Master’s thesis

Visualization of Regional Liver Function with Hepatobiliary Specific Contrast Agent Gd-EOB-DTPA

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LiTH-IMH/RV-A--11/002--SE

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Liver biopsy is a very common, but invasive procedure for diagnosing liver disease. However, such a biopsy may result in severe complications and in some cases even death. Therefore, it would be highly desirable to develop a non-invasive method which would provide the same amount of information on staging of the disease and also the location of pathologies. This thesis describes the implementation of such a non-invasive method for visualizing and quantifying liver function by the combination of MRI (Magnetic Resonance Imaging), image reconstruction, and image analysis, and pharmacokinetic modeling. The first attempt involved automatic segmentation, functional clustering ($k$-means) and classification ($k$NN) of in-data (liver, spleen and blood vessel segments) in the pharmacokinetic model. However, after implementing and analyzing this method some important issues were identified and the image segmentation method was therefore revised. The segmentation method that was subsequently developed involved a semi-automatic procedure, based on a modified image forest transform (IFT). The data were then simulated and optimized using a pharmacokinetic model describing the pharmacokinetics of the liver specific contrast agent Gd-EOB-DTPA in the human body. The output from the modeling procedure was then further analyzed, using a least-squares method, in order to assess liver function by estimating the fractions of hepatocytes, extracellular extravascular space (EES) and blood plasma in each voxel of the image. The result were in fair agreement with literature values, although further analyses and developments will be required in order to validate and also to confirm the accuracy of the method.
Abstract

Liver biopsy is a very common, but invasive procedure for diagnosing liver disease. However, such a biopsy may result in severe complications and in some cases even death. Therefore, it would be highly desirable to develop a non-invasive method which would provide the same amount of information on staging of the disease and also the location of pathologies. This thesis describes the implementation of such a non-invasive method for visualizing and quantifying liver function by the combination of MRI (Magnetic Resonance Imaging), image reconstruction, and image analysis, and pharmacokinetic modeling. The first attempt involved automatic segmentation, functional clustering \((k\text{-means})\) and classification \((kNN)\) of in-data (liver, spleen and blood vessel segments) in the pharmacokinetic model. However, after implementing and analyzing this method some important issues were identified and the image segmentation method was therefore revised. The segmentation method that was subsequently developed involved a semi-automatic procedure, based on a modified image forest transform \(\text{(IFT)}\). The data were then simulated and optimized using a pharmacokinetic model describing the pharmacokinetics of the liver specific contrast agent Gd-EOB-DTPA in the human body. The output from the modeling procedure was then further analyzed, using a least-squares method, in order to assess liver function by estimating the fractions of hepatocytes, extracellular extravascular space \(\text{(EES)}\) and blood plasma in each voxel of the image. The result were in fair agreement with literature values, although further analyses and developments will be required in order to validate and also to confirm the accuracy of the method.
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Chapter 1

Introduction

The diagnostic tool used today to characterize liver pathologies is liver biopsy. However a biopsy only reveals information about the area where it is taken from. Biopsy used together with for example ultrasound method can assess which area or areas to take biopsies from. Yet, a biopsy represents a very small area of suspected pathology and the spread of it, meaning that something could be missed while using this method. Not just the risk of missing pathologies or the severity of the disease play a part, also the risks of complications and the discomfort for the patient by doing a liver biopsy. Therefore, it is of great interest to develop a non-invasive method to visualize and quantify functional patterns in the whole liver. A new diagnostic method which presents differences in functionality between regions in the liver. The aim of using such a method is not just to replace a liver biopsy with a non-invasive method, but to evolve it. Results should reveal information about staging of pathologies, where functional impairments are located. The method could possibly also be a tool to assess when the best time for liver resection is for a specific patient, but most important if a restriction is possible at all, meaning if it is possible to remove all damaged tissue but however leave enough liver tissue to manage the essential functions after surgery. Thus, the method should help medical staff to estimate the functionality in the liver before and after a possible liver resection and also show exactly which areas to remove during liver surgery.
1.1 Aim

The aim of this thesis was to produce and test a work-flow scheme leading to a three dimensional visualization and quantification of regional liver function.

1.2 Outline of the project

The main goal with the thesis was to implement and analyze a work-flow scheme, combining image acquisition, image analysis and pharmacokinetic modeling in order to visualize and quantify regional liver function. The different steps in the chain towards visualization and quantification of liver function are presented in Figure 1.1.

**Figure 1.1.** Outline of the project, where SI is signal intensity, R is relaxation rate, C is concentration and EES is extracellular extravascular space
After implementing and testing the steps some important issues were identified in the segmentation step, further reading and discussion in sections 4.1 and 5. Therefore a new work-flow scheme was developed, implemented and analyzed. Changes to the segmentation step can be seen in Figure 1.2.

**Figure 1.2.** A new approach to the project, due to some important issues the automatic segmentation method was replaced with semi-automatic segmentation method.
Chapter 2

Background

2.1 Basics of the liver

The liver is the largest organ in the body and also the only one that can regenerate. [2] The diagnose of liver disease can in most cases be performed by physical examinations, medical history and some laboratory test. However in some cases additional information is needed and a biopsy, which is considered as the "gold standard" to characterize liver disease and further information about grading and staging of the disease can be assessed. [19] However, a liver biopsy includes both discomfort and risks to the patient and reveals information of just that small appointed area. To do a biopsy multiple times to obtain further information about other areas in the liver are not desirable because of the risks. Following sections will further describe the liver and its function and non-invasive imaging methods.

2.1.1 Fundamental liver anatomy and physiology

By representing about 1.5-2.5 % of human lean body mass, the liver is the largest organ in the body with a weight of 1-1.5 kg. The liver is located in the upper right abdomen and is kept up by ligamentous attachments to the diaphragm, peritoneum, great vessels and upper gastrointestinal organs. It obtains dual blood supply from the hepatic artery and the portal vein. [19] The liver is often divided into two lobes and eight segments. [2]

The majority of the cells in the liver are hepatocytes, about two-thirds of the mass of the liver. Other cell types in the liver are Kupfer cells, stellate cells, endothelial cells, blood vessel, bile ductular cells and supporting
Background

structures. One side of the hepatocyte is (see Figure 2.1) directed to the space of Disse and are richly lined with microvilli and both passive and active uptake of nutrients, proteins and other molecules can occur. The other side of the hepatocytes forms the cannnicular membranes through which bile components are secreted. The hepatocytes have an important role in the human body and regulate many different functions including synthesis of most essential serum proteins, production of bile, the regulation of nutrients and metabolism of lipophilic compounds for excretion in the bile or urine. [19] There are several liver specific contrast agent, which can be used to enhance MR images. Details about contrast enhancement function of the liver are further described in section 2.4.2.

Figure 2.1. A detailed view of the liver parenycma, image from [10]

2.1.2 Imaging the liver

In order to detect and characterize a liver disease tests are needed. Liver biopsy was first performed by Paul Ehrlich in 1883 and is considered as the "gold standard" to assess the functionality of a liver today. However a biopsy is an invasive procedure and include risks, both complications and death, even though the risk statistics are low, a non-invasive method would be preferred.

To further investigate an indication of pathology, an examination of the liver can be done by using an imaging tool. Scanning of the liver is an essential step in the diagnostic process, for detection, characterization and for helping surgeons planning liver resection. Imaging methods that could be used are ultrasound (US), computer tomography (CT) and magnetic resonance imaging (MRI). US is the most inexpensive method however, MRI shows great potential in three dimensional characterizations and planning of liver surgery and do not subject the examining body for radiation as in CT. [28]
2.1 Basics of the liver

MRI of the liver

When performing a MRI of the liver the patient is asked to fast for seven hours before the examination. The MRI examination starts by taking a pre-contrast image, during the examination the patient is asked to breath in and then out and hold their breath for about 20-23 s. Breath-holding imaging is used in order to avoid respiratory motion problems, like movement and deformation of the liver. When the pre-image, at time 0, the contrast agent (CA) is injected through a manual bolus injection. Bolus-tracking follows the CA and when it reaches the aorta the zero in the image time series is set and the artery phase imaging begins. The venous phase image follows 30 s after the artery phase. Thereafter acquisitions at 3, 5, 10, 20 and 30 minutes are obtained, however in the data set some patients lacks a fully time series. An example of an image series is shown in Figure 2.2 (same patient is used as an example throughout the thesis).

![Figure 2.2. An example of magnetic resonance images at 0, 3, 10, 20 and 30 minutes.](image)

Problems with imaging of the liver

When examining the liver with magnetic resonance imaging respiratory motion problems can appear. The liver moves and deform while breathing. To prevent this, a breath-holding technique is used during imaging, however since patients are asked to hold their breath for about 20-23 s it is often hard to get perfect images. Often further reconstruction and registration of the images are needed to get every point in an image to be represented by the same area during the time series.
2.2 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is an imaging technique used to produce high quality images from anatomical structures of the human body. The technique is based on the electromagnetic interactions of the hydrogen nuclei in the human body. An image from MRI is generated by magnetic resonance and not by ionization radiation as in conventional X-rays. MRI is a technique based on the nuclear magnetic resonance (NMR) principles, which is a method that is used to extract chemical and physical information about molecules. [15] The principles of NMR were proposed by Bloch and Purcell for which they received the Nobel prize in 1952.[8]

In 1973 Lauterbur proposed a method of producing images by NMR [25], since the first commercial MRI scanners were introduced the technique has evolved greatly and is today often called magnetic resonance imaging (MRI).[34]

Magnetic resonance images consist of several image objects called slices, which contains image elements called pixels. The intensity in each of these pixels is proportional to the NMR signal intensity. The composed slices consists of multiple voxels, volume elements, and each voxel corresponds to a signal intensity, i.e. how bright or dark the volume is in the grayscale image at a certain time.[15]

Bloch and Purcell discovered that if nuclei are placed in a strong magnetic field and pulsed with radio frequency energy it would resonate and emit a signal. The amplitude and frequency distribution of the emitted signal then yields information about the samples chemical composition.[34]

In magnetic resonance imaging the majority of the MR signal comes from the hydrogen molecule of water molecules, however lipid protons in fatty tissue also contributes to the signal. Fat and water molecules resonate at different frequencies, i.e. difference in chemical shift. In some areas in the body tissues of high amounts of both fat and water which can create chemical shift artifacts [12], in order to avoid this a reconstruction, which divides the image into separate fat and water images, was performed, see section 2.3.

2.2.1 MRI scanner

A magnetic resonance image scanner consists of a superconducting electromagnetic coil which produces a stationary magnetic field, $B_0$. There are also gradient coils, which produce a gradient in $B_0$ in the x-, y- and
z-directions. Within the gradient coils a radio frequency (RF) coil is placed which produces a magnetic field $B_1$ which rotates the net magnetization by a given pulse sequence. There are three types of RF coils; transmit and receive coils, receive only coils and transmit only coils. The receiver RF coils can detect a signal from the spins from the imaged subject, which then by sampling is converted to a digital format.[15]

### 2.2.2 Obtaining an MRI signal

In a nucleus each neutron and proton spins around its axis, which yields a magnetic moment. Normally the neutrons and protons in a nucleus align, their net spins and magnetizations are therefore canceled. In substances containing one or more unpaired protons or neutrons the dipoles orient randomly and no net magnetization are obtained. When applying a magnetic field to such a substance the dipoles will be aligned along and against the magnetic field. If the magnetized sample then is exposed to a low energy pulse of a proper frequency the sample will absorb some of the energy and its magnetic moment tilts out of alignment from the magnetic field, as described in Figure 2.3. In its tilted orientation the magnetic moment rotates slowly in a motion called precession. The rotation causes emission of a radio signal from the sample. The frequency from a sample’s radio signal is proportional to the precessional frequency, which is determined by the Larmor frequency ($\nu$), equation 2.1. The Larmor frequency is given by the product of the magnetic field strength ($B$) and the gyromagnetic ratio ($\gamma$) of the nuclei in the sample:

$$\nu = -\gamma B$$  \hspace{1cm} (2.1)

Each type of nucleus has a unique Larmor frequency for a given magnetic field, therefore all identical nuclei emit a signal of the same frequency. The frequency of this signal only varies with the magnetic field strength, which is important in encoding the signal with spatial information in the process of forming a magnetic resonance image. [34]
In order obtain contrast in an image and visualize pathology or a specific tissue, a contrast agent and/or a difference in signal intensity between different compartments in the body is needed. For each voxel the signal intensity is defined from translation of the original RF signals. The signal intensity is determined by:

- $T_1$, Spin-Lattice relaxation time [s].
- $T_2$, Spin-Spin relaxation time [s].
- $\rho$, Spin density.
- $T_2^*$, Transversal relaxation time incorporating $B_0$ inhomogeneities effects [s].

The spin density component is the concentration of signal bearing spins; this component is decided by instrumental variables:

- TR, Repetition time, time between the RF-pulses
- TE, Echo time
- TI, Inversion time
- $\theta$, Rotation angle, the angle which the net magnetization is rotated to the static magnetic field direction, due to application of RF-pulses at the Larmor frequency.
• $T_2^*$, Transversal relaxation time incorporating $B_0$

which are determined for each experiment in order to obtain separation or depending on what part of the body that is being examined.[15]

### 2.2.3 $T_1$ and $T_2$

$T_1$ and $T_2$ are time constants, where $T_1$ describes the time to reduce the difference between the longitudinal magnetization, $M_z$, and its equilibrium value and $T_2$ is the time it takes for the transversal net magnetization, $M_{xy}$, to return to its equilibrium. When the magnetization vector lies along the direction of the applied magnetic field, $B_0$, is the magnetization at equilibrium and is then called equilibrium magnetization, $M_0$. [15]

#### $T_1$ recovery

The recovery of $T_1$, also known as the spin-lattice relaxation time, arise by interaction between protons and the electromagnetic fields in the surrounding structures. The time constant $T_1$ represents the time it takes for $M_z$ to reach 63 % of the longitudinal magnetizations maximum value, see Figure 2.4. The $T_1$ relaxation time is constant for a particular tissue and occurs between one excitation pulse and the next or the TR (repetition time). [3]

![Figure 2.4](image-url)

**Figure 2.4.** Relaxation time constant, $T_1$, is the time it takes for the longitudinal magnetization to reach 63 % of its maximum value.
$T_2$ relaxation

Interactions between the magnetic fields of neighboring spins cause $T_2$ decay and is called spin-spin relaxation time. It is a result of the intrinsic magnetic fields of the nuclei interacting with each other. This process produces a loss of phase coherence or dephasing and results in a decay of the net magnetization vector in the transverse plane. The time it takes for $M_{xy}$ to fall back to 37 % of its original level is represented by $T_2$, see Figure 2.5.

Figure 2.5. The relaxation time constant, $T_2$, is the time it takes for 37 % of the transversal magnetization vector to decay to its original value.

The relaxation rates ($R$) are described by the inversed time constants respectively [5]:

\[
R_1 = \frac{1}{T_1} \quad \text{and} \quad R_2 = \frac{1}{T_2}
\]  

(2.2)

2.2.4 Dynamic contrast-enhanced MRI

Dynamic contrast-enhanced MRI (DCE-MRI) is an imaging method where MR images are obtained after an intravenous injection of a contrast agent to describe the dynamic effect of it. DCE-MRI can for example be used to examine the liver non-invasively. The contrast in an image is obtained by the different $T_1$ relaxation times in the tissues in the images. If the difference in $T_1$ relaxation times between different biological tissues is not large enough to get a good enough contrast in the image, a contrast agent that enhances the difference can be used. An example of a contrast agent
that enhances the liver parenchyma is Gd-EOB-DTPA, which is further described in sections 2.4.1 and 2.4.2. Tissue specific agents, as Gd-EOB-DTPA, are specially designed to target a specific organ, in this case the liver. A contrast agent can affect the signal intensity in two ways, it can either be positive, increase in signal ($T_1$-enhancement) or negative, signal reduction ($T_2$-enhancement).

In this study $T_1$-based dynamic imaging is used. One great advantage of measuring $T_1$ is that the transverse relaxation effects and signal saturation are completely eliminated. The change in relaxation rate is proportional to the concentration of the contrast agent as given by the following equation:

$$\Delta R_1(t) = R_1(t) - R_1(0) = C_t(t) r_1$$

where $C_t$ is the concentration in a specific tissue at time $t$, $R_1(0)$ is the relaxation rate before injection of the contrast agent and $r_1$ is the $T_1$-relaxivity of the contrast agent.[1]

### 2.3 Image analysis and reconstruction

Each voxel in an MR image of the human body consists of one or more different tissues. These different tissues contains both water and fat, which also both contains several hydrogen atoms. MR images which contains both water and fat can give both similar and different diagnostic information. However they often interfere with each other which do the MR images more difficult to interpret from a diagnostic point of view. The solution is to image the water and fat separately.[32] To divide MR images into water and fat images, a technique called two-point Dixon [31] is used. Due to a difference in chemical shift between water and fat they can be separated in each voxel by signal acquisition using two different echo times ($T_E$) in a gradient echo imaging sequence. There are two different images taken at each of the echo times, at $T_{E1}$ the water and fat signal are measured $180^\circ$ out-of-phase (OP) and at $T_{E2}$ they are detected aligned, in-phase (IP). The observed in phase and out-of-phase images can be described as [27]:

$$IP = (w + f)e^{i\phi_1(x,y,z)}$$  \hspace{1cm} (2.4a)

$$OP = (w - f)e^{i\phi_2(x,y,z)}$$  \hspace{1cm} (2.4b)
where the water and fat contributions to the signal in a pixel are represented by $w$ and $f$ respectively, $\phi_1$ and $\phi_2$ are the spatially varying phase fields.[16]

The phase needs to be corrected, due to the Larmor frequency offset in the magnetic field. After the phase correction the water and fat images can be calculated. In the in-phase image correcting the phase is a straight forward calculation, since both the water ($w$) and fat ($f$) components are positive and therefore are the sum of them also always positive. The corrected image can be obtained simply as the magnitude of $IP$: $IP = |IP| = w + f$.

The out-of-phase image cannot be calculated in the same way, since the sign of $(w - f)$ is unknown. However the phase field $\phi_2$ can be estimated with the inverse gradient method as describe in Rydell et. al. (2007). The water and fat images are calculated as shown in equations (2.5a) and (2.5b) respectively.

$$2 \cdot w = IP + OP \quad (2.5a)$$

$$2 \cdot f = IP - OP \quad (2.5b)$$

## 2.4 Hepatobiliary-specific contrast agents

In the past couple of decades, several different liver specific contrast agents for MRI have been tested and evaluated with the aim of increasing performance in liver MRI.[20] Generally conventional contrast agents are metal chelates with unpaired electrons and works by shortening both $T_1$ and $T_2$ relaxation times of surrounding water protons to produce a signal-enhancing effect.[14]

Contrast agents used in MRI are indirectly detected by their effect on the relaxation time constants, $T_1$, $T_2$ and $T_2^*$. Hepatobiliary-specific contrast agents can be used in MRI for imaging of the liver. Functional hepatocytes can accumulate hepatobiliary-specific contrast agents and then excrete it through both the renal and biliary system.[22] A liver specific contrast agent as hepatobiliary contrast agents (CA) accumulate in the liver and functional information about the liver parenchyma can be obtained if the contrast uptake is related to the liver function.[30] The paramagnetic properties of hepatobiliary-specific agents shortens the $T_1$ relaxation time in both liver and bile. Because of the shortening of $T_1$ relaxation time the contrast-to-noise ratio between non-functioning or partly functioning hepatocytes and normal liver increases. The hepatobiliary-specific contrast agents approved
2.4 Hepatobiliary-specific contrast agents

by the Food and Drug Administration (FDA) in the United States are both based on Gadolinium, Gd; gadobenate dimeglumine (Gd-BOPTA) and gadolinium ethoxybenzyl dietylenetriamine pentaacetic acid (Gd-EOB-DTPA), where the later one is used in this study.[22]

2.4.1 Gd-EOB-DTPA

The hepatocyte specific contrast agent was mainly developed to improve detection and characterization of focal liver lesions in MRI.[23] Gd-based compounds as Gd-EOB-DPTA (gadolinium ethoxybenzyl dietylenetriamine pentaacetic acid, Primovist® 0.25 mmol/mL, Bayer Schering Pharma, Berlin, Germany) are paramagnetic agents, due to Gd’s seven unpaired electrons and are used to enhance the liver parenchyma in MR images. The principle for Gd contrast agents is the shortening of $T_1$ relaxation time and this is accomplished by the dipole-dipole interactions between Gd and protons.[22] Gd-EOB-DTPA produces a strong relaxation enhancement in water, blood plasma and liver tissue. Gd-EOB-DTPA is highly specific to the hepatobiliary system and is eliminated both through the urinary tract and the biliary system in about equal amount.[13]

Gd-EOB-DTPA are administrated via an intravenous bolus injection and initially distributed in the extracellular compartments and thereafter subsequently taken up by the hepatocytes in the liver.[22] In the hepatobiliary phase, when the CA has been taken up by the hepatocytes, after injection the enhancement in functioning hepatocyte are distinct and possible hepatic lesions can clearly be seen as darker spots in the image.[20]

2.4.2 Uptake of contrast agent into hepatocytes

The contrast agent (CA), Gd-EOB-DTPA, is highly water-soluble with conventional properties as a non-specific extracellular CA, with the additional property of hepatocyte specificity. The uptake of Gd-EOB-DTPA into the hepatocytes is a highly complex mechanism and is not yet fully known. Since Gd-EOB-DTPA has a lipophilic ethobenzyl group the CA is transported through an anion transport system. There are several different types of anion transport systems placed in the hepatocytic membrane. About 50 % of the injected contrast agent is taken up by the hepatocytes and then excreted via an ATP driven process through the biliary system, the rest of the CA is excreted via the renal system.[23]
2.5 Image segmentation

To be able to separate data in the data segmentation of the MR images is needed. A segmentation could be automatic, semi-automatic or manual. An automatic segmentation method is \textit{k means}, which is a widely used and simple unsupervised clustering method, as for example in [33]. The same applies for the supervised classification method \textit{k-nearest neighbor}, a simple and often used classification tool, as in [18]. However, to my knowledge they have not been used together to automatically segment images, which probably would be an easy first attempt towards an image segmentation in this case. The large data sets and the pre-classified small areas in the images do these two methods appropriate. A semi-automatic segmentation is a method which involve human interaction as described in [21] this gives an accurate segmentation, however the method is more time consuming than an automatic segmentation but more rapid then a manual segmentation. In the following chapters the methods used in this thesis explained.

2.6 Functional clustering method

In a multidimensional data set it can be interesting to find a set of vectors that describes a similar behavior in the multidimensional data. Clustering means that observations or cases are grouped together with objects of similar character, i.e. different clusters consists of several observations that have similar pattern and structure. In this case, data sets with similar function and differs from the properties the observations in another cluster have. [7]

Clustering algorithms are generally used in an unsupervised fashion. In unsupervised clustering algorithms a data set is divided into groups according to some notion of similarity.[33] Clustering algorithms do not classify or estimate the data, instead the algorithm attempt to segment the data set into relatively homogeneous subgroups, clusters. [7]

2.6.1 K-means clustering

K-means clustering is an algorithm where \( n \) data points in an I-dimensional space are divided into \( K \) clusters [4]. By selecting a number of initial cluster centers \( k \), i.e. how many clusters the data set should be divided in to, the iterative process can begin as described below:
1. User decides how many clusters $k$ the data should be divided into.

2. Randomly assignment of $k$ data points to be the initial cluster center locations.

3. For each data point, find the nearest cluster center. In a sense the cluster center owns a subset of the data, thereby representing a partition of the data set and each $k$ cluster, $C_1, C_2, \ldots, C_k$.

4. For each cluster $k$, find the cluster centroid, i.e. the new cluster center with the new data points in the cluster, and update the location of each cluster center to the new value of the centroid.

5. Repeat steps 3 to 5 until convergence or termination.

### 2.7 Functional classification method

In contrast to clustering, classification methods define a set of data into labeled groups. In most cases the methodology used is supervised learning modeling. In this type of method the classification algorithm is provided with a so-called training set of data, which includes pre-classified values of the target. The groups the data should be divided into are decided by the training data, for example a training data set in which the data already has been classified manually with the knowledge of function and what kind of tissue it is, as in this case study. The classification model uses the information about structure and patterns from the training data set to compare it with an unknown data set to classify the data set. By probability each data point is given a label that describes which group it is most likely to belong to.\[7\]

#### 2.7.1 k-NN classification

The classification method used in this thesis was kNN, $k$ nearest neighbor, where $k$ represents how many nearest neighbors the data point will be compared to. Each data point is compared to its neighbors and is assigned to the same class as its nearest neighbor(s). $k$-Nearest neighbor is a classification method-example of instance-based learning, which means that the training data set is stored. Classification of new data sets can be found by comparing similarities in the observations from new unclassified data sets with the training data set. To measure the similarity, distance metrics or a distance function is used, which is a real-valued function $d$, for any
coordinate $x$, $y$ and $z$. The most common and straightforward distance function used is euclidean distance, as shown in (2.6).

$$d_{Euclidean}(x, y) = \sqrt{\sum [i](x_i - y_i)^2}$$

(2.6)

where $x = x_1, x_2, ..., x_m$ and $y = y_1, y_2, ..., y_m$ represents the $m$ attributes of two different records.

### 2.8 Semi-automatic image segmentation

In image analysis it is interesting to do an image segmentation i.e. dividing the image into regions, which later can be investigated separately. To identify and separate different objects and structures in an image, different approaches of image segmentation can be used. The process is divided into two tasks: recognition and delineation. The recognition is deciding where in the image the interesting object is located. Delineation determines the extent of that object, i.e. the outer lines of the object. Since a fully automatic segmentation of an image can be difficult to perform, a semi-automatic method is preferred, depending on the implementation. In interactive or semi-automatic segmentation methods, the human ability to recognize the desired object and the computer’s ability to do a delineation are combined.[21]

#### 2.8.1 Image Foresting Transform (IFT)

The image segmentation method used in this thesis is a modified Image Foresting Transform (IFT) presented by Malmberg (2009). This method allows the user to mark the background and the desired object, as shown below in Figure 2.6 where the background is marked in red and the object in green. The computer then performs the delineation and then user can do changes and new settings of background and object in an iterative process until the image segmentation is completed. Further reading about the algorithm can be found in Filip Malmberg thesis [21] and a conference contribution to IWCI'A'09 (In proceedings of the 13th International Workshop on Combinatorial Image Analysis) [9].
2.9 Pharmacokinetic analysis

To describe the dynamics of the contrast agent in the human body, a pharmacokinetic model is used. This model uses mathematical statements, implemented and simulated in Systems Biology Tool Box (SBTB) in MATLAB (Mathworks, Natick, MA, USA), to describe and analyze the biological data. For this purpose, a modified version of a minimal model describing the pharmacokinetics of Gd-EOB-DTPA throughout the human body is used, the original model is described in the master thesis by Forsgren (2011). A short introduction of the original model, the simulation and optimization process is presented shortly in the following subchapters for further reading see [10].

Figure 2.6. Image segmentation method with a modified IFT, the background is marked with red and the desired object is marked in green.
2.9.1 The pharmacokinetic model

The model used in this thesis is the so-called $M_{bf}$ in the thesis by Forsgren, the model is shown in Figure 2.7

![Figure 2.7.](image)

**Figure 2.7.** The figure represents the model equations where the states are presented as rounded rectangles, output as circles, transports with their base equation as arrows, input function as pointed rectangles and the measured DCE-MRI signals as the shaded gray areas. Figure was redrawn and modified from Forsgren (2011).

There are four different states presented in the model; however it is known that there is no uptake of CA in the spleen, which means that only three states (hepatocytes, EES and blood plasma) are affected by the flux of CA and this is used in further investigations in this thesis.

2.9.2 Simulation and evaluation of the model

The evaluation of the model is based on the residuals. For a given data set and a model, the difference between the measured data $y(t)$ and the predicted data, $\hat{y}(t,p)$, in the model simulation, is the residuals here denoted
2.9 Pharmacokinetic analysis

\[ e(t, p) = y(t) - \hat{y}(t, p) \]  

(2.7)

Large residuals are a sign that the combination of the model and parameter set cannot explain the data. In order to reject faulty models a statistical \( \chi^2 \)-test was created. Based on the residuals and assuming that the experimental data and data from the model for a certain set of parameters behave similar, a null hypothesis was created. The null hypothesis assumes that the residuals have the same probability distribution as the systems noise. Assuming a zero mean normal distribution a statistical \( \chi^2 \)-test can be used:

\[
T_{\chi^2} = \sum_{[i,j]} \left[ \frac{(y_i(t_j) - \hat{y}_i(t_j, p))^2}{\sigma_i^2(t_j)} \right] \in \chi^2(d)
\]  

(2.8)

where \( d \) is the degrees of freedom which is equal to the number of time points in the experimental data. If the model fails the statistical test it is rejected.

The optimization of the model parameters was performed by using a pseudorandom global search algorithm, called simulated annealing, implemented in MATLAB. Following equation describes the objective function for optimization:

\[
\hat{p} = arg_{p} \min V(p)
\]  

(2.9)

where \( V(p) \) is equal to \( T_{\chi^2} \) in (2.8).

All parameter vectors that pass the statistical test are stored for further evaluation. The test score in this case referred to as the cost, i.e. the closer the cost is to zero, the better the model is able to describe the data.[11]
Chapter 3

Materials and Methods

The material and methods used in order to fulfill the aim of visualization and quantification of liver function are described in this chapter.

3.1 Subjects

The data presented in this thesis is from an on-going study from the NILB (Non-invasive liver biopsy) project. This is a prospective cohort study that started in 2008 in which 100 patients are going to be included. So far data from about 50 patients has been collected. The patients are included based on suspected or established liver disease. These patients have by clinical reasons been assessed to need a conventional liver biopsy for histopathological diagnosis, either to make a diagnosis or decide level of pathology. Except from the MRI evaluation the patients also will have a conventional liver biopsy and several blood samples taken for further analysis.

3.2 Contrast agent

In this study the liver specific contrast agent Gd-EOB-DTPA (Primovist®, 0.25 mmol/mL, Bayer Schering Pharma AG, Berlin, Germany) was used. Each subject was examined with a dose of 0.025 mmol/kg via a manual bolus injection at the rate of approximately 1 mL/s followed by an injection of equal amount of physiologic saline.
3.3 Data acquisition

The data from all examinations were collected using a 1.5 Tesla (T) magnetic resonance system (Achieva, Philips Medical Systems, Best, The Netherlands), with a four-channel phased array SENSE body coil. Also the subjects had to fast at least seven hour before the examination.

The MRI protocol consisted of one pre-contrast and seven post-contrast acquisitions of an axial breath-hold gradient-echo fat-saturated $T_1$-weighted 3D sequence (THRIVE, $T_R = 6.5360$ ms, $T_{E_1} = 2.3$ ms and $T_{E_2} = 4.6$ ms, flip angle $\alpha = 13^\circ$, scan time 30-40 s, FOV 350-400 x 250-280 mm$^2$, matrix 256 x 115, reconstructed voxel size 1.6 x 1.6 x 1.6 mm$^3$). The acquisitions post-contrast were performed at arterial and portal venous phases, 3, 5, 10, 20, 30 minutes after injection of the contrast agent.

3.4 Data processing

First the data was divided into fat and water images as described in section 2.3. The signal intensity (SI) time curves from each voxel in liver and spleen from the examinations were normalized and converted into relaxation rates. The relaxation rates are then comparable to concentration of contrast agent in each voxel, shown by the following expression:

$$\frac{1}{T_1(t)} = \frac{1}{T_1(0)} + C(t) \cdot r_1$$

(3.1)

where $\frac{1}{T_1(t)} = R_1(t)$, which describes the relaxation rate in a specific tissue at the time $t$ and $R_1(0)$ is the relaxation rate before contrast injection. $C$ is the contrast agent in the same specific tissue at the time $t$, and $r_1$ is the relaxtivity in that organ.[24] The next sections show how the SIs are recalculated to relaxation rates.

3.4.1 Normalization of signals

Each voxels SI time series were normalized by dividing $SI(t)$ with the pre-contrast signal intensity, $SI(0)$. By normalization of the SI time series all constant background effects were removed. Following equation shows the normalized signal intensity (S):

$$S(t) = \frac{SI(t)}{SI(0)}$$

(3.2)
3.4.2 Recalculation of SI values to relaxation rates

The normalized SI values were then recalculated to relaxation rate values, this assuming fixed pre-contrast $T_1$ values, $T_{1\text{pre}} = T_1(0)$, which were 586, 1057 and 1200 ms in liver, spleen and vessels respectively. The nonlinear effects from the chosen $T_R$ and $\alpha$ was also corrected for by the following relation [17]:

$$\frac{1}{T_1(t)} = -\frac{1}{T_R} \left( \ln \left( \frac{SI(t)}{SI(0)} \frac{1 - E(0)}{1 - \cos(\alpha)E(0)} - 1 \right) - \ln \left( \cos(\alpha) \frac{SI(t)}{SI(0)} \frac{1 - E(0)}{1 - \cos(\alpha)E(0)} - 1 \right) \right)$$

(3.3)

where SI(t) is the signal intensity at the time t, SI(0) is the signal intensity pre-contrast i.e. at time 0 and

$$E(t) = e^{-\frac{T_R}{T_1(t)}}$$

(3.4a)

$$R_1(t) = \frac{1}{T_1(t)}$$

(3.4b)

3.5 Data analysis

The data was analyzed first by recognizing data sets where the patient had been as still as possible between the different time points when the data was collected in the MRI scan. In order to make this approach usable in the future a voxel-to-voxel registration must be done, so that the analyses become accurate, the knowledge that each voxel in the time series represents the same area at all times is necessary. However, for the time frame of this thesis the registration step was left out.

3.6 Pharmacokinetic model

To analyze the image data a pharmacokinetic analysis of the time series was made by a two-compartment model, which is described in more detail in section 2.9, is used. The model describes the contrast agents way through the human body. Compared to the original model $M_{bf}$ described by Forsgren (2011) [10], some changes were made to adapt the model to patient specific data. First the data had to be divided into data from liver, spleen
and vessels. Partition of the data into the different tissues is needed to get input into the pharmacokinetic model. The first approach was to do an automatic segmentation from the image data with clustering and classification tools in MATLAB. The second approach was to do a semi-automatic segmentation developed by Filip Malmberg at Centre for Image Analysis in Uppsala Sweden, as presented in his Ph.D thesis Graph-based Methods for Interactive Image Segmentation (2011) [21]. This constructed two different hypotheses:


how this is done in more detail are described in sections 3.7 and 3.8.

As mentioned the model consists of two compartments, liver and spleen. The spleen is in the model represented by spleen parenchyma, splenic blood plasma and extracellular extravascular space (EES). The liver is represented by hepatocytes, blood plasma in liver and EES. Further data that are set as constants in the model and needed for calculations are shown in the table below:

### 3.6.1 Model changes due to patient specific data

The original model is based on mean valued data from human test subjects and assumptions e.g. liver volume and EES volume based on a "standard human", a 70 kg male with 20 % fat. To accommodate the original model to data from specific patient some changes are needed:

- Liver volume.
- EES volume.
- Blood volume.
- Mean valued $R_1(t)$ from liver, spleen and vessel for optimization.

and this is done through some different equations and methods. The liver volume is decided with a segmentation method that in a way separates the liver data from the rest of the data in the MR image. With the separation of data, the volume can be calculated. By knowing each voxel size, the spacing between them and which voxels that contains liver tissue, the liver volume can be calculated with following equation:
where $V_L$ is the liver volume and it is calculated from the segmented liver, the space between the pixels, the slices thickness and gap.

The EES volume and blood volume [29] are calculated depending on the gender of the patient and are estimated by the following equations:

$$V_{Total\ \text{body\ water}} = k \cdot (\text{hight in cm}) + l \cdot (\text{weight in kg}) - m$$  \hspace{1cm} (3.6)

where $V_{Total\ \text{body\ water}}$ describes the volume of the water in the body, where about 45% is extracellular fluid or as estimated in this thesis the EES volume.[26] The calculations are done depending on the gender where $k$, $l$ and $m$ are constants different for the genders, see table 3.1.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>0.344547</td>
<td>0.194786</td>
</tr>
<tr>
<td>$l$</td>
<td>0.183809</td>
<td>0.296785</td>
</tr>
<tr>
<td>$m$</td>
<td>35.270121</td>
<td>14.012934</td>
</tr>
</tbody>
</table>

$$V_{Blood} = s \cdot (\text{hight in m})^3 + t \cdot (\text{weight in kg}) + r$$  \hspace{1cm} (3.7)

where the volumes are describe in liters, the different constants used for calculations are different depending on sex and are shown in table 3.2.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s$</td>
<td>0.3561</td>
<td>0.36690</td>
</tr>
<tr>
<td>$t$</td>
<td>0.03308</td>
<td>0.03219</td>
</tr>
<tr>
<td>$r$</td>
<td>0.1833</td>
<td>0.60410</td>
</tr>
</tbody>
</table>
3.7 Hypothesis 1: Automatic classification of compartments

With an automatic segmentation or classification of the MR images from the abdomen the steps towards a functional visualization of the liver can be done directly without involving human decisions, other than pre-classified training data which the unknown data from examinations are compared to and classified after. This hypothesis was based on the following steps:

1. Data acquisition like described in 3.3.
2. Data processing as described in 3.4.
3. Automatic separation of data from different organs.
4. Data from liver and spleen are used into the model to show the dynamics of the contrast agent.
5. Analysis of the results from the model to show functionality in the liver, by dividing each voxel into fractions of hepatocytes, blood plasma and EES, for more in detail see section 3.8.2

3.7.1 K-means clustering

The K-means clustering method was used to divide the different image data into functional clusters. The time series in each voxel was compared with each other and then paired together with voxels that had similar signal intensity time curves. How many different clusters the data was divided into was decided beforehand and different numbers were compared to each other so that the clusters were not overlapping. The aim with this was to divide the different data into functional clusters and then by a classification tool clarify what each cluster was compared to a data set that a radiologist have had ROIs (region of interest) manually put out into the images. These ROIs are place in liver parenchyma, spleen, bile and different vessels.

3.7.2 K-nearest neighbor classification

As mentioned previously, in section 2.7.1 the k nearest neighbour classification tool are used to give the functional clusters a label. The labels are set on the basis of image data which a radiologist has pointed out ROIs which are small areas placed in liver, spleen, bile and different vessels. The data
from the ROIs are then used as a training data set which the unknown data from patients different functional clusters are compared to and depending on which ROI data the cluster are most similar to, that is the label the cluster data gets.

### 3.8 Hypothesis 2: Semi-automatic classification of compartments

The second hypothesis were constructed due to problems with segmentation of data in the first hypothesis, this is further described in section 4.1. In this case the separation of data into liver, spleen and vessels are done by a segmentation method that are described in section 2.8. This hypothesis is then based on the following steps in order:

1. Data acquisition like described in section 3.3.
2. Data processing as described in section 3.4.
3. Semi-automatic separation of data from different organs.
4. Data from liver and spleen are used into the model to show the dynamics of the contrast agent.
5. Analysis of the results from the model to show functionality in the liver, by dividing each voxel into fractions of hepatocytes, blood plasma and EES, for more in detail see section 3.8.2

as seen the only step that differs from the first hypothesis are the third step, this step are now done manually and described further for this application below.

#### 3.8.1 Segmentation of compartments

The segmentation method works as described in section 2.8. At an in-house workstation the graphic segmentation program is used to semi-automatic segment the liver, spleen and large vessels from different patient image data. This was done manually simply recognizing the different structures in the MR images. By a few repetitions of pointing out with green inside of the organ and red in areas were the organ is not found, the segmentation were accepted to used as in-data to the mathematical model describing the pharmacokinetic mechanisms in the specific organ.
3.8.2 Calculation of fractions

By investigating fractions of hepatocytes, blood plasma and EES in each voxel in the liver, some understanding of functionality in the liver is gained. Together the fractions are a sum of one, so that the result can be seen as a per cent for each fraction, which then can be compared to literature values. If the fractions are similar to literature values the patient would be considered to have healthy liver tissue, this is further discussed in section 5.

To visualize the function in the liver each voxel of the segmented liver was analyze. Each voxel was assigned to consist of fractions of hepatocytes, EES and blood plasma of total 100%. The fraction calculations were performed by using lsqmin in MATLAB, which is a least-squares method, where $A = Bx + C$. By comparing the observed signal intensities (A) with the simulated values (B), $C = 0$, a combination of the states or compartments (x) (concentration of CA in hepatocytes, EES and blood plasma) was calculated to match the simulated and observed data, perhaps better shown by following equation system, for each voxel:

$[\text{time } \times \text{ SI}] = [\text{time } \times C(\text{state})] \cdot [C(\text{state})]$

where $C$ is the concentration and the states are $state = \begin{bmatrix} \text{Hepatocyte} \\ EES \\ \text{Blood plasma} \end{bmatrix}$
Chapter 4

Results

In this chapter the results from the two different approaches that appeared during the project are presented. The results from each step in these different hypotheses and the final result from the visualization is also presented in the following subchapters.

The data was collected and reconstructed at CMIV in Linköping to an ongoing research project. NILB (Non-Invasive Liver Biopsy) started in 2008 and I was given data from this study. The data was three-dimensional (x,y,z) images at several separate time points. First the data was assembled into a four-dimensional data set which described the image in (x,y,z,t), i.e. the position in the 3-dimensional image (which voxel its referring to) at a certain time, t. The analyses that were to be done needed information throughout the time series, it is therefore important that the x-, y- and z-components represent the same position in the actual body at all time points. However that is not often the case, patients are told to use a breath-holding technique during imaging, but movement and deformation of the liver often occur. To solve the problem, voxel-to-voxel registration of the images at each time point could be used. This project shows a first draft of a series of steps with the aim to visualize regional liver function, at this point the patients with the least movement between the time points were used. To identify these patients the difference in the z-direction was analyzed, there was not any of the patients that had been completely still during the examination, but a few had move quite little. To move on towards regional liver function visualization, the different steps believed to be needed, shown in Figure 1.1, was implemented. To visualize the results, they were divided into the two hypotheses that appeared during the time of the project.
4.1 Hypothesis 1

The first hypothesis, was as declared in section 3.7 the first approach of the project. Assumption was that the images could be automatically divided into functional clusters, which then could be classified and analyzed by the pharmacokinetic model. The first step was to do a functionality analysis; this was performed using a clustering algorithm called \textit{k-means} and the \textsc{Matlab} command \textit{kmeans}. This clustering method was applied on the image data time series to obtain a clustering depending on SI time curves. The time series in the different voxels were compared to each other and then assembled into groups with a similar appearance throughout the time series. The number different clusters that were to be constructed had to be decided before each use of the algorithm. To make this decision, the value of \( k \) started low and was increased to a point when there were at least two clusters that appeared to be overlapping or be very similar. An example of functional clustering in patient data is shown in Figure 4.1. The figure shows the clusters in different colors at a slice, a cross section, of the volume. The liver contours are visible to the left in the image. The spleen cannot be seen in this particular slice. However the \textit{k-means} algorithm can only cluster the data with similarities and cannot predict what the data point represents. To be able to set a label to each cluster, the results from the clustering method are classified with an algorithm called \textit{k-nearest neighbour}. 
The *k-nearest neighbour* algorithm is applied directly to the result from the clustering. As explained in section 2.7 an unknown data set is classified, i.e. compared to a training data set which is a pre-classified data set, and are labeled from that. The training data set was constructed from the regions of interest (ROIs) that was placed in the data by a Radiologist. The ROIs were placed in different tissues for example in liver, spleen and aorta in the abdomen area, this was then used to compare the unknown data sets to. Since patients are different, the signal intensity between patients can be varying a lot. To avoid mistaking a healthy patient from a patient with pathology the data was normalized due to its highest signal intensity value, so that the signal intensity always varies between 0 and 1. The attempt of separating different tissues by a classification method was performed with the aim that the result could be used into the pharmacokinetic model, which describes the contrast agents dynamics the human body, which then could be related to the liver function. The Figure 4.2 shows the result from classification of the clusters from the same patient as above.
Figure 4.2. Result from classification with k-NN algorithm, for a patient in one slice.

- Background
- Liver 1
- Liver 2
- Liver 3
- Spleen
- Kidney
- Common bile duct
- Hepatic artery
- Muscle tissue

The same classifier were then used on another patient image data, which first have been divided into clusters as described above, the result is shown in Figure 4.3
4.2 Hypothesis 2

As the Figure 4.3 shows, with the classification method used the in-data needed into the pharmacokinetic model, was not fully separated. The figure also shows that when the method was used on other patient data sets that have a different function pattern the classification method have problems classifying the data. Since the simple method did not perform the segmentation as well as needed at the moment, a new way of identifying liver, spleen and large vessels had to be found. The alternative a semi-automatic segmentation, a segmentation method described by Malmberg, see section 3.8.1, that was available on a computer at CMIV was chosen. The results from a segmentation of liver and spleen are shown in Figure 4.4, the segmentations are shown in one slice, the segmented areas varies in the different slices according to the positioning of the liver or spleen. The segmentation result is then used to localize the liver, spleen and blood vessels in the image data.

Figure 4.3. Result from classification with the same classifier as used above but on another patient data.
The segmentation method must however be performed separate for each patient, which could be time consuming.

From the segmented and separated liver, spleen and vessels the data from each organ was mean valued and recalculated from SI values to relaxation rates as shown in section 3.4.2. These relaxations rates were then used to fit the simulated data to in the model optimization.

### 4.3 Pharmacokinetic analysis

As mentioned above the mean valued data from liver and spleen respectively were recalculated into relaxation rate as shown by equation (3.3). The relaxation rates were calculated at each time point in the time series, which created a time series with relaxation rates.

The model simulates the contrast agent (CA) dynamics through the body from injection to excretion, with ordinary differential equations describing the flux of the CA in the human body. Optimization of the model was performed by fitting the simulated data to real data, in this case the mean value data from liver, spleen and blood vessels. How well the model fits to the real data is shown by a cost function, the lower cost the better fit. The cost function consists of a $\chi^2$-test, with a test statistic variable calculated in MATLAB ($\text{chi2inv}$), 0.95 confidence interval and 15 degrees of freedom. The degrees of freedom represents the time points in the experimental data.

The model was simulated and the parameters, which are the main components in the state equations, were optimized. The optimized parameter sets forms the output from the model. From the model an output of the relax-
4.4 Liver function analysis

The aim with the thesis was to implement a first draft towards visualization of regional liver function. The model output has given knowledge about the CAs concentration in the liver, spleen, blood plasma and EES state over the simulated time and since

\[ SI(t) = k \frac{1 - e^{-\frac{T_R}{T_1(t)}}}{1 - \cos(\alpha)e^{-\frac{T_R}{T_1(t)}}} \]  

(4.1a)

\[ \frac{1}{T_1(t)} = \frac{1}{T_1(0)} + r_1 C_t \]  

(4.1b)

where \( k \) is a scaling constant, which are used to take in consideration that the concentration is proportional to the signal intensity but are not exactly the same and also the sensitivity of the MR-scanner. \( T_R \) is the repetition time, \( T_1 \) is the relaxation at the time \( t \), \( \alpha \) is the flip angle, \( r_1 \) is the tissue specific relaxivity and \( C_t \) is the concentration in a specific tissue, in this case the different states.

By combining the two equations 4.1a and 4.1b an equation without being time dependent and takes in consideration of the concentration of CA in the tissue instead was constructed.

\[ SI(t) = k \frac{1 - e^{-T_R \left( \frac{1}{T_1(0)} + r_1 C_t \right)}}{1 - \cos(\alpha)e^{-T_R \left( \frac{1}{T_1(0)} + r_1 C_t \right)}} \]  

(4.2)

Since equation 2.3 shows that the relaxation rate is proportional to the concentration of CA and with the equation described above (4.2) the signal intensity can be related to the concentration of CA in the different states, which is given by the model.

Calculations of the amount of each state fraction that each voxel consisted of was performed according to the system described in section 3.8.2. The fractions of hepatocytes, EES and blood plasma are the calculated with
lsqin in MATLAB. A comparison between the simulated and observed SI time curves, in one voxel, which is used to calculate the fraction are shown in Figure 4.5.

Figure 4.5. The two time curves, the observed signal intensity (the upper figure) and the simulated signal intensity (the lower figure), which are compared in the fraction concentration analysis. The observed signals shown are taken from liver parenchyma from the same voxel area at different slices, which are all separately compared to the simulated signal. The stars in the upper figure marks the measured time points. Both the observed SI and the simulated SI had to be recalculated to be compared, also there is a scaling factor involved to be able to compare them. The simulated signals which are shown are one for hepatocytes, one for EES and one for blood plasma.
To visualize the result the fractions of hepatocytes, EES and blood plasma in the liver are shown separately, where the fractions is shown within a scale of 0 to 1, where 0 means that the liver consists of 0 % of that fraction and 1 means 100 %. For the patient that moved the least during the examination the visualization result from the optimal parameter set are shown in Figure 4.6.

As the figure shows, especially in the blood plasma figure, there is some problems that the voxels have not been registered against each other in the time series. There is a line at the edge of the liver which probably depends on movements between the image takes in the MRI.

The fractions found in literature for hepatocytes, EES and blood plasma in the liver are, of the total liver volume, 64 % hepatocytes, 20 % EES and 12 % blood plasma for a "standard" healthy male. [6] By comparing these literature values to the results from the fraction calculations that literature values is a bit lower than the calculated values.

4.4.1 Further analyses of the fractions evaluation

Instead of using the most optimal parameter set to perform the fraction analysis the ten most spread parameter sets, but still acceptable due to the $\chi^2$- test was used. This to investigate if there is any difference in how the fractions are optimized due to different acceptable parameter set which all can describe the data in an acceptable way, i.e. they have passed the $\chi^2$- test. Below figures of the ten most spread but still acceptable parameter sets are calculated to fractions, each of the different states (hepatocytes, EES and blood plasma) is shown separately however they all corresponds to each other.

In the figure it is seen that there is an uncertainty in the chain towards the visualization. With the optimal parameter set the patient have fractions comparable to a healthy person; however with other but yet acceptable parameter set the patient seems to have both sets which have a higher fraction of hepatocytes and a lower fraction of hepatocytes than the optimal solution. Since the fractions all add up to in total one this pattern follow in both EES and blood plasma.
Figure 4.6. Result from least squares optimization from the most optimal parameter set for one patient. The first figure shows the fraction of hepatocytes in the liver (in each voxel, shown in one slice). The middle figure show the fraction of EES and the last figure shows the fraction of blood plasma.
Figure 4.7. Visualization results for fraction of hepatocytes in the liver at the same slice in the same patient with the ten most spread but still acceptable parameter sets.
Figure 4.8. Visualization results for fraction of EES in the liver at the same slice in the same patient with the ten most spread but still acceptable parameter sets.
Figure 4.9. Visualization results for fraction of blood plasma in the liver at the same slice in the same patient with the ten most spread but still acceptable parameter sets.
Chapter 5

Discussion

It is of a great interest for liver surgeons to have a fully visualization of the function in the liver, to be able to remove all possible pathological tissue, but also to plan if when and how to perform liver restriction. As mentioned earlier this thesis is a first output of trying to do this. Each step towards visualization of liver function will be discussed separately at first.

5.1 Preprocessing and registration

No registration of the material was performed before this thesis and the data available for evaluation was limited. By registration means that each voxel between each time point corresponds to the same position. This is of great interest of performing for further development since it is difficult to do any comparison or evaluations of the time series if they are not corrected. In the data available, not even one of the patients were completely still during the imaging in the MRI. However a few had moved very little, especially one patient that only varied at one time point, except from the time points in the dynamic phases. These patients were used for further experiments. Consideration of removing time points which varied were made, but since most had only 5-6 time points (without the dynamic phases), they were not removed since the movement were quite small, one voxel difference in z-direction for the patient that were mostly still. The data in the Figure 4.6 shows that perhaps some movement had appeared according to the outer line, especially visible in the blood plasma figure, around the liver. Another source of error could be a poor segmentation.
5.2 Segmentation of data to model input function

In order to produce significant data into the pharmacokinetic model the
data from liver, spleen and some vessels had to be separated. The first
approach was to do this process automatically, using functional clustering
and classification, which would save time and money. The approach, later
called hypothesis 1, was to use an ordinary clustering method in this case
$k$ means and voxels with similar functionality were paired. So far the ap-
proach seemed to do what was intended, except for some initial difficulties
with the data handling, due to lack of experience. However, when applying
the classification method ($k$NN) it became clear that the process would not
go as simple as that. The classification revealed some issues with separa-
tion of liver, spleen and blood vessels from the rest of the intestinal. For
example, as viewed in Figure 4.3, some parts of the abdomen were labeled
with the same classification label as liver or spleen. Another problem that
appeared was that the classifier probably would fail if the patient has a lack
of function in most of the liver. Since the model is dependent on in-data
from liver, spleen and blood vessels separately, the method simply failed
to deliver that in this initial state. However with a better or more devel-
oped classification method and perhaps even clustering method the problem
might be solved. Instead the problem was revised and hypothesis 2 was de-
scribed. But since hypothesis 2 segments the data semi-automatically, it
would save time and also removed possible mistakes when segmenting due
to the human error but also the segmentation methods precision to be able
to use an automatic segmentation method.

Retrospectively, these problems probably could have been avoided, since
it now seems obvious that a method like this would not work so easy in
such a complex data. Also it would probably become troublesome to apply
this method on patients with different range of pathology. To do that the
classification method would have to recognize the pathological tissue in for
example the liver but still classify it as liver to get the right kind of in-data
to the pharmacokinetic analysis and further visualization analysis. It is
maybe possible that a more advanced training data set could solve this.
A more advanced clustering/classification method, that not only considers
the functionality aspect which still is important for the analysis, but also
the form of the organ. For example an organ could contain several different
functional clusters, but are then classified as one organ by the classification
method, due to the organs natural boundaries which is needed to move
forward into the pharmacokinetic analysis.

The semi-automatic segmentation method could also be a source of the
line shown in the blood plasma visualization Figure 4.6, since this was the first time I have used this method a human error could explain some of the problems with both the model and thereby also the fraction optimization. Since the output from the segmentation is the direct input into the model and that the fractions optimization evaluation is based on the output from the model. Another disadvantaged with the semi-automatic segmentation compared to an automatic segmentation, is that for every new patient a separate segmentation has to be done, which could be costly.

5.3 Pharmacokinetic analysis

As already mentioned in the master thesis by Forsgren, the model still needs some improvements and further development. For example a better incorporation of the biliary system in the model is necessary, and would also perhaps give a better view of the function in the liver. The bile ducts could then also be implemented as a part of the fraction analysis, which would probably then give a more accurate overall view of the functionality in the liver.

Another already mentioned problem is the variety in the acceptable parameter sets, in order to get a better and more accurate view of the fractions in the liver, the model must give a less wide result. Possible by punishing parameter sets that appears far from the optimal solution, or by pre-setting the possible range of the parameters more narrow.

In this study the model has been converted to fit patient specific data, not mean-valued data as has been done before. Also some constants have been replaced with equations to calculate the specific value for that patient. However there is still some constants which are based on the healthy "standard human", for example the estimated fractions of what the liver consists of in the model. A more accurate set of fractions could also possibly give a better result.

5.4 Visualization of liver

As for now the visualization of the function is a straight forward technique, the expected fractions of, at this stage, hepatocytes, blood plasma and EES are compared to calculated fractions in a patient liver. The expected fractions can be found in literature and by comparing the fractions an indication of functionality in the liver could be done. Since functioning
hepatocytes are expected to take up the contrast agent and by that the signal intensity increases, which mean that non-functioning or partly functioning hepatocyte gives a lower signal intensity compared to functioning hepatocytes. By comparing the concentration changes over time of the contrast agent in hepatocytes (and blood plasma and EES) can be simulated by the pharmacokinetic model, which then can be recalculated to signal intensities, with the original signal intensity a fraction of how much of each compartment that have to take part in a specific voxel to match the both signal intensities, the result is then easy to visualize.

While using spread parameter sets it is obvious that the method has an uncertainty. There are some rather large differences in the optimized fractions between the parameter sets. As discussed with a further developed model some of the problems might be solved. The optimization of fractions is also a highly simplified, the liver does not only consist of hepatocytes, EES and blood plasma another component in the CAs pharmacokinetics is for example the biliary system. The incorporation of the biliary tract in both the model and the fraction optimization would be preferable to do in further analyses.

5.5 Future work

This is only a first version of liver function visualization, there is a lot left to consider and to develop in the different steps in the chain. To be able to do these kinds of examinations on patients, the motion artifacts must be corrected for by registration of each voxel in the images. This is important because if a voxels time series is not represented correctly, the one voxel have to correspond to the same area in the body at all measured times. Also, the segmentation method could be improve, a fully automatic segmentation could possibly be done. The model also needs further development as the visualization part, this is a project just in the starting blocks, however its a start.
Chapter 6

Conclusions

In the thesis a series of steps combining image analysis, image reconstruction and pharmacokinetic analysis, was implemented and analyzed. The output was compared to literature values and seemed to be in right interval, however since this only is a first version further analyses and development are needed in order to validate and confirm the accuracy with this method. All steps needs to be controlled and validated to ensure a correct visualization and quantification of regional liver function, however a first proposal was able to be produced in this thesis.
Bibliography


Appendix A

Appendix

********** MODEL NAME
Liver CA pharmacokinetic model

********** MODEL NOTE
Patient specific model

********** MODEL STATES
\[
d/dt(Ch) = v3-v2+(-v1)/(Vl*Fh)
\]
\[
d/dt(Cp) = ((v2-v3)*Vl*Fh-v4+(v6-v5)*Vees+v8)/(Vb*(1-Hct))
\]
\[
d/dt(Cees) = v5-v6
\]
\[
d/dt(Csyr) = 0
\]
\[
d/dt(Dose) = v8
\]
\[
d/dt(Bile) = v1
\]
\[
d/dt(Urine) = v4
\]
\[
Cp(0) = 0
\]
\[
Ch(0) = 0
\]
\[
Cees(0) = 0
\]
\[
Csyr(0) = 0.25
\]
\[
Dose(0) = 0
\]
\[
Bile(0) = 0
\]
\[
Urine(0) = 0
\]
****** MODEL PARAMETERS

Km = 1e-2
vm = 3e-04
Ktrans = 0.23
Kph = 1
Khp = 1

****** MODEL VARIABLES Fb = 0.12

Flees = 0.20
Fbd = 0.0043
Fh = 1-(Fb+Flees+Fbd)
Fs = 0.35
Fsees = 0.2
Alb = 0.9
Vl = 1.8165
Vb = 5.5733
Vees = 21.2704
Hct = 0.41
CLr = 0.1165/60
Rw = 4.7e3
Rbl = 7.3e3
Rbi = 5.4e3
Rl = 10.7e3
Rpl = 6.9e3
Ksyr = 6e-2

****** MODEL REACTIONS

v1 = (vm*Ch)/(Km + Ch)
v2 = Khp*Ch
v3 = Kph*Cp*Alb
v4 = CLr*Cp*Alb
v5 = Ktrans*Cp*Alb
v6 = Ktrans*Cees
v8 = Csyrt*Ksyr
Cl = Ch*Fh*Rl+Cp*Fb*(1-Hct)*Rbl+Cees*Flees*Rw
Cs = Cp*Fs*(1-Hct)*Rbl+Cees*Fsees*Rw
Cb = Cp*(1-Hct)*Rbl

********** MODEL EVENTS
Syringe_done = ge(Dose, 3e-3),Csyrt,0