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Magnetic resonance tagging or the mouse heart
a feasibility study

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Award date:
2002

Link to publication
Magnetic Resonance Tagging of the mouse heart: a feasibility study
MRL/FIK 2002-03 AV
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18 October 2002

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Graduation Report

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Abstract

The availability of genetically modified mice offers unique opportunities to measure the effects of an increase or a decrease in the expression levels of specific genes, or to investigate the effect of targeted gene mutations. The phenotyping of such models calls for reliable and non-invasive tools for the in-vivo measurement of the consequences of the genetic modification. Magnetic Resonance Imaging (MRI) is rapidly becoming the most important imaging modality for measuring organ and tissue function of these genetically engineered mice.

This graduation report deals with the use of MRI to measure the mouse heart function. A so-called "tagging MRI" imaging sequence is implemented, which allows for the determination of displacement and strain fields of the mouse heart muscle. This sequence introduces a modulation to the longitudinal spin magnetization, which results in a characteristic stripe pattern as a function of the position in the image. The movements of this stripe pattern can then be followed by using a fast readout imaging sequence.

The sequence was tested on a rotating phantom. It is concluded that MR tagging experiments in vivo on mouse hearts are within experimental reach with the current MR instrument, provided the heart rate of the mouse can be lowered, by well controlled anaesthesia, to about 300-400 beats/min. In vivo experiments on mice were not performed yet.
Acknowledgments

This graduation report is written at the Eindhoven University of Technology (TU/e) in the Magnetic Resonance Laboratories. With this report I end my academic education at the Applied Physics department specialization Clinical Physics.

I would thank some people, who made this project possible. First prof. dr. K. Nicolay and dr. ir. G. Strijkers, my supervisors, who gave me a great room of freedom and confidence. Dr. A v.d. Toorn and dr. F. Prinzen, both from the University of Maastricht where I started this final project, for interesting me in magnetic resonance tagging.

The members of the Magnetic Resonance Laboratories (MRL) group for their helpfulness and good atmosphere. And the MRL catering for their tasty Cup-a-Soup and the use of the microwave oven for my pizzas, cake and other ready-made meals.

From Philips Research Natlab, ing. F. Vossen for helping me with the electronic scheme, used for triggering the scanner, and the discussions during our snooker games.

Finally, I would thank my girlfriend Angelique, who takes good care of me.

Eindhoven, October 2002

Edwin Heijman
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Chapter 1

Introduction

In modern medical science it is getting more and more important to measure accurately the functionalities and physiological parameters of organs in humans and animals. One of these measuring methods is Nuclear Magnetic Resonance (NMR) or Magnetic Resonance Imaging (MRI). To improve treatments of patients more refined knowledge is gathered with this measuring method.

Nowadays one can measure many physiological parameters with a MRI scanner, such as:

- the perfusion of the brain
- pump function of the heart
- fatigue of muscles

The parameters above are measured on a regular or experimental base in clinics and can be translated into mathematical, chemical or genetical models. With the progress in genetic science over the last decade, the interest in genetically engineered mice or other small animals is growing fast. Particularly the result of the expression of the genes in animals, phenotyping, is studied and also new treatments for (genetical) diseases. Using recombinant DNA techniques on embryos and embryonic stem cells, it is possible to insert new genes (transgenic animals), to delete specific genes (knock-out animals) and even to modify endogenous genes with knock-in mutations ([34]). MRI is in this development an ideal tool, because of the different measurable parameters in a living animal without influencing the biological processes. Especially in the cardiovascular area, MRI has capabilities of imaging and measuring 3D flow and motion.

In the world there is great research effort to study the cardiovascular system. The clinical interest is triggered by the fact, that the heart diseases and failures are one of the major mortalities in the western world. Only in the Netherlands in 2000, more than a third of the causes of death was due to disfunction of the cardiovascular system (reference CBS). To gain insight into heart diseases more knowledge is needed of heart physiology. Therefore a lot of mouse models are now studied to explore the possibilities of simulating human heart diseases or failure. The MRI technique is already used to image the heart of mice ([35, 34]).
This final project is the start of a bigger project to measure the strain in the left ventricle of the mouse at the Eindhoven University of Technology in corporation with the University of Maastricht. The research goal of this final project was: "to investigate how strain must be measured in the myocardium of mice with a tagging technique. And build an experimental setup with a phantom, for testing and verifying sequences".

1.1 The outline of this report

In the next chapter the function and geometry of the heart and heart muscle is described. The term strain is explained in chapter three, together with the analysis methods to translate image data into strain data. The analysis method determines the optimal tagging pattern and is described before the image and tagging sequences (chapter four). The experimental setup and some results are shown in the fifth chapter. This report ends with a discussion, conclusion and recommendations.
Chapter 2

The heart of a mouse

The heart is one of the characteristics of the warm-blooded animals, mammals. It is composed of a three-dimensional continuum, the myocardium, which is essentially made up of rod elements. The assumption is that the structure and the functioning of the mouse heart is comparable to the human heart. A mouse heart contains four chambers (figure 2.1), two ventricles and two atria, the same as with humans.

![Figure 2.1: A drawing and a photo of a mouse heart (LV: left ventricle; RV: right ventricle; LA: left atrium; RA: right atrium; A: aorta; T: trachea)](image)

The rod elements, the cardiac muscle cells, have one-dimensional actuators, which only contract. This contraction is induced by an action potential pulse at the cell membrane. Fast sodium channels and slow calcium-sodium channels are opened ([1]). The calcium ions enter the structure of myosin and actin filaments, the actuator. The calcium induces a coupling between these two filaments, cross-bridges, and releases adenosine diphosphate (ADP) and a phosphate ion. After the release of ADP the myosin and actin
filaments telescope. The muscle contracts and tension is build up in the muscle fibre and muscle skeleton. To release these cross-bridges adenosine triphosphate (ATP) is needed and bounded to the myosin filaments. The filaments extend by the tension in the myocardium. After the binding of the myosin and actin filaments, calcium is pumped back. The ADP will be continuously converted in ATP by the mitochondria by consuming oxygen and producing water and carbon dioxide. The cycle can start again by releasing the calcium after the refractive period, the period where the cell is insensible for new electric stimulations.

It is clear that the contraction of a muscle cell is a complex process. The amount of contraction and force development can depend on electric stimulation, perfusion or other physiological conditions, intra- or extracellular. The force or tension in the myocardium can not be measured in vivo. Only the pressure in the ventricles and the contraction of the myocardium can be directly determined. This means that a change in contraction is an indirect indicator of a change in the surroundings of the muscle cell. To discriminate, which process is the provoker, experimental results from contraction measurements must be verified with other techniques or the experimental conditions must be well controlled.

Figure 2.2: (Left) the helical pathway of a single fibre from the base to the apex in the endocardium and back in the epicardium. The right image is a dissected dog heart ([26]), where the fibre arrangement in planes and their oblique change through the myocardium can be clearly seen.

Cells are arranged in fibres ([18]), papillary muscles, describing a helical path from the base to the apex and back. One part of this path is in the endocardium and the other in the epicardium (see figure 2.2). This structure causes the complex heart motion. This motion is a combination of a torsion and a longitudinal translation. The fibre bundles form fibre planes. These planes can explain the phenomenon of heart wall thickening. In the left ventricle, the shortening generally is about 15%. For a cylindrical fibre this would result in a thickening of 8%. However, the thickening of the wall is 40%, which is explained by the structural morphology of the wall [12]. The papillary muscle change in their oblique orientation in the epicardium to
circumferential (midwall) to reverse oblique in the endocardium (see figure 2.3). This basic structure of the myocardium is present in mice, but it is not known if the same values, fibre angles and thickening apply as for humans.

Figure 2.3: The arrangement of the fibres in planes causes extra wall thickening by their change in oblique orientation through the myocardium ([12]).

At the end of this chapter some terms used are explained of the left ventricle. The base of the heart is the atroventricular fibrous tissue, an electrically isolated layer between the atria and the ventricles. The lowest point of the left ventricle on the long axis is the apex. This long axis is the "rotating symmetrical axis" of the left ventricle. The equator lies in the short axis plane, perpendicular to the long axis, where the left ventricle has its largest diameter. A long axis image is a longitudinal section of the heart wall parallel to the long axis. An example of a long axis image of the mouse heart in an ex vivo mouse preparation is displayed in the right image of figure 2.4. The left image is a short axis image of a section perpendicular to the long axis.

Figure 2.4: (Left) A short axis MR image and (right) a long axis image through ex vivo mouse heart ([8]). The arrows indicate the left ventricle.
Chapter 3

From strain to harmonic phase tracking

Strain is the physical term of the physiological parameter contraction. The contraction area of the myocardium is represented with a strain tensor. This strain tensor is determined with data from the displacement field.

The displacement in the myocardium can be followed with a tag pattern. These tags are imaged and tracked in time. The creation of a tag pattern in a MRI image is described in the next chapter.

The tracking method largely determines the optimal tag creation technique and the optimal shape of the pattern. Therefore, we first describe a tag tracking method, which is based on the phase of a modulated pattern in the image. This chapter ends with a condition of the wavelength of this modulated pattern.

3.1 Displacement field and strain tensor

The objective of the displacement field is to obtain the six components of the strain tensor in every point in the myocardium during the heart cycle. Measured data is translated into a displacement field. First is the relation between the displacement field and the strain tensor described.

The definition of strain is:

The deformation resulting from a stress measured by the ratio of the change to the total value of the dimension in which the change occurred ([9]).

The stress is generated by the contraction of the muscle cells and is transferred in the heart muscle structure.

There are a few strain descriptions and one of them is the material or Lagrangian description ([22]):

\[ \bar{\tau} = \bar{\tau}(\bar{R}, t) \]  (3.1)

Small letters are the current position (\(\bar{\tau}\)) and time (\(t\)). Capitals (\(\bar{R}\)) are the position at time \(T = 0\). Each point at \(T\) is uniquely labelled by its coordinates (\(\bar{R}\)), the points are bijective. To get an unique solution of
equation 3.1, the Jacobian \((J)\) is non zero. The Jacobian represents the norm expansion of a voxel:

\[
J = \left| \begin{array}{ccc}
\frac{\partial x}{\partial r} & \frac{\partial x}{\partial y} & \frac{\partial x}{\partial z} \\
\frac{\partial y}{\partial r} & \frac{\partial y}{\partial y} & \frac{\partial y}{\partial z} \\
\frac{\partial z}{\partial r} & \frac{\partial z}{\partial y} & \frac{\partial z}{\partial z}
\end{array} \right| \tag{3.2}
\]

This requirement is based on the mass conservation law. In equation 3.3 the density functions \((\rho\) and \(\rho_0)\) are positive, which implies that the Jacobian is also positive. This can be used to control the strain calculations in a volume.

\[
\rho[\vec{r}(\vec{R}, t), t]J = \rho_0(\vec{R}) \tag{3.3}
\]

Another description for deformable bodies is the spatial description or Euler velocity field. The velocity of a point \((\vec{v})\) is a function of the current position and time.

\[
\vec{v}(\vec{r}, t) = \hat{V}[\vec{R}(\vec{r}, t), t] \tag{3.4}
\]

This description is easier to use for fluid mechanics or stress calculations. For calculations of the displacement field \(\vec{U}\), the material description is commonly used.

\[
\vec{U}(\vec{R}, t) = \vec{r}(\vec{R}, t) - \vec{R} \tag{3.5}
\]

When a second point is defined, \(d\vec{X}\) away from \(\vec{R}\) (see figure 3.1), the displacement field of this second point with respect to the first point is:

\[
\vec{U}(\vec{R} + d\vec{X}, t) = \vec{r} + d\vec{x}(\vec{U}(\vec{R}, t), t) - \vec{R} + d\vec{X} \tag{3.6}
\]

The deformation between these points is described with both displacement fields.

\[
d\vec{x} = d\vec{X} + \vec{U}(\vec{R} + d\vec{X}, t) - \vec{U}(\vec{R}, t) \tag{3.7}
\]

Figure 3.1: A material point \(\vec{R}\) in a deformable material is displaced to a new position \(\vec{r}\). Deriving the local deformation of the material a second point is defined \(d\vec{X}\) from the first one. After deformation the second point position is \(\vec{r} + d\vec{x}\).
The displacement of the second point \((\mathbf{U}(\mathbf{R}, t))\) can be written as a Taylor expansion. It is assumed, that the displacement field and the distance \((d\mathbf{X} \text{ and } d\mathbf{\bar{X}})\) are small. With the square products of \(d\mathbf{X}\) neglected in the Taylor expansion, the deformation is written with 3.7 as:

\[
dx_i = \left( \delta_{ij} + \frac{\partial U_i}{\partial X_j} \right) dX_j
\]

(3.8)

The deformation tensor is defined as \(\frac{\partial U_i}{\partial X_j}\). This tensor can be split in two parts ((4)). One is a second rank rotation tensor, which rotates a rigid body around an axis in three dimensional space. This rotation tensor is the anti-symmetric part of the deformation tensor. It does not deform the material.

The symmetric part is the strain tensor (\(\varepsilon\)). The strain tensor is defined in formula 3.10.

\[
d\mathbf{\bar{X}} = \varepsilon d\mathbf{\bar{X}}
\]

(3.9)

\[
\varepsilon_{ij} = \frac{1}{2} \left( \frac{\partial U_i}{\partial X_j} + \frac{\partial U_j}{\partial X_i} \right)
\]

(3.10)

The strain tensor is used to quantify myocardial performance, because it is the mathematical expression that isolates the local deformation from the bulk transformation and rotation. The translation and rotation of the entire heart may actually dominate the displacement field and velocity field, but these are of no value to the local myocardial performance (\([27]\)).

The components, \(\varepsilon_{ij}\), with \(i \neq j\), describe the shear between the two points. The other components, \(\varepsilon_{ij}\), with \(i = j\), represents the strain along the axes of the defined coordinate system. The longitudinal strain along the axis \(i\) is written in formula 3.11.

\[
\frac{dx_i - X_i}{dX_i} = \sqrt{1 + 2\varepsilon_{ii}} - 1
\]

(3.11)

When the six components of strain tensor are known, the principal axes of the tensor can be calculated.

An easier method is to determine the simple strain or elongation \(e\) ([31]):

\[
e = \frac{\|\mathbf{\bar{r}} + d\mathbf{\bar{X}}(\mathbf{\bar{R}} + d\mathbf{\bar{X}}, t) - \mathbf{\bar{r}}(\mathbf{\bar{R}}, t))\|}{\|d\mathbf{\bar{X}}\|} - 1
\]

(3.12)

When the parameter \(e\) value is displayed as a color, the areas of function or disfunction in the myocardium can be readily located.

### 3.2 Harmonic phase tracking

In the first tagging experiments, the aim was to generate sharp tag patterns ([3] [39]). These patterns are easy to track as material points in time and a displacement field can readily be determined. Combining these points in a 2 or 3 dimensional grid the strain field is calculated.

A dense sharp tag pattern demands a high resolution image. The tag resolution is lower than the scanned...
resolution. Not every pixel contributes to a tag or material point. At least four pixels should be used for generating enough contrast and counting with the dynamics in the heart wall.

An improvement is to estimate the tag position with subpixel accuracy. The precision of the tag position can be made independent from the pixel resolution by using a tag fitting method described by Atalar ([2]).

The detection and tracking of a sharp tag pattern needs some intervention from the operator ([38]). "Real-time" strain determination of the imaged myocardial wall is not possible. To solve this, algorithms are developed to track the tag pattern automatically. Kumar ([24]) describes an energy-minimizing algorithm based on lines and edges in an image. The algorithm determines where the material points have moved to in the next time frame. This method however requires a lot of computer power.

When using a modulated tag pattern, a sine function (sections 4.3), each pixel contributes to the position of a material point and automatic tracking of the pattern is possible. The phase of the modulation is filtered from the pattern. By given a material point a harmonic phase, it can be tracked by searching its new position in the next image. The filtering of the harmonic phase from the modulated pattern works as follows:

A) An image is recorded in the Fourier domain, called k-space. The recorded echoes are copied directly as k-lines into k-space. K-space is converted by an inverse Fourier transformation to a phase and magnitude image. This magnitude image will only be used in the next step (left image in figure 3.2).

B) For analyzing the magnitude image, it is transferred in a complex Fourier domain, called the magnitude Fourier domain. The tag pattern creates copies of the zero order spectral image information in the magnitude Fourier domain (see the right figure in 3.2). These copies are centered around the tag pattern frequency or higher harmonics of this frequency. These copies are a result of the multiplication of the modulated pattern with the image.

C) One of the main advantages of using a harmonic tag pattern is the possibility of filtering in the magnitude Fourier domain. This reduce the noise level and only the relevant information after conversion is used. The filtering in the magnitude Fourier domain is done by a mask. The wanted area, region of filtering (ROP), in the mask is filled with ones, the other pixels with zeros. This mask is multiplied with the magnitude Fourier image. To prevent ringing in the image smoothing is done on the edges of the ROP. Zhang ([15]) used a low pass filter in the ROP but a Hanning filter over the ROP is also possible.

D) After filtering the new magnitude Fourier data is subjected to an inverse Fourier transformation, resulting in a magnitude image and a phase image (see images in figure 3.3). The phase image is called a HARP image by Osman ([31]) and the pixel values of the HARP image HARP angles. HARP is the abbreviation of "HARmonic Phase". Only the HARP image is now used for tracking the material points.
Figure 3.2: The modulated pattern of the sample container filled with agar in the left image, has been imaged with a spin echo image sequence. The right image is the three dimensional representation of the magnitude Fourier domain. The center peak contains the magnitude image information with the modulated pattern. The outer peaks are a result of the multiplication of the modulated pattern with the image. These are scaled copies of the center spike. For clarity the figure has been rotated. A region filter (ROF) is drawn in the figure. By selecting this region the image data is removed and only the tag pattern remains.

Figure 3.3: After filtering the image in the magnitude Fourier domain (see figure 3.2) with a mask, an inverse Fourier transformation generates a new magnitude image, image left, and a new phase image (right). This phase image is called the HARP image. The magnitude image (left) is a copy of the left image in figure 3.2 without the modulation. The smooth edges arise from the rectangular mask. The dark region in the left image is caused by a fungus in the agar gel.
Intensity ramps in the HARP image (see the right image figure 3.3) are visible in the direction of the modulated pattern interrupted by sharp transitions. These transitions are caused by wrapping of the HARP angle. The HARP angle is defined as a value in the range \([-\pi, \pi]\).

A normal procedure is to unwrap the phase, which is also done in MR thermometry ([20]). A problem of unwrapping is, that there is no reference of the HARP angle between image columns. The image columns are defined along the unwrap direction, the direction of the ramps. Every column has its own start phase resulting in a different unwrapped HARP angle offset. A displacement along the columns is carrying the same unwrapped HARP angle offset. The offset is then cancelled out by the displacement field calculation in equation 3.5.

A movement perpendicular to the columns leads to wrong displacement calculations. To avoid this problem Osman ([31]) has used the ramps as reference points. He does not unwrap the image, except when a HARP angle is searched in the neighbourhood of a transition. This sets an extra limit on the maximum displacement between two time steps, which is equal to the wavelength of the modulated tag pattern.

The location of a HARP angle is not an intersection or pixel location ([31]). The pixels in a HARP image contain values, which represent the mean phase value in these pixels. By interpolating between the pixels the HARP angle position can determined on the subpixel level.

This principle can also be used to make a dense grid for the strain field. The number of pixels in the grid can be higher than in the scanned image, by defining positions between the image pixels. The representation of the strain smooths by this method. Whether this will lead to a subpixel strain measurement depends on the interpolation function and mathematical models of the heart.

The variables \(\vec{R}\) and \(\vec{r}(\vec{R}, t)\) of one material point are tracked with the conditions in 3.13. \(\xi\) is the phase of the material point position. With these parameters the displacement can be calculated. When this is done with more material points a displacement field can be determined.

\[
\xi(\vec{R}) = \xi(\vec{r}(\vec{R}, t)) \tag{3.13}
\]

This condition holds for a one-directional displacement. In a multi-dimensional displacement, the HARP angle is not unique anymore. For a two-dimensional displacement, two HARP images with perpendicular modulated tag patterns are needed.

A material point in the myocardial wall \((\vec{R}(\xi_x, \xi_y))\) has two HARP angles, \(\xi_x\) in the x direction and \(\xi_y\) for the y direction. Osman uses a Newton-Raphson method as tracking algorithm. The Newton-Raphson method is restricted to one pixel. Every HARP angle displaces less than one pixel from its original position. This restriction is not practical for mouse tagging. In the next section a new tracking strategy is proposed.

### 3.3 Wavelength of the modulated tag pattern in mouse

The wavelength is defined in units of a number of pixels. The first condition is the lower limit of the wavelength. The lower limit is defined by the Nyquist theorem which states that:

a signal should be sampled at a rate, which is twice the frequency.
The minimum wavelength is at least two pixels long, when the strain is at its highest value. The maximum displacement should not be larger than the wavelength of the modulation between two following images. In practice more than four pixels are used to have a save margin for aliasing. A higher resolution and a short wavelength of the tag pattern has immediate consequences for the time between the images.

The upper limit is not easy to define. A long wavelength has a low \( \frac{\partial \xi}{\partial x} \) or \( \frac{\partial \xi}{\partial y} \). This induces a higher sensitivity for noise in the HARP angle position calculation (see figure 3.4).

![Graph showing noise influence on the accuracy of the HARP angle position between two pixels.](image)

Figure 3.4: Noise influence on the accuracy of the HARP angle position between two pixels. \( \sigma \) is the noise level in the HARP angle pixels. For a short wavelength (\( \Delta x_{\text{short}} \)) (left) the deviation of the position is smaller, than with a longer wavelength (\( \Delta x_{\text{long}} \)).

The lower limit prescribes the wavelength of the pattern. In practice a wavelength of 4 or 5 pixels can be used. This implies that the restriction of the tracking method is equal to this wavelength, which prevents aliasing of the material points.
Chapter 4

MR tagging

After the description of the tag image analysis, which generates the displacement field for the strain tensor, the tag creation and imaging is now described. This procedure is called MR tagging. The tags are not made in the image sequence, but in a preparation sequence. Reichek ([33]) describes short the relation between the tagging and image sequence as follows:

"myocardial tagging methods are based on deposition of planes of presaturation intersecting the myocardium prior to the playout of the MRI sequence itself".

The MR tagging technique prepares the longitudinal magnetization, which is visualized with an image sequence. The advantage is that the both parts can be optimized separately.

![Tag preparation sequence and Image sequence](image)

Figure 4.1: A schematic representation of the MRI tagging sequence. First a preparation of the longitudinal magnetization is done by a tag preparation sequence. This preparation is then followed by an image sequence.

The best way to start developing a MR tagging sequence, is to start with the image sequence. When the parameters and pitfalls of the image sequence are known, the judgement of the tag quality can be made easier and the development can go faster. In the first section of this chapter a gradient echo image sequence is explained, followed by two adaptations of this sequence that are usable for mouse heart imaging (see figure 4.1).

Two tag preparation sequences have been developed through time. The first method is based on a RF, radio frequency, preparation of the longitudinal magnetization and is described in the first paragraph in section two. The second paragraph of section two describes the spatial modulation of the longitudinal magnetization (SPAMM) method based on a gradient field.
This chapter ends with a summary of developments of two dimensional MR tagging generating a three dimensional displacement or strain field.

### 4.1 Image sequence: Gradient echo

A fast image sequence is needed to image the heart of the mouse (600 beats/min). The most frequently used image sequences are based on the formation of a gradient echo. After excitation of the spin vector with a RF pulse, an echo of the signal is created by a gradient pair with opposite sign (see figure 4.2). Formula 4.1 describes the condition of forming an echo with a gradient pair.

\[
\int_{t_1}^{t_2} G(\tau) d\tau = 0 \tag{4.1}
\]

\(G(\tau)\) is the gradient strength in time \(\tau\). The value of \(G\) is chosen such, that dephasing of the spins occurs within each voxel. This reduces the influence of magnetic field inhomogeneities on the phase of the spin vector. During the rephasing part (opposite gradient sign), the spins are coming into phase when condition 4.1 is reached. An echo is created.

![Figure 4.2](image)

*Figure 4.2: A RF pulse followed by a bipolar gradient pair generates a gradient echo. RF is the radio frequency channel and G is the magnetic field gradient axis. The excitation flip angle of the RF pulse is \(\theta\). The excitation flip angle is the angle between the longitudinal axes \((\vec{B}_0)\) and the spin vector after the RF pulse.*

With this principle it is easier to make fast scans than with a spin echo sequence. Another difference with a spin echo sequence is, that a variable \(\vec{B}_1\) field strength can be used. This leads to a different excitation flip angle \((\theta)\), the angle between the spin vector and the longitudinal axis \((\vec{B}_0)\) after a RF pulse. A gradient echo sequence is shown in figure 4.3.
Figure 4.3: A gradient echo sequence. The RF pulse is represented with a line on the RF channel ($\theta$ is the excitation flip angle). The gradient echo is created when the readout gradient reaches the condition 4.1. RF: RF channel; $G_{SS}$: slice selection gradient; $G_{PE}$: phase-encoding gradient; $G_R$: readout gradient; $T_E$: echo time; $T_R$: repetition time.

With a very short echo-time ($T_E$), the repetition time ($T_R$) can also be short. A short $T_R$, ($T_R \ll T_2^*$) needs extra attention. The magnetization of the first pulse has not decayed and is influenced by the second, third, RF pulse. When a certain number of these pulses have passed, a steady state is reached in the longitudinal magnetization (see figure 4.4). This steady state develops if the decay of the longitudinal magnetization is equal to the flip angle change by the next RF pulse. The extent of the decay is a function of the $T_1$ of the tissue and the $T_R$ of the sequence.

Figure 4.4: The formation of steady state longitudinal magnetization ($M_z$) during a train of RF pulses. This state is reached when the longitudinal decay is equal to the flip angle change of the next RF pulse. $T_R$ is the repetition time, i.e. the time between successive RF pulses. $M_0$ is the equilibrium magnetization.

In a fast gradient echo sequence, the echoes become a complex system of spin ensembles. The spin vector represents different spin ensembles with their own histories. Echoes are formed, when one of the spin ensembles is coming into phase.
Dephasing and rephasing is not only accomplished by gradient fields, but RF pulses and the relaxation mechanisms $T_2$ and $T_2^*$ also interact with the phase difference in a spin ensemble. This makes it more complex.

A method to study a fast gradient echo sequence is described by Woessner ([37]) and Kaiser ([23]). This method, called configuration theory, calculates the contribution of each RF pulse to the formed echoes. Part of this theory is described in this section. For the full theory and applications see Vlaardingerbroek ([36]) and Nijsten ([30]).

The formulas used in this section are all derived for the rotating frame of reference. This reference frame rotates with the reference frequency or Larmor frequency ($\omega_0$), calculated with the formula 4.2.

$$\omega_0 = \gamma B_0$$  \hspace{1cm} (4.2)

$\gamma$ is the gyromagnetic constant of the observed nucleus.

A RF pulse is accepted as an instantaneous transformation field of the spin vector. The effect of a RF pulse can be described as a rotation around the $x$-axis (see equations 4.3 and 4.4). The superscript "-" represents the moment before the RF pulse and the superscript "+" the moment after the pulse.

$$\tilde{M}^+ = R_x(\theta) \tilde{M}^-$$  \hspace{1cm} (4.3)

$$R_x(\theta) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos(\theta) & \sin(\theta) \\ 0 & -\sin(\theta) & \cos(\theta) \end{pmatrix}$$  \hspace{1cm} (4.4)

The transformation induced by a RF pulse, is written per magnetization component in the rotating frame of reference:

$$M_x^+ = M_x^-$$  \hspace{1cm} (4.5)

$$M_y^+ = M_y^- \cos(\theta) + M_z^- \sin(\theta)$$  \hspace{1cm} (4.6)

$$M_z^+ = -M_y^- \sin(\theta) + M_z^- \cos(\theta)$$  \hspace{1cm} (4.7)

These equations are changed with goniometric formulas.

$$M_x^+ = M_x^- \left( \cos^2 \left( \frac{\theta}{2} \right) + \sin^2 \left( \frac{\theta}{2} \right) \right)$$  \hspace{1cm} (4.8)

$$M_y^+ = M_y^- \left( \cos^2 \left( \frac{\theta}{2} \right) - \sin^2 \left( \frac{\theta}{2} \right) \right) + M_z^- \sin(\theta)$$  \hspace{1cm} (4.9)

$$M_z^+ = M_z^- \left( \cos^2 \left( \frac{\theta}{2} \right) - \sin^2 \left( \frac{\theta}{2} \right) \right) - M_y^- \sin(\theta)$$  \hspace{1cm} (4.10)

The transverse magnetization can be imagined as two vectors rotating in the opposite direction. The subscript "-" is used for the anti-clockwise rotating magnetization, the subscript "+" for the clockwise rotating
magnetization. Both vectors are written in equations 4.12 and 4.11. The vector $M_+$ can be observed, because it rotates in the natural direction. The natural direction in a positive magnetic field is clockwise ([19]). $M_-$ is the non-observable component.

$$M_+(t) = M_x(t) + i M_y(t)$$
$$M_-(t) = M_x(t) - i M_y(t)$$

Combining the equations 4.8 to 4.10 with 4.11 and 4.12 we can conclude that each RF pulse changes the spin system into four spin populations $M_z$, $-M_z$ and $(M_+, M_-)$. These populations represent in same order the three flip angles $\theta = 0^\circ$, $180^\circ$ and $90^\circ$.

![Figure 4.5: A spin vector in the rotating frame of reference.](image)

Figure 4.5: A spin vector in the rotating frame of reference. $\beta$ is phase in the $xy$-plane and $\theta$ is angle with $z$-axis (flip angle). The $z$-axis is the direction of the main magnetic field $\vec{B}_0$.

$$M_+^z = M_+^z \cos^2(\frac{\theta}{2}) + M_-^z \sin^2(\frac{\theta}{2}) + i M_-^z \sin(\theta)$$
$$M_-^z = M_-^z \cos^2(\frac{\theta}{2}) + M_+^z \sin^2(\frac{\theta}{2}) - i M_+^z \sin(\theta)$$
$$M_+^z = M_+^z \left( \cos^2(\frac{\theta}{2}) - \sin^2(\frac{\theta}{2}) \right) - \frac{i}{2} (M_-^z - M_+^z) \sin(\theta)$$

Important is that the measurements are based on longitudinal magnetization ($M_z^+$) and two components of transverse magnetization ($M_-^z - M_+^z$). The RF field makes it possible to convert anti-clockwise (non-observable) rotating magnetization into clockwise rotating magnetization and the other way around.

One of the interesting parts of the theory, is to visualize the formation of echoes from the different RF pulses and spin ensembles. This is done by a phase diagram (figure 4.6). The diagram has a horizontal time axis and on the vertical axis the transverse phase difference ($\Delta \beta$) from the spin ensemble is indicated. The phase difference is the dispersion of phases in the spin ensemble itself. The transverse angle $\beta$ and the flip angle $\theta$ of the spin ensembles are drawn in figure 4.5.

A spin ensemble is defined as spins, who have been together created or transformed by a RF pulse. The
Oblique phase lines are spin ensembles changing their phase difference in time. Spin ensembles directed along the longitudinal axis, the z-axis (figure 4.5), describe a horizontal phase line in the phase diagram. These spin ensembles are not affected by a gradient field or dephasing by $T_2$ or $T_2^*$ effects. These ensembles maintain their transversal phase difference, which they had, just before they were transferred to the z-axis.

To simplify the phase computing of the phase lines, the time and the phase are divided in intervals. A fraction of the repetition time ($T_R$) or $T_R$ itself can serve as a time interval width ($t_{int}$). The phase interval, i.e., in the readout direction, is defined by formula 4.16 ([36]). The parameter $x_R$ is the dimension of the object in the readout direction and $G_R$ the gradient strength of the readout gradient.

$$
\Delta \beta(t_{int}) = \gamma x_R \int_0^{t_{int}} G_R(\tau) d\tau
$$

(4.16)

Each RF pulse transforms a phase line in new phase lines, which are described by the formulas 4.13 to 4.15. In the phase diagram only the clockwise spin ensembles are drawn, because they are measurable. An echo is created, when a phase line crosses the $\Delta \beta=0$ axis, because then the spin ensemble comes into phase and has the maximum signal intensity or spin vector length.

In figure 4.6 a phase diagram of the zeroth k-line of a gradient echo sequence is drawn for a couple of RF pulses. The phase changes by the slice selection gradient are cancelled out. The phase gradient is also without effect, because this gradient is not used for the zeroth k-line in k-space.

![Figure 4.6: A phase diagram of the zeroth k-line in a gradient echo sequence (see figure 4.3). $\Delta \beta$ is the phase deviation in a spin ensemble. The different spin ensembles, generated by the RF pulses, are drawn as phase lines. Oblique phase lines are influenced by gradient fields. Each RF pulse splits a spin ensemble and generates a fresh spin ensemble. The dots on the $\Delta \beta=0$ axis are formed echoes.](image)

When echo formation is close together, the recording window may contain more than one echo. This will lead to a modulation of the object, just a little bit shifted in the image (banding [36]). A method to
prevent this, is to use a steady state incoherent gradient echo (SSI) sequence described in the paragraph below (4.1.1).

A lot of phase lines in a gradient echo sequence are not used for forming echoes. To recycle, or combine spin ensembles in one echo, a steady state coherent gradient echo (SSC) sequence has been developed and is described in 4.1.2. This SSC sequence is also called a balanced gradient echo sequence.

The SSI and SSC methods are explained and their steady state excitation flip angle for maximum signal conditions are derived. At the end of each paragraph, it is discussed, whether the sequence is suitable for mouse heart imaging.

4.1.1 Steady state incoherence gradient echo sequence

In a steady state incoherence gradient echo (SSI) sequence a large part of the remnant transverse magnetization, is spoiled prior to the occurrence of each new RF pulse. This is done by phase cycling of the RF pulses with $\beta = n \times 117^\circ$ (19).

The principle is that the echo, $T_E$ after the RF pulse, exists only of a phase line generated by this RF pulse, while the other phase lines are spoiled. The phase cycling angle $(117^\circ)$ is determined by simulation of the formation of echoes with different phase angles during a fast gradient echo sequence similar to figure 4.6 (30). A theoretical explanation is given by Vlaardingerbroek (36). The SSI sequence is drawn in figure 4.7.

![Diagram of SSI sequence]

Figure 4.7: Steady state gradient incoherent echo sequence. The RF pulse is represented with a line on the RF channel ($\theta$ is the excitation flip angle). The following RF pulses each have a transverse phase increment of $\beta = 117^\circ$. The gradient echo is created when the readout gradient reaches the condition 4.1. A rewinder, phase coding gradient with a downwards arrow, preserves the transverse phase of the RF pulse. RF: RF channel; $G_{SS}$: slice selection gradient; $G_{PE}$: phase-encoding gradient; $G_R$: readout gradient; $T_E$: echo time; $T_R$: repetition time.

The excitation flip angle for a steady state is derived below. The steady state of a SSI sequence is defined as:

$$M_x(mT_R) = M_{x,ss} \quad m \geq N([19])$$

(4.17)
where a constant magnetization \((M_{z,ss})\) is developed at the \(N^{th}\) RF pulse of the SSI sequence. This steady state criterion can be written as a function of the flip angle. The Bloch equation is used for the transverse magnetization \(M_t\) and longitudinal magnetization \(M_z\). Solutions of this equation are ([19]):

\[
M_z((n + 1)T_{R}^+) = M_z(nT_{R}^+ \cdot \cos(\theta) \cdot e^{-\frac{T}{T_1}} + M_0(1 - e^{-\frac{T}{T_1}})
\]

\[
M_t((n + 1)T_{R}^+) = M_z(nT_{R}^+ \cdot \sin(\theta) \cdot e^{-\frac{T}{T_2}})
\]

\(T_{R}^+\) is defined as the time just after the RF pulse while \(n\) is the number of pulses before this RF pulse.

Inserting the criterion (4.17) in the last two formulae we get:

\[
M_{z,ss} = M_0(1 - e^{-\frac{T}{T_1}})
\]

\[
1 - \cos(\theta) \cdot e^{-\frac{T}{T_1}}
\]

\[
M_{t,ss} = M_{z,ss} \cdot \sin(\theta) \cdot e^{-\frac{T}{T_2}}
\]

with \(M_0\) the longitudinal magnetization in thermal equilibrium.

The Ernst angle is the excitation flip angle \((\theta)\) of the RF pulse, where the transverse magnetization \(M_{t,ss}\) is maximized and thus the signal intensity. The longitudinal magnetization \(M_{z,ss}\) (4.20) is inserted in 4.21.

The Ernst angle \((\theta_E)\) for a steady state incoherent sequence becomes:

\[
\theta_E = \arccos(e^{-\frac{T}{T_1}})
\]

The advantage of this sequence compared with the normal gradient echo sequence is, that k-lines are not contaminated with spurious echoes. The SSI sequence generates a good \(T_1\)-contrast, which is a good property for imaging a tag pattern.

The biggest disadvantage of the SSI sequence is, that a great part of the total magnetization is wasted. The signal conditions are not optimal. Only a spin ensemble excited by the last RF pulse contributes to the echo.

### 4.1.2 Steady state coherence gradient echo sequence

One of the solutions to get a better signal intensity, is recycling of the magnetization. This is done by generating a second echo, when the next RF pulse is given. On that moment the free induction decay is composed of components from "fresh" longitudinal magnetization and recycled magnetization from the last pulse. This sequence is therefore called a steady state coherent gradient echo (SSC) sequence or balanced gradient echo sequence. The coherence is created by extra balancing gradients (see figure 4.8). The transverse phase is preserved as defined in formula 4.1 at the moment of the following RF pulses. This criterion not only applies to the readout and slice selection gradient, but also to the phase encoding gradient. The correcting phase encoding gradient is called a rewinder.

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Figure 4.8: The steady state coherence gradient echo (SSC) sequence. The RF pulse is represented with a line on the RF channel ($\theta$ is the excitation flip angle). The recording echo is created when the readout gradient reaches the condition 4.1. All the gradients fulfill this condition at the moment of the next RF pulse. RF: RF channel; G$_{SS}$: slice selection gradient; G$_{PE}$: phase-encoding gradient; G$_R$: readout gradient; T$_E$: echo time; T$_R$: repetition time.

To determine the optimal excitation flip angle for the steady state, we can use a solution from the Bloch equation proposed by Haacke ([19]). The spin vector rotation, induced by the RF pulse, is only around the $x$-axis of the rotating frame of reference. Superscript $'-'$ is before the RF pulse and the superscript $'+'$ refers to the time after the RF pulse. The equations calculate only the steady state situation for $t \rightarrow \infty$:

$$M_{x,ss}^- = M_0(1 - e^{-\frac{T_R}{T_1}}) \times \frac{e^{-\frac{T_R}{T_2}} \sin(\theta) \sin \beta}{d} \tag{4.23}$$

$$M_{y,ss}^- = M_0(1 - e^{-\frac{T_R}{T_1}}) \times \frac{e^{-\frac{T_R}{T_2}} \sin(\theta)(\cos \beta - e^{-\frac{T_R}{T_2}})}{d} \tag{4.24}$$

$$M_{z,ss}^- = M_0(1 - e^{-\frac{T_R}{T_1}}) \times \frac{(1 - e^{-\frac{T_R}{T_2}} \cos \beta) - e^{-\frac{T_R}{T_2}} (\cos(\beta) - e^{-\frac{T_R}{T_2}} \cos(\theta)) \cos(\theta)}{d} \tag{4.25}$$

$$M_{x,ss}^+ = M_{x,ss}^- \tag{4.27}$$

$$M_{y,ss}^+ = M_0(1 - e^{-\frac{T_R}{T_1}}) \times \frac{\sin(\theta)(1 - e^{-\frac{T_R}{T_2}} \cos(\beta))}{d} \tag{4.28}$$

$$M_{z,ss}^+ = M_0(1 - e^{-\frac{T_R}{T_1}}) \times \frac{e^{-\frac{T_R}{T_2}} (e^{-\frac{T_R}{T_2}} \cos(\beta)) + (1 - e^{-\frac{T_R}{T_2}} \cos(\beta)) \cos(\theta)}{d} \tag{4.29}$$

$$d = (1 - e^{-\frac{T_R}{T_1}} \cos(\theta))(1 - e^{-\frac{T_R}{T_2}} \cos(\beta)) - e^{-\frac{T_R}{T_2}} (e^{-\frac{T_R}{T_1}} \cos(\theta))(e^{-\frac{T_R}{T_2}} \cos(\beta)) \tag{4.30}$$
The angle $\theta$ is the excitation flip angle and $\beta$ is the phase of the RF pulse in the transverse plane of the rotating frame of reference (figure 4.5).

The maximum magnetization $M_{y,ss}$ is reached for a flip angle of:

$$
\cos(\theta_{opt}) = \frac{e^{-\frac{T_R}{T_1}} e^{-\frac{T_B}{T_2}} \left( \cos(\beta) - e^{-\frac{T_B}{T_2}} \right)}{1 - e^{-\frac{T_B}{T_2} \cos(\beta)}} + \frac{\frac{e^{-\frac{T_B}{T_2} \cos(\beta)}}{1 - e^{-\frac{T_B}{T_2} \cos(\beta)}}}{1 + \frac{\frac{e^{-\frac{T_B}{T_2} \cos(\beta)}}{1 - e^{-\frac{T_B}{T_2} \cos(\beta)}}}{1 - e^{-\frac{T_B}{T_2} \cos(\beta)}}}
$$

(4.31)

For large excitation flip angles ($\theta$) the transverse angle $\beta$ of the RF pulses, must be alternated between $0^\circ$ and $180^\circ$ for getting maximum signal intensity in the transverse plane ([19]). On the other hand small excitation flip angles need a $\beta$ of $0^\circ$.

The SSC sequence is favoured for mouse heart imaging, because of its good signal intensity. This good signal condition can only be reached by a perfect balance between the different gradients.

Now we know the image sequence the next section describes the tag preparation sequence.

### 4.2 Tagging preparation sequence

The HARP tracking method needs a modulated pattern. In this section two tag pattern generating methods known from the literature are described. Both descriptions ends with a discussion whether the method is suitable for MR tagging of the mouse heart.

![Figure 4.9: A modulation pattern in a phantom (a sample container containing agar gel) a SPAMM pattern imaged with a spin echo sequence.](image)
4.2.1 RF pattern

An early solution for RF pattern generation, is described in an article from Zerhouni ([39]). He has used selective saturation pulses orthogonal to the image plane. The selection is made with a gradient field and a RF saturation pulse with a certain central frequency and bandwidth. A rotating slice selection gradient with RF saturation pulses generated a star pattern around the orthogonal axis of the image plane. For each line a saturation pulse is needed, which takes a lot of time. For mouse heart tagging this method is unsuitable. The method is too slow and the image resolution is poor.

Another RF pattern generation method is called DANTE (Delays Alternated with Nutations for Tailored Excitation). This method is based on a train of RF pulses, separated by a constant time delay. These pulses are "hard" RF pulses, which mathematically can be represented as "delta functions". The amplitude of these pulses is controlled with a second function (a multiplication of the pulse train), which results in the Fourier domain in a convolution product. The Fourier components of this pulse schedule are projected in the image by using a selection gradient ($G_x$) within the image plane. The selected regions are saturated by this procedure (see figure 4.10).

![Figure 4.10: A DANTE pulse train is drawn, in which the "hard" RF pulses are represented by arrows. The top drawing shows the Fourier components of the $B_1$ field of this pulse train ([19]). The Fourier components of the $B_1$ field are projected on the image plane by the simultaneous application of a magnetic field gradient.](image-url)
In figure 4.10 a sinc function is chosen as a multiplication with the pulse train. This results in a rectangular pattern. $B_1$ is the magnetic field strength of the RF field and is chosen high enough to saturate the selected regions.

The width ($x_w$) and the spacing ($x_t$) of the pattern are controlled with the pulse duration ($T_p$), the time between the "hard" pulses ($t_p$) and the magnetic gradient field ($G$). These relations are written in the formulas 4.32 and 4.33.

$$T_p = \frac{2\pi}{\gamma * G * x_w}$$  \hspace{1cm} (4.32)

$$t_p = \frac{2\pi}{\gamma * G * x_t}$$  \hspace{1cm} (4.33)

Because of the finite duration of the pulse train, an extra convolution product in the Fourier domain takes place. This leads to blurring of the sharp edges of the rectangular saturation pattern.

The pulse train will generate a stripe pattern in the image plane, perpendicular to the gradient field, which converts the frequency components to a spatial pattern.

To make a grid, the procedure is repeated with the magnetic gradient field perpendicular to the first one. This second magnetic gradient field is also in the image plane.

With the DANTE pattern method a tagging pattern can be generated fast. However a modulated pattern is not possible with this method. The DANTE tagging image cannot be analyzed by harmonic phase tracking method (see section 3.2).

### 4.3 SPAMM

Axel and Dougherty ([3]) have first described the method of creating a sinusoidally modulated tag pattern. They used two RF pulses combined with a magnetic gradient field between them, to wrap the phase along the gradient field. There are many variations based on this principle, which are known together as SPAMM (SPAtial Modulation of Magnetization).

The basics of the SPAMM sequence (figure 4.11) exists of a non-selective RF pulse, which rotates the longitudinal magnetization over a certain flip angle ($\theta_z$). The magnetization becomes with the same convention used in section 4.1.

$$M_x^+(t_2) = 0$$  \hspace{1cm} (4.34)

$$M_y^+(t_2) = M_0 \sin(\theta_z)$$  \hspace{1cm} (4.35)

$$M_z^+(t_2) = M_0 \cos(\theta_z)$$  \hspace{1cm} (4.36)

The times $t_1$ to $t_4$ are defined in figure 4.11.
Figure 4.11: The SPAMM preparation in the rotating frame of reference. A RF pulse ($\theta_x$) is used to rotate all spin vectors around the $x$-axis. The gradient $G_x$, along the $x$-axis, dephases the spin vectors. The vectors are individual spin vectors at different positions on the $x$-axis each with their own transverse phase. A second RF pulse rotates the spin vectors again. To generate only a longitudinal magnetization pattern a crusher gradient spoils all the transverse magnetization. The result is a modulation of the longitudinal magnetization.

A constant magnetic gradient field is switched on to generate wrapping of the phase. The gradient time is $t_{SPAMM}$. The transverse phase of the magnetization, after the gradient, is changed into:

$$M_x^-(x, t_3) = M_0 \sin(\theta_x) \sin(\beta(x))$$
$$M_y^-(x, t_3) = M_0 \sin(\theta_x) \cos(\beta(x))$$

The transverse phase $\beta(\bar{x})$ can be calculated from the formula 4.39. For a $x$-gradient ($G_x$) the phase is linear to its position $x$:

$$\beta(x) = \gamma \int_{t_1}^{t_2} ||x|| G(x, \tau) d\tau$$
$$= \gamma x G_x(t_{SPAMM})$$

for a rectangular gradient pulse.

When combining 4.36 to 4.38 a vector $\vec{M}$ is formed, which can be used in formula 4.3. When both RF pulses generate the same excitation flip angle, the longitudinal magnetization $M_z^+(x, t_4)$ becomes:

$$M_z^+(x, t_4) = -M_0 \cos(\beta(x)) \sin^2(\theta_x) + M_0 \cos^2(\theta_x)$$

After the last RF pulse a crusher gradient is used to destroy any residual transverse magnetization. This prevents unwanted echo formation during the image sequence.
The SPAMM sequence is the only solution for creating a modulated pattern and can be used for mouse MR tagging. This section continues with an improved SPAMM sequence and ends with some practical information for imaging the modulated tag pattern.

An improvement on SPAMM is CSPAMM (Complementary SPAtial Modulation of Magnetization). This method is developed by Fischer ([17]). This method consists of two SPAMM acquisitions (with the modulation in the same direction). First a normal SPAMM acquisition is done. In the second acquisition the second RF pulse, with the same excitation flip angle as all the other RF pulses, has an opposite transverse phase compared to the first one. This results in a negative flip angle \(-\theta_z\) in figure 4.11. The modulation of the tag pattern is then inverted, compared with the first 'normal' acquisition. These two acquisitions can be added or subtracted from each other. When the images are added, a normal image is formed. Subtraction results in a modulated pattern without any offset, which is easier for analyzing with HARP tracking (see section 3.2). Filtering in the magnitude Fourier domain is not necessary, because there is only a modulation. This can reduce computation time during the analysis. The subtraction improves also the signal to noise ratio (SNR) with a factor \(\sqrt{2}\).

The fading of the tagging pattern is also described by Fischer ([17]). Formula 4.41 is valid for \(t_d\) after the last RF pulse (\(t_d > t_4\) see figure 4.11).

\[
M_z(t_d) = (M_z^+ - M_0)e^{-\frac{t_d}{T_1}} + M_0
\]  

(4.41)

When a fast gradient echo sequence is used this formula is not valid anymore.

For a SPAMM pattern with a modulation between \(M_0\) and \(-M_0\), the longitudinal magnetization is written as \(M_0 \cos(x)\). In a magnitude image, this leads to a \(M_0 \cos(x)\) function. The coil only receives the transverse component of the spin vector. To transfer this pattern in a \(\cos\) function the transverse phase of the spin vector can be used. Otherwise it is difficult to analyze these images automatically with the HARP tracking. To prevent this, RF pulses with a flip angle of 45 degrees or less can be used.

The readout direction in k-space is set perpendicular to the tag lines, which results in fast recording of the tag pattern information ([28]). The resolution in the readout direction can be raised, without introducing extra scanning time. This will result in a better sampling of the harmonic function.

To minimize ghost artifacts, arising by a varying R-R interval, the order of k-space filling is done from the zero k-line outward and alternating between the positive and negative phase k-lines (see figure 4.12).
The SPAMM technique can also be used in two directions creating a grid pattern. This is done by repeating the basic SPAMM sequence, but changing the gradient direction in the second part. This is normally not done, because it is more sensitive to motion and will not allow us to use selective k-space sampling ([28]).

According to Mc. Veigh ([27]) the minimum contrast-to-noise ratio (CNR) of 5 is useful for image analysis for SPAMM. The CNR is defined in formula 4.43.

\[
C_{AB} = S_A - S_B \quad (4.42)
\]
\[
CNR = \frac{C_{AB}}{\sigma_0} = \frac{S_A - S_B}{\sigma_0} \quad (4.43)
\]
\[
= SNR_A - SNR_B \quad (4.44)
\]

The contrast difference between two pixels \((C_{AB})\) is the subtraction of the pixel intensities \((S_A \) and \(S_B)\). These intensities are the pixel values in the magnitude image. The standard deviation \((\sigma_0)\) is defined by Haacke ([19]) as the mean value \((\overline{S_{ns}})\) of a region without any MR signal in the magnitude image times a factor for the Rayleigh distribution.

\[
\overline{S_{ns}} = 1.25\sigma_0 \quad (4.45)
\]

The difference of the signal-to-noise ratio (SNR) can also be used. The minimum CNR is now calculated between the lowest value of the modulated tag pattern and its maximum.
4.4 Three dimensional displacement field with two dimensional MR tagging sequence

The heart moves in the three-dimensional space. Measuring in the third dimension is possible by using 3D MRI scan techniques, where two dimensions are used for phase encoding. The measured 3D k-space is converted by a three dimensional inverse Fourier transformation to a three dimensional image. It is technically possible to make a three dimensional SPAMM grid, but the analysis methods of this grid are still under development.

MR tagging images of the heart are normally scanned as a stack of 2D parallel planes (multi slice). This multi slice experiment is packed in a cine sequence. The cine sequence is an image sequence generating images of the contracting heart as a movie. The advantage of this combined sequence is, that it is possible to serially scan k-lines from different image planes and times, without losing time on delays.

To measure from these stacks the three dimensional displacement field of the heart in time (yielding the three dimensional strain), several options have been proposed in the literature:

1) Using 2D displacement field for a 2D analysis of the strain and calculate with anatomical data the 3D strain along the fibre direction. The fibres are organized into a branching laminar structure ([25]). This structure is, when unrolled during an anatomical study, like a rope. At a distance of one diameter of the rope, the orientation of the fibres is inverted. Mid ventricular, where the curvature is large, the rope appears flattened from both sides. The rope-like structure appears as a single roof-like undercrossing layering. Near the apex where the rope surrounds a smaller lumen, the rope structure is well preserved ([26]).

Using a transformation matrix the 2D strain vector in the 2D image can be translated to a 3D structure of the heart. There is enough data of the fibre geometry in the human and canine heart ([10]), which are comparable to that of the mouse.

Important is the position and the orientation of the image in the heart structure. During the heart cycle the heart moves through the plane and tilts with respect to the long axis. Both must be accurately known.

To segregate between the different laminar structures through the wall, the resolution of the strain vector must be very high. To make this less critical a mean direction of the fibre orientation in a voxel must be determined.

2) Using a 3D biomechanical model of the heart for fitting the displacement field. With the displacement field the 3D myocardial strain can be calculated. The fitting points are material points in the myocardium, which are tracked in time. These material points (nodes) are generated by guide points, which are defined manually in the first time frame of the multi slice data set ([38]). Papademetris described in his thesis ([32]) interactive segmentation methods and mathematical models of automatic tracking of points.

Three types of images are used. Two short axis image stacks with perpendicular tag patterns and a third long axis image stack with the tag pattern perpendicular to the long axis of the heart ([16]).

This method requires a lot of human intervention in the calculation of the 3D strain and with the use of predefined material points the resolution of the image sets is decreased.
3) Velocity encoding for through plane motion during the acquisition of the tagging images. With this method a image stack of short axis images is made between the base and the apex of the left ventricle. With an extra bipolar gradient (for this method the slice selection gradient) along the motion direction, the phase of the spins in the image plane is a function of the through plane velocity. This method is described by Kuijer ([14]). The spins around material points accumulate phase, during a gradient field \( G_s(\tau) \) (see formula 4.46). The position of the material point \((\vec{r}(\tau))\) can be written as a Taylor expansion, formula 4.47, at time \( t \) with velocity \( \vec{v}_t \) and acceleration \( \vec{a} \) ([36]).

\[
\varphi = \gamma \int_{t_1}^{t_2} G_s(\tau) \vec{r}(\tau) d\tau \tag{4.46}
\]

\[
\vec{r}(\tau) = \vec{r}_i + \vec{v}_t (\tau - t) + \frac{1}{2} \vec{a}(\tau - t)^2 \tag{4.47}
\]

The initial position and the acceleration can be cancelled out by using opposite gradient fields of equal strength. This gradient couple is called a dipolar gradient. The accumulated phase of a bipolar gradient becomes then:

\[
\varphi(t) = 2\gamma \int_{t_1}^{t_2} G_s(\tau) \vec{v}_t (\tau - t) d\tau \tag{4.48}
\]

when rectangular gradients are used and both parts of the bipolar gradient have the same shape. The integral in formula 4.48 becomes a multiplication between the gradient field strength \( (G_s) \) and half the switch on time of the gradient \((t_2 - t_1)\).

The phase is defined in the range \([-\pi, \pi]\), \(\pi\) in both velocity directions along the gradient field.

A certain gradient field strength and a maximum velocity should be considered to prevent wrapping of the phase. This velocity \( v_{enc} \) is the maximum velocity, which can be measured in the through plane direction. The encoded velocity as a function of the gradient field strength is defined in formula 4.49.

\[
v_{enc} = \frac{\pi}{\gamma G_s(t_2 - t_1)} \tag{4.49}
\]

The phase is linearly dependent on the through plane velocity \( v_m \) (formula 4.50, which must be lower than the defined \( v_{enc} \) ([14]).

\[
v_m = \frac{\Delta \varphi}{\pi} v_{enc} \tag{4.50}
\]

\(\Delta \varphi\) is the subtraction of the phase in a pixel with and without a bipolar gradient. With this procedure the phase offset is eliminated. This means that at least four acquisitions must be taken, to get all the information needed for analysis, two images with a modulated pattern \((x\ and\ y\ direction)\) with a velocity encoding and two without a velocity encoding.

Another point of interest is the deviation of the phase, this deviation is a function of the length of the spin vector. Especially when a pixel a very low signal intensity (e.g. in the noise) the phase poorly defined. Kuijer ([14]) removes these pixels, when they are below a predefined threshold to prevent large errors in the analysis of the through plane displacement.
Chapter 5

Phantom Experiments

The experiments, described in this chapter, are designed to test the pulse sequences and tagging imaging parameters on a well controlled moving phantom. In-vivo experiments on mice have not been performed yet, because animal housing facilities and anaesthesia equipment were not available at the time. All experiments were carried out at the 6.3 Tesla superconducting magnet (270 MHz for protons).

5.1 Experimental setup

The phantom sample consists of a rotating container, filled with agar gel. The goal of the experiment is to assess whether a well controlled rotation can be measured and quantified with the MR tagging sequences. The experimental setup consists of the components named in Table 5.1 and drawn in Figure 5.1. The experimental setup can be divided into four groups of components. The first group consists of the scan components, the superconducting magnet (6.3 T), the gradient system (180 mT/m), probe, amplifiers, and console. The console, the control and measure unit of the scanner were from the company Varian with the software "VNMR" release 6.1B. The second group is the rotating sample container described below. Group three is the air turbine system. The air turbine receives pressurized air from the air preparation and pressure reduction valve. The valve controls the rotation frequency of the sample container. To reduce variations in the rotation frequency and give the turbine enough power, a transmission is placed between the turbine and the container. The fourth group is the triggering unit. To synchronize the scanner and the rotation of the sample container, laser light is coupled in a optical fibre (see Figure 5.1) and is detected by a light diode. For measuring the rotation frequency, a shutter disk is positioned between the laser and the fibre. To produce squared trigger pulses for the scanner, amplification and thresholding is done by an electronic circuit. Technical details of this circuit are given in appendix C.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Air turbine</td>
</tr>
<tr>
<td>B</td>
<td>Turbine blades</td>
</tr>
<tr>
<td>C</td>
<td>Sample container</td>
</tr>
<tr>
<td>E</td>
<td>Electronic converter</td>
</tr>
<tr>
<td>F</td>
<td>Glass fibre (tip)</td>
</tr>
<tr>
<td>G</td>
<td>Gradient system of the MRI scanner</td>
</tr>
<tr>
<td>H</td>
<td>Holes through which air is blown to the turbine blades</td>
</tr>
<tr>
<td>I</td>
<td>Birdcage coil</td>
</tr>
<tr>
<td>L</td>
<td>Laser</td>
</tr>
<tr>
<td>M</td>
<td>Superconductive magnet</td>
</tr>
<tr>
<td>P</td>
<td>Probe</td>
</tr>
<tr>
<td>R</td>
<td>Air preparation and pressure reduction valve</td>
</tr>
<tr>
<td>S</td>
<td>Shutter disk</td>
</tr>
<tr>
<td>T</td>
<td>Transmission (turbine:container=9:1)</td>
</tr>
</tbody>
</table>

Table 5.1: List of the experimental setup components drawn in figure 5.1.

Figure 5.1: A schematic drawing of the experimental setup for the rotating sample container. The suspension is left out for clarity. The letters are explained in table 5.1.
Some of the components of the experimental setup (S,F,C,A,T) are fixed to the probe (P). This probe is a long tube, which can be inserted in the scanner. The probe also contains a receiver and transmitter birdcage coil.

The air turbine, the transmission and the suspension of the sample container were made from Lego© components (see figure 5.2). The advantage of Lego© is that it allows one to quickly reliable experimental setup, which is MRI compatible. No susceptibility artifacts due to the Lego© components were found. To prevent the Lego© components from wearing away 'White Grease' was used for the moving parts.

![Figure 5.2: Picture of the Lego© components in the probe. A: the shutter disk and optical fibre; B: birdcage coil; C: the air turbine; D: pressurized hose.](image)

The sample container (figure 5.3) was made from a tube with a cap on both ends. A Perspex plate in the middle of the container was included to provide a reference for the rotation angle. The caps and the tube were made from Perspex to allow visible inspection of the substance in the container. For good sealing two O-rings (2x21 mm) were placed between the caps and the tube. The materials can withstand a temperature of 80°C. After closing the sample container, the air can vent out of the container through a hole in the middle of one of the caps. This opening will help to prevent the formation of air bubbles in the gel (agar). In drawing B.1, appendix B, the technical details of the sample container are shown.
The sample container was filled with agar gel (Sigma Agar EC no. 232-658-1). First water was cooked to remove air from the water. The agar powder (1.5% of mass) was stirred in the water at a temperature of 85°C. Before the mixture was cooled down it was poured into the container.

5.2 Results

5.2.1 Composition of the agar gel

The NMR specific $T_1$ and $T_2$ relaxation times of the agar gel were measured and adjusted with the addition of MnCl₂, to the relaxation times of the heart muscle. The agar gel contained 1.5% (w/v) agar powder and 2.35 mg/l MnCl₂.

The left graph in figure 5.4 shows the inversion recovery $T_1$ measurement ([19]) of the agar gel, resulting in a $T_1=1647\pm46$ ms. $T_1$ depends on the magnetic field strength. Only little data is available of the $T_1$ for cardiac muscle at high fields. Bottomly ([6]) has collected $T_1$ values for different tissues at NMR frequencies up to $f=100$MHz and postulated the empirical formula:

$$T_1 = 0.00130 f^{0.3618}$$

(5.1)

for $T_1$ as function of the resonance frequency. For a magnetic field of 6.3 T $\sim f=268.24$ MHz this results in a $T_1$ of 1456±233 ms. Considering the lack of experimental in vivo data, above $T_1=1647$ ms seems a reasonable value.

$T_2$ of the agar gel has been measured with a normal spin-echo sequence. The right graph in figure 5.4 shows signal intensity versus echo time, resulting in a $T_2$ of approximately 32 ms. The $T_2$ of heart tissue is $57\pm16$ ms according to Bottomley, somewhat higher than the value for the agar.
Figure 5.4: $T_1$ (left) and $T_2$ (right) measurements of agar gel. The agar contained 1.5% (w/v) agar powder and 2.35 mg/l MnCl$_2$. $T_1$ is measured with an adiabatic inversion pulse followed by a spin echo sequence with a constant echo time. The adiabatic pulse is insensitive for $B_1$ inhomogeneities and inverts the longitudinal magnetization in a large area. The repetition time of the sequence was 9 seconds, allowing the longitudinal magnetization to return to its thermal equilibrium. The $T_2$ was measured with a normal spin echo sequence with a varying echo time. The repetition time was kept at 9 seconds).

5.2.2 Imaging of the rotating phantom

The rotating sample container was scanned with different echo times to assess the influence of phase accumulations during motion in a gradient field. These data is shown in figure 5.5. From the figure, it is clear that the echo time should be kept as short as possible to obtain an undistorted image.

Figure 5.5: Rotating sample container imaged with different echo times. From left to right $T_E = 3.1, 5.0, 10, 20, 30$ ms. The images were made with a SSC sequence and a repetition time of 300 ms. The rotation frequency was 3 Hz. Implying that the outer velocity of the cylinder was 235 mm/sec.

To measure the images during different phases of the rotation cycle, a number (N) of k-space lines were recorded sequentially after each trigger pulse. Each k-space line belongs to a different image at a different phase in the rotation cycle. To test this procedure and test the synchronization between the scanner and the rotating sample container multi phase experiments were done. A typical result is displayed in figure 5.6. Some images show motion artifacts. This is probably due to the fact that the stability of the rotation
frequency of the air turbine was not sufficient.

Figure 5.6: The Rotating sample container was imaged at intervals of 0.033 seconds, clockwise starting in the left upper corner. The container was imaged with a SSC sequence with: $T_E=1.8$ ms; $T_R=300$ ms; matrix=128x128; in-plane resolution=100 $\mu$m, slice thickness= 1 mm. The rotating frequency was 3 Hz. The outer velocity of the cylinder was 235 mm/sec.

To test the combination of the MR tagging sequence, a rotating sample container is tagged and imaged. This data set is displayed in figure 5.7 and figure 5.8. After every tag preparation sequence 16 images were measured with a $T_R$ of 10 ms. To decay the longitudinal magnetization a pause of 4.5 seconds was taken before the tag preparation was started. This can of course be improved for faster scan times.
Figure 5.7: SPAMM tagging of the rotating sample container, imaged at intervals of 10 ms from the left upper corner to the right corner below. The container was imaged with a balanced gradient echo sequence with: $T_E=1.4$ ms; $T_R=10$ ms; matrix=256x256; in-plane resolution=100 $\mu$m; slice thickness= 1 mm. The rotation frequency is 1.7 Hz. The tagging pattern was applied parallel to the reference plate.

Figure 5.8: SPAMM tagging of the rotating sample container. The experimental conditions were as described in the legend to figure 5.7, except that the tagging pattern was applied perpendicular to the reference plate, the black line, in the rotating container.
Chapter 6

Discussion

The heart cycle can be divided in two periods, i.e. the relaxing phase of the myocardium (diastolic period) and the contraction phase (systolic period). The heart cycle of a mouse is about 0.1 seconds (600 beats/minute) (see appendix A).

In a human heart the systolic period time is circa one third of the heart cycle ([7]). Extrapolating this information, the systolic period is 0.033 seconds and the diastolic period 0.066 seconds for mice.

The diameter of the heart is approximately 5 mm and the heart wall thickness 1 mm (1-2 mm thickness [21]). During the systolic period the human myocardium thickness increases with 40% ([7]). The ejection fraction of the mouse heart is estimated at 80%.

With these parameters a rough estimation can be made for the expected velocities of the mouse heart. The velocities during the systolic period are higher than the velocity in the diastolic period, because of its shorter duration and higher activity.

Three systolic velocities can be distinguished and estimated as follows:

1) The wall thickening velocity: \( v_w = \frac{0.4}{0.033} = 120 \text{mm/sec} \)
2) The radial displacement velocity: \( v_r = \frac{0.6}{0.033} = 180 \text{mm/sec} \)
3) The twisting velocity: \( v_t = \frac{0.13}{0.033} = 52 \text{mm/sec} \)

Radial displacement is the displacement of a point towards the center of gravity, for a sphere the center point. The radial displacement velocity is calculated as the radius change of a sphere between start and end systolic period. The heart volume changes from \(1.41\cdot10^{-2} \text{ ml} \) to \(8.76\cdot10^{-3} \text{ ml end systolic}\).

The twisting, rotation along the long axis, of a mouse heart between base and apex is 2 degrees in the systolic period ([21]). When this velocity is projected on the equator plane, the outer radius moves \(2\pi\cdot2.5\cdot2^\circ/180^\circ\) mm.

These estimated velocities are important for the evaluation of the imaging sequence, but for the tagging sequence other criteria are concerned. The spatial resolution of the images must provide enough pixels across the myocardium, from which at least two parallel tags can be detected ([27]). In the mouse a tag spacing of 0.5 mm would allow 1-2 tag lines across the myocardial wall. This means that with the lowest limit defined in section 3.3, the wavelength is approximately 5 pixels. With 2 periods in the myocardial
wall, the image resolution should be around 100x100 $\mu$m.

With the SPAMM sequence these tagging criteria are feasible. The RF pulses can be made short (< 200 $\mu$s) and enough power is released to have enough contrast in the modulated pattern. The gradient, for wrapping the phase, has not been used to its full capabilities. And it can be switched on for a very short time creating a wavelength of 500 $\mu$m. A SPAMM preparation within 1 ms is achievable.

The imaging sequence has two important parameters that have to be optimized, the echo time ($T_E$) and the repetition time ($T_R$). A short $T_E$ reduces the motion sensitivity ([28]). For an image of 64x64 (resolution 400 $\mu$m), $T_E=1.4$ ms is feasible.

The repetition time is important for preventing aliasing of the modulated tag pattern. Taking the maximum velocity and the derived wavelength of 500 $\mu$m, a $T_R$ of approximately 2.8 ms is needed. This is possible, because the RF pulse time and the gradient times are both around 500 $\mu$s.

The only limiting factor is the echo acquisition time ($t_{acq}$). This time is defined by the number of pixels ($np$) in the readout direction and the spectral width ($sw$).

$$t_{rec} = \frac{np}{sw}$$

(6.1)

When an image has the dimensions 256x256 (resolution 100 $\mu$m), the minimal echo acquisition time will be 2.5 ms. This lead to a minimal $T_E=2.1$ ms and minimal $T_R=4.1$ ms for the present hardware ($sw = 100$ kHz).

The balance between the echo acquisition time and the wavelength needs further investigation. The experiments show that tagging of the mouse heart is within experimental reach, provided we can lower the heart rate of the mouse somewhat (300-400 beats/min). This is possible with well controlled anaesthesia.

Only a few articles describe image protocols used for imaging mouse hearts. Ruff([35]) has used a FLASH (Fast Low Angle SHots) sequence with $T_E=2.4$ ms and $T_R=4.6$ ms. Other articles reported longer times, Henson ([21]): $T_E=5$ ms; $T_R=10$ ms and Ross ([34]): $T_E=3.9$ ms; $T_R=10.8-16.7$ ms. Both did MRI at a more favorable heart rate of 300-450 beats/min instead of the typical 600 beats/min.

MR Tagging in mice was very recently reported by Epstein ([13]). He used one or two heartbeats per k-line instead of a multi phase scan and an echo time of 5.5 ms. The problem is that scans are long lasting and averaging for better signal quality is difficult. Epstein et al. reported a heart rate of 390-428 beats/min.
Chapter 7

Conclusion and recommendations

The main conclusion is that tagging of the mouse heart is within experimental reach with the present 6.3 Tesla MR scanner. The sequences SPAMM and a steady state coherent gradient echo image sequence have enough parametric freedom for optimizing them for mice hearts. The first optimization, is to find the balanced between the wavelength of the modulated tag pattern and the echo time. To make it easier, the sample rate of the scanner should be improved for shorter echo and repetition times. The presently available 100 kHz sample rate is very low. Systems of 1 MHz sample rate and higher are commercially available.

Practical experience with mice is needed to obtain more knowledge on the heart parameters, such as the heart rate and the $T_1$ and $T_2$ relaxation times, in order to obtain optimal parameter settings for mouse heart MRI. Some research groups report heart rates below 600 beats/min (see appendix A). A lower heart rate will help minimizing motion artifacts. This can be done with well controlled anaesthesia.

The experimental setup with the synchronization between the scanner and the rotating sample container works well. It may be necessary in the future to build a controlled, deformable phantom. Verification of the tag measurement and the strain analysis can then be done. The goal of the continuing project must be, to develop a three dimensional strain measurement, which will generate more knowledge on the function of the mouse heart. This will provide a better tool for biomedical research of mouse models of cardiovascular diseases.
Appendix A

Mouse physiology

General:
- Body weight\(^a\): average \(\sim 20\) g (highly variable)
- Chromosome number\(^b\): 40
- Body Temperature\(^a\): \(\sim 36.9^\circ C\)
- Lifespan\(^a\): 1.3 - 3 years (highly variable)

Respiration and circulation:
- Respiratory rate\(^a\): \(\sim 163\) per minute
- Respiratory rate\(^b\): 90 - 180 per minute
- Tidal volume\(^a\): \(\sim 0.15\) ml
- Oxygen consumption\(^b\): 1.7 ml/gr body weight/ hour
- Total serum protein\(^a\): \(\sim 4-7\) g/100 ml
- Blood volume\(^a\): \(\sim 5.5\) ml per 100 g body weight
- Heart rate\(^a\):
  - Adult\(^a\): \(632 \pm 51.3\) beats per minute;
  - Newborn\(^a\): \(286 \pm 56.8\) beats per minute
- Systolic pressure\(^a\): 83-164 mm Hg

Blood parameters:
\begin{align*}
  p\text{H}\(^b\): & \quad 7.35 \pm 0.09 \quad \text{pCO}_2\(^b\): \quad 42 \pm 5.7 \text{ mmHg} \\
  p\text{O}_2\(^b\): & \quad 80 - 100 \text{ mmHg} \quad \text{ABE}\(^b\): \quad 2 \text{ mmol/l} \\
  \text{HCO}_3\(^b\): & \quad 25.5 \text{ mmol/l} \quad \text{tCO}_2\(^b\): \quad 40 - 55 \text{ Vol. %} \\
  \text{Na}\(^+\): & \quad 128 - 145 \text{ mmol/l} \quad \text{K}\(^+\): \quad 4.8 - 5.8 \text{ mmol/l} \\
  \text{tHB}\(^b\): & \quad 11.1 \text{ g/dl} \quad \text{Hct}\(^b\): \quad 41.8 \% \\
  \text{sO}_2\(^b\): & \quad 95 - 99 \% \quad \text{O}_2\text{HB}\(^b\): \quad 94 - 99 \% \\
\end{align*}

Note: Strain variation can affect these values significantly.

\(^a\)[11]  
\(^b\)[29]
Appendix B

Technical drawing of the sample container

Figure B.1: Technical drawing of the sample container.
Appendix C

Electronic circuit of the triggering group

The scheme of the electronic circuit is drawn in figure C.1.
The voltage over the photodiode (D), 'Infineon SFH 250 V' photodiode, remains constant by the op-amp (O1). This op-amp is a TL072, which has JFET-inputs for a high input impedance and low bias and low noise level. The voltage over D is controlled by the voltage over resistance R2. The photo current through D, reversed bias, is supplied by O1 to maintain the voltage over D. This current produces a voltage over R1. The output voltage of O1 is the addition of the voltages over R1 and R3. This leads to converting and amplification of the current when R1 has a large value (for instance 10 MΩ).
Thresholding is done by the op-amp O2. O2 compares the voltage from the voltage divider R3 and the output voltage from O1. The binary voltage output of op-amp O2 is between 1.42 and 16.58 [V] and is reduced with a factor 10.
The reduced signal from the trigger generator is connected with the base of a commonly used NPN bipolar junction transistor, which creates a short-circuit in the trigger line of the MR scanner at 0.5 [V]. The resistance values are given in table C.1.

<table>
<thead>
<tr>
<th>Resistances</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>10 MΩ</td>
</tr>
<tr>
<td>R2</td>
<td>1.1 kΩ</td>
</tr>
<tr>
<td>R3</td>
<td>1 kΩ + 100 Ω divider</td>
</tr>
<tr>
<td>R4</td>
<td>10 kΩ</td>
</tr>
<tr>
<td>R5</td>
<td>1 kΩ</td>
</tr>
</tbody>
</table>
Figure C.1: Electronic scheme of the trigger generator.
Appendix D

Sequence code

Program code D.1 Source code of the SPAMM and gradient echo sequence for the Varian console.

/*******************************************************************************
tagging_1_004.c

Spamm tagging + Gradient echo image part

This file is a mixture of the Puls Sequence Files: spamm_flash_gs.c and gems.c

original written by Varian
    adapted by MARIJN KRUISKAMP (spamm_flash_gs.c)
    adapted by Gustav Strijkers (spamm_flash_gs.c)
    adapted by Edwin Heijman (tagging_1_*.c)
    Magnetic Resonance Laboratory
    Technical University Eindhoven

*******************************************************************************

History:

    see spamm_flash_gs.c and gems.c files

15/1/2002 creation of this sequence (version 1.0)

*******************************************************************************
Edwin Heijman

Needs:

- shaped gradients: rampup.GRD, rampdown.GRD
- RF pulses: bir4.0.1.RF, bir4.0.2.RF,
  bir4.0.3.RF, gauss.RF, ahp_tt.RF
- phase encoding table: centric128, centric256
- macros: gotag, flashtag

*****************************************************************************

SPAMM

RF |---[bir4.0.1]---[bir4.0.2]---[bir4.0.3]---

GSS

GPE

GRO |

  \///\_____\///\_

  "
tagname='S' or tagname='s'

QSPAMM

RF |-----[tagpat]-----[tagpat]-----[preppuls]-----

|<---->  |<------>
| pretagdelay  | tagpw

GSS

|<-->  |<-->
| tagdelay

GPE

|<--| tagtime

GRO |\///\_\///\_

Eindhoven University of Technology

Magnetic Resonance Laboratories
```c
#include <standard.h>
#define OFFSETDELAY 26e-6
#define RFSPOILDELAY 50e-6
#define GRISE 0.0005
extern double rint();

/* for a constant crusher pulse: */
static int gr2[16] = {1,1,1,1, 1,1,1,1, 1,1,1,1, 1,1,1,1};

pulsesquence() {

    /* Internal variable declarations ***********************/
    double predelay, seqtime, tref, risetref;
    double trefmin, tssmin, trmin, tpemin, temin, tA;
    double agro, agss, sgpe, rgpe, grate, gssrint, grorint, gpeint;
    double reffreq, nextphase, spoilfreqs[1024];
    int i, table=0;
```
/* tagging */
double gtag, tagpw, tagtpwr, tagdelta, tagtime;
double pretagdelay, posttagdelay;
int igtag;
char tagpat[MAXSTR], tag[MAXSTR], tagname[MAXSTR];
char gread, gphase, gslice;
int orient_error;

/* additional features */
double pptpwr, turns, dticks, ppl, dstim, tcrush, gcrush;
char prepuls[MAXSTR], stim[MAXSTR], rewind[MAXSTR], gcrusher[MAXSTR];

/*****************************/
initparms_sis();
loop_check();
if (strcmp(petable, "n") && strcmp(petable, "N") &&
    strcmp(petable, "")) { loadtable(petable);
table = 1;
}

/***** PARAMETER READ IN FROM EXPERIMENT***********/
tagpw=getval("tagpw");
pretagdelay=getval("pretagdelay");
posttagdelay=getval("posttagdelay");
tagtpwr=getval("tagtpwr");
tagdelta=getval("tagdelta");
gtag=getval("gtag");
tagtime=getval("tagtime");
getstr("tagname", tagname);
getstr("tagpat", tagpat);
getstr("tag", tag);

grof=getval("grof");
gssf=getval("gssf");
dstim=getval("dstim");
turns=getval("turns");
dticks=getval("dticks");
ppl=getval("ppl");

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Edwin Heijman

Graduation report

pptpwr=getval("pptpwr");
gcrush=getval("gcrush");
tcrush=getval("tcrush");
getstr("stim",stim);
getstr("rewind",rewind);
getstr("prepuls",prepuls);
getstr("gcrusher",gcrusher);

orient_error=getorientation(&gread,&gphase,&gslice,"sorient");
igtag=(int)gtag;

if (grof == 0.0 || !var_active("grof",1) ) /*gems*/
grof = 1.0;
if (gssf == 0.0 || !var_active("gssf",1) ) /*gems*/
gssf = 1.0;

grate = trise/gmax;
agro = fabs(gro);
agss = fabs(gss);

/* GRADIENT*TIME integrals for slice selection and readout */
grorint = agro*(at/2.0 + tspoil + grate*agro/2.0);
gssrint = agss*(pl/2.0 + rofl + agss*grate/2.0);
gpeint = B0/(lpe*sfrq*le6);

/****************************
* Calculate minimum time for phase-encoding, slice refocussing
* and readout prefocussing block. Trapezoidal if GMAX is exceeded.
* TSSRMIN does not include the gradient fall time. RISETREF is
* the gradient fall time for the PE block.
****************************/

if (trise*gmax >= gssrint)
tssrmin = sqrt(grate*gssrint);
else
  tssrmin = gssrint/gmax;
if (trise*gmax >= grorint)
  trormin = sqrt(grate*grorint);
else
  trormin = grorint/gmax;
if (trise*gmax >= gpeint*nv/2.0)
  tpemin = sqrt(grate*gpeint*nv/2.0);
else
tpemin = gpeint*nv/2.0/gmax;

/* Minimum refocussing block time is the longest of calculated minima */
trefmin = (tssrmin > trormin) ? tssrmin : trormin;
trefmin = (trefmin > tpemin) ? trefmin : tpemin;

/*******************************************************
* If TREFMIN implies trapezoidal PE block, minimum RISETREF
* is RISETIME, otherwise minimum RISETREF is TREFMIN.
*******************************************************/
risetref = (trefmin > trise) ? trise : trefmin;

/* Check minimum TE *******************************/
temin = pl/2.0 + rofl + trefmin + risetref + tspoil + at/2.0 + grate*(agss + agro);
if (te < temin) {
    printf("%s: te too short. Minimum te = %f\n",seqfil,temin);
    abort(1);
}

/*******************************************************
* TREF is the refocussing block length, not including gradient fall
* time. TREF is scaled up to a maximum value consistent with TE
*******************************************************/
tref = trefmin + 0.5*(te - temin);
tref = (tref > 4.0*trefmin) ? 4.0*trefmin : tref;

/***** Calculate GPE, adjusting for integral dac value, then adjust TREF */
sgpe = gpeint/tref;
sgpe = gmax/gradstepsz*floor(sgpe*gradstepsz/gmax + 0.5);
tref = gpeint/sgpe;
if (rewind[0] == 'y')
    rgpe = sgpe;
else
    rgpe = 0.0;

/***** Calculate GSSR and GROR with new TREF **************/
gror = -grof*grorint/tref*gro/fabs(gro);
gssr = -gssf*gssrint/tref*gss/fabs(gss);
fprintf(stderr,"%s: gror = %8.4f gssr = %8.4f\n", seqfil,gror,gssr);

/***** Determine PE block gradient fall time with new TREF */
risetref = fabs(gssr) > fabs(gror) ? fabs(gssr) : fabs(gror);
risetref = risetref > fabs(sgpe*nv/2.0) ? risetref : fabs(sgpe*nv/2.0);
risetref *= grate;

/* tA is the delay between refocussing and readout ****/
tA = te - (p1/2.0 + rof1 + tref + risetref + tspoil + at/2.0
 + grate*(agss + agro));

/* Relaxation delay *******************************************/
seqtime = te + p1/2.0 + rof1 + at/2.0 + grate*(agss + agro);
if (gspoil || rewind[0] == 'y')
    seqtime += tref + risetref;
if (rfspoil[0] == 'y' || rfspoil[0] == 'Y')
    seqtime += RFSPOILDELAY;
seqtime = ns*seqtime;
if (tr < seqtime) {
    printf("%s: Requested tr too short. Min tr = \%f\n", seqfil, seqtime);
    abort(1);
}
predelay = (tr - seqtime)/ns;

************************************************************************
* Calculation of frequencies to get phase shifts for optional
* RF spoiling. Phase shifts are obtained by hopping to variable
* frequency for fixed time.
************************************************************************
if ((rfspoil[0] == 'y' || rfspoil[0] == 'Y') && nv > 0) {
    reffreq = resto + sfrq*le6/B0*gro*pro;
    for (i=1; i<= (int)(nv+0.5); i++) {
        nextphase = i*rfphase;
        nextphase = nextphase - 360.0*floor(nextphase/360.0);
        spoilfreqs[i] = reffreq + nextphase/RFSPOILDELAY/360.0;
    }
    create_offset_list(spoilfreqs, nv, OBSch, 0);
}

/* PULSE SEQUENCE **********************************************/

/* Phase cycle *****************************************************/
mod2(ct,v3); /* v3: 0 1 0 1 0 1 0 1 */
dbl(v3,v3); /* v3: 0 2 0 2 0 2 0 2 */
hlv(ct,v4); /* v4: 0 0 1 1 2 2 3 3 */
mod2(v4,v4);  /* v4: 0 0 1 1 0 0 1 1 */
add(v3,v4,v1);  /* v1: 0 2 1 3 0 2 1 3 */
assign(v1,oph);  /* oph: 0 2 1 3 0 2 1 3 */

initval(turns,v13);
initval(nv/2.0,v7);
settable(t6,16,gr2);  /* initialize real time crusher table t2 */

status(A);

/* Begin phase-encode loop ***************************************/
pelooop(seqcon[2],nv,v5,v6);
  if (nv == 0)
    assign(zero,v8);
  else if (table)
    getelem(t1,v6,v8);
  else
    sub(v6,v7,v8);

/************************** TAGGING PART ******************************/
loop(v13,v14);
  delay(dticks);
  xgate(ticks);
endloop(v14);

sploff();  /*sticks=20pare gate 1 off*/
if ((tag[0] == 'y') || (tag[0] == 'Y'))
{
  delay(pretagdelay);  /* Varying the tag creation moment*/
}

/************************** SPAMM *************************************/
if ((tagname[0] == 'S') || (tagname[0] == 's'))
{
  offset(tof,TODEV);
  observepower(tagtpwr);
  shaped_pulse("bir4.0.1",0.25*tagpw,zero,rof1,rof2);
  delay(tagdelta);

  oblique_shapedgradient("rampup",","",GRISE,gtag,0,0,spsi,sphi,
                        stheta,1,WAIT);
  delay(tagtime);
  oblique_shapedgradient("rampdown",","",GRISE,gtag,0,0,spsi,sphi,
                        stheta,1,WAIT);
delay(tagdelta);
shaped_pulse("bir4.0.2", 0.5*tagpw, zero, rofl, rof2);
delay(tagdelta);

oblique_shapedgradient("rampup", ",", ",", GRISE, -gtag, 0, 0, spsi, sphi, stheta, 1, WAIT);
delay(tagtime);
oblique_shapedgradient("rampdown", ",", ",", GRISE, -gtag, 0, 0, spsi, sphi, stheta, 1, WAIT);
delay(tagdelta);
shaped_pulse("bir4.0.3", 0.25*tagpw, zero, rofl, rof2);
} /* end SPAMM*/

/****** QSPAMM *****************************/

if ((tagname[0] == 'Q') || (tagname[0] == 'q'))
{
    offset(tof, TODEV);
    observepower(tagtpwr);
    shaped_pulse(tagpat, tagpw, zero, rofl, rof2);
delay(tagdelta);

    oblique_shapedgradient("rampup", ",", ",", GRISE, gtag, 0, 0, spsi, sphi, stheta, 1, WAIT);
delay(tagtime);
oblique_shapedgradient("rampdown", ",", ",", GRISE, gtag, 0, 0, spsi, sphi, stheta, 1, WAIT);
delay(tagdelta);
shaped_pulse(tagpat, tagpw, zero, rofl, rof2);
} /* end QSPAMM*/

/****** CRUSHER ***************************/

if ((gcrusher[0] == 'Y') || (gcrusher[0] == 'y'))
    /* crusher gradient after tagging part*/
{
    getelem(t6, v6, vl2);
    pe3_gradient(0, 0, 0, 0, gcrush, zero, zero, vl2);
delay(tcrush);
    zero_all_gradients();
delay(trise);
delay(posttagdelay);
    /*Varying the time between tag pattern and scan sequence*/
}

/****** END OF TAGGING ****************************************************/

if ((stim[0]=='y')||(stim[0]=='Y')) splon(); /* spare gate 1 on*/

/* Begin multislice loop ****************************************************/
msloop(seqcon[l],ns,vll1,vl2);

/* Relaxation delay **********************************************************/
poffset_list(pss,gss,ns,vl2);
ifzero(vl2); /* first image without a delay*/
elseenz(vl2);
    delay(predelay);
endif(vl2);

/* RF pulse ***************************************************************
if ((prepuls[0]=='y')||(prepuls[0]=='Y')) /* prepuls for fast Steady State*,
{
    ifzero(vl2);
        /* first pulse at a large flip angle*/
        obl_gradient(0.0,0.0,gss);
        delay(grate*agss);
        obspower(pptpwr);
        shaped_pulse(plpat,ppl,vl,rofl,rofl);
        zero_all_gradients();
        delay(grate*agss);
    elseenz(vl2); /* second and further low flip angle*/
        obl_gradient(0.0,0.0,gss);
        delay(grate*agss);
        obspower(tpwrl);
        shaped_pulse(plpat,pl,vl,rofl,rofl);
        zero_all_gradients();
        delay(grate*agss);
    endif(vl2);
} else

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{ 
obl_gradient(0.0, 0.0, gss);
delay(grate*agss);
obspower(tpwr1);
shaped_pulse(plpat, pl, vl, rofl, rofl);
zero_all_gradients();
delay(grate*agss);
}

/* Read & Slice refocus and phase encode pulse ***********/
pe_gradient(gror, 0.0, gssr, -sgpe, v8);
delay(tref);
zero_all_gradients();
delay(risetref);

/* Pre-acquire delay *******************************************/
delay(tA);

/* Acquire echo *******************************************/
poffset(pro, gro);
obl_gradient(gro, 0.0, 0.0);
delay(grate*agro + tspoil);
acquire(np, 1.0/sw);
zero_all_gradients();
delay(grate*agro);

if (gspoil || rewind[0] == 'y')
{
    pe_gradient(0.0, 0.0, gspoil, rgpe, v8);
delay(tref);
    zero_all_gradients();
delay(risetref);
}
endmsloop(seqcon[1], v12);

/**********************************************
* Optional RF spoiling. REMINDER: Do not place any events
* after this block or before poffset at the start of ploop.
***********************************************/
if ((rfspoil[0] == 'y' || rfspoil[0] == 'Y') && nw > 0)
{
    voffset(0, v6);
delay(RFSPOILDELAY - OFFSET_DELAY);
endpeloop(seqcon[2],v6);

} /*End sequence*/
Bibliography


