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"Optimization of Myocardial First-Pass Perfusion MR Imaging using Gadolinium and Differences in FiO_2"

Marta María Llordén de Paz

Dr. Manuel Desco Menéndez

Dr. Daniel Calle Hernández

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Abstract

The correct perfusion of the myocardium, the heart's muscle, is a key parameter that has been proven to be a powerful tool for the diagnosis of cardiovascular diseases. In general, perfusion is defined as the delivery of oxygen and nutrients by the circulatory system to any tissue or organ. When an organ like the heart receives insufficient blood supply, an ischemic disease is likely to take place.

So far, several image techniques have been used for the study of myocardial perfusion such as CT, PET, SPECT or MRI. Due to its high spatial resolution and the avoidance of limitations offered by ionizing radiation the following project specifically addresses the MRI technique. The use of MRI as diagnostic readouts in patients suffering from cardiovascular diseases is currently growing in the clinics. Therefore, having this protocol optimize in a research lab will be of great interest

This work was carried out using a 7 Tesla MRI scanner. This scanner is made for preclinical research with small animal models which is the first step performed in every research project before it can be translated into humans. For the study of perfusion in MRI a contrast agent (commonly gadolinium-based) must be intravenously administered to the patient. However, this approach offers many drawbacks regarding secondary effects with gadolinium posing health risk in patients.

In this context, this project investigates the possibility of avoiding such contrast agent by using intrinsic blood oxygenation level instead. This idea relies on the Blood Oxygenated Level Dependent (BOLD) principle that describes the magnetic characteristics of oxyhemoglobin and deoxyhemoglobin. To this end, the first step was the implementation of a first-pass perfusion in rat's myocardium using gadolinium as contrast agent. The change produced by the passage of the gadolinium bolus can be screen with T1 weighted images. A Graphic User Interface was developed using MATLAB so as to easily asses the results and furtherly compared both contrast agents.

The paramagnetic characteristics of deoxyhemoglobin locally alters the main magnetic field of the MRI scanner if its concentration is increased. For this purpose, a gas mixer with two different mixtures, one with 100% Oxygen and the other with 100% Nitrogen, was used so as to produce changes in the fractions of inspired oxygen (FiO_2). Therefore, this method studies the relationship between the MRI signal created by the deoxyhemoglobin bolus and the changes of FiO_2 recorded by a pulse-oximeter.

After several MRI sequence adjustments, the scanner was capable of recording the signal produced by the deoxyhemoglobin bolus. Even though the gadolinium signal is more precise and accurate, our new proposal is clearly a focal point for further research since it overcomes health inconvenience offered by the contrasts in used.

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Acronyms

AIF: Arterial Input Function

ASL: Arterial Spin Labeling

BOLD: Blood oxygenated level dependent CBF: Cerebral Blood Flow

Bpm: Beats per minute

B0: Main magnetic field

CT: Computed Tomography

DCE: Delayed Contrast Enhancement

DICOM: Digital Imaging and Communication On Medicine

DSC: Dynamic Susceptibility Contrast

ECG: Electrocardiogram

EPI: Echo planar imaging

F0: specific frequency

FID: Free Induction Decay

FiO2: Fraction of Inspired Oxygen

Fisp: Fast Imaging with steady state precession

GRE: Gradient Echo

GUI: Graphical User Interface

MRI: Magnetic Resonance Imaging

M: Net magnetization vector

NMR: Nuclear Magnetic Resonance

RF: Radio Frequency

ROI: Region of Interest

SE: Spin Echo

SpO2: Saturation of oxygen

TR: Repetition Time

TE: Echo Time

T1: Longitudinal relaxation time

T2: Transversal relaxation time

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1. Introduction and theoretical framework

1.1 Medical Imaging

Medical Imaging is the term that refers to different technologies or techniques (referred to as 'modalities') employed to create representations (acquire, process and display) of the interior of the human body in order to assist diagnosis or therapeutic procedures [1]. In the last decades special efforts have been made on the field by engineers or scientist towards the idea of diagnosing or treating patients avoiding side effects or surgical interventions.

The most widespread modalities regarding diagnosis are: Radiography (X-ray), CT (Computed Tomography), Nuclear Medicine, Ultrasound or MRI (Magnetic Resonance Imaging). Among these, the first three make use of ionizing radiation providing enough energy that can remove electrons from the orbit of an atom, therefore, making the atom be ionized or charged [2]. This type of radiation can be harmful and cause damage to tissues depending on the amount of radiation that is absorbed, measured in Gray (Gy).

X-ray imaging is the most ancient one and is based on the physical principles of electromagnetic radiation. A beam of X-rays is projected towards the target and depending on the object´s density part of the radiation is absorbed. The denser tissues would absorb more X-rays photons, such as bone. The remaining radiation is captured by the detector and a 2D image is created [3]. Likewise, Computed Tomography uses X-ray beams and several detectors that take measurements on different angles so as to create tomographic images or "slices" of the area under study. The third modality using ionizing radiation is Nuclear Medicine. This technique makes use of radioactively labeled substances, such as 18F-Fluordeoxyglucose (FDG), that are introduced in the body either by injection, swallowing or inhalation [4]. After the radiotracer has been introduced, it moves and travels through the body while releasing energy in the form of gamma-rays which are detected by a detector (gamma cameras). In this case, the radiation is being emitted from within the body and not created externally (unlike in CT or radiography), therefore, this diagnostic analysis let us study physiological functions rather than anatomical structures.

Other techniques that do not make use of ionizing radiation are considered "safe" for imaging sensitive patient groups such as pregnant women or children. Ultrasound imaging uses high frequency sound waves (20 kHz or higher) whose echoes are registered to create real-time images of different tissues and organs. Nowadays it is mainly used for different vascular diseases, visualization of muscles

or tendons, tumors or fetal monitoring. Finally, Magnetic Resonance Imaging (MRI) uses strong magnetic fields and radiofrequency energy creating the perfect combination for the study of soft tissue in any part of the human body. It can be used for the study of either structural anatomy or physiological processes thanks to all the parameters that can be set and modified. As stated before, it does not make use of ionizing radiation, it is non-invasive, safe, painless and the final image created by the scanner has good spatial resolution and contrast. On the contrary, the usage of strong magnets prevents the use of metals nearby the machine, therefore excluding any patient wearing prostheses made out of such material. Moreover, the acquisition tends to take longer time than other modalities and the scanner produce a loud noise that can be unpleasant for the patient. All in all, this technique has become one of the most versatile modalities used in many hospitals. To be able to understand how MRI works, some concepts regarding electromagnetism had to be explained and this would lead us to understand the wide scope of possibilities offered by this technique.

1.2 Magnetic Resonance Imaging

1.2.1 The NMR phenomenon: Spin, Precession, Resonance, Excitation and Relaxation, Acquisition Parameters, Typical Sequences

The Nuclear Magnetic Resonance (NMR) is a physical phenomenon explained by two models: the classical model and the quantum mechanical model (less intuitive)[5]. However, for our purposes in most part we will make use of the classical model to explain the generation and manipulation of NMR signal.

Spin

Every object that rotates has a property known as angular momentum that depends on mass, size, shape or velocity. The analogous term for atomic or subatomic particles is known as spin, which interacts with electromagnetic fields the same way angular momentum interacts with gravitational fields. In contrast to angular momentum, spin only takes discrete integer or half-integer units[6]. Nuclear spin values are from 0 to 8, and the electron, neutron and proton spin values are always ½. Thus, NMR depends on the interaction between a magnetic field and a nucleus with a non-zero nuclear spin. Some of the best candidates for the production of NMR signals are: ^{1}H, ^{3}He, ^{13}C, ^{17}O, ^{19}F, ^{23}Na and ^{31}P. Since Hydrogen is the most abundant (located in water molecules and fat) and has the largest magnetic moment, it is the most common nucleus used for NMR signal production [4]. As the hydrogen nucleus is composed by a single proton, the words spin, nucleus and protons are usually used as synonyms in the magnetic resonance field.

Precession

The same way a spinning top precesses around a gravitational field, nuclei precess around a magnetic field. They both have the tendency of maintaining this motion unless a force act against it. The magnetic field creates a twisting force or torque perpendicular to the field and the resulting circular motion is called precession. This movement occurs at a specific frequency (f_0) which is proportional to the strength of the magnetic field (B_0) and the gyromagnetic ratio (γ). This relation is expressed by the Larmor equation [7]–[9]:

$$f_0=\gamma B_0$$

As explained before, we won´t focused on a specific nuclei behavior (quantum model), instead we would take into account the sum of the magnetic properties that would create what we call net magnetization, denotated with letter M (Figure 1). This vector M follows the behavior dictated by the classical model. When magnetic perturbations are not present, this net magnetization it is aligned with the main magnetic field created by the scanner (B_0). Nonetheless, whenever we apply a radiofrequency pulse, the global magnetization is altered flipping the net magnetization vector so is no longer align with B_0 and precesses on its new direction.

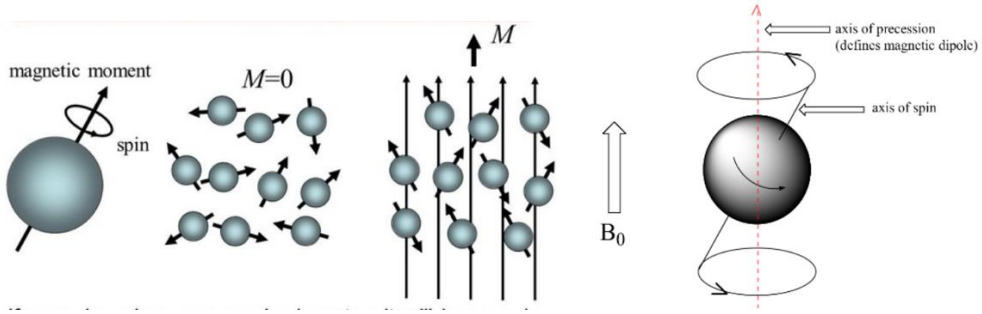

***Figure 1**. Nuclei under a magnetic field (B0). Nuclei Precession.* [10]

Resonance and Excitation

Even though all particles with non-zero spin undergo precession under a magnetic field (Earth´s magnetic field is always acting) not all of them experience what we call resonance. This event is defined as an oscillating response to an external input energy over certain frequencies, close to the Larmor frequency. In other words, a transfer of energy takes place.

The protons of a sample precess in both, parallel and antiparallel directions (Figure 2). Since there are more nuclei precessing in the parallel direction the net magnetization vector would follow the direction of the magnetic field whenever the system is at equilibrium. Subsequently, when we proceed with the acquisition of the study, the

patient is irradiated with an electromagnetic RF energy pulse with a frequency equal to the Larmor frequency (resonance). This event makes some of the nuclei that were precessing on the parallel direction (low-energy) flip to the antiparallel direction. Consecutively, the net magnetization vector decreases with time on the longitudinal axis (B_0 direction) and starts growing on the transversal axis (M_{xy}) (Figure 2). After some time, the RF energy is released at the same frequency while the nuclei adopt their initial state. Both recoveries of equilibrium by the magnetization vector, the longitudinal and transversal, are independent events. The emission of energy due to the relaxation process is recorded by the antennas of the MRI scanner constituting the MR signal. This physical process, which occurs when nuclei placed in a magnetic field absorbs and emits energy is what is denoted as **nuclear magnetic resonance (NMR).**

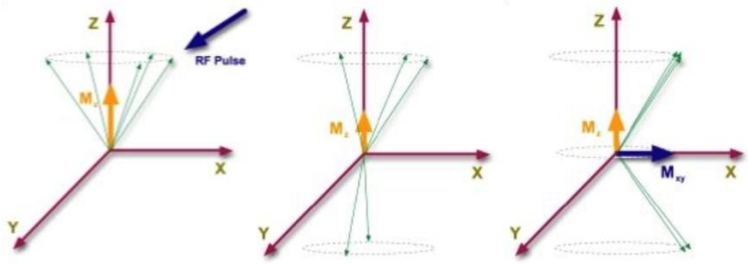

Figure 2. *RF Excitation* [11].

Longitudinal Relaxation

Longitudinal Relaxation or T1 relaxation is the process where the net magnetization vector (M) recovers its initial value (M_0) parallel to the main magnetic field (B_0). This process is described with an exponential function where T1 is a first-order time constant of this function. It can be seen as the time need to recover 63% of the magnetization vector initial value (Figure 3).

This relaxation occurs due to the energy transference from the nuclei to the external environment in the form of heat. This transfer of energy needs to be done with molecules of the surrounding that moves with a frequency close to the Larmor Frequency. This explains why different tissues have different T1 values, since not always the energy is transferred from the nuclei to the surroundings with the same efficiency. In the case of solids, the movement is limited, and liquids exceed the Larmor frequency because their molecules move to fast. A good example for short T1 is fat, since the speed of the molecules that form it move at a frequency close to Larmor frequency [8][12].

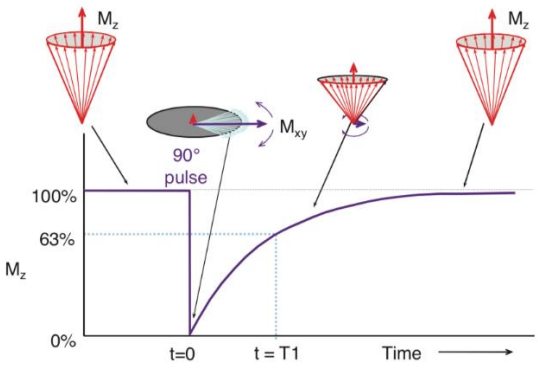

***Figure 3**. T1 Relaxation* [4].

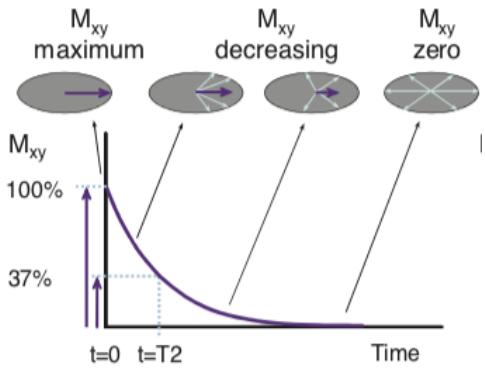

***Figure 4.** T2 Relaxation* [4].

Transversal Relaxation

Transversal relaxation or T2 relaxation is the process where the transverse component is lost with time. The transverse component of the magnetization vector (M) is denoted as M_{xy}. The T2 value is the time required by a specific tissue to lose 37% of the transversal magnetization vector (Figure 4) [13]. This type of relaxation takes place at a faster rate than Longitudinal Relaxation

After the RF pulse is released, maximum transversal magnetization is accomplished, and a sinusoidal signal know as free induction decay (FID) is created and recorded by the antenna. Both, FID and M_{xy} decay with time due to magnetic inhomogeneities created by the sample (intrinsic) and the phase coherence is lost. T2 is the decay time caused by intrinsic magnetic inhomogeneities and T2* is the one created by intrinsic and extrinsic magnetic inhomogeneities since the main magnetic field is not perfectly homogeneous.

Acquisition parameters: Repetition Time (TR) and echo time (TE)

Repetition Time (TR) is the time between two RF excitation pulses where a T2 decay and a T1 recovery takes place. Depending on the type of sequence used, the TR value will range from a couple of milliseconds to more than 10.000 milliseconds. A long repetition time will allow the spins of the tissue to align with the main magnetic field. On the contrary, a short RT won´t let the spins to fully relax to their initial state before another measurement takes place [4]**.**

Echo Time (TE) is the time between the RF excitatory pulse (90^0) and the peak of an induced echo by a 180^0 RF inversion pulse (or gradient field with a polarity reversal). At time TE the readout takes place [4]**.**

To generate a **T1-weighted** image a short TR and a short TE are needed. While the nuclei are recovering the longitudinal relaxation a maximum difference in that recovery needs

to be achieve, and this is possible with a short TR. A short TE helps minimizing the T2 decay [14].

In contrast, to generate a **T2-weighted** image, a long TR and a long TE are needed. Using a long TR helps minimizing the effect of the T1 recovery. The T2 difference is achieved by using a long TE so the dephasing can take place [14].

Figure 5.*TE and TR parameters* [15] .

Typical Sequences: Spin echo and Gradient Echo

Spin Echo (SE) is a sequence that make use of a secondary RF excitatory pulse that induce a 180 degrees inversion in the protons in order to achieve the generation of an echo of the signal. The first 90^0 RF pulse converts the net magnetization vector M_0 to M_{xy} with a phase coherence that starts to decay (T2* relaxation). At time equals TE/2 a secondary RF excitatory pulse is applied (180^0), and it leads to phase coherence at time TE. The usage of this inverting pulse causes the nuclei to only experience the intrinsic inhomogeneities and its dephasing effects, therefore it only depends on T2 and not T2*[4].

Unlike SE, **Gradient Echo (GRE)** sequence only makes use of a unique RF pulse followed by the application of gradient which accelerates the dephasing. Subsequently, a rephrasing gradient is applied (same strength but different polarity) and the FID signal or echo is generated. In this case the T2* effect is not cancelled out and the acquisitions are normally weighted in T2* or T1 [16].

1.2.2 Perfusion in MRI

Perfusion is the flow of a fluid, blood in this case, through the circulatory system to an organ or tissue. Depending on the organ or tissue the perfusion measurements diverge, and it might differ its result for different pathologies. For this purpose, contrast agents such as gadolinium (for MRI) or iodine (for CT) are injected in the patient. These molecular compounds have specific magnetic characteristics, paramagnetism in the case of gadolinium, different from the surroundings that can be detected by the MRI scanner increasing the contrast in tissues where it accumulates.

1.2.2.1 *Myocardial perfusion assessment*

Myocardial perfusion is usually assessed by the calculation of parameters that characterize the first pass of a bolus (contrast agent) in the myocardium. Through mathematical modeling it is possible to obtain myocardial perfusion parameters quantitatively; however, this quantitative analysis implies many barriers such as time-intensive processing or different assumptions that are not fulfilled by every patient.

For these reasons, many researchers have proposed semi-quantitative measurements of myocardial perfusion directly obtained from the Signal Intensity-time curves. These measurements may offer a rapid diagnosis since a visual assessment is possible without the need of difficult quantifications. Among different semiquantitative measurements, the upslope generated by the contrast first pass is easily calculated with the aid of a linear fitting performed on the curve. This method has shown great accuracy and reproducibility [17]. In order to correct for any difference caused by factors like different injection velocity, animal's weight or bolus compactness, two upslopes are calculated, one from the myocardium and other one from the left ventricle. An example is depicted of Figure 6. Subsequently, the myocardial upslope is divided by the left ventricle upslope, thus obtaining a normalized myocardial perfusion slope [18]–[20].

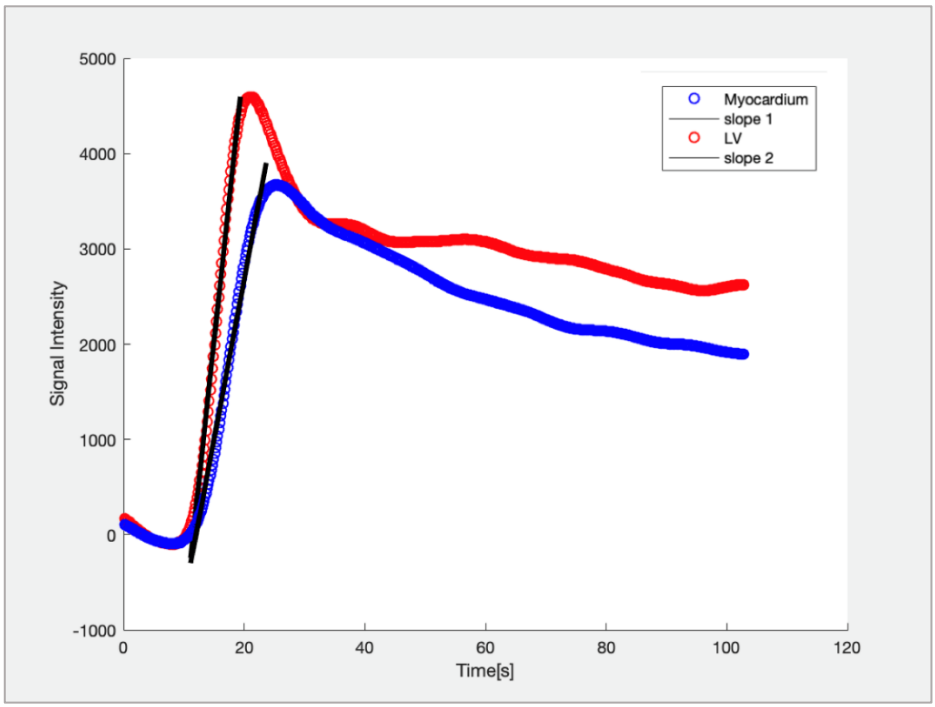

Figure 6. *Semi-quantitative method for myocardial perfusion assessment.*

1.2.2.2 *Perfusion imaging methods: DSC, DCE and ASL* [21]

There are three methods used in MRI for the study of perfusion: Dynamic Susceptibility Contrast (DSC), Dynamic Contrast Enhanced (DCE) and Arterial Spin Labeling (ASL). The first two methods make use of an exogenous intravenous contrast agent such as gadolinium, while the third one does not make use of any intravenous bolus.

DSC is a technique where the first-pass of a contrast agent is recorded as a result of a T2 or T2* weighted image acquisition, meaning that the signal intensity decays when the exogenous contrast agent arrives. This is achieved with the usage of sequences such as gradient or spin echo. Data acquired with this technique can be used to generate parametric maps (BF or BV) by converting the signal-intensity time curves into contrast-concentration time curves in every pixel of the image.

DCE technique acquires T1 weighted images and generates a signal-intensity time curve conveying information about permeability, perfusion, surface area and extracellular space. This technique is based on a two-compartment pharmacokinetic model where the intensities are converted into concentration.

ASL technique is characterized by the use of an endogenous contrast consisting of labeled blood's water molecules with RF pulses. When these labeled molecules arrived at the organ under study, the signal intensity is reduced. Normally, the procedure is carried out by acquiring two images, one previous to the magnetization labeling and other one after the molecules have been labeled. Then, both images are subtracted. Even though this technique is non-invasive, it offers low Signal-to-Noise ratio, contrast resolution and time resolution.

	DSC	DCE	ASL
BOLUS HANDLING	Bolus tracking	Bolus passage	Bolus tagging
ACQUISITION POINT	First pass of contrast agent	Acumulation of contrast agent	Accumulation of tagged blood
EXOGENOUS OR ENDOGENOUS	Exogenous method	Exogenous method	Endogenous method
CONTRAST MEDIA	Intravenous bolus injection of Gd-based contrast agent	Intravenous bokus injection of Gd-based contrast agent	Without contrast agent
TRACER	Non-difusible blood pool tracer	Flow or permeability-limited difusible tracer	Diffusible tracer
RELAXATION MECHANISM	T_2/T_2* relaxation	T_1 relaxation	Magnetic labeled blood T_1 relaxation
EFFECT	Increased susceptibility effect	T_1 shortening effect	Blood magnetization inversion
SIGNAL BEHAVIORS	Decreased signal	Increased signal	Subtracted signal

***Table 1**. DCE, DSC, and ASL, perfusion techniques [22].*

1.2.2.3 *Blood Oxygenation Level Dependent (BOLD) contrast*

Blood Oxygenated Level Dependent (BOLD) contrast arises from oxy-hemoglobin and deoxy-hemoglobin regional concentration. The first paper describing magnetic properties of oxy- and deoxy- hemoglobin by Pauling et al. stated that oxyhemoglobin has no unpaired electrons and is diamagnetic [23]. On the other hand, deoxyhemoglobin has unpaired electrons and presents paramagnetism. The unpaired electrons of the iron center caused by the released of oxygen explains why this molecule becomes highly paramagnetic. The idea of using oxygenation level as a measurable contrast in MRI was developed the first time by Owaga et al. in 1990 [24].

The deoxyhemoglobin concentration leads to the lowering of T2 and T2* relaxation times. The local field changes caused by the presence of deoxyhemoglobin triggers frequency changes and phase shifts of the spins responsible for T2 shortening [25][26].

1.2.3 MRI applications in the heart

1.2.3.1 *First-Pass Perfusion MRI of the myocardium*

Myocardial perfusion is a great marker of cardiac pathologies. Many heart diseases relate to the lack of myocardial perfusion due to poor functioning of the coronary arteries, responsible for myocardial irrigation. MRI is widely used in routine clinical applications for assessing heart perfusion using an image acquisition method known as first-pass perfusion.

This technique makes use of an exogenous contrast agent with certain magnetic characteristics that can be detected by the MRI scanner. Particularly, gadolinium with its paramagnetic features is a good choice for this technique. Once the contrast agent has been injected intravenously, the first pass of the contrast bolus through the myocardial tissue will be recorded by a T1-weighted image acquisition. Regions of the myocardium with reduced perfusion will be characterized by lower intensities in the image [17].

Some important features regarding this technique are: high temporal resolution, short acquisition time and good in-plane resolution. Is important to acquire images every one or two beats to properly record the perfusion. Since the heart is responsible for pumping blood through the circulatory system it has a characteristic movement caused by the continuous relaxation (Diastole) and contraction (Systole) of its chambers (ventricles and atria) shown in Figure 7. In order to avoid the artifacts caused by this continuous movement, a short acquisition time is required. Moreover, a good in-plane resolution is important, no more than 2.5 mm, so the myocardial layers are well characterized.

The appropriate way to study myocardial perfusion is by acquiring images of the short axis of the heart where the different chambers and the muscle are well delineated.

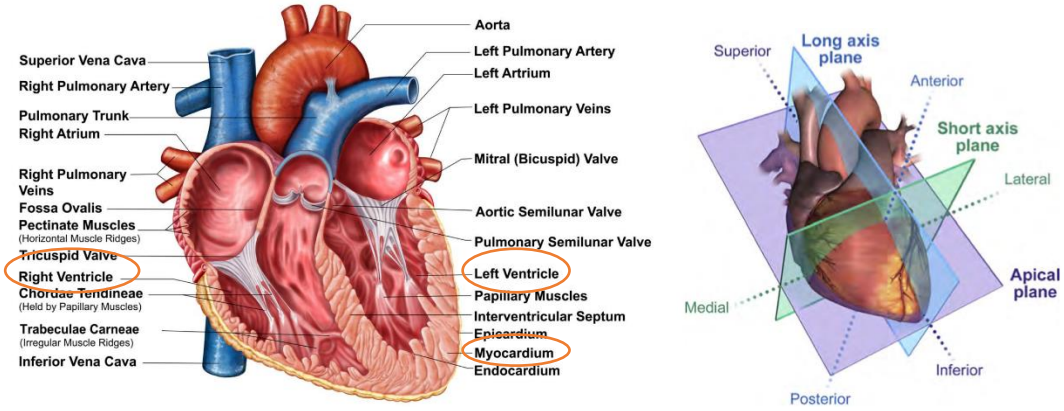

Figure 7. *Hearts Anatomy (A) and Heart's Planes (B)* [27][28].

1.3 Antecedents and state of the Art

So far, myocardial perfusion is approached invasively using Gadolinium as main contrast agent. This technique is the preferred option for cardiac function and structural anatomy assessment since it offers accuracy and spatial resolution [29]. This type of paramagnetic contrast was first approved for clinical studies in 1988, when the first gadolinium-based contrast agent (gadopentetate dimeglumine) was available particularly for MRI scanner and marketed as Magnevist [30]. From that date, many clinical and research groups have taken advantage of contrast-enhance MRI using gadolinium for functional studies of organs such as heart or brain. Regarding myocardial perfusion, it was first practice in 1990 by Atkinson et al. using a GRE sequence and a gadolinium-based contrast agent (Gd-DTPA) in a rat animal model [31]. Since then, improvements have taken place on the scanner hardware, such as magnetic field strength or gradient coils, different contrast agents with diverse magnetic properties and pulse sequences [32].

Up to now, the Medical Imaging Laboratory at Hospital General Universitario Gregorio Marañón does not have any protocol regarding the study of myocardial perfusion for small animals. To the best of our knowledge, there are no implemented protocols for myocardial perfusion using a 7 Tesla scanner BioSPec 70/20 USR (ultra-shielded) from Bruker Corporation. For this reason, one of the major goals of the study was the implementation of such protocol based on papers like those published by Nierop et al. and Coolen et al. [33], [34] as guidance for our project. Both investigations focused on mouse myocardial first-pass perfusion making use of a Bruker scanner.

Once this first protocol was implemented, we intended to test other innovative techniques based on the BOLD principle. Some previous methods have been proposed using BOLD signal in preclinical studies for the study of ischemic strokes[35]. Cristina Sainz, in her final thesis, used BOLD principle for perfusion assessment with DSC or DCE techniques in rat's brain [36]. Taking her positive results as motivation, this project aims to translate this innovative endogenous contrast agent to a non-static organ such as the heart. Therefore, this project will study the feasibility of the new contrast based on FiO_2 changes.

Many research projects are conducted regarding the study of myocardial perfusion using BOLD MR imaging. So far, their main goal was the study of medical conditions related to blood supply in the myocardium like scar tissue, infarct, edema, ischemia or coronary artery disease [37]–[41]. Most of the investigations regarding BOLD imaging focused on the detection of T2 and T2* changes in response of the administration of pharmaceutical stress agents or vasodilatory drugs such as DIP (dypiridamole) and adenosine in cardiac stress test [42][40]. Since BOLD implies very low signal to noise ratio, high magnetic field inhomogeneities or motion artifacts improvements regarding this technique are needed so as to translate this innovative technology to the clinical field. For this reason, new research show the utility of making use of apnea periods that generate measurable BOLD signal changes [43]. Preclinical studies are being performed using small animals, like rats, or big animal models in canine and porcine studies with increasingly positive results. Nowadays the main goal is to detect small changes in the oxygenation of the cardiac muscle (different regions), difficult to achieve with low SNR and contrast resolution. One of the latest contributions (May 2019) has been performed in canines using carbon dioxide as oxygen substitute (hypercapnia) to detect myocardial oxygenation through BOLD imaging [44].

To our knowledge, there are no previous studies performed on small animals where a bolus of deoxygenated blood is produced with the short inhalation of Nitrogen for the assessment of the first pass perfusion in the myocardium. As previous studies regarding BOLD principle where SSFP sequence (steady state free precession) is used, this project focuses on True Fisp sequence.

2. Motivation and objectives

2.1 Motivation

Among all the organs where perfusion has proven to be a powerful tool for diagnosis, this project will focus on the myocardium (heart's muscle). According to the World Health Organization, approximately 17.9 million people die every year due to cardiovascular diseases which is a 31% of all deaths [45]. For this reason, many efforts are now being put on its prevention and diagnosis, since an early assessment makes a huge impact on the outcome of this type of medical condition.

This project was performed at Hospital General Universitario Gregorio Marañón at the Unit of Experimental Medicine and Surgery. In particular, the Medical Imaging Laboratory of such unit has one of the most innovative and complete resources for preclinical imaging diagnosis nationwide [46]. Apart from fluorescence tomography, PET, CT-PET, CT or optical imaging, this research lab holds a 7 Tesla MRI scanner. This device has been the key for studies related mainly to neuroimaging, so embarking a research project about the heart and its cardiovascular diseases would open up the range of possibilities offered by this entity to other research groups or laboratories. At the moment, this laboratory had only acquired multiplanar cine studies of the heart, but nothing related to its perfusion. For this purpose, the first motivation of the project was to implement the first-pass of a gadolinium contrast agent through the heart myocardium in rats. The protocol was based on previous studies performed by other research groups detailly explained in papers published by Nierop et al. and Coolen et al. [33], [34]. Both projects used a scanner from Bruker corporation but none of them made use of the same device model. Therefore, an implementation of the acquisition process with all its parameters was necessary for the Experimental Medicine and Surgery unit at hospital.

The usual contrast agent used for myocardial perfusion in MRI studies contains gadolinium. This type of contrast is administrated intravenously, and it has a very high financial and economic cost. In addition to this, many adverse effects are nowadays being proven such as brain depositions of the contrast [47]. Likewise, vulnerable patients like pregnant women and patients suffering from kidney disorders are not recommended to go through this type of diagnosis technique. In order to overcome all these untoward effects and go a step further on this investigation, some studies were performed using an innovative endogenous contrast agent, the patient's own blood. This new method relies on the BOLD principle where the paramagnetic characteristic of the deoxyhemoglobin is stated. Consequently, the bolus of endogenous contrast agent is created by changing the fraction of inspired oxygen (FiO_2) by short periods of time that will be screen by the scanner due to the changes produced in the overall magnetic

field. This new approach can be harnessed to bring benefits such as non-invasiveness or the availability for the vulnerable groups of patients previously mentioned. This is further explained in section 7.1 where there is a detail explanation of the socio-economic impact of this new approach.

At the present time, this innovative contrast agent was tested by Cristina Sainz in her bachelor thesis where different FiO_2 were exanimated for the study of brain perfusion [36]. Her job performed at the same facilities had obtained highly positive results in brain, which motivated us to translate this technique to other organ, the heart. It is highly convenient for a research institute like the Medical Image Laboratory at Hospital General Universitario Gregorio Marañón to have a research branch dedicated to such new contrast agent. This is intended to fill gaps in the existing set up and introduce new expertise on the field by implementing this protocol in this laboratory with small animal models using Nitrogen as oxygen substituent for the apnea process in order to produce BOLD signal.

2.2 Alternative Designs

This section is intended to offer an overview of different alternative techniques or measurements that could have been used in the project. Therefore, none of the concepts and ideas explained in the following paragraphs have been implemented on our study mainly due to its high computational cost or the side effects that could have been caused by other modalities.

The most common parameters studied in perfusion are: Blood volume (BV), Blood Flow (BF), Mean Transit Time (MTT) and Time to Peak (TTP). These measurements are normally represented in parametric maps, where different colors are used as a code for the study of the organ's activity [48].

- **Blood Volume** is defined as the amount of blood circulating or arriving to a tissue in milliliters divided by the mass of the tissue that is being perfused (mL/100g).
- **Blood flow** is the movement of blood through a tissue, measured as blood volume per unit time divided by the mass of the tissue (mL/s/100g).
- **Mean Transit Time** is the amount of time blood is stablished in the tissue under study measured in time units (seconds).
- **Time to Peak** is the time between the injection of a contrast and the maximum signal measured in time units (seconds).

Mean Transit Time (MTT), Blood Volume (BV) and Blood Flow are related by the following equation:

$$Blood\ Flow\ (BF) = \frac{Blood\ Volume\ (BV)}{Mean\ Transit\ Time\ (MTT)} \quad [49]$$

All these parameters can be calculated developing tough mathematical models. However, this quantitative analysis offers many drawbacks like time-intensive processing or several assumptions that are not fulfilled by every patient. In addition, these parameters are mainly obtained for static organs such as brain since motion presented by organs like the heart would make the calculations inaccurate and difficult (a perfect registration would be required). Because of this, this project has focused on the calculation of semi-quantitative measurements suggested by many researchers and obtained directly from the intensity curves.

In terms of image modality, a common choice for perfusion studies in the heart is SPECT or PET (nuclear medicine). This technique makes use of a radioactive isotope, such as technetium, to study the heart's muscle function and its perfusion.

Even though this modality is used in many hospitals on a daily basis, we have decided to perform our study using magnetic resonance imaging (MRI). Apart from the organ's function, MRI offers the option of studying structural anatomy with the same acquisition, being one of the most accurate and precise techniques used nowadays. Another drawback of nuclear medicine is related to the application of a radioactive isotope with its high economical cost and the secondary effects produced by the ionizing radiation.

2.3 Objectives

The main objectives of this work are to implement the use of gadolinium as a contrast agent for first-pass perfusion of the myocardium and study the viability of a novel approach to evaluate perfusion using deoxyhemoglobin as an endogenous contrast agent by reducing oxygen saturation. The specific aims are:

- Implement a first-pass perfusion protocol using Gadolinium as contrast in MRI.
- Quantify the curve up-slope of different regions as a semiquantitative measurement for perfusion using Gadolinium as contrast.
- Design a GUI implemented on MATLAB software for the interactive assessment of perfusion parameters.
- Set up a convenient MRI sequence for the acquisition of images using FiO_2 as an endogenous contrast agent.
- Validate FiO_2 contrast studies against the standard: Gadolinium perfusion.

3. Materials and methods

3.1 Planning and Overall Design

The principal objective of this project is to study the feasibility of the usage of deoxyhemoglobin as an endogenous contrast agent for myocardial perfusion by implementing first a first-pass perfusion protocol using gadolinium-based contrast agent. Before explaining each material used up to this end, two brief workflows are presented in Figure 8 and 9. The first figure represents the setting related to the Gadolinium implementation process. The second one refers to the usage of FiO_2 change as contrast agent.

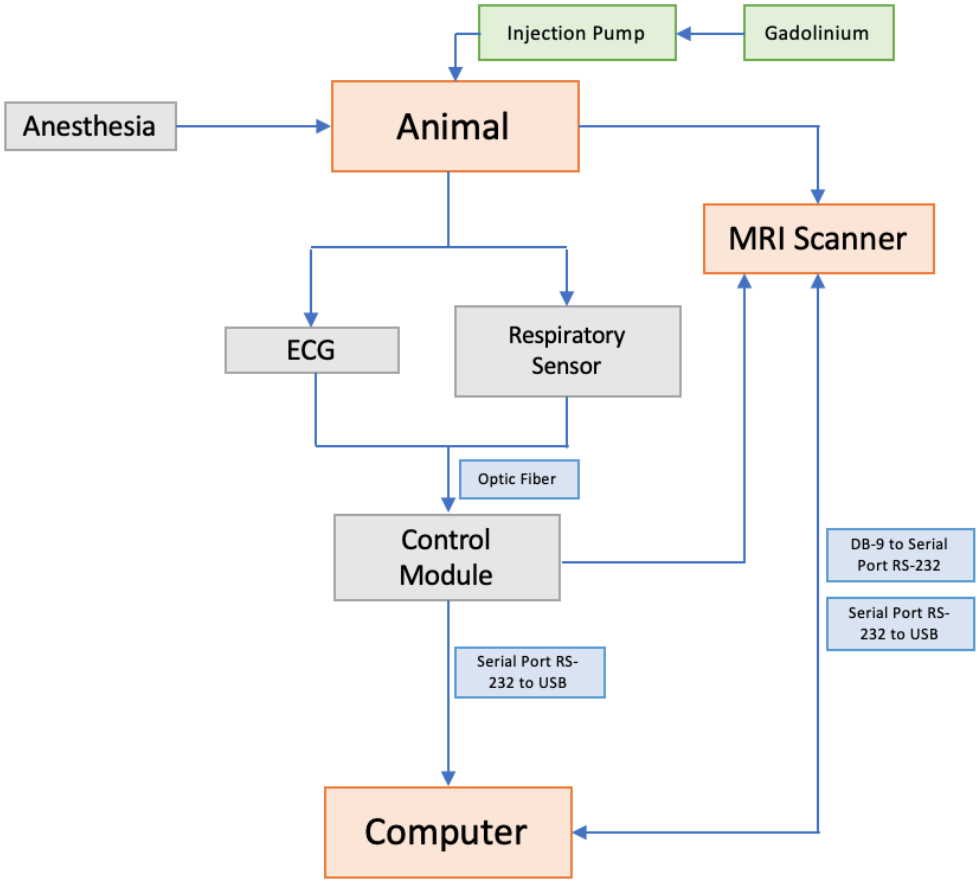

Figure 8. *Workflow of Gadolinium-based contrast agent acquisition.*

The process using Gadolinium is described in Figure 8, where an animal, rat in this case, is placed inside the MRI gantry covered with a surface coil wrapping its chest to record the MRI signal (will be used to reconstruct the image) with the aid of a volume coil that creates the excitatory pulses. The images are acquired by the MRI scanner over this process so myocardial perfusion can be studied. The animal is constantly being monitored by an ECG and respiratory sensors that are connected to a Control module

by optic fiber, so the respiratory rate and the electrocardiogram are recorded. The ECG signal is furtherly used for the MRI triggering mode explained in the following sections. Moreover, this module is connected to a computer which stores and processes all this information.

To study the myocardial perfusion, a gadolinium bolus is intravenously injected in the rat's tail ten seconds after the beginning of the acquisition. More details of the contrast agents used and the animal model are described in sections 3.2.3 and 3.2.6.

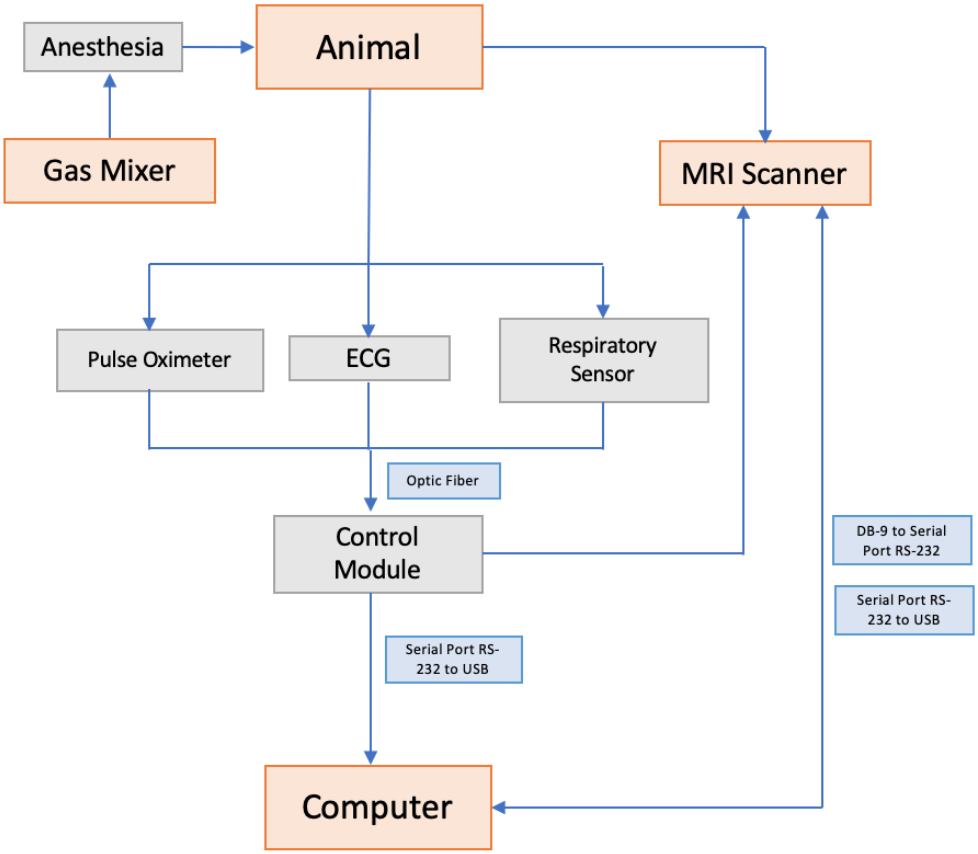

Figure 9. *Workflow of deoxyhemoglobin endogenous contrast agent acquisition.*

The scheme in Figure 9 describes the general design of the second experiment, where the FiO_2 was used as contrast agent for the study of myocardial perfusion. There are two additional devices with respect to Gd experiment: the pulse oximeter and a gas mixer. The pulse oximeter is connected to a second control module by optic fiber and it is used to monitor the animal and to record the FiO_2 changes produced by the gas mixer. The gas mixer uses the *GSM-CommVS* software [50] for the O_2/N_2 adjustment. At the beginning of the acquisition the animal inhale 100% Oxygen. Afterwards, the software changes to 100% Nitrogen automatically for 25/60 seconds. Finally, the animal is administered 100% Oxygen recovering the initial value of FiO_2.

3.2 Materials

3.2.1 MRI Scanner

The MRI scanner employed for this project was a 7 Tesla BioSPec 70/20 USR (ultra-shielded) from Bruker Corporation (Figure 10).

Figure 10. *MRI scanner.*

3.2.2 MRI Coils

For this project, two types of coils were used: surface and volume coils. Like the scanner, these two radiofrequency coils are from Bruker Corporation. The volume coil can either serve as receiver or emitter, and in this case was used as a transmitter coil placed inside the scanner surrounding the animal. This type of coils in emitter mode offer good homogeneous signal in the entire sample.

On the other side, the surface coil was used as a receiver coil since it covers the animal's chest perfectly, offering higher signal to noise ratio than a volume coil. Furthermore, it has a four-coil phased array configuration which enables a parallel acquisition of the image that will improve time resolution.

3.2.3 Animal Model

The animal model used for the study is a Wistar Rat, an albino breed commonly used for scientific research. A total of four healthy adult rats were used: two males and two females. The Regulatory Framework related to animal experimentation is explained in section 7.3.

***Figure 11**. Wistar Rat used for the experiments* [51], [52] .

3.2.4 Animal monitoring: Pulse-Oximeter, Cardiac monitoring, Respiratory monitoring, Temperature monitoring

During MRI acquisition the animal was precisely monitored. The following components from SA Instruments, Inc. (manufacturers of imaging compatible devices for small animals) were used:

Pulse Oximeter

A Pulse Oximeter is a noninvasive device used to measure the animal's pulse and its oxygen saturation (SpO_2). The SpO_2 refers to the proportion of oxyhemoglobin and deoxyhemoglobin carried out by red blood cells. This is done with the aid of two different light wavelengths, red and infrared, and a light detector mounted on a clamp. The light absorbance at two different wavelengths (red and infrared) is measured, and the arterial SpO_2 is obtained by using Beer's Law as a function of the ratio of the two absorbances [53].

The device is properly adjusted to the animal's foot as shown in Figure 12.

Cardiac monitoring

The cardiac monitoring device makes use of three electrodes placed intracutaneously in the animal's limb as shown in Figure 13. These electrodes record the electrocardiogram (ECG).

In addition, the output of this device is used for cardiac triggering. This means that the MRI scanner will only acquire data at certain time window after the R-wave of the ECG, thus obtaining static images at a precise moment of the cardiac cycle.

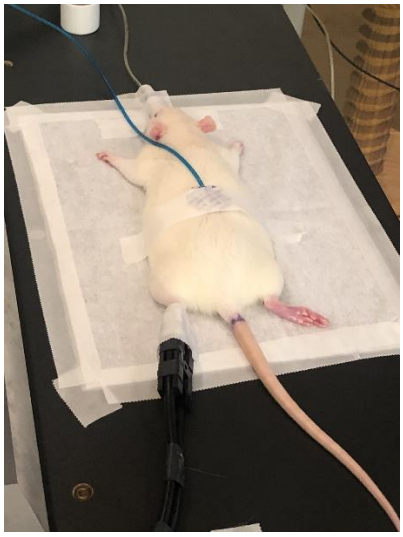

Figure 12. Pulse Oximeter on the rear foot (left).

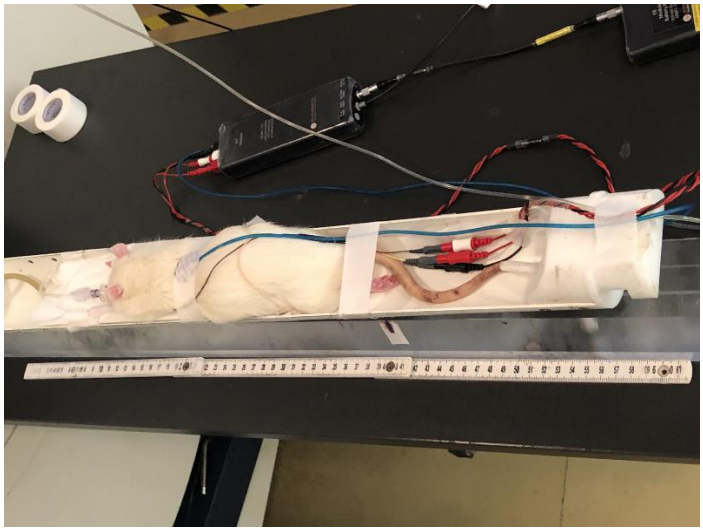

Figure 13. ECG electrodes.

Respiratory monitoring

The respiratory sensor is based on a pressure pad placed at the animal's back (Figure 12 and 13 blue cable). It is a noninvasive method of obtaining the breathing rate and controlling if the animal is being properly anesthetized.

Temperature monitoring

The animal's temperature was also recorded by a sensor placed at the pressure pad used for breathing monitoring. To keep temperature, a Circulating Warm Water Pump is used (Figure 14).

Figure 14. Circulating warm water pump (white and blue) and heat blanket (green) placed on the animal.

Anesthesia System

The anesthesia system is composed by an anesthesia vaporizer from SurgiVet brand connected to a methacrylate box where the animal is placed and anesthetized at the beginning of the experiment. Once the animal is fully anesthetized, it is moved to the scanner bed where it remains anesthetized with a tube coming from the vaporizer and connected to a mouthpiece directly placed on the animal's mouth.

The parameters for the animal anesthesia system on Figure 15 are explained on section 3.3.1.2.

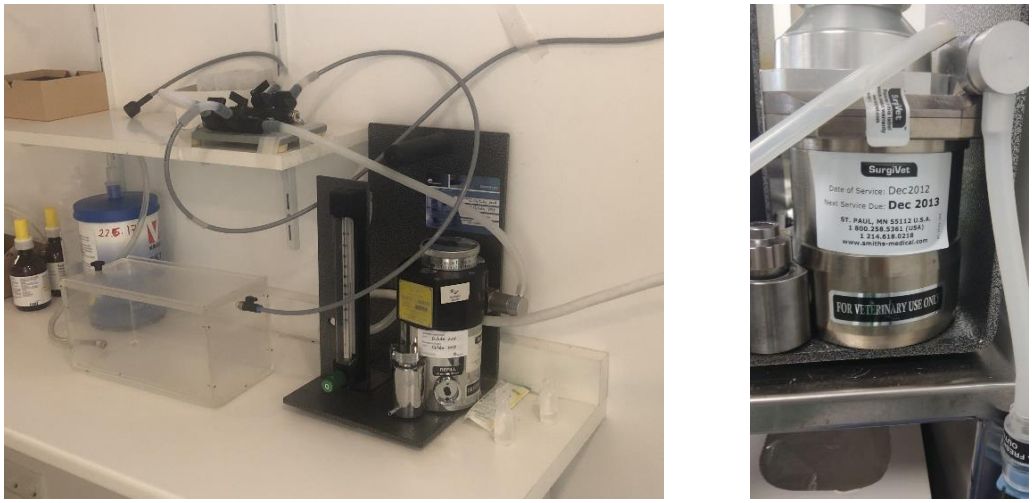

Figure 15. *Animal anesthesia system.*

3.2.5 Gas Mixer

The percentage of Oxygen inhaled by the animal is set with the gas mixer shown in Figure 16. This is the GSM3-Gas Mixer (CWE, Inc.). The device has two control modes: Local and Remote. For this project, the Remote mode was more convenient so changes between different mixtures were done automatically by setting some parameters in its own software. A RS-232 cable was used to connect the gas mixer to the computer for the remote mode. For our purposes we used two mixtures of two gases: 100% Oxygen and 0% Nitrogen, 100% Nitrogen and 0% Oxygen.

Figure 16. *GSM3-Gas Mixer.*

3.2.6 Gadolinium and Injection Pump

Gadoteridol (0.5 mmol/mL) (ProHance, Bracco, Italy) is contrast agent used. This contrast agent is intravenously injected to the animal with the aid of an injection pump (Harvard apparatus, USA) that allows the user to set parameters such as volume and flow (Figure 17).

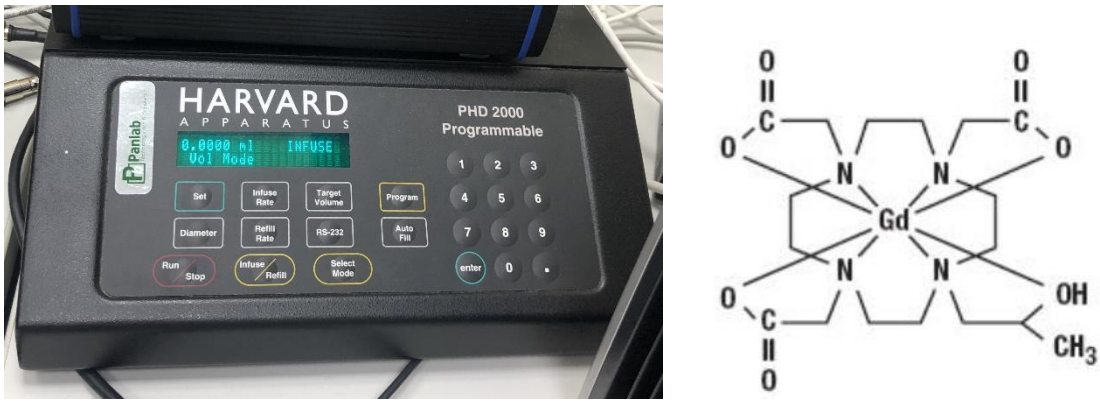

Figure 17. *Injection Pump by Harvard Apparatus. Gadoteridol (ProHance)* [54].

3.2.7 Software: MATLAB, gas mixer, monitoring, MRI scanner

Four different software platforms were used for the project: MATLAB, PC-SAM, ParaVision software and GSM-CommVS software.

MATLAB

MATLAB was the chosen platform for creating Graphic User Interfaces (GUI) that will be in charge of the image postprocessing and the perfusion parameters assessment. The version used for this study was R2018B from MathWorks, Inc. corporation.

ParaVision Software

ParaVision software is a preclinical software from Bruker manufacturer for setting all the acquisition parameters such as Echo Time, Repetition Time, Image Size and Field of View. The version used in this study is: ParaVision 6.0.1.

PC-SAM

The devices in charge of the animal monitoring (cardiac, respiratory and pulse oximeter monitoring, SA Instruments Inc., USA) are controlled by the software, PC-SAM, shown in Figure 18.

Figure 18. *PC-SAM software.*

GSM-CommVS software

Figure 19 shows the software used by the Gas Mixer settings in remote mode control. On the top of the screen, MIX 1 and MIX 2 represent our two mixtures model for Nitrogen and Oxygen. On the left down corner, there is a sequencer where the time

settings of each mixture can be stablished. The version used for the project is GSM-CommVS v3.41 from CWE, Inc.

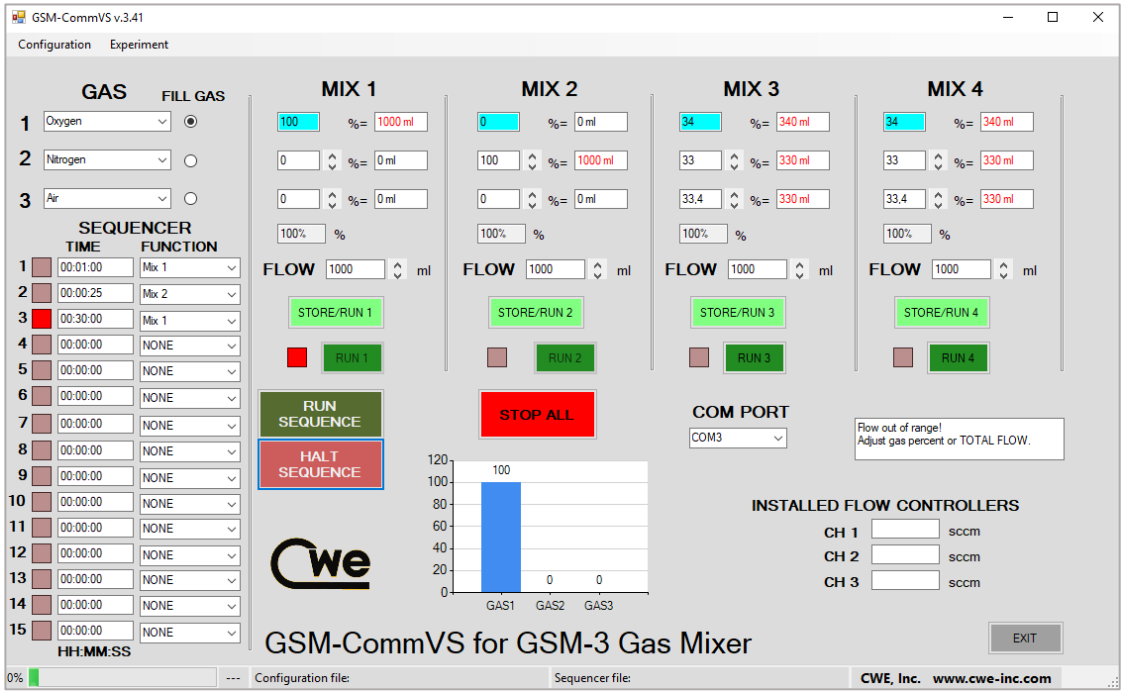

Figure 19. *GSM-CommVS software.*

3.3 Methods

The project is divided into two subprojects: the implementation of the use of Gadolinium for a first-pass perfusion and the feasibility of the usage of different FiO_2 as contrast agent by comparing both results. The first part is used as general basis for the endogenous contrast agent (second part).

3.3.1 Implementation of a first-pass perfusion protocol using Gadolinium as contrast agent

3.3.1.1 *Image Sequence*

In this project two sequences were studied: True FISP and EPI. The EPI sequence was tested at first. Echo Planar Images (EPI) is a fast gradient echo sequence where data for an entire 2D plane is obtained only with one RF-excitation, meaning that one entire image will be made up with one repetition time.

For the use of the EPI sequence, a T2/T2*-weighting was implemented. Nevertheless, this sequence was rapidly discarded since it lacks temporal resolution.

After some literature research the True FISP sequence was considered. The parameters selected for this sequence were based on two papers: “Mouse Myocardial First-Pass Perfusion MR Imaging by Coolen et al. and “Quantitative First-Pass Perfusion MRI of the Mouse Myocardium” by Nierop et al. [17], [33].

The True FISP sequence is a Gradient Echo sequence (GRE) that refocuses both, FID and Echo signals. This sequence is weighted on T1, therefore our contrast is expected to increase the intensity of the image (shortens time t1). In order to implement the process several parameters were changed and tested using five different acquisitions. These parameters were: trigger, quantity of gadolinium and slice thickness. All the parameters including TR, TE and number of frames are shown on Table 2. All the acquisitions took approximately 100 seconds (it depends on the heart rate of the animal) with a FOV of 30x30 mm.

Since one of the main goals of the project is implementing a new sequence for the study of myocardial perfusion in a 7 Tesla BioSPec 70/20 USR MRI scanner, different parameters such as the gadolinium, triggering and slice thickness of the image are tested. This is explained in section 3.3.1.3.

Echo Time	0.6/1	(ms)	*
Repetition Time	1.2/2	(ms)	*
Number of Slices	1		
Slice Thickness	1.5/2	(mm)	*
Field of View	30x30	(mmxmm)	
Image Size	32x64		
Number of frames	600		
Duration of the sequence	100	(s)	
Parallel Acceleration factor	1.60		

Table 2. *Proposed sequence parameters for True FISP. (*) These parameters have two values since they were tested for the implementation.*

3.3.1.2 *Study protocol*

The studies and acquisitions carried out in this part of the project were done with two male Wistar rats (300-400 grams). At the beginning of the process the animal was placed in the induction chamber so it could be easily anesthetized. The gas used for this purpose was a mixture of pure oxygen with Sevoflurane (7%), highly used in inhalational anesthesia, with a flux of 600 cc/min. After the induction was completed the animal was moved to the scan bed and the flux was reduced to 300 cc/min with Sevoflurane (2-3%).

The anesthesia was kept up to the end of the experiment so the respiratory rate could be maintained between 40-50 rpm.

After the animal was well positioned (prone position) at the scan bed, three needle electrodes were placed on three of its limbs (two front legs and one rear leg), the respiratory sensor was placed on its back and the temperature sensor was placed on its rectum. At this point all constants (temperature, cardiac rate and respiratory rate) could be recorded. All these steps were carefully followed for every acquisition.

A total of five acquisitions took place. The first one was done based on the parameters specified on those two papers previously mentioned by Coolen et al. and Nierop et al. with cardiac triggering, a slice thickness of 1.5 mm and 0.3 mL of Gadolinium. The second acquisition was performed using the same rat, adding a second trigger: respiratory triggering. Finally, the remaining acquisitions were performed using the last animal. Here the amount of Gadolinium was changed from 0.3 to 0.23 mL and the slice thickness was increased to 2 mm.

After every acquisition, the images obtained with the MRI were processed and studied as explained in section 3.3.1.5.

3.3.1.3 *Signal Intensity-time curves characterization (acquisition parameters)*

All 600 time frames of each study are represented for both data in graphs where the x-axis represents the time in seconds and the y-axis shows the signal-intensity values. The first-pass peak of the signal enhancement was obtained for every acquisition. The up-slope of the Signal Intensity-time curves was used as semiquantitative measurements of perfusion dividing the myocardial up-slope by the left ventricle lumen up-slope.

The implementation process of the Gadolinium protocol includes many parameters that can be modified and subsequently tested. Among the great variety of parameters, three of them are compared in this project: trigger, gadolinium and slice thickness. These three parameters are studied with ROIs that enclosed the entire area of the myocardium and left ventricle respectively. Moreover, signals of four regions of the myocardium are presented so as to assess the perfusion depending on the myocardial region.

Parameter 1: Cardiac and Respiratory Triggering

First acquisition was performed using cardiac triggering. The MRI scanner acquired images after the detection of the R-wave of each cardiac cycle recorded in the ECG. The second one makes use of both, cardiac and respiratory triggering. Apart from acquiring right after each R-wave, the MRI scanner does not obtain images during the respiration.

Parameter 2: Gadolinium

Secondly, the amount of Gadolinium was tested. The concentration of Gadolinium was kept constant among all the acquisitions, with a value of 0.5 mmol/mL. Two different amounts of Gd were compared: 0.3 mL (0.15 mmol) and 0.23mL (0.115 mmol). The experiment was performed with the third rat (acquisitions 3 and 5, further information in Table 3).

Parameter 3: Slice Thickness

Slice thicknesses of 1.5 mm and 2 mm were tested. Both acquisitions were performed with the same animal (see Table 3 acquisition 3 and 4 for further information) with a period of 15 minutes between each acquisition.

Regional perfusion quantification

For this analysis the Myocardium was divided into four segments previously explained in Figure 20, Section 3.2.1.3. Once again, myocardial and left ventricle Signal Intensity-time curves were depicted for each region: septum, lateral, inferior and anterior myocardium wall.

RAT\SPEC.	**TRIGGER**	**GADOLINIUM** (0.5mmol/mL)	**SEQUENCE**	**SLICE THICKNESS**	**TR**	**TE**	**NUMBER OF FRAMES**
Rat 1 (Acq. 1) 305 gr	ECG triggering	0.3 mL (0.15 mmol)	True FISP	1.5 mm	1.2 ms	0.6 ms	600
Rat 1 (Acq. 2) 305 gr	ECG + respiratory triggering	0.3 mL (0.15 mmol)	True FISP	1.5 mm	2 ms	1 ms	600
Rat 2 (Acq. 3) 411 gr	ECG triggering	0.23 mL (0.115 mmol)	True FISP	1.5 mm	1.2 ms	0.6 ms	600
Rat 2 (Acq. 4) 411gr	ECG triggering	0.23 mL (0.115 mmol)	True FISP	2 mm	1.2 ms	0.6 ms	600
Rat 2 (Acq. 5) 411 gr	ECG triggering	0.3 mL (0.15 mmol)	True FISP	1.5 mm	1.2 ms	0.6 ms	600

***Table 3**. Different acquisitions for the implementation of the Gadolinium study.*

3.3.1.4 *Image Display and Anatomy Interpretation*

First image on Figure 20 shows both, the left and the right ventricles. The left ventricle myocardium, which is the one we are interested in, is the darker ring that surrounds the left ventricle, depicted on the third image in the same figure.

Additionally, for our study purposes, the heart myocardium was divided into four regions (second image on Figure 20): Anterior Wall (A), Septum (S), Lateral Wall (L), and

Inferior Wall (I). A comparative study will be done related to the different data obtained for each region of the heart myocardium.

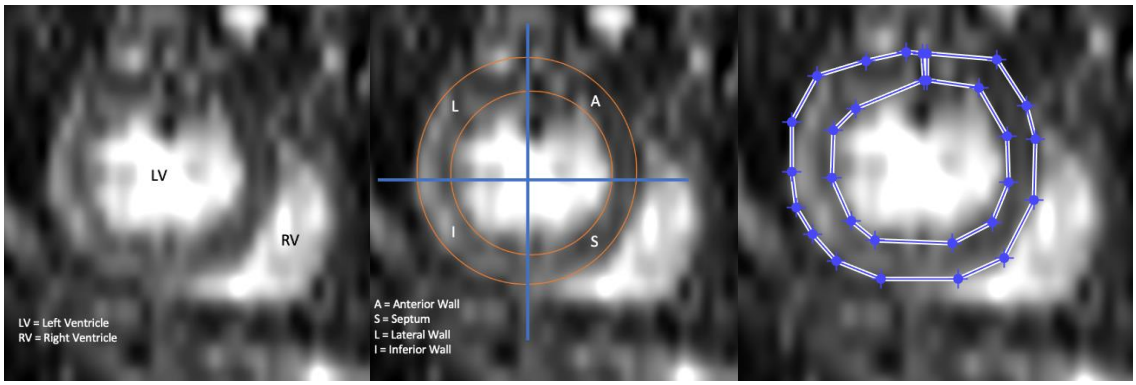

Figure 20. *Midventricular short-axis image.*

3.3.1.5 *Computing perfusion parameters*

A MATLAB code was developed so as to carry out image post-processing. Signal-intensity time curves were calculated, and the up-slope of each curve was used as semiquantitative measure for perfusion.

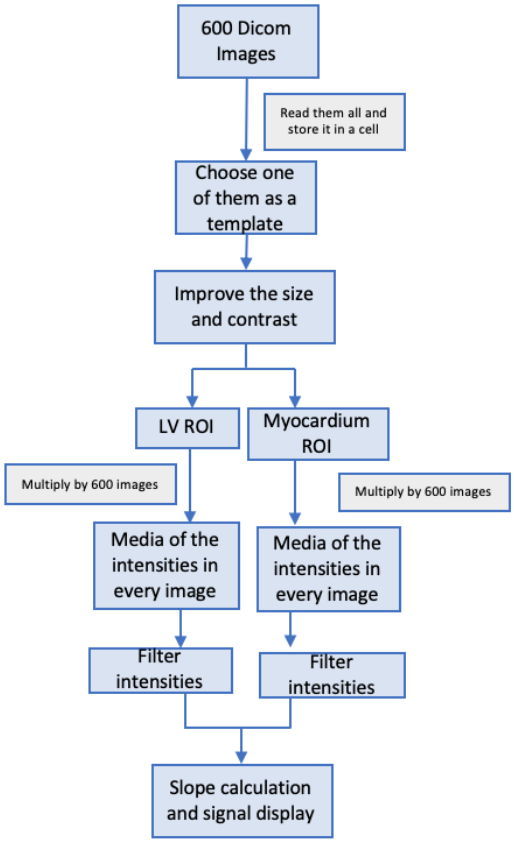

Figure 21. *Calculation of Perfusion Parameters.*

The workflow followed for the post processing is depicted in Figure 21. The images exported from the MRI scanner have DICOM format (Digital Imaging and

Communication On Medicine). The number of repetitions for this study was 600 to ensure that the signal generated by the contrast was well recorded. The size of each image is 32 by 64 pixels with a field of view (FOV) of 30x30 mm.

After images are load in the software, one of the images is chosen as a template. Before displaying it, the template image is rescaled by multiplying the y-axis by 2 transforming it to a 64 by 64 image. The reason why it was acquired as a flattered image is because it improves temporal resolution and rescaling is a simple step that can be performed in the post-processing steps. Intensity values are adjusted improving the contrast resolution and making the ROI selection easier for the user. After the both ROIs are selected, one for the myocardium and the other one for the Left Ventricle lumen, two masks filled with 1 and 0 were formed representing both ROIs. All of these steps are represented in Figure 22.

Once we have both ROIs, the masks are rescaled to the original size and multiplied by the 600 images. The results are two sets of 600 images, one having intensity information of the myocardium and the other set about the left ventricle. See **appendix A** for further information using a visual example.

The average of the non-zero values is performed and a single intensity value is obtained per image.

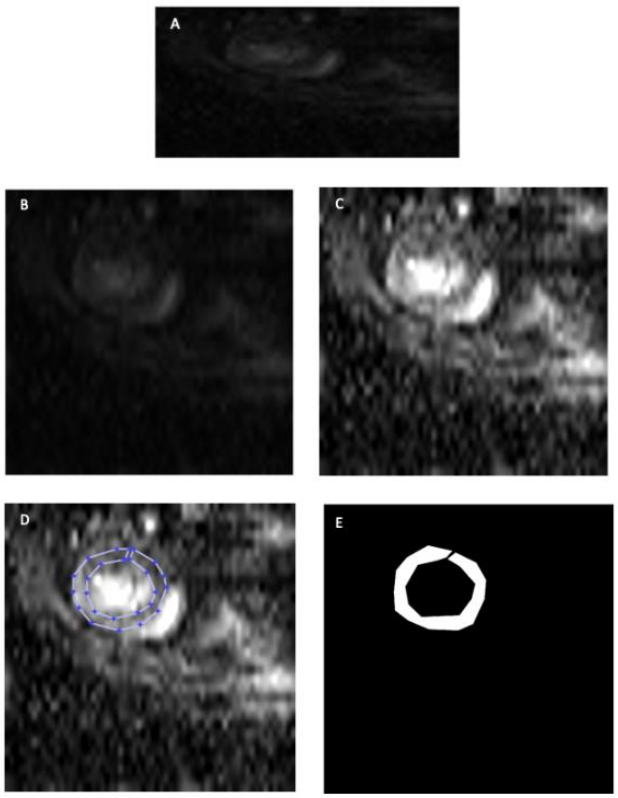

Figure 22. *A. Original Image. B. Rescale Image. C. Adjust Intensities. D. ROI selection. E. Myocardium Mask.*

Filtering

Once the intensities are represented, periodic outliers of high intensities are quite noticeable in the graph (specially for the myocardium). This artifact is caused by the respiration and was eliminated by using a filter that removes intensities that exceed by a certain percentage he median intensity value. Both values, the number of intensities forming each group and the strength of the filter, can be set by the user. Groups of less than 20 are recommended since the up-slope of the signal changes rapidly and the filter could consider those values as outliers getting rid of important information for perfusion measurements.

In Figure 23 both signals (left ventricle and myocardium) are displayed, and the outliers are represented by the green dots. In this example the intensities were divided in groups of 11.

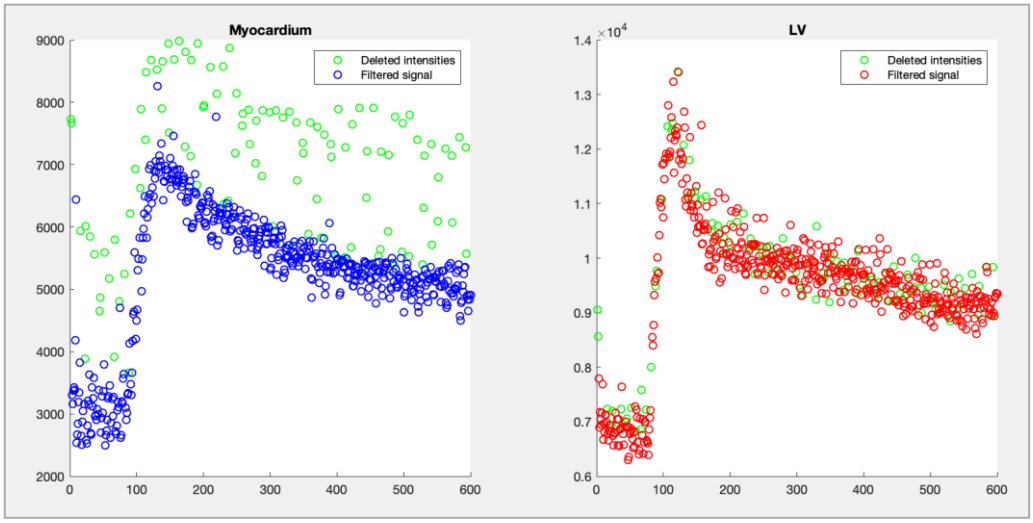

Figure 23. *Myocardium and Left Ventricle Signal Intensity-time curves with the outliers in green.*

Slope Calculation

Once the signal is filtered, the base-line intensity value (before the injection) is subtracted from the signal by calculating the average value of the first 50 images. This rectifies any kind of inhomogeneity that could have been created by the surface coil.

For the slope calculation two points from each signal were selected, one from the baseline (point B(x_2, y_2))and the other one close to the first-pass peak (point A(x_1, y_1)). Knowing the equation of a straight line, the slope "m" was calculated as follows:

$$y = mx + n \qquad m = \frac{(y_1 - y_2)}{(x_1 - x_2)}$$

A good estimation of our curves related to the up-slope created by the contrast agent would be the following equation. S(t) represents the value of our signal at any time, t_0 is the starting point of signal rising. The slope is represented by letter "m".

$$\begin{cases} S(t) = 0 & t < t_0 \\ S(t) = m(t - t_0) & t > t_0 \end{cases} \quad [17]$$

The up-slope of the myocardium is normalized with the up-slope of the left ventricle lumen. This result is used as a semiquantitative measurement for perfusion.

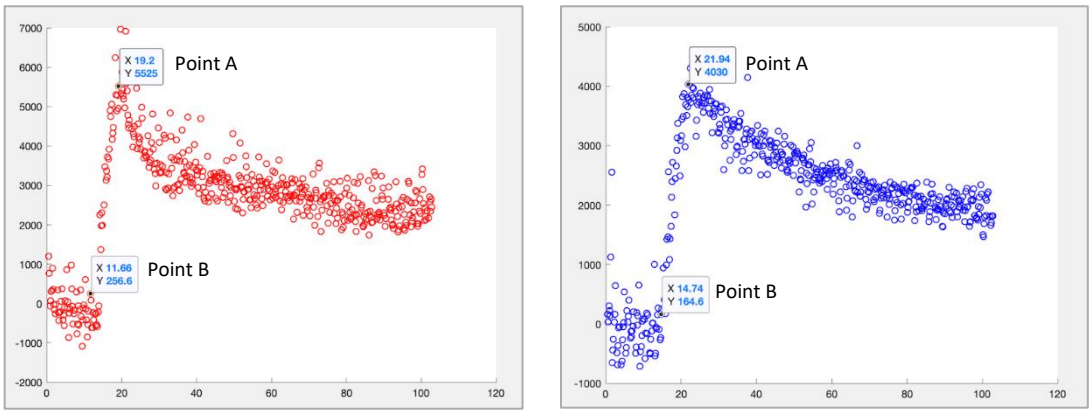

Figure 24. *Example of two points selected for the slope calculation. Both figures represent Signal Intensity-time curves. Myocardium (right) and Left Ventricle (left).*

3.3.1.6 *Graphical User Interface (GUI)*

A MATLAB Graphical User Interface (GUI) was created. All the steps can be seen in Appendix B.

The steps followed by the GUI are the following:

1. First, a pop-up window would let you navigate through the computer's documents.
2. A second pop-up window will show four different images that could be chosen as template. The one with higher contrast resolution would be the best choice.

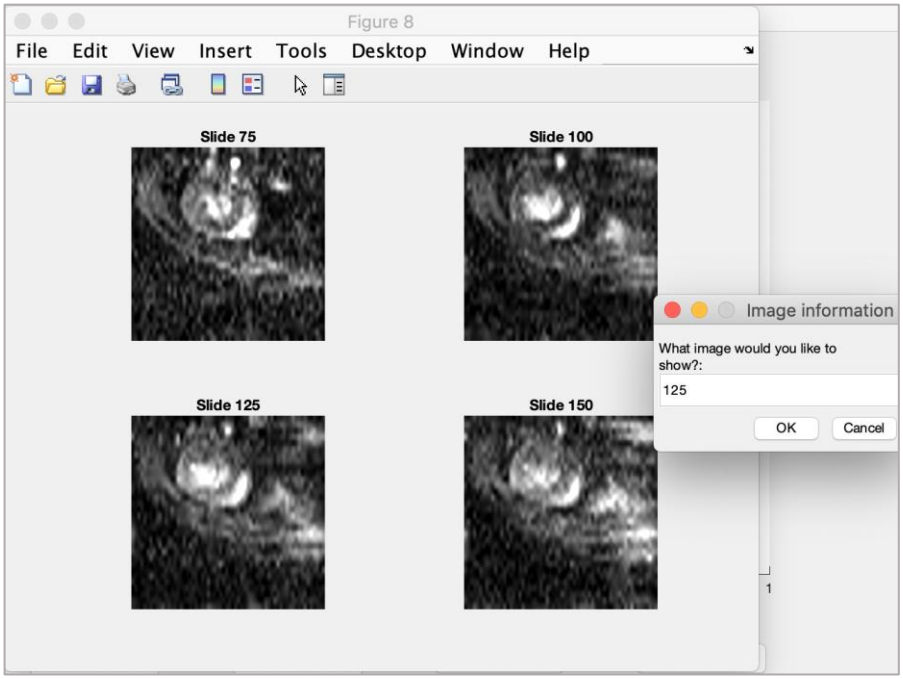

Figure 25. *Four different images that can be chosen as a template. On the right the pop-up window where you select the image.*

3. Now is the time for selecting ROIs. The INSTRUCTION window would tell you to select the Myocardium at first and then the Left Ventricle.

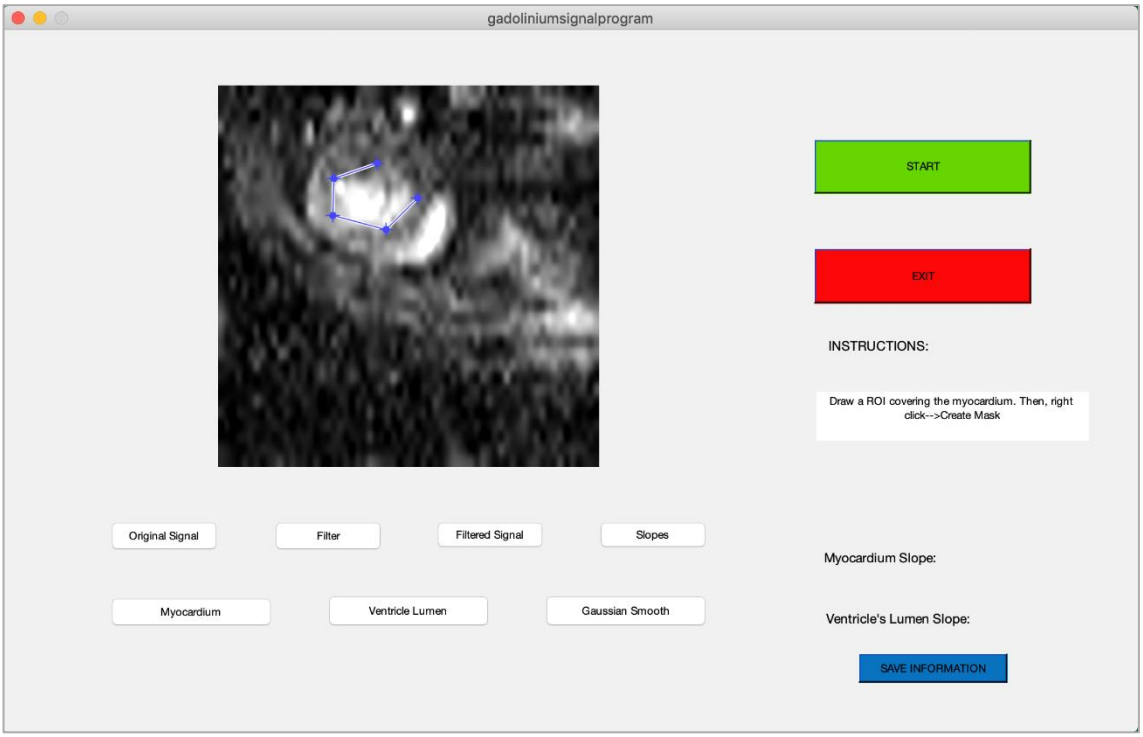

Figure 26. *ROI selection. The instructions can be seen in the white panel.*

4. Filter: a popup window will appear where the filter parameters should be specified.
5. Once these parameters are chosen, both signals will be displayed. The green intensities are the outliers that will be deleted.
6. For the up-slope calculation both signals will be displayed, and points A and B have to be selected manually. The slopes will be displayed on the right down corner of the interface.
7. Seven options for representing the Signal Intensity-time curves are available: original signal, Original signal showing the outliers that have been deleted in green, filtered image (no outliers), signals with the slopes, myocardium signal, left ventricle signal and both signals with a smooth filter. All this information can be saved as a txt file.

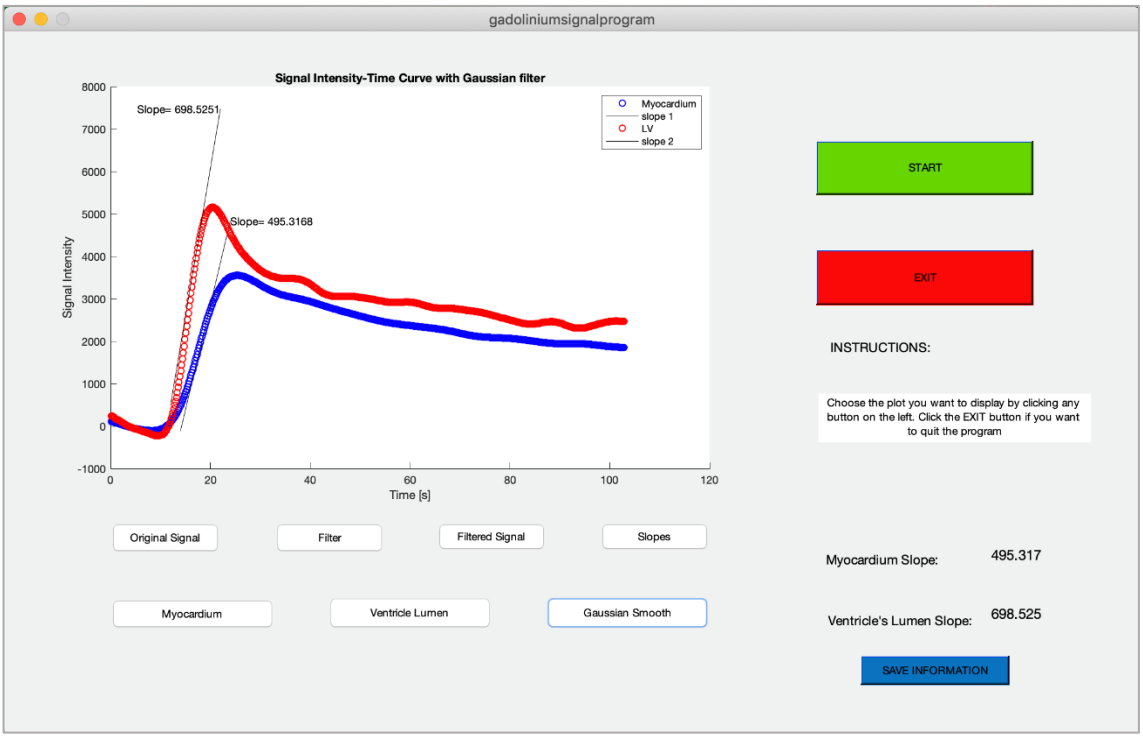

Figure 27. *Interface output. The Left Ventricle and the Myocardium Signal Intensity-time curves are displayed. The START (green) and EXIT (red) button are located on the upper right corner. The SAVE INFORMSTION (blue) button is located on the down right corner.*

3.3.2 Implementation of a first-pass perfusion protocol using changes of FiO_2 as endogenous contras agent

As we have implemented the process using gadolinium-based contrast agent, the first approach for the second implementation was to use the same sequence with its corresponding parameters.

3.3.2.1 *Hardware synchronization*

Unlike True Fisp sequence, EPI sequence offers the opportunity of synchronizing the MRI scanner and the gas mixer, meaning that the FiO_2 start changing at the moment the MRI scanner begins the acquisition. For this purpose, the option "trigger out" is available in the EPI sequence, where a TTL signal of 5 volts will be continuously sent to a computer. Once the acquisition begins, this signal turned into 0 volts.

However, the True Fisp sequence does not include this option, and the synchronization was performed manually using GSM-CommVS software, proposed by the manufacturer of the gas mixer, increasing the error caused by human delay.

3.3.2.2 *Pulse-oximeter characterization*

The signal produced by the pulse-oximeter must be perfectly characterized in order to implement the new protocol. This curve is produced by changes of FiO_2 that create a bolus of deoxygenated blood. Parameters such as the time of 100% nitrogen, the tube's length and the flow rate were tested by Cristina Sainz in her Bachelor Thesis [36]. She came to the conclusion that the tube's length must be kept long (usual tube), a flow rate of 1l/min and 25 seconds of 100% N_2. These variables were kept as stated for the first acquisition where True Fisp was used.

Since the results obtained with the previous values were not optimal for this organ, the duration of the nitrogen was also modified. Different times were tested from 25 seconds to one minute.

3.3.2.3 *Image Sequence*

True Fisp

The first study performed for the use of FiO_2 as endogenous contrast agent for myocardial perfusion were carried out with a True Fisp sequence. This sequence is explained in section 3.3.1.1 and the parameters are described in section 5.1 Table 4. In addition, previous BOLD imaging studies show a great interest on SSFP sequence since it overcomes low SNR and offers high CNR with high temporal resolution [42].

EPI sequence used for the brain in previous studies was tested with N_2-based contrast agent in the heart with poor results. Therefore, this sequence was discarded due to its low temporal resolution.

3.3.2.4 *Study protocol*

The studies and acquisitions carried out in this part of the project were done with two female Wistar rats. All of them were adults (over six months) with a weight close to 250-350 grams. The first part of the process remains unchanged from the experiment using Gadolinium. The animal was placed in the induction chamber so it could be easily anesthetized. The gas used was a mixture of pure oxygen with Sevoflurane (7%), highly used in inhalational anesthesia, with a flux of 600 cc/min. After the induction was completed the animal was placed to the scan bed and the flux was reduced to 300 cc/min with Sevoflurane (2-3%). The anesthesia was kept up to the end of the experiment so the respiratory rate could be maintained between 40-50 rpm. Animal position and monitoring was similar to the previous experiment with Gd-contrast.

A total of three acquisitions took place (one with EPI sequence and two with Fisp sequence). The last two were done based on the parameters specified by the implementation of Gadolinium. The acquisition perform with EPI sequence was rejected at the very beginning since it was not possible to obtain any signal from it due to its low temporal and spatial resolution.

After every acquisition, the images obtained with the MRI were processed and studied as explained in section 3.3.1.5.

4. Results

4.1 First-pass perfusion protocol using gadolinium as contrast agent

4.1.1 Structural Images

An example of a time-series of first-pass perfusion is shown in Figure 28. This series of images obtained from our first acquisition (see Table 3 in Section 3.3.1.5 for more information) represents the right ventricle arrival, left ventricle, myocardium influx and washout. Five out of the 600-time frames were selected. The gadolinium was injected ten seconds after the MRI scanner started acquiring the study. The first image (time = 0.5 seconds) represents an image before Gadolinium was injected. The contrast reaches the right ventricle at time 12.6 s and to the left one at 13.8 seconds. Myocardial influx occurs at time 16 seconds and the washout process takes place after 40 seconds till the end of the acquisition.

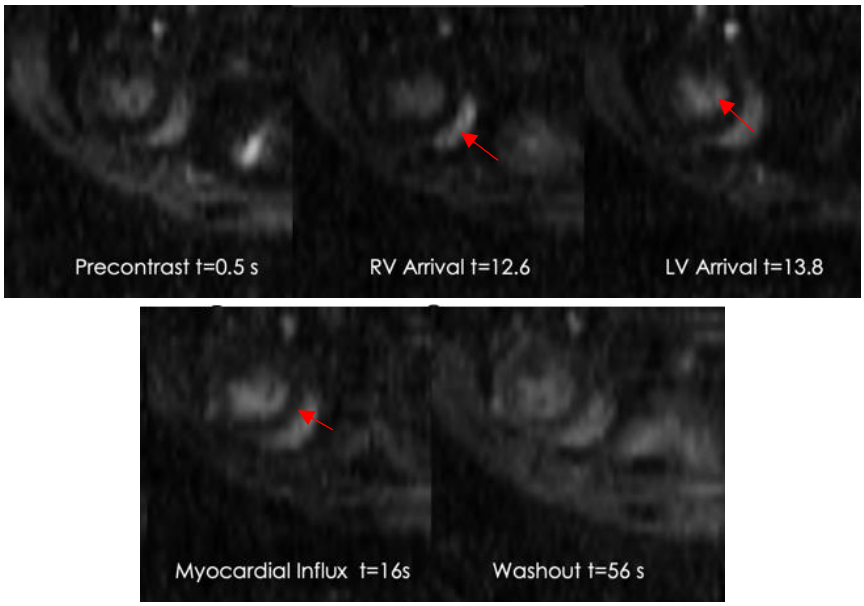

Figure 28. *Time series of cardiac images during the first-passage of a Gadolinium bolus.*

4.1.2 Analysis of the MRI signal change using Gadolinium

The mean signal intensity values for the heart myocardium (blue signal) and the left ventricle (red signal) were calculated for every study. All 600 time frames are represented for both data in the following graphs where the x-axis represents the time in seconds and the y-axis shows the signal-intensity values.

As stated before, three parameters are compared in this project: trigger, gadolinium and slice thickness. These three parameters are studied with ROIs that enclosed the entire area of the myocardium and left ventricle respectively. All the following graphs represent the results are obtained with a True Fisp MR sequence after all the post-processing.

Parameter 1: Cardiac and Respiratory Triggering

First acquisition (Figure 29) was performed using cardiac triggering. The second one (Figure 30) makes use of both, cardiac and respiratory triggering.

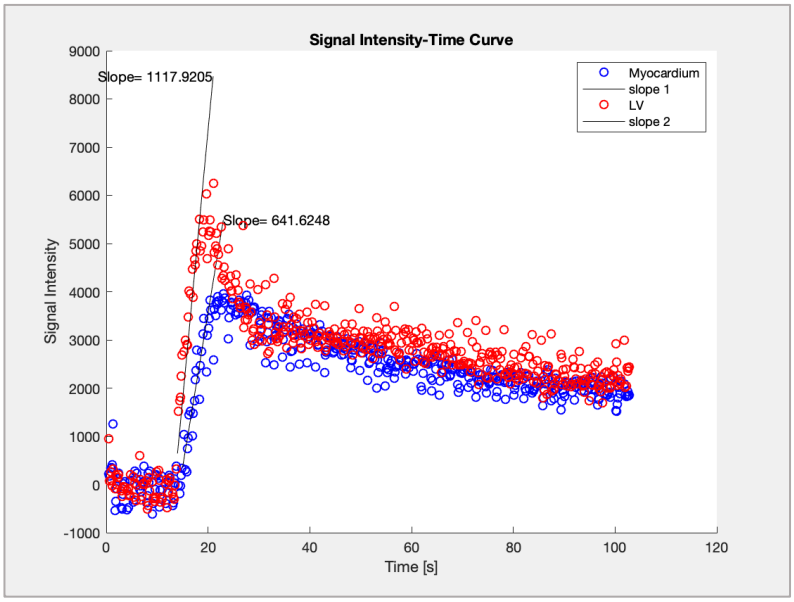

Figure 29. *Signal Intensity-time curve using Cardiac Triggering.*

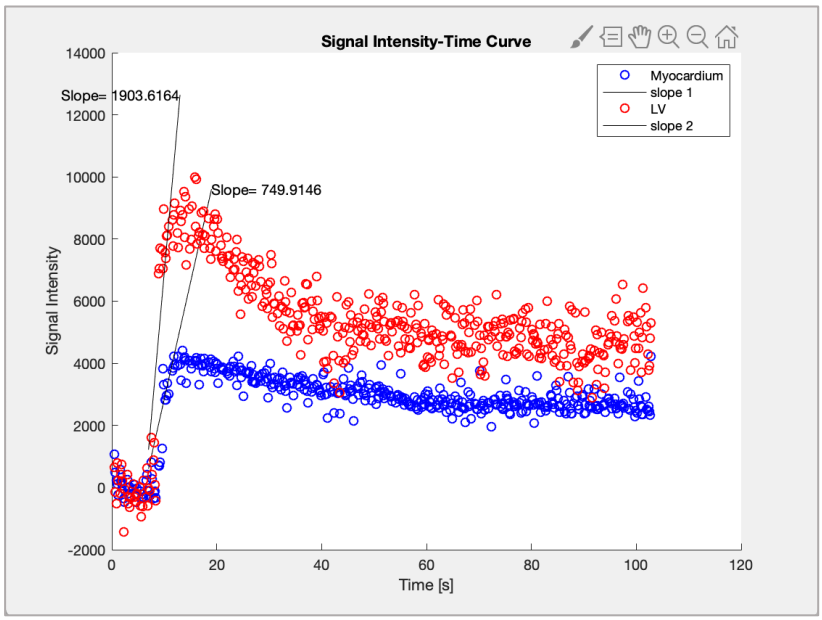

Figure 30. *Signal Intensity-time curve using Cardiac and Respiratory Triggering.*

Parameter 2: Gadolinium

Two different amounts of gadolinium were compared: 0.3 mL (0.15 mmol) and 0.23mL (0.115 mmol). The results can be observed in Figure 31 (0.23 mL) and Figure 32 (0.23 mL). The concentration was kept constant among all the experiment with a value of 0.5 mmol/mL

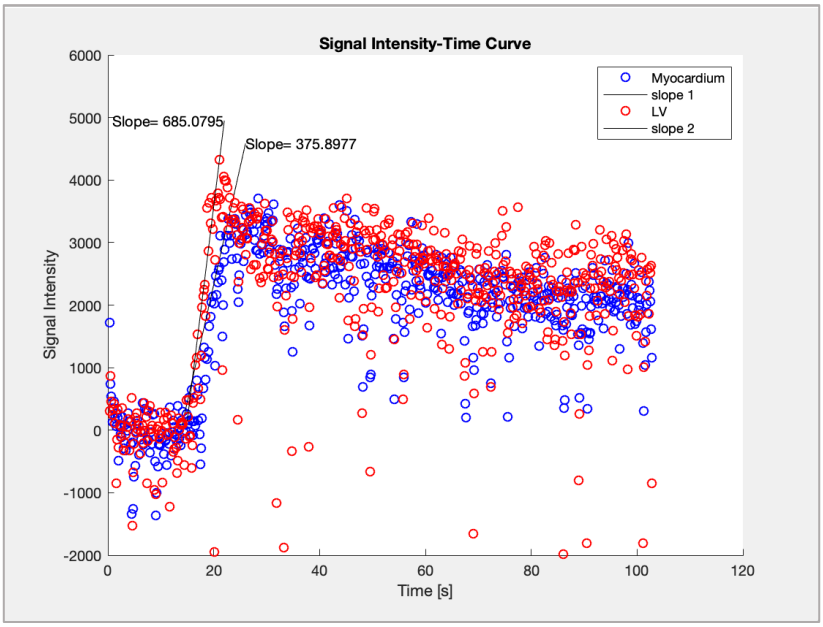

Figure 31. *Signal Intensity-Time curve injecting 0.3mL of Gadolinium (0.5 mmol/mL).*

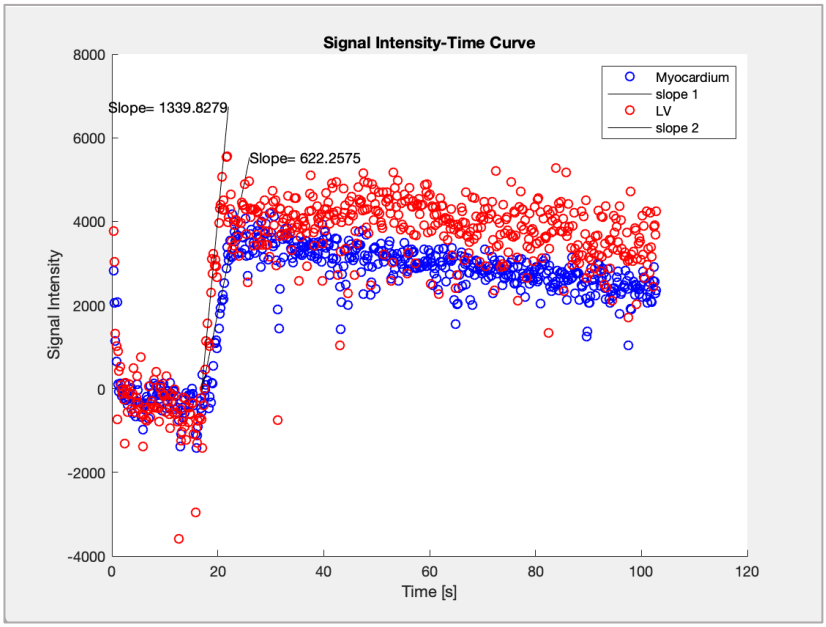

Figure 32. *Signal Intensity-Time curve injecting 0.23mL of Gadolinium (0.5 mmol/mL).*

Parameter 3: Slice Thickness

Both curves, ventricle and myocardium, are once again represented as Signal Intensity-time curves in Figure 33 (slice thickness 1.5 mm) and Figure 34 (slice thickness 2 mm).

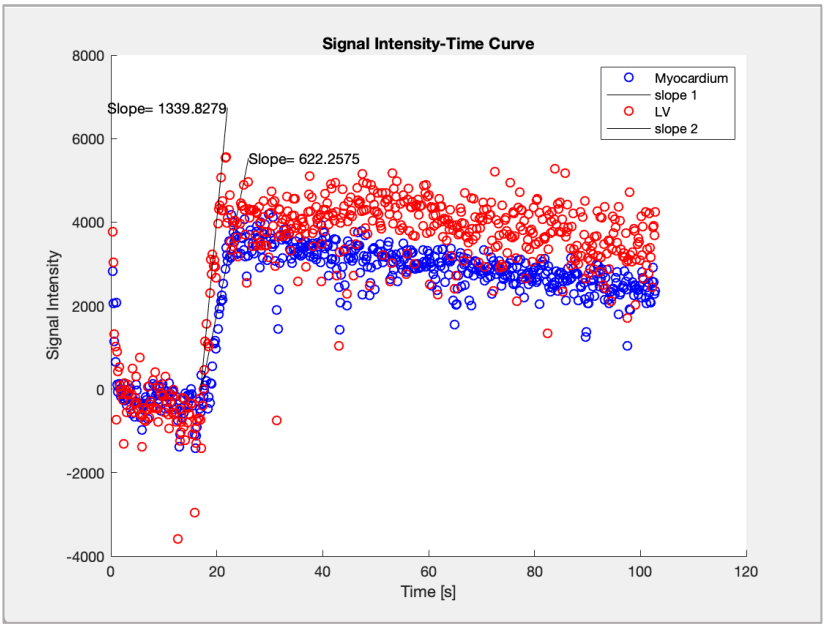

***Figure 33**. Signal Intensity-time curve with slice thickness of 1.5 mm.*

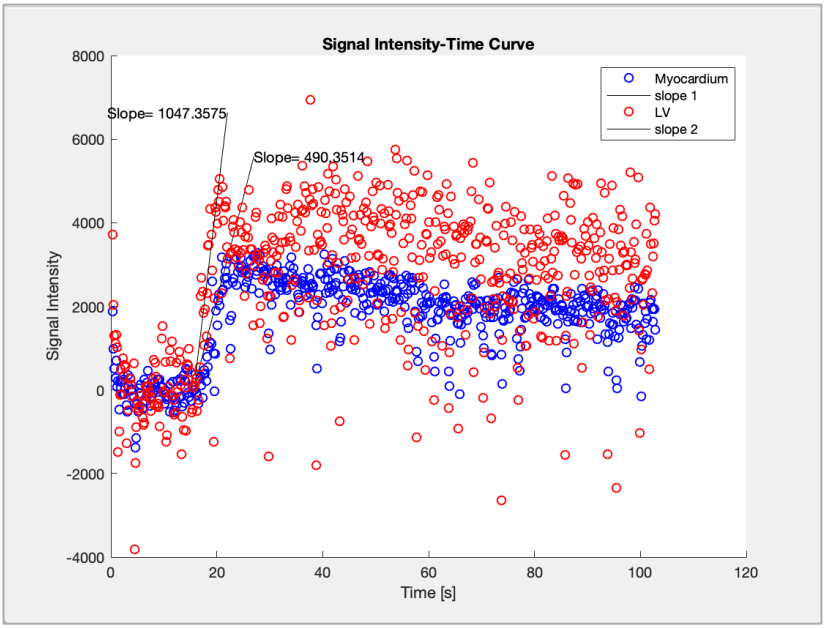

***Figure 34.** Signal Intensity-time curve with slice thickness of 2 mm.*

Regional perfusion quantification

In the following figures (Figure 35 and 36) myocardial and left ventricle Signal Intensity-time curves were depicted for each region: septum and lateral, inferior and anterior myocardium wall. For this step, the first acquisition was used (described in table 3).

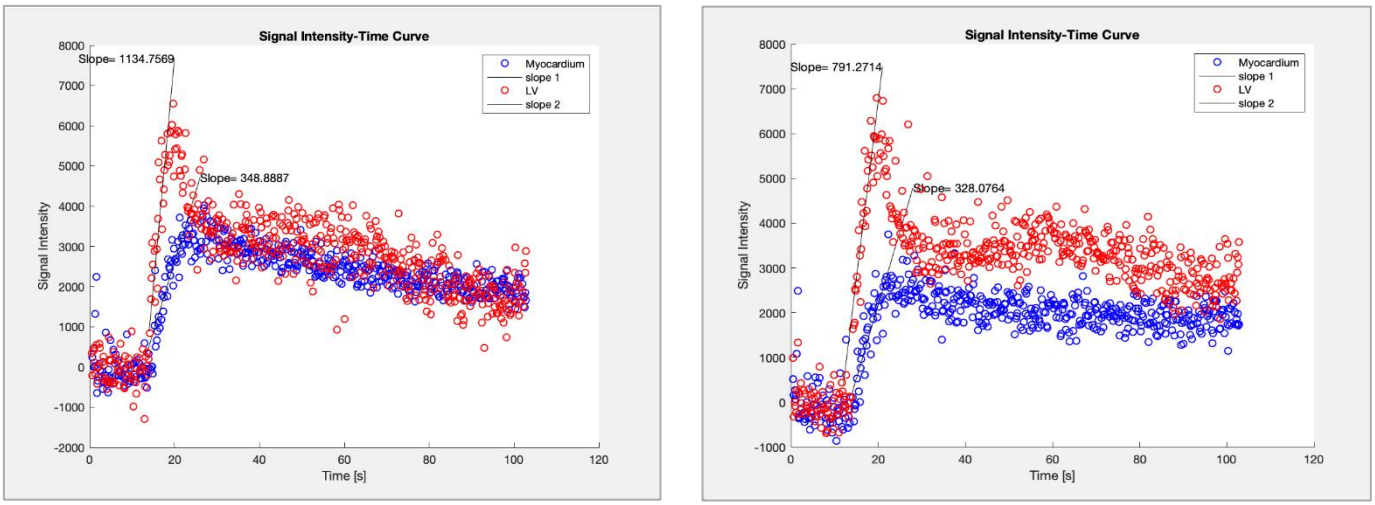

Figure 35. *Signal Intensity-time curve of the Lateral Wall (Image on the left) and Anterior Wall (Image on the right).*

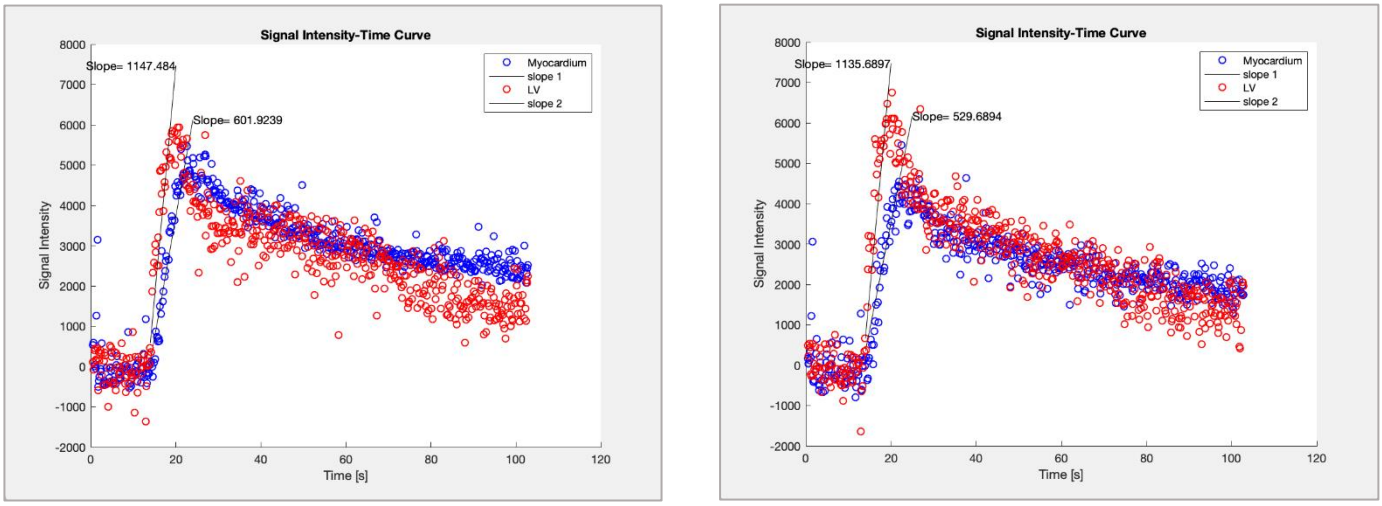

Figure 36. *Signal Intensity-time curve of the Inferior Wall (Image on the left) and Septum (Image on the right).*

4.1.3 Validation of the technique with the slope ratios

The program explained in previous sections was used to obtain graphs from the four different parts of the myocardium: left wall, anterior wall, inferior wall and septum. Since the measurement of both slopes (myocardium section and left ventricle lumen) are highly dependent on the ROI chosen, a total of twelve measurements per region were obtained by different technicians. Then, a normalization step took place, where every myocardial slope was divided by the left lumen slope. The results for all these 48 ratios (twelve per region) are depicted on Figure 37 with a box plot.

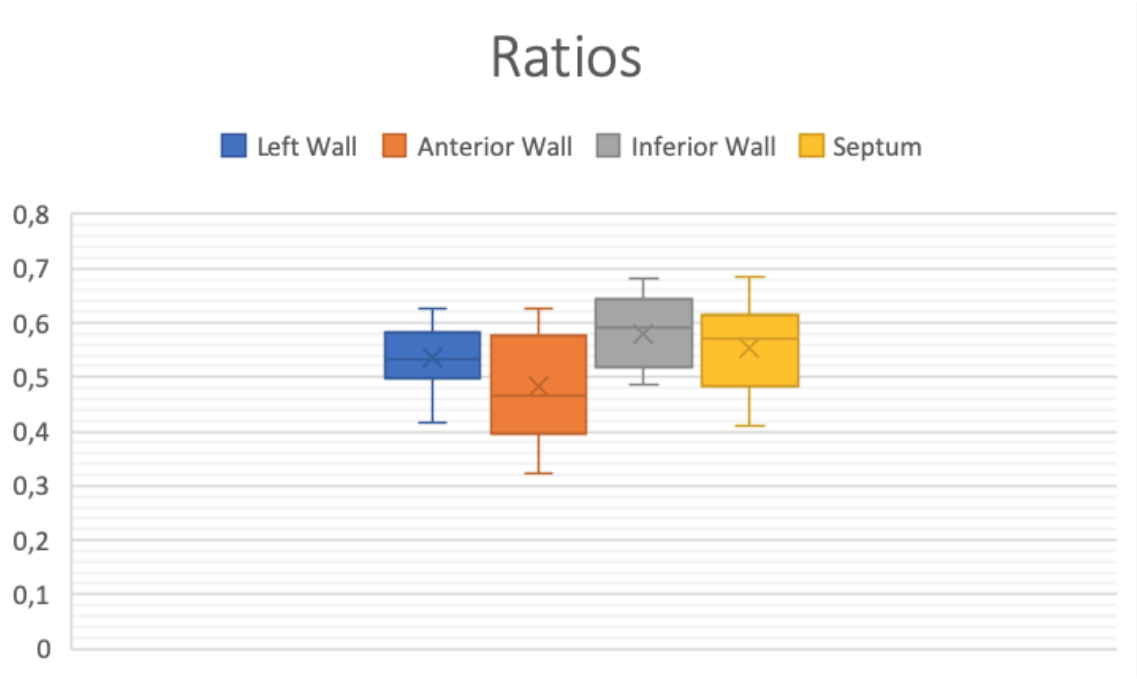

Figure 37. *Ratios obtained from different parts of the myocardium.*

4.2 First-pass perfusion protocol using changes of FiO_2 as endogenous contrast agent

4.2.1 Pulse-Oximeter Curve

Before acquiring images using FiO_2 as contrast agent, the curve created by the pulse-oximeter must be characterized. This curve is created when the bolus of deoxygenated blood passes through the circulatory system (in this project this is measured in the rear limb of the animal). Figure 38 represents the signal obtained when the gas mixture of 100% N_2 is maintained for 25 seconds. Y-axis is the oxygen saturation (SpO_2) and the x-axis represents the time in seconds.

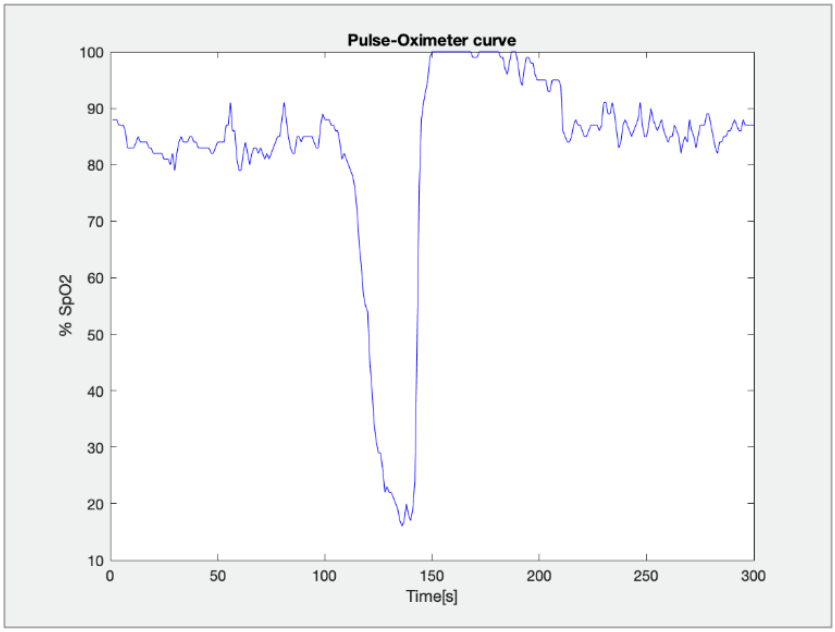

Figure 38. *Pulse-Oximeter curve for 25 seconds of N_2*

Since the amount of signal recorded by the MRI scanner with 25 seconds of deoxygenated blood was quite low, another acquisition was performed increasing to 60 seconds the amount of Nitrogen inspired by the animal. This is depicted in the following figure.

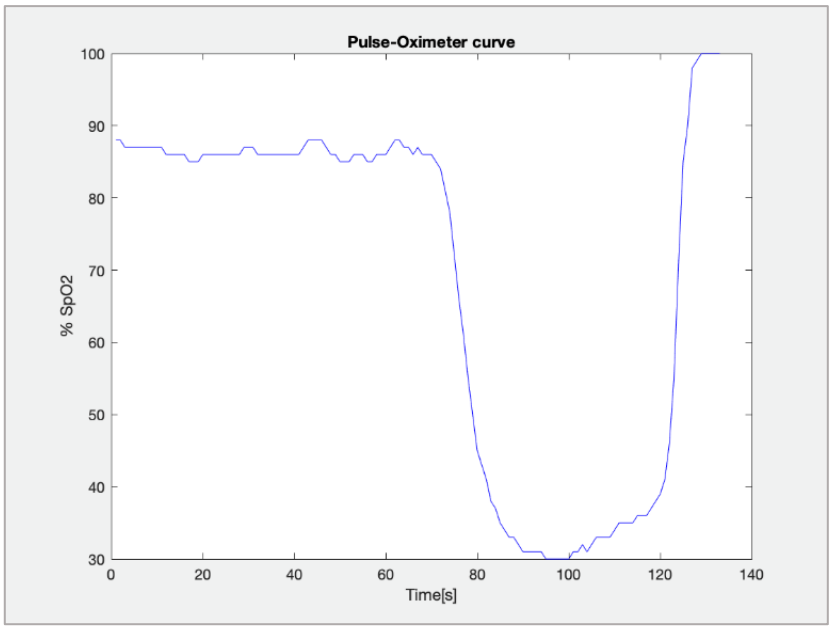

Figure 39. *Pulse-Oximeter curve for 60 seconds of N2.*

4.2.2 Analysis of the MRI signal change using FiO_2

As the previous experiment, the mean signal intensity values for the heart myocardium (blue signal) and the left ventricle (red signal) were calculated for every study.

The same sequence with different amounts of inspired nitrogen are compared in this part of the project: 25 and 60 seconds of N_2. The main goal is to proof if the MRI scanner is able to record alterations in the magnetic field produced by changes of FiO_2.

True Fisp sequence: 25 seconds N_2

All 1200 time frames are depicted for both data in the graphs of APENDIX C where the x-axis represents the time in seconds and the y-axis shows the signal-intensity values.

True Fisp sequence: 60 seconds N_2

In the previous acquisition, a total of 1200 time frames were acquired so as to ensure that the signal was correctly recorded by the scanner. Since the endogenous contrast agent is inhale (not injected) it is thought to arrive more slowly to the tissue of interest. Even though, we have realized that 600 time frames were enough, so the later acquisition was performed with less frames. This information is depicted in Figure 40.

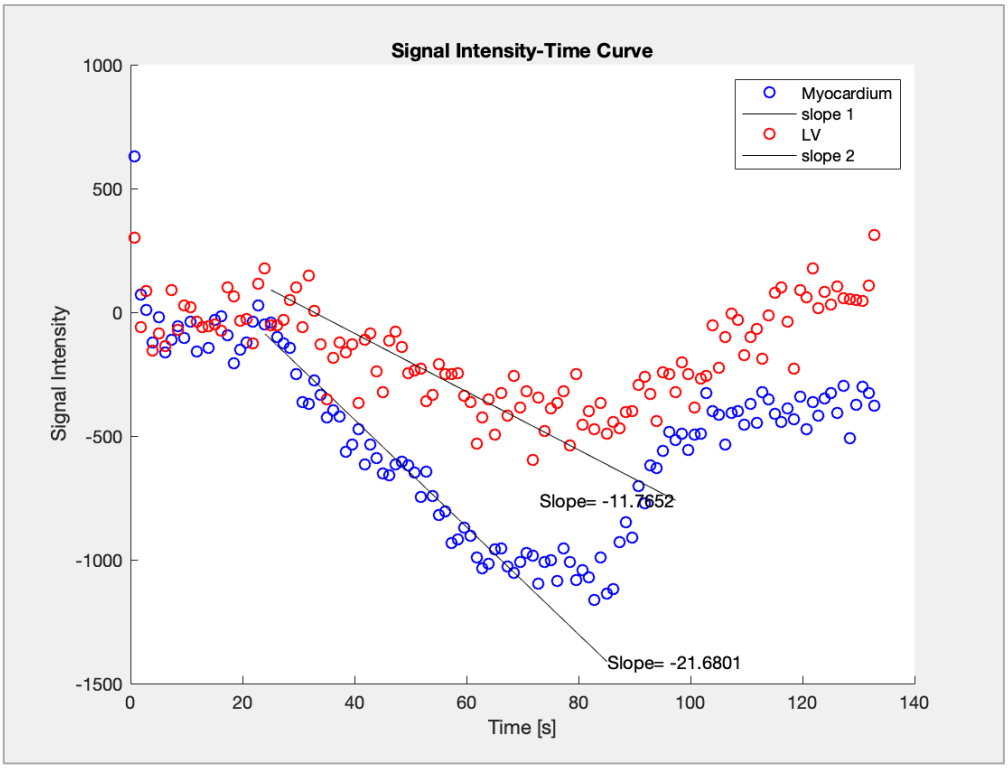

Figure 40. *Signal Inensity-time curve with N2-based contrast agent. (60 seconds)*

The following graph (Figure 41) represents both, the data recorded by the pulse-oximeter and the one from the MRI scanner scale to oxygen saturation values.

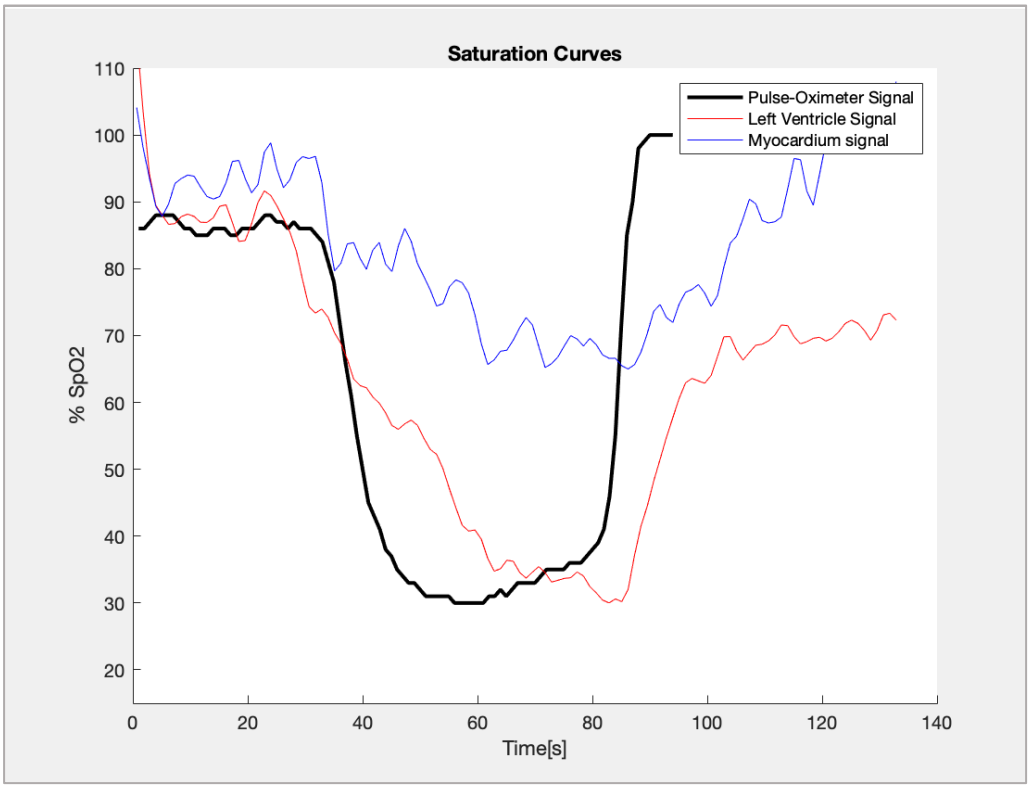

Figure 41. *MRI and pulse-oximeter signals using the endogenous contrast agent.*

5. Discussion

5.1 First-pass perfusion protocol using Gadolinium as contrast agent

According to the objectives of this project, one of its goals is the implementation of a First-pass perfusion protocol using Gadolinium as exogenous contrast agent in a rat model. The method uses an ultrafast imaging sequence for MRI known as True Fisp, so the first pass of a bolus is captured correctly. The fast passage of Gadolinium contrast and the high heart rate of the animal are not compatible with the acquisition of high contrast resolution images (Figure 28). Therefore, for the assessment of myocardial perfusion time resolution is prioritized over contrast resolution.

After two ROIs were selected for each acquisition, the time course of the signal enhancement was depicted. All the results discussed for this section are in the form of Signal Intensity-Time curves of the Left Ventricle lumen and the Myocardium before, during and after the arrival of the contrast.

5.1.1 Analysis of the MRI signal change using Gadolinium

The Signal Intensity-time curves were studied to establish how different parameters may affect its shape and the data that will determine the semiquantitative measurement for perfusion and will successively validate the technique. Among all the variables that could have been analyzed, three of them were selected: the slice thickness, gadolinium and cardiac and respiratory triggering.

Parameter 1: Cardiac and Respiratory Triggering

The first variable to be examined is the triggering option offered by the MRI scanner. Many papers such as that one by Coolen et al. [17] make use of cardiac triggering, meaning that the machine will only acquire at a certain moment of the cardiac cycle monitored by the ECG. Apart from this option, the software offers the opportunity of using respiratory triggering on top of the cardiac one. In order to improve contrast resolution and to avoid movement artifacts cause by the respiration, one of our acquisitions was performed using both types of triggering. The result for cardiac triggering and cardiac plus respiratory triggering are depicted in Figure 29 and 30 respectively. Both acquisitions were performed using the same animal in different dates (no intensities in the second acquisition show recirculation of the contrast agent due to the first acquisition).

Both graphs show the same pattern: left ventricle signal reaches higher values than myocardium signal after contrast agent arrival. After the peak that represents the first

pass of the bolus, continuous recirculation of the contrast agent is recroded in both regions of interest (ventricle and myocardium). Apart from this, what is more striking is the fact that many points are lost on the up-slope of Figure 30. The missing data points may render it impossible to calculate the semiquantitative measurements of perfusion. Moreover, unlike the cardiac rate, the respiration is not completely periodic and varies greatly over time. Therefore, the time scale will be lost since is not possible to predict those data points deleted by the respiration triggering.

For these reasons, the use of respiratory triggering was discarded. As predicted by Nierop et al. and Coolen et al. [17], [33]the correct way of acquiring this type of studies is using the ECG-triggering option.

Parameter 2: Gadolinium volume

The main purpose of this experiment was to test lowest amount of Gadolinium so as to avoid injecting greater amount of exogenous contrast agent than the necessary. The gadolinium-based contrast agent used for the implementation was Gadoteridol (0.5 mmol/mL) marketed as ProHance. The molar concentration was kept constant over the entire experiment and changes were performed on the volume administrated to the animal. The fact that the administration was done intravenously increased greatly the speed of the contrast arrival to the area of interest.

The same rat (rat number 3) was used for the study of this parameter and the following one. A total of three acquisitions were performed with 15 minutes in between. The reason for this lies in the fact that the contrast agent will recirculate after the first arrival provoking changes in the intensity of the acquired images. Figures 31-34 show a great amount of noise caused by this since the time was not probably enough to complete the washout of the contrast. Without taking this into account, the signals depicted in the images are sufficiently good to draw firm conclusions regarding these parameters.

Two different volumes of Gadolinium were compared: 0.3 mL (0.15 mmol) and 0.23 mL (0.115 mmol). The results are shown in Figure 31 (0.3 mL) and Figure 32 (0.23 mL).

A slight variation can be observed between both graphs: Figure 31 shows a more remarkable peak after the first bolus arrives to the left ventricle, meaning that a higher accuracy is achieved. This peak is important, since the semiquantitative measurements is based directly on the graph’s data. For this reason, a reduction of the contrast agent will not be performed, volume of 0.3 mL is the preferred one.

Parameter 3: Slice Thickness

By increasing thickness more tissue is included in the same voxel thus increasing signal. Two different slice thicknesses were tested. At the beginning a slice thickness of 1.5 mm was set (following Nierop et al. [33] parameters), followed by a second acquisitions increasing the slice thickness to 2 mm. The results are shown in Figure 33 and 34.

No significant change was observed between both plots. Since the enlarge of the slice doesn´t show any improvement, the thickness was kept at 1.5 mm.

Echo Time	0.6 (ms)
Repetition Time	1.2 (ms)
Number of Slices	1
Slice Thickness	1.5 (mm)
Field of View	30x30 (mmxmm)
Image Size	32x64
Number of frames	600
Duration of the sequence	100 (s)
Gadolinium (0.5 mmol/mL)	0.3 (mL)
Trigger	ECG Triggering

Table 4. *Final parameters used for the First-pass perfusion protocol using Gadolinium as contras agent.*

5.1.2 Validation of the technique with the slope ratios

Since the technique puts strain on the ability of the technician in terms of ROI selection, a total of twelve measurements per region of myocardium were performed by different technicians of the department. The comparison among all these regions is carried out so as to validate two things: perfusion is taking place through all the myocardium and the parameter given by the slopes is consistent.

First, we assume that all the animals in this project do not suffer any pathological condition. For this reason, we expect to obtain similar values from all four regions. In Figure 37, a graphic method was implemented to show the information through their quartiles.

From the graphical representation we know the following results:

- The average value for each region is: 0.5357, 0.4827, 0.5800, 0.5541.
- The median value for each region is: 0.5319, 0.4646, 0.5901, 0.5700.

The first value corresponds the left wall, then the anterior wall, inferior wall and finally the septum. Accordingly, our technique is quite consistent since both average and median are close to the same value (0.54 ± 0.04) in all cases. Furthermore, this result

also indicates that the perfusion is being correctly recorded by the scanner and this protocol can be used for further research.

5.2 First-pass perfusion protocol using changes of FiO_2 as endogenous contrast agent

Apart from implementing the protocol of myocardial perfusion with gadolinium-based contrast agent, one of its main goals is the implementation of a First-pass perfusion protocol using changes in FiO_2 as endogenous contrast agent in a rat model. This method uses the same ultrafast imaging sequence used for the experiment of gadolinium. Furthermore, other MRI sequences such as EPI were taken into consideration so as to improve the technique and obtain greater results.

5.2.1 Analysis of the MRI signal change using FiO_2

True Fisp

Signal Intensity-time curves were obtained for both regions, the myocardium and the left ventricle lumen.

The first study was performed with the EPI sequence originally proposed by Cristina Sainz [36]. As results were not optimal, we decided to use our previous setting with the gadolinium sequence as a template. First, apnea was induced for 25 seconds by inhalation of 100% Nitrogen gas. The signal generated by True Fisp sequence in the left ventricle lumen did not show any significant growth (APENDIX C), meaning that no semiquantitative measurements can be made with this sequence using such intrinsic contrast agent (time of 100% N_2 = 25 seconds)

Then, a change of the amount of nitrogen was performed. Since the signal obtained with 25 seconds of 0% O_2 and 100% N_2 was not enough to produce a signal change useful for perfusion analysis, it was increased to 60 seconds.

The results for this study are shown in Figure 40, on which we can observe that the signal change could be detected. Figure 41 shows the MRI signal scaled up to the values recorded by the pulse-oximeter. The minimum SpO_2 achieved by the apnea induction was in the range of 30-35% with a duration of approximately 30 seconds. This value was proven to be enough for the MRI to record the change.

5.3 Validation of the technique comparing both contrast agents: changes in FiO_2 and gadolinium-contrast image

The validation of the new contrast agent was performed by comparing it with gadolinium results, both were post-processed using the same MATLAB software. Signal Intensity-time curves from gadolinium reached values up to 5000-6000 (Figure 29), four times greater to what we have obtained with our new endogenous contrast agent (around 1100). However, the signal recorded by the MRI scanner is high enough to assess perfusion and all the drawbacks posed by gadolinium could be overcome with this innovative contrast.

The semiquantitative measurement for myocardial based on the Gd curve slope ratio showed a value of 0.54 ± 0.04, very similar to the value obtain with the example of FiO_2-based contrast agent in Figure 40: 0.5427, thus supporting the validity of the approach.

6. Limitations and future improvements

Regarding image quality and resolution, it would be interesting to try new sequences or change parameters used for True Fisp. Some papers related to BOLD imaging suggest that FLASH sequence, a gradient echo sequence used for fast imaging acquisitions, is a good alternative for perfusions studies in the myocardium [38]. These improvements lead to an image of the heart where the anatomical structures are easily recognized by the physician. Other alternatives rely on image postprocessing, where a new software could be developed for the spatial co-registration of the heart. This would avoid inaccurate results derived from the respiratory motion.

Since True Fisp does not offer any synchronization signal output during acquisition, it is of a great interest to find other alternatives for the synchronization of the MRI scanner with the gas mixer. This would reduce delay errors due to the contrast injection. There are several fast sequences that could be tested or either modify software given by the manufacturer so as to create the missing option.

Finally, we have developed a Graphic User Interface for gadolinium-based contrast agent that generates the signal produced after a ROI is selected. It would be interesting to further improve such software including parametric maps of the myocardium.

Besides all these improvements, an excellent way to demonstrate the feasibility of our approach would be the implementation in a pathological animal model. Currently there are animal models with cardiovascular disease that would directly affect myocardial perfusion. The comparison of healthy and pathological subjects could be the main goal of future experiments regarding this type of contrast agent.

7. Socio-economic impact and Regulatory Framework

7.1 Socio-economic impact

Social Impact

Among all the objectives of this project, the most important one relies on the social impact that these studies may have in the near future. For the moment this project is performed as a preclinical study, that is intended to understand all the results and mechanisms that will decide if the new method is ready for clinical research with humans. The proposed contras agent, changes in FiO_2, is intended to offer a new alternative for the study of myocardial perfusion using the MRI as the acquisition technique.

In addition, this new contrast agent will address the drawbacks and hazards offered by the Gadolinium. Patients with renal misfunction are not capable of completely filtering gadolinium out of their bodies causing nephrotoxicity with the risk of developing nephrogenic systemic fibrosis (NSF) [55]. In the same way, this type of contrast agent is contraindicated for pregnant woman, since the risks for the correct development of the fetus increase considerably with its use [56]. Most importantly, a paper highlighting the fact that gadolinium-based contrast agent may remain in the body as depositions in certain parts of the brain (nucleus and Globus pallidus) was published in 2014 by Kanda T. et al. [57]. The cause of this accumulation relies on prior gadolinium contrast administrations.

Economic Impact

The Gadolinium contrast agent employed for the study marketed as ProHance 279.3 mg/ml by an Italian multinational know as Bracco costs 280.89 euros for a bottle of 50 ml. The proposed endogenous contrast agent produced with changes of FiO_2 reduces greatly the costs incurred by the gadolinium. In addition, the costs of the injection procedure are removed since the new contrast agent is directly inhaled by the patient using the mouthpiece used for the anesthesia.

7.2 Budget estimation

The costs of this Thesis can be divided into: Materials, Services from the Gregorio Marañón Health Research Institute.

Materials

MATERIAL	*cost/unit (€)*	*quantity*	*total cost (€)*
MATLAB	800	1	800
Laptop	900	1	900
RS-232-USB	15	2	30
Gadolinium (prohance) 1 mL	5.6	1.5	8.4
		TOTAL	1738.4

Services

SERVICE	*cost/hour (€)*	*time (hours)*	*total cost (€)*
BRUKER MRI SCANNER	91	12	1092
TECHNICIAN	17	12	204
ANESTHESIA	10	12	120
		TOTAL	1416 + IVA (21%)

Human Resources

HUMAN RESOURCES	*cost/hour (€)*	*time (hours)*	*total cost (€)*
BIOMEDICAL ENGINEER	15	600	9000
Supervisor	33	170	5610
		TOTAL	14610

Total

COST SOURCE	*cost (€)*	*iva (21%)*	*final cost (€)*
materials	1738.4		1738.4
services	1416	297.36	1713.36
human resources	14610		14610
		TOTAL	18061.76

7.3 Regulatory Framework

The entire project was performed using small laboratory animals. Therefore, different ethics and regulatory concerns involving the usage of this model must be taken into account throughout the experimental procedures. All parts of the study involving these animals were performed according to the National regulation (RD 53/2013 and Orden ECC 566/2015) and EU regulation (2010/63/EU) [58]–[60].

Furthermore, every research protocol and procedure that implies laboratory animals in the center, must be approved by the Comité de Ética de Experimentación Animal (CEEA) of the Hospital General Universitario Gregorio Marañón (ES280790000087) [61].

8. Final Conclusion

After all the work performed in this project it can be conclude that all its objectives were achieve with highly positive results. The implementation of the first-pass perfusion of the myocardium using gadolinium as contrast agent was well assessed since the signal change was fully recorded by the MRI scanner and its completely ready to be tested with animal models suffering from ischemic diseases. For such protocol, a GUI was developed using MATLAB code allowing the physician to visualize the resulting signal in a less complicated way. This can be used as a diagnostic tool.

Apart from gadolinium, we were able to use FiO_2 as an endogenous contrast agent by proving how the scanner is capable of recording changes in FiO_2 as a signal in the heart's myocardium and lumen. The signal is not as precise and accurate as the one produced by gadolinium, but it can be furtherly improved in the future so as to be able to translate this type of experiments to humans. This innovative contrast agent is a focal point for further research since it overcomes drawbacks offered by the contrast agents in used.

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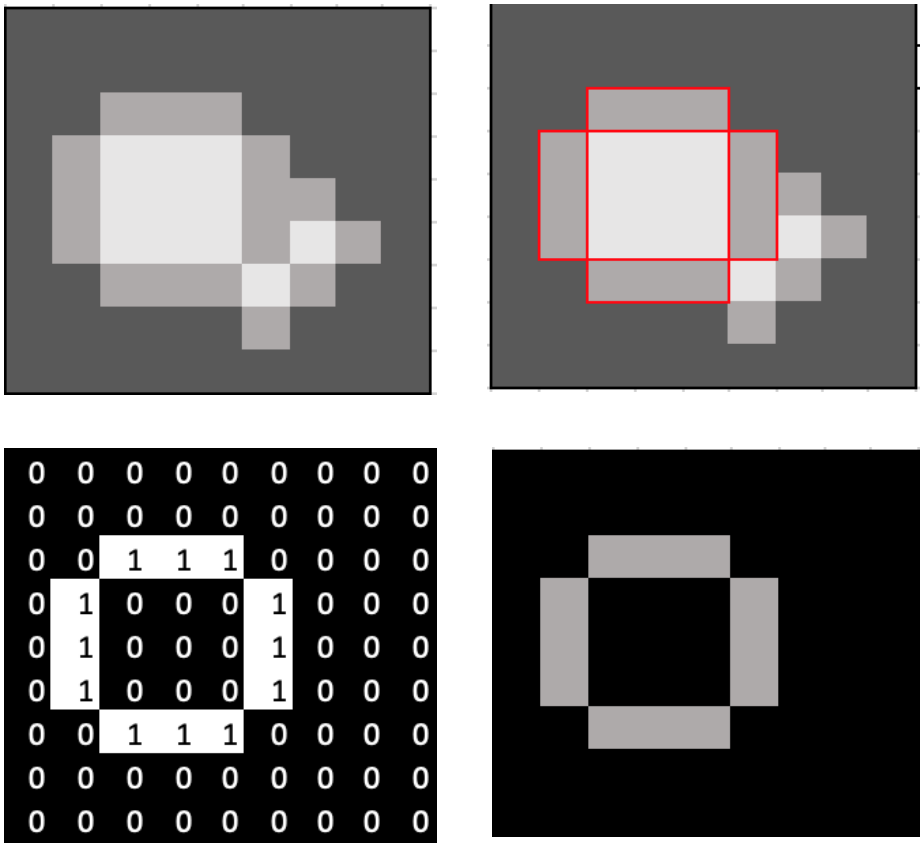
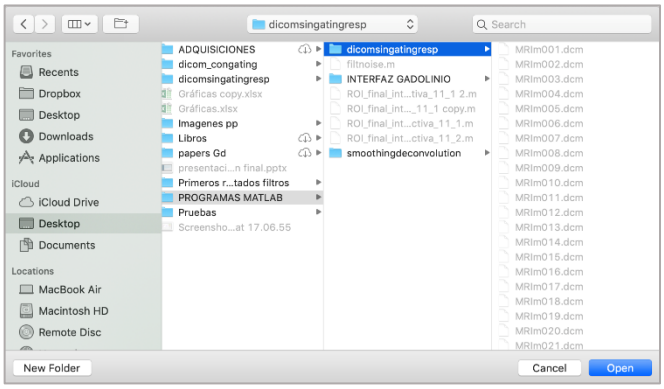
APENDIX A



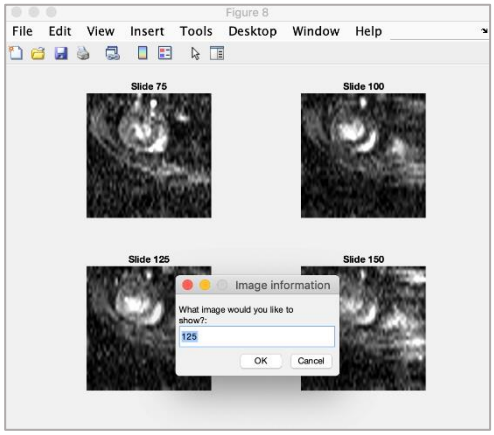
First image represents our template image chosen from the original data. Image on the upper right corner includes the myocardium ROI (in red) selected by the user. The ROI selected is based on the first image and will be used for the mask. Third image represents the mask created with the ROI selection composed by 1s and 0s. Our ROI is filled with 1s and the rest of the image will be considered to be 0s. This mask is now multiplied by all the images of our study, therefore from now on we will only take into account the values of the ROI, in this case the myocardium

APENDIX B

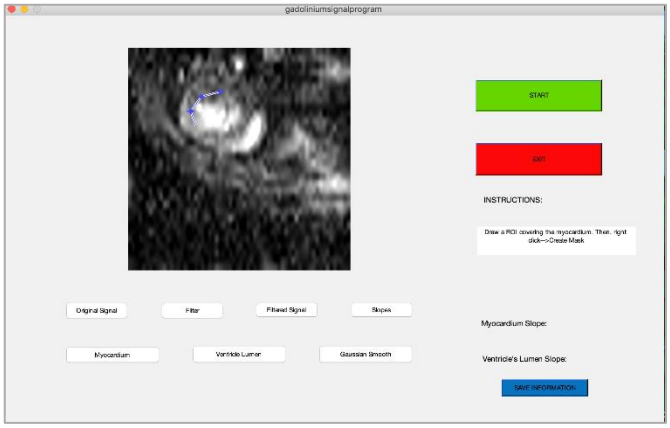
MATLAB interface for Gadolinium



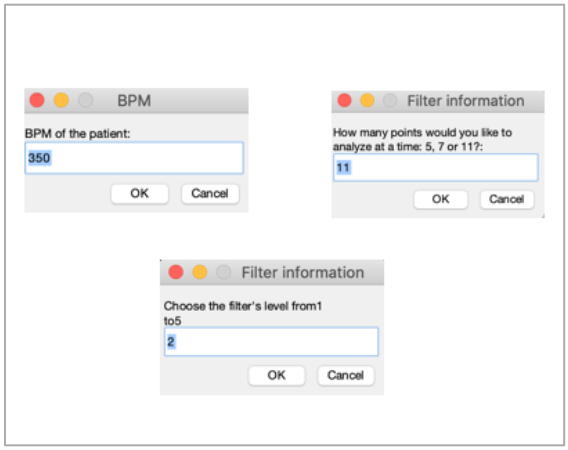
Pop-up window showing all the documents in the computer



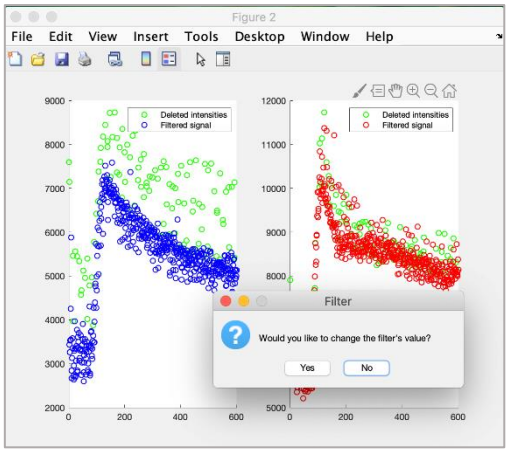
Pop-up window showing the four options given as template



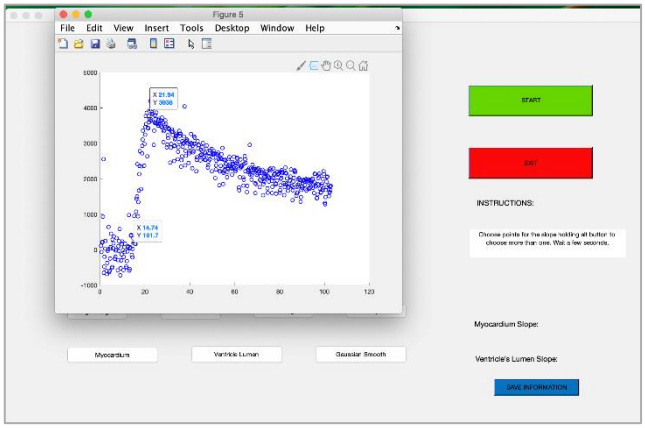
Interface showing the image chosen as a template and the ROI selection



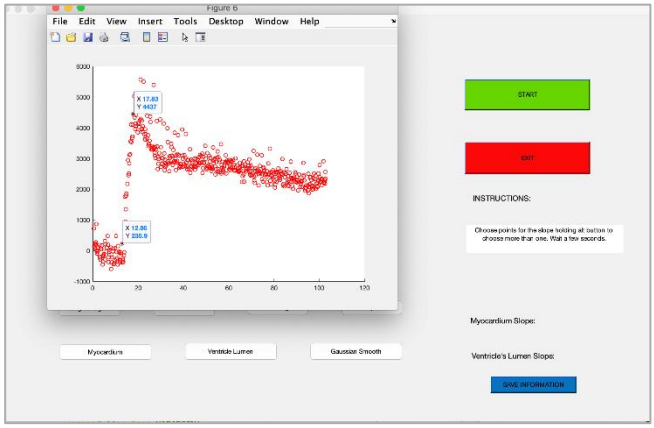
The filter's parameters and the Heart rate chosen by the user



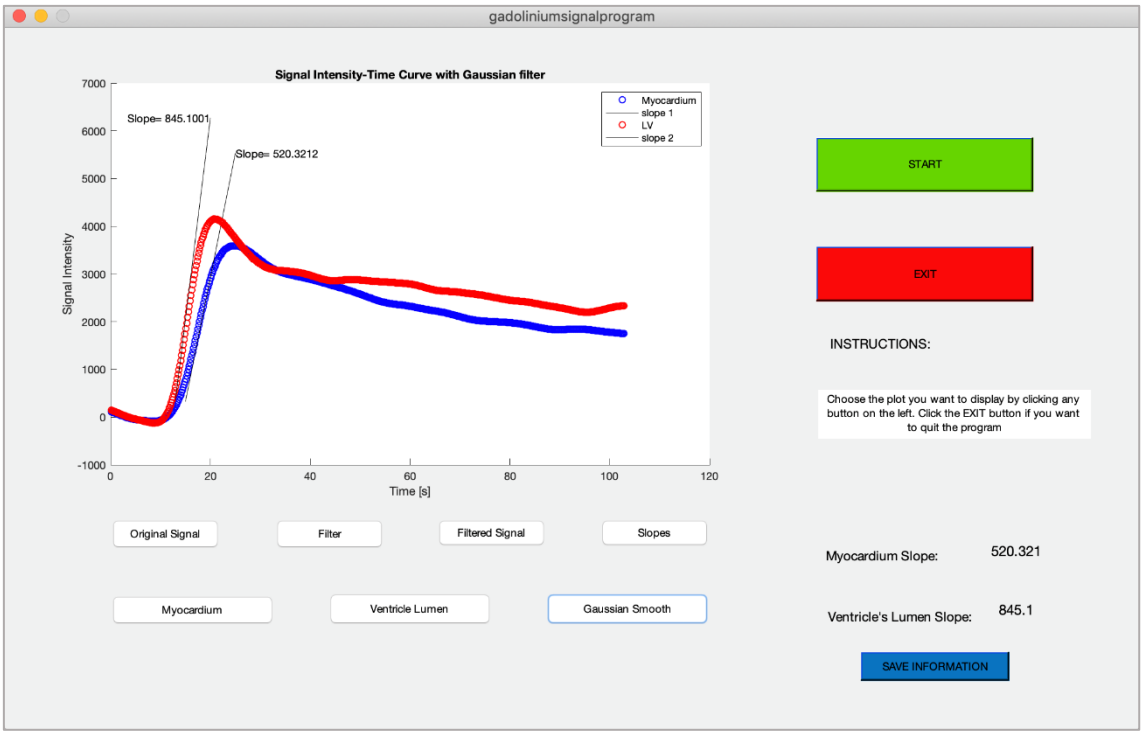
The output from the filter.



Points selected for the myocardium slope.



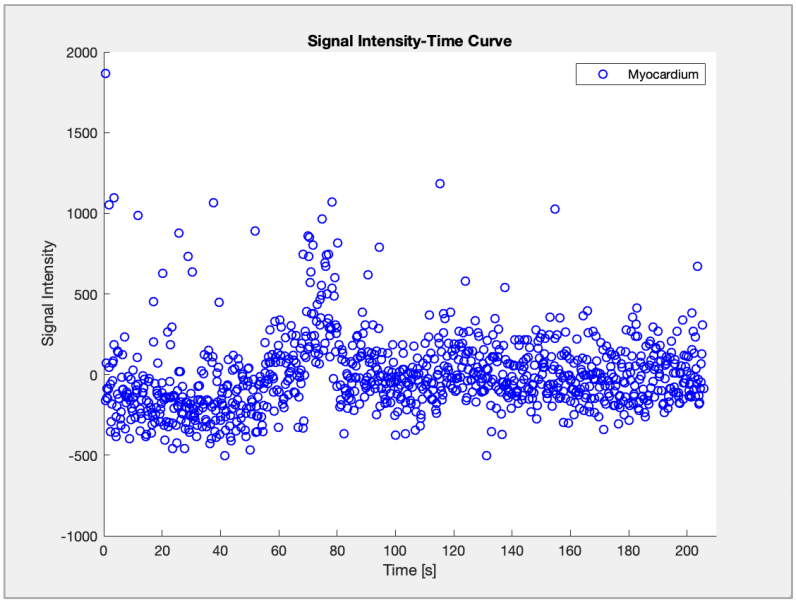
Points selected for the Left Ventricle slope.



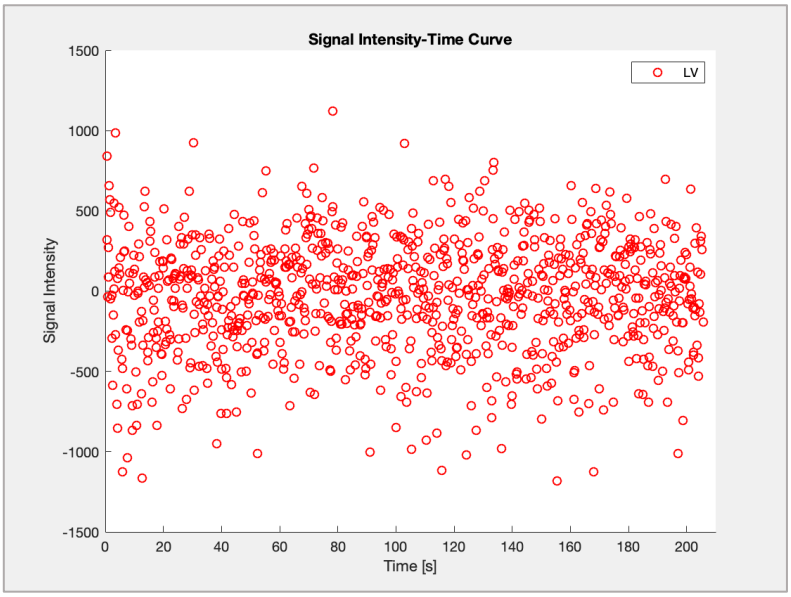
Final display of the GUI.

APENDIX C

Signal Intensity-time curves after 25 seconds of 100% N_2



Signal Intensity-time curve of the myocardium



Signal Intensity-time curve of the Left Ventricle