harmful than the external fat (SAT).

References

- [1] K. S. Meacham, The MRI Study Guide for Technologists, Springer, 1st edition, 1995.
- [2] Rabi, II, Zacharias JR, Millman S, Kusch P. Milestones in magnetic resonance: a new method of measuring nuclear magnetic moment'. 1938. J Magn Reson Imaging 1992; 2:131-133.
- [3] Lauterbur PC. Image formation by induced local interactions. Examples employing nuclear magnetic resonance. 1973. Clin Orthop Relat Res 1989:3-6.
- [4] Mansfield P, Maudsley AA. Medical imaging by NMR. Br J Radiol 1977; 50:188-194.
- [5] Z. P. Liang and P. C. Lauterbur, Principles of Magnetic Resonance Imaging: A Signal Processing Perspective. New York: IEEE Press, 2000.
- [6] S. Webb, The Physics of Medical Imaging, Taylor & Francis, 1st edition, 1988.
- [7] Stark DD, Bradley WGJ.
- [8] S. A. Huettel, A. W. Song, and G. McCarthy, Functional Magnetic Resonance Imaging, Sinauer Associates, Inc, 2004.
- [9] ACR's White Paper (combined 2002 and 2004), White Paper on MR Safety.
- [10] P. Bassar, J. Mattiello, D. L. Bihan (1994). MR Diffusion tensor spectrography and imaging. Biophys. J. pp. 259–267.
- [11] P. Hagmann et al. (2003). DTI mapping of human brain connectivity: Statistical fibre tracking and virtual dissection. NeuroImage.
- [12] L. Jonasson et al. (2003). White matter mapping in dt-mri using geometric flows. Eurocast: 9th International Workshop in Computer Aided Systems Theory pp. 80–82.

- [13] <http://www.nationalmssociety.org/> "About MS", Visited 11/04/2009.
- [14] T. Kohonen, Self-Organizing Maps, Springer, 3rd edition, 2001, Editors: Thomas S. Huang, Teuvo Kohonen, Manfred R. Schroeder.
- [15] F. Dangond, "Multiple sclerosis", eMedicine Neurology, vol. <http://www.emedicine.com/neuro/topic228.htm>, 2005.
- [16] R.I. Grossman and J.C. McGowan. Perspectives on multiple sclerosis. AJNR, 19:1251–1265, August 1998.
- [17] D. H. Miller, P. S. Albert, F. Barkhof, G. Francis, J. A. Frank, S. Hodgkinson, F. D. Liblin, D. W. Paty, S. C. Reingold, and J. Simon, "Guidelines for the Use of Magnetic Resonance Techniques in Monitoring the Treatment of Multiple Sclerosis," Annals of Neurology, vol. 39, no. 1, Jan. 1996.
- [18] B. Larsson, K. Svardsudd, L. Welin, L. Wilhelmsen, P. Björntorp, and G. Tibblin, "Abdominal adipose tissue distribution, obesity and risk of cardiovascular disease and death: 13 y follow up of participants in the study of men born in 1913," Br Med J Clin Res, Vol. 288, pp. 1401- 1404, 1984.
- [19] K. Tokunaga, Y. Matsuzawa, K. Ishikawa, and S. Tarui, "A novel technique for the determination of body fat by computed tomography," Int. J. Obes., Vol. 7, 5, pp. 437-445, 1983.
- [20] W. H. Press, S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery, Numerical Recipes in C: The Art of Scientific Computing. Cambridge University Press, Second Edition, 1992.
- [21] J. Kobayashi¹, N. Tadokoro¹, M. Watanabe and M. Shinomiya, "A novel method of measuring intra-abdominal fat volume using helical computed tomography," International Journal of Obesity, Vol. 26, pp. 398–402, 2002.

- [22] A. N. Bandekar, M. Naghavi, and I. A. Kakadiaris, "Performance evaluation of abdominal fat burden quantification in CT," IEEE-EMBS, pp. 3280-3283, 2005.
- [23] <http://www.mr-tip.com> (Magnetic Resonance Technology Information Portal), Visited 11/04/2009
- [24] W.T. Dixon. Simple proton spectroscopic imaging. Radiology, vol. 153(1), pp. 189–194, 1984.
- [25] J. Ma. Breath-hold water and fat imaging using a dual-echo two-point dixon technique with an efficient and robust phase-correction algorithm. Magn. Reson. Med., vol. 52, no. 2, pp. 415–419, 2004.
- [26] S. M.-H. Song et al. Phase unwrapping of MR phase images using Poisson equation. IEEE Trans. Im. Proc., vol. 4, no. 5, pp. 667– 676, May 1995.
- [27] K. Lim, A. Pfefferbaum, "Segmentation of MR brain images into cerebrospinal fluid spaces, white and gray matter," J. Comput. Assist. Tomogr., vol. 13, pp. 588±593, 1989.
- [28] F. A. Lucas-Quesada, U. Sinha, S. Sinha, "Segmentation strategies for breast tumors from dynamic MR images," JMRI, vol. 6, pp. 753±763, 1996. York: Plenum Press, 1984.
- [29] N. Shareef, D. L. Wand, R. Yagel, "Segmentation of medical images using LEGION," IEEE Trans. Med. Imag., vol. 18, no. 1, pp. 74±91, 1999.
- [30] G. J. Awcock, R. Thomas, Applied Image Processing. New York: McGraw-Hill, Inc., 1996.
- [31] P. Suetens, E. Bellon, D. Vandermeulen, M. Smet, G. Marchal, J. Nuyts, L. Mortelman, "Image segmentation: methods and applications in diagnostic radiology and nuclear medicine," European Journal of Radiology, vol. 17, pp. 14±21, 1993.

- [32] J. C. Rajapakse, J. N. Giedd, J. L. Rapoport, "Statistical approach to segmentation of single-channel cerebral MR images," *IEEE Trans. Med. Imag.*, vol. 16, no. 2, pp. 176±186, 1997.
- [33] P. K. Sahoo, S. Soltani, A. K. Wond, Y. C. Chen, "A survey of thresholding techniques," *Comput. Vision, Graph, Image Process.*, vol. 41, pp. 233±260, 1988.
- [34] M. Sonka, V. Hlavac, R. Boyle, *Image Processing, Analysis, and Machine Vision*. CA: PWS Publishing, Pacific Grove, 1999.
- [35] N. Otsu. A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man and Cybernetics*, 9(1):62{66, 1979.
- [36] W. K. Pratt, *Digital Image Processing*. New York: John Wiley & Sons, 1991.
- [37] H. R. Singleton, G. M. Pohost, "Automatic cardiac MR image segmentation using edge detection by tissue classification in pixel neighborhoods," *Mag. Res. ed.*, vol. 37, pp. 418±424, 1997.
- [38] S. E. Umbaugh, *Computer Vision and Image Processing: A Practical Approach Using CVIP tools*. Upper Saddle River, 6. D. G. Ballard, C. M. Brown, *Computer Vision*, Englewood Cliffs: Prentice Hall, 1982.
- [39] I. N. Bankman, T. Nizialek, I. Simon, O. B. Gatewood, I. N. Weinberg, W. R. Brody, "Segmentation algorithms for detecting microcalcifications in mammograms," *IEEE Trans. Inform. Techn. Biomed.*, vol. 1, no. 2, pp. 141±149.
- [40] S. W. Zucker, "Region growing: childhood and adolescence," *Comp. Graphics Image. Proc.*, vol. 5, pp. 382±399, 1976.
- [41] K. S. Fu, J. K. Mui, "A survey on image segmentation," *Pattern Recognition*, vol. 13, no. 1, pp. 3±16, 1981.
- [42] J. C. Russ, *The Image Processing Handbook*. Boca Raton: CRC Press, 1999. pp.

242±248, 1995.

- [43] M. Kass, A. Witkin, and D. Terzopoulos. Snakes: Active contour models. *International Journal of Computer Vision*, 1(4):321–331, 1987.
- [44] C. Xu and J.L. Prince. Snakes, shapes, and gradient vector flow. *IEEE Transactions on Image Processing*, 7(3):359–369, 1998.
- [45] V. Caselles, R. Kimmel, and G. Sapiro. Geodesic active contours. *International Journal of Computer Vision*, 22(1):61–79, 1997.
- [46] T. McInerney and D. Terzopoulos. Topologically adaptive snakes. *IEEE International Conference on Computer Vision*, pages 840–845, 1995.
- [47] S. Osher and J.A. Sethian. Fronts propagating with curvature-dependent speed: Algorithms based on Hamilton-Jacobi formulations. *Journal of Computational Physics*, 79(1):12–49, 1988.
- [48] N. Paragios and R. Deriche. Geodesic active contours and level sets for the detection and tracking of moving objects. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 22(3):266–280, 2000.
- [49] T. McInerney and D. Terzopoulos. Deformable models in medical image analysis: A survey. *Medical Image Analysis*, 1(2):91–108, 1996.
- [50] B. Leroy, I. Herlin, and L.D. Cohen. Multi-resolution algorithms for active contour models. *Lecture Notes in Computer Science*, 219, 1996.
- [51] L.D. Cohen. On active contour models and balloons. *Computer Vision, Graphics, and Image Processing: Image Understanding*, 53(2):211–218, 1991.
- [52] D. Cremers, F. Tischhuser, J. Weickert, and C. Schnorr. Diffusion snakes: Introducing statistical shape knowledge into the Mumford-Shah functional. *International journal of computer vision*, 50(3), 2002.

- [53] C. Xu and J. Prince. Gradient vector flow: A new external force for snakes. IEEE Conference on Computer Vision and Pattern Recognition, pages 66–71, 1997.
- [54] J. Tang, S. Millington, S.T. Acton, J. Crandall, and S. Hurwitz. Ankle cartilage surface segmentation using directional gradient vector flow snake. IEEE international Conference on Image Processing, pages 2745–2748, 2004.
- [55] J. Cheng and S.W. Foo. Dynamic directional gradient vector flow for snakes. IEEE Transactions on Image Processing, 15:1563–1571, 2006.
- [56] L. Ford and D. Fulkerson. Flows in networks. Princeton University Press, 1962.
- [57] Z. Wu and R. Leahy. An optimal graph theoretic approach to data clustering: Theory and its application to image segmentation. IEEE Transactions on Pattern Analysis and Machine Intelligence, 15(11):1101–1113, 1993.
- [58] S. Wang and J.M. Siskind. Image segmentation with minimum mean cut. IEEE Conference on Computer Vision and Pattern Recognition, pages 517–524, 2001.
- [59] J. Shi and J. Malik. Normalized cuts and image segmentation. IEEE Conference on Computer Vision and Pattern Recognition, pages 731–737, 1997.
- [60] Y. Boykov and M. Jolly. Interactive graph cuts for optimal boundary and region segmentation of objects in n-d images. IEEE International Conference on Computer Vision, 1:105112, 2001.
- [61] N. Xu, R. Bansal, and N. Ahuja. Object segmentation using graph cuts based active contours. IEEE Conference on Computer Vision and Pattern Recognition, 2:46–53, 2003.
- [62] Anbeek, P., K. L. Vincken, et al. (2004). ‘Automatic segmentation of different-sized white matter lesions by voxel probability estimation.’ Med Image Anal 8(3): 205-15.
- [63] Pachai C, Zhu YM, Grimaud J, Hermier M, Dromigny-Badin A, Boudraa A, Gimenez

- G, Confavreux C, Froment JC. A pyramidal approach for automatic segmentation of multiple sclerosis lesions in brain MRI. *Computerized Medical Imaging and Graphics* 1998;22:399–408.
- [64] Stamatakis EA, Tyler LK. Identifying lesions on structural brain images-Validation of the method and application to neuropsychological patients. *Brain and Language* 2005;94:167–177.
- [65] Jack, C.R.J., et al., FLAIR histogram segmentation for measurement of leukoaraiosis volume. *Journal of Magnetic Resonance Imaging*, 2001. 14(6): p. 668-676.
- [66] Hirono N, Hajime K, Kazui H, Hashimoto M, Mori E. Impact of white matter changes on clinical manifestation of Alzheimer's disease a quantitative study. *Stroke* 2000;31:2182 – 2188.
- [67] Miki Y, Grossman RI, Udupa JK, Samarasekera S, van Buchem MA, Cooney BS, Pollack SN, Kolson DL, Constantinescu C, Polansky M, et al. Computer-assisted quantitation of enhancing lesions in multiple sclerosis: correlation with clinical classification. *American Journal of Neuroradiology* 1997;18(4):705–710.
- [68] Udupa JK, Wei L, Samarasekera S, Miki Y, van Buchem MA, Grossman RI. Multiple sclerosis lesion quantification using fuzzy-connectedness principles. *IEEE Trans Med Imaging* 1997;16: 598 – 609.
- [69] Wu, M., C. Rosano, et al. (2006). 'A fully automated method for quantifying and localizing white matter hyperintensities on MR images.' *Psychiatry Res* 148(2-3): 133-42.
- [70] Admiraal-Behloul, F., D. M. van den Heuvel, et al. (2005). 'Fully automatic segmentation of white matter hyperintensities in MR images of the elderly.' *Neuroimage* 28(3): 607-17.

- [71] Dyrby, T. B., E. Rostrup, et al. (2008). 'Segmentation of age-related white matter changes in a clinical multi-center study.' *Neuroimage* 41(2): 335-45.
- [72] DeCarli, C., B. L. Miller, et al. (1999). 'Predictors of brain morphology for the men of the NHLBI twin study.' *Stroke* 30(3): 529-36.
- [73] Wen, W. and P. Sachdev (2004). 'The topography of white matter hyperintensities on brain MRI in healthy 60- to 64-year-old individuals.' *Neuroimage* 22(1): 144-54.
- [74] Fazekas, F., J. B. Chawluk, et al. (1987). 'MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging.' *AJR Am J Roentgenol* 149(2): 351-6.
- [75] Payne, M. E., D. L. Fetzter, et al. (2002). 'Development of a semi-automated method for quantification of MRI gray and white matter lesions in geriatric subjects.' *Psychiatry Res* 115(1-2): 63-77.
- [76] F. Kruggel et al. Texture-based segmentation of diffuse lesions of the brain's WM. *NeuroImage* 39 (2008) 987–996.
- [77] Smith S. Fast robust automated brain extraction. *Human Brain Mapping*. 2002; 17 (3), 143-155.
- [78] J. Russ, *The image processing handbook* (2nd ed.), CRC Press, Boca Raton,
- [79] Kenchaiah S, Evans JC, Levy D, et al. Obesity and the risk of heart failure. *N Engl J Med* 2002;347:305–313.
- [80] Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch Intern Med* 2002;162:1867–1872.
- [81] Sharma AM, Chetty VT. Obesity, hypertension and insulin resistance. *Acta Diabetol* 2005;42(Suppl 1):S3–S8.

- [82] Van der Kooy K, Leenen R, Seidell JC, Deurenberg P, Droop A, Bakker CJ. Waist-hip ratio is a poor predictor of changes in visceral fat. *Am J Clin Nutr* 1993;57:327–333.
- [83] C.S. Fox et al. Abdominal visceral and subcutaneous adipose tissue compartments. *Circulation*, 116(1):39-48, 2007.
- [84] Zhao B, Colville J, Kalaigian J, et al. Automated quantification of body fat distribution on volumetric computer tomography. *J Comput Assist Tomogr* 2006; 30(5):777-783.
- [85] Kim S, Lee GH, Lee S, et al. Body fat measurement in computer tomography image. *Biomed Sci Instrum* 1999; 35: 303-308.
- [86] Gronemeyer SA, Steen RG, Kauffman WM, Reddick E, Glass JO. Fast adipose tissue (FAT) assessment by MRI. *Magn Reson Imaging* 2000;18:815–818.
- [87] Shen W, Punyanitya M, Chen J, et al. Visceral adipose tissue: relationships between single slice areas at different locations and obesity-related health risks. *Int J Obes* 2007;31:763–769.
- [88] Lancaster JL, Ghiatas AA, Alyassin A, Kilcoyne RF, Bonora E, De Fronzo RA. Measurement of abdominal fat with T1-weighted MR images. *J Magn Reson Imaging* 1991;1:363–369.
- [89] Gronemeyer S, Steen R, Kauffman W, Reddick W, Glass J. Fast adipose tissue (FAT) assessment by MRI. *Magn Reson Imag* 2000;18: 815–818.
- [90] Pichiecchio A, Uggetti C, Egitto MG, Berardinelli A, Orcesi S, Gorni KO et al. Quantitative MR evaluation of body composition in patients with Duchenne muscular dystrophy. *Eur Radiol*. 2002; 12 (11): 2704–2709.
- [91] Shen W, Wang Z, Punyanita M, Lei J, Sinav A, Kral JG et al. Adipose tissue quantification by imaging methods: a proposed classification. *Obes Res* 2003; 11: 5–16.

- [92] Seidell JC, Bakker CJ, van der Kooy K. Imaging techniques for measuring adipose-tissue distribution – a comparison between computed tomography and 1.5-T magnetic resonance. *Am J Clin Nutr* 1990; 51: 953–957.
- [93] Poll LW, Wittsack HJ, Koch JA, Willers R, Scherer A, Kapitza C et al. Quantification of total abdominal fat volumes using magnetic resonance imaging. *Eur J Med Res* 2002; 7: 347–352.
- [94] Elbers J, Haumann G, Asscheman H, Seidell J, Gooren L. Reproducibility of fat area measurements in young, non-obese subjects by computerized analysis of magnetic resonance images. *Int J Obes Relat Metab Disord* 1997; 21: 1121–1129.
- [95] Gronemeyer S, Steen R, Kauffman W, Reddick W, Glass J. Fast adipose tissue (FAT) assessment by MRI. *Magn Reson Imag* 2000; 18: 815–818.
- [96] Poll LW, Wittsack HJ, Koch JA, Willers R, Scherer A, Kapitza C et al. Quantification of total abdominal fat volumes using magnetic resonance imaging. *Eur J Med Res* 2002; 7: 347–352.
- [97] Accurate quantification Diane et al; Armao D, Guyon JP, Firat Z, Brown MA, Semelka RC: Accurate quantification of visceral adipose tissue (VAT) using water-saturation MRI and computer segmentation: preliminary results. *J Magn Reson Imaging* 2006, 23(5):736-741.
- [98] Kullberg J, Ahlström H, Johansson L, Frimmel H. Automated and reproducible segmentation of visceral and subcutaneous adipose tissue from abdominal MRI. *Int J Obesity (Lond)* 2007; 31: 1806–1817.
- [99] P. S. Jørgensen. Segmentation of male abdominal fat using MRI. Master's thesis, Informatics and Mathematical Modelling, Technical University of Denmark, DTU, Richard Petersens Plads, Building 321, DK-2800 Kgs. Lyngby, 2006.

- [100] Positano V, Gastaldelli A, Sironi AM, Santarelli MF, Lombardi M, Landini L. An accurate and robust method for unsupervised assessment of abdominal fat by MRI. *J Magn Reson Imaging* 2004; 20: 684–689.
- [101] Gastaldelli A, Sironi AM, Ciociaro D, et al. Visceral fat and beta cell function in non-diabetic humans. *Diabetologia* 2006;48:2090–2096.
- [102] Racette S, Weiss E, Villareal DT, et al. One year of caloric restriction in humans: feasibility and effects on body composition and abdominal adipose tissue. *J Gerontol A Biol Sci Med Sci* 2006;61:943–950.
- [103] Weiss EP, Holloszy JO. Improvements in body composition, glucose tolerance, and insulin action induced by increasing energy expenditure or decreasing energy intake. *J Nutr* 2007;137:1087–1090.
- [104] Demerath EW, Ritter KJ, Couch WA, et al. Validity of a new automated software program for visceral adipose tissue estimation. *Int J Obes* 2007;31:285–291.
- [105] Liou TH, Chan WP, Pan LC, Lin PW, Chou P, Chen CH. Fully automated large-scale assessment of visceral and subcutaneous abdominal adipose tissue by magnetic resonance imaging. *Int J Obes (London)* 2006; 30: 844–852.
- [106] S.A. Sadananthan, Z. Weili and V. Zagorodnov. “Improved skull stripping using graph cuts”, *International Society for Magnetic Resonance in Medicine (ISMRM)*, 2009.
- [107] Sled JG, Zijdenbos AP, Evans AC. A non-parametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998; 17:87-87

- [108] Ahmed S. Abutableb, Automatic thresholding of gray-level pictures using two-dimensional entropy, Computer Vision, Graphics, and Image Processing, v.47 n.1, p.22-32, July 198.