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**Evaluation of Quantitative Susceptibility
Mapping Algorithms on Gel Phantoms
and Preclinical Models**

Tesi per la Laurea Magistrale di:

Martina Bellino

Relatore:

Prof. Alessandro Lascialfari

Correlatrici:

Dr.ssa Francesca Brero

Dott.ssa Silvia Megalizzi

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Abstract

The magnetic susceptibility χ is a fundamental physical property that reflects how materials respond to an external magnetic field.

Magnetic Resonance Imaging (MRI) is a non-invasive technique used to visualize tissue composition and microstructure, including soft tissues, by exploiting differences in magnetic properties. In MRI, susceptibility variations between tissues induce local phase shifts in the signal, providing additional contrast that can reveal subtle structural and compositional features. Clinically, MRI plays a crucial role in the diagnosis and monitoring of neurological, cardiovascular, and oncological conditions by delivering detailed anatomical and physiological information.

Quantitative Susceptibility Mapping (QSM) is an advanced MRI post-processing technique that reconstructs spatial maps of tissue magnetic susceptibility from phase measurements, solving an inherently ill-posed inverse problem. This approach has found increasing applications in biomedical research for the detection and quantification of iron deposition, calcifications, demyelination and the spatial distribution of contrast agents. Despite its potential, QSM reconstruction is sensitive to noise, artifacts and orientation effects, which has led to the development of multiple algorithms aiming to optimize accuracy and robustness.

In this work, it is presented a comprehensive analysis and comparison of the QSM algorithms proposed by Wang et al., all implemented in the Sepia Toolbox (developed in MATLAB), to evaluate their relative performance and applicability. The algorithms are tested on a gel phantom containing nine different concentrations of the superparamagnetic contrast agent VivoTrax, as well as on a set of high-resolution rat brain acquisitions, serving as pre-clinical models. MRI acquisitions were performed at 7 Tesla using a gradient-echo sequence with optimized parameters for phase contrast. Reconstruction quality was assessed in terms of linearity of susceptibility versus concentration, noise amplification and artifact suppression.

Preliminary results indicate that certain algorithms achieve higher sensitivity at low contrast concentrations (such as TKD), while others demonstrate superior artifact mitigation in complex geometries (such as iLSQR and MEDI). These findings provide valuable insights into the trade-offs between reconstruction accuracy and robustness. Ultimately, this study aims to guide the selection of optimal QSM reconstruction methods for pre-clinical applications, with potential implications for future clinical translation, particularly in the quantitative assessment of pathological changes in neurological and oncological diseases.

Abstract (Italian Version)

La suscettività magnetica (χ) è una proprietà fisica fondamentale che descrive come i materiali rispondono a un campo magnetico esterno.

La Risonanza Magnetica (MRI) è una tecnica non invasiva utilizzata per visualizzare la composizione e la microstruttura dei tessuti, inclusi i tessuti molli, sfruttando le differenze nelle proprietà magnetiche. Nella MRI, variazioni di suscettibilità tra tessuti inducono spostamenti di fase locali nel segnale, fornendo un contrasto aggiuntivo che può rivelare microvariazioni strutturali e compositive. Dal punto di vista clinico, la MRI svolge un ruolo cruciale nella diagnosi e nel monitoraggio di condizioni neurologiche, cardiovascolari e oncologiche, offrendo informazioni anatomiche e fisiologiche dettagliate.

La Quantitative Susceptibility Mapping (QSM) è una tecnica avanzata di post-elaborazione della MRI che ricostruisce mappe spaziali della suscettività magnetica dei tessuti a partire da misure di fase, risolvendo un problema inverso intrinsecamente mal posto. Questo approccio ha trovato applicazioni sempre più numerose nella ricerca biomedica per la rilevazione e la quantificazione di depositi di ferro, calcificazioni, processi di demielinizzazione e per la mappatura della distribuzione di mezzi di contrasto. Nonostante il suo potenziale, la ricostruzione QSM è sensibile al rumore, agli artefatti e agli effetti di orientamento, il che ha portato allo sviluppo di molteplici algoritmi volti a ottimizzare accuratezza e robustezza.

In questo lavoro, viene presentata un'analisi comparativa degli algoritmi QSM proposti da Wang et al., tutti implementati nella Sepia Toolbox, con l'obiettivo di valutarne le prestazioni relative e l'applicabilità. Gli algoritmi sono stati testati su un fantoccio in gel contenente nove differenti concentrazioni del mezzo di contrasto superparamagnetico VivoTrax, e su un set di acquisizioni ad alta risoluzione di cervelli di ratto, utilizzati come modelli pre-clinici. Le acquisizioni MRI sono state effettuate a 7 Tesla utilizzando una sequenza gradient-echo con parametri ottimizzati per il contrasto di fase. La qualità della ricostruzione è stata valutata in termini di linearità della suscettività rispetto alla concentrazione, amplificazione del rumore e soppressione degli artefatti.

I risultati preliminari indicano che alcuni algoritmi raggiungono una maggiore sensibilità a basse concentrazioni di contrasto (ad esempio, TKD), mentre altri mostrano una migliore mitigazione degli artefatti in geometrie complesse (per esempio, iL-SQR e MEDI). Questi risultati forniscono indicazioni utili sui compromessi tra accuratezza e robustezza della ricostruzione. In definitiva, questo studio mira a guidare la scelta dei metodi ottimali di ricostruzione QSM per applicazioni pre-cliniche, con potenziali implicazioni per una futura traduzione clinica, in particolare nella valutazione quantitativa delle alterazioni patologiche in malattie neurologiche e oncologiche.

Introduction

This thesis was developed as part of a broader research project focused on improving the imaging and therapeutic applications of extracellular vesicles (EVs) in neurodegenerative diseases. Specifically, it aimed to enhance the sensitivity and accuracy of EV tracking by exploiting advanced magnetic resonance imaging (MRI) techniques, with particular emphasis on Quantitative Susceptibility Mapping (QSM).

Extracellular vesicles are nanoscale particles secreted by cells, capable of transporting proteins, lipids and nucleic acids. They have emerged as promising candidates for both diagnostic and therapeutic applications. [1] In the context of neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS) and Multiple Sclerosis (MS), EVs may serve as vehicles for targeted drug delivery or as biomarkers reflecting pathological changes in the brain. Several neurodegenerative conditions are characterized by abnormal iron accumulation in specific brain regions, which may contribute to oxidative stress and disease progression. Detecting such changes can provide valuable diagnostic and prognostic insights. However, *in vivo* tracking of EVs remains a major challenge, primarily due to their small size and the lack of reliable non-invasive imaging tools.

MRI is widely used in neurological research due to its excellent spatial resolution and soft-tissue contrast. Among emerging MRI-based techniques, QSM has gained attention for its ability to quantify tissues and magnetic susceptibility, offering insight into the distribution of iron-containing compounds and magnetic tracers. Susceptibility alterations in brain tissues have been shown to correlate with pathological processes such as demyelination, gliosis, and abnormal iron deposition, commonly observed in neurodegenerative diseases. [2] This makes QSM a potentially powerful tool for detecting and quantifying EVs labeled with iron-based contrast agents.

Beyond neurology, QSM is actively being developed for applications in other body regions such as the breast, extremities, liver and kidney. These include studies of hemorrhage, metabolic oxygen consumption, mineral distribution and contrast agent kinetics. For example, QSM can detect calcifications in breast tissue and characterize vascular and fat structures in the liver. [3]

This work investigates the applicability of QSM in preclinical EV imaging, through both controlled phantom experiments and *ex vivo* murine brain studies. It also explores the biodistribution of EVs internalizing magnetic nanoparticles, enabling the identification of their accumulation sites within biological tissues. QSM is particu-

larly valuable in this context due to its high sensitivity, which allows the detection of extremely low susceptibility values, often undetectable with conventional imaging techniques.

A detailed evaluation of multiple QSM reconstruction pipelines was carried out to assess their influence on susceptibility quantification. The insights gained from the basis for a robust methodological framework for EV tracking in future studies. Preliminary quantitative results show that TKD achieves the highest sensitivity in detecting susceptibility variations at low contrast concentrations (down to 0.1 mg/mL), whereas iLSQR and MEDI provide more robust reconstructions, with lower RMSE and reduced streaking artifacts in complex geometries.

The experimental work was conducted with the support and collaboration of the Centro Grandi Strumenti (CGS) at the University of Pavia, which provided access to the imaging facilities for the phantom acquisitions, and Dr. Edoardo Micotti from the Istituto di Ricerche Farmacologiche Mario Negri that kindly supplying the mouse brain data.

Thesis Overview

- Chapter 1 provides a general overview of Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI), outlining the fundamental physical principles and their relevance in biomedical imaging.
- Chapter 2 focuses on Quantitative Susceptibility Mapping (QSM), beginning with a detailed explanation of magnetic susceptibility and its significance in MRI.
The chapter then provides a comprehensive overview of the various algorithms implemented in the SEPIA toolbox, covering each step of the QSM reconstruction pipeline including phase unwrapping, background field removal, and dipole inversion.
- Chapter 3 presents a detailed description of the phantom models and mouse acquisitions used in this study. It includes information on the experimental setups, acquisition protocols and the equipment utilized for MRI scanning.
- Chapter 4 summarizes the main findings of the study and discusses the conclusions drawn from the experimental results, highlighting their implications for future research and applications.
- Chapter 5 provides the final conclusions of the thesis, summarizing the overall achievements and discussing the limitations.

Chapter 1

Basic Principles of NMR and MRI

This chapter focuses on the Nuclear Magnetic Resonance (NMR) principles which constitute the basis of the Magnetic Resonance Imaging (MRI) technique.

MRI is a non-invasive technique used to generate detailed images of organs and tissues within the human body.

1.1 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is a physical phenomenon involving atomic nuclei which provides information on multiple scales. It represents a process of energy absorption and emission by nuclei with a magnetic dipole in the presence of an applied external magnetic field.

The angular frequency at which nuclei oscillate is the Larmor frequency ($\vec{\omega}_0$) and it is proportional to the magnetic field $\vec{B}_0$ in which the nuclei are immersed

$$\vec{\omega}_0 = \gamma \vec{B}_0 \quad (1)$$

where γ is the gyromagnetic ratio specific to each type of nucleus.

1.1.1 Nuclear Spin Interaction with a Magnetic Field

NMR phenomenon is based on the properties of the majority of atomic nuclei possessing an intrinsic angular momentum $\vec{I}$, also called spin. A nuclear magnetic moment is associated to each nucleus with spin

$$\vec{\mu} = \gamma \frac{h}{2\pi} \vec{I} \quad (1.1)$$

where $h = 6.62618 \cdot 10^{-34} J \cdot s$ is the Plank constant.

NMR can be observed only in atomic species with a non zero nuclear spin $\vec{I}$.

When a nuclear spin is immersed in a static magnetic field $\vec{B}_0$, it experiences the effect of a torque $\vec{N}$ and it begins to precess around the external magnetic field axis.

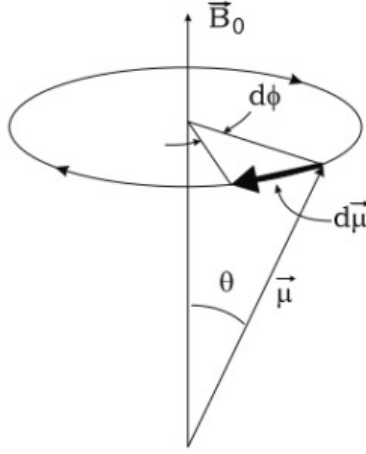


Figure 1.1: Clockwise precession of a nuclear spin around a static magnetic field $\vec{B}_0$. [4]

The spin vector precession is clockwise due to the magnetization vector attempt to align along the $\vec{B}_0$ direction in order to minimize the potential energy

$$U = -\vec{\mu} \cdot \vec{B}_0 = -\mu B_0 \cos(\theta) \quad (1.2)$$

where θ is the angle between $\vec{B}_0$ and $\vec{\mu}$.

The torque generated by the magnetic field is

$$\vec{N} = \vec{\mu} \wedge \vec{B}_0 \quad (1.3)$$

and it can be calculated as

$$\vec{N} = \frac{d\vec{I}}{dt} \quad (1.4)$$

By combining equations (1.1) and (1.4), the spin's motion equation can be written as

$$\frac{d\vec{\mu}}{dt} = \gamma \vec{\mu} \wedge \vec{B}_0 \quad (1.5)$$

The Cartesian representation of this precession, assuming B_0 aligned along the z axis, can be written as

$$\frac{d(\mu)_x}{dt} = \gamma B_0 (\mu)_y \quad (1.6)$$

$$\frac{d(\mu)_y}{dt} = -\gamma B_0 (\mu)_x \quad (1.7)$$

$$\frac{d(\mu)_z}{dt} = 0 \quad (1.8)$$

When a nucleus precessing in an (applied) static magnetic field $\vec{B}_0$ is exposed to an

electromagnetic radiation with a frequency ω_0 that matches its Larmor frequency, the resonance condition is satisfied. To generate the resonance condition, it is necessary the application of a radiofrequency (rf) pulse B_1 produced by a coil tuned at the Larmor frequency ($\omega_{rf} = \omega_L$).

1.1.2 Relaxation Times and Bloch Equations

Up to this point, it was considered the case of an isolated spin subjected to a combination of external magnetic fields. The NMR signal is generated by all nuclei in a system, so it is useful to introduce the concept of magnetization, defined as the sum of all the magnetic moments within a volume (V) divided by it

$$\vec{M}(r, t) = \frac{1}{V} \sum_{i=1}^N \vec{\mu}_i(r, t) \quad (1.9)$$

When the static magnetic field B_0 is applied, all $\vec{\mu}_i$ precess around the z axis. At equilibrium, the magnetization will be

$$M_x = 0 \quad (1.10)$$

$$M_y = 0 \quad (1.11)$$

$$M_z = M_0 \quad (1.12)$$

When the rf pulse B_1 is turned on, the total magnetization vector could be fully tilted on the (x-y) transverse plane. Once the rf pulse is turned off, spins realign along the B_0 axis emitting their excess of energy. Spins can interact both with the surrounding lattice (which represents the environment) and among themselves, generating two different relaxation processes.

Phenomenologically, Bloch supposed that the continuous presence of $\vec{B}_0$ is responsible for the M_z relaxation to its equilibrium value, with a constant rate proportional to $M_0 - M_z$

$$\frac{dM_z}{dt} \propto \frac{1}{T_1} (M_0 - M_z) \quad (1.13)$$

where T_1 is called longitudinal relaxation time and it describes the time needed by the spins to realign along the longitudinal z-axis. Moreover, it is also called spin-lattice relaxation time because it refers to the time it takes for the spins to give back to the lattice the energy obtained by the rf pulse, in order to reach the equilibrium. The complete motion equation for the M_z component is

$$\frac{dM_z}{dt} = \gamma(M \wedge B_0)_z + \frac{M_0 - M_z}{T_1} \quad (1.14)$$

The M_{xy} decay, similarly to (1.14), can be written as

$$\frac{dM_{xy}(t)}{dt} = \gamma(M \wedge B_0)_{xy} - \frac{M_{xy}}{T_2} \quad (1.15)$$

where T_2 is called transverse relaxation time and it describes the rapidity to M_{xy} to return to zero. It characterizes all the non-equilibrium processes due to the local randomly fluctuating field variation generated (most times) by the neighboring nuclei, so that T_2 is also called spin-spin relaxation time, and it is shorter than T_1 ($T_2 < T_1$).

The $\vec{B}_0$ inhomogeneities contribute to an additional dephasing of the magnetization; if T_2' defines the decay time due to the inhomogeneity of B_0 , then M_{xy} decays at zero with a time constant T_2^*

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \quad (1.16)$$

where $T_2^* > T_2$ is always valid.

Under resonance condition and after an applied $\pi/2$ -pulse, Bloch's equations become

$$M_z(t) = M_0(1 - e^{-\frac{t}{T_1}}) \quad (1.17)$$

$$M_{xy}(t) = M_0 e^{-\frac{t}{T_2}} \quad (1.18)$$

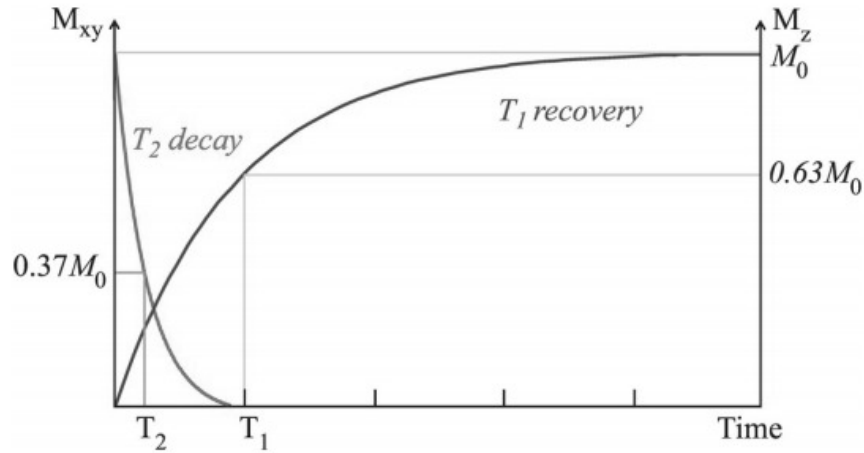


Figure 1.2: Recovery of the longitudinal magnetization M_z and decay of the transverse magnetization M_{xy} (in the co-rotating frame) after the application of a rf pulse.

[5]

1.1.3 NMR Experiment

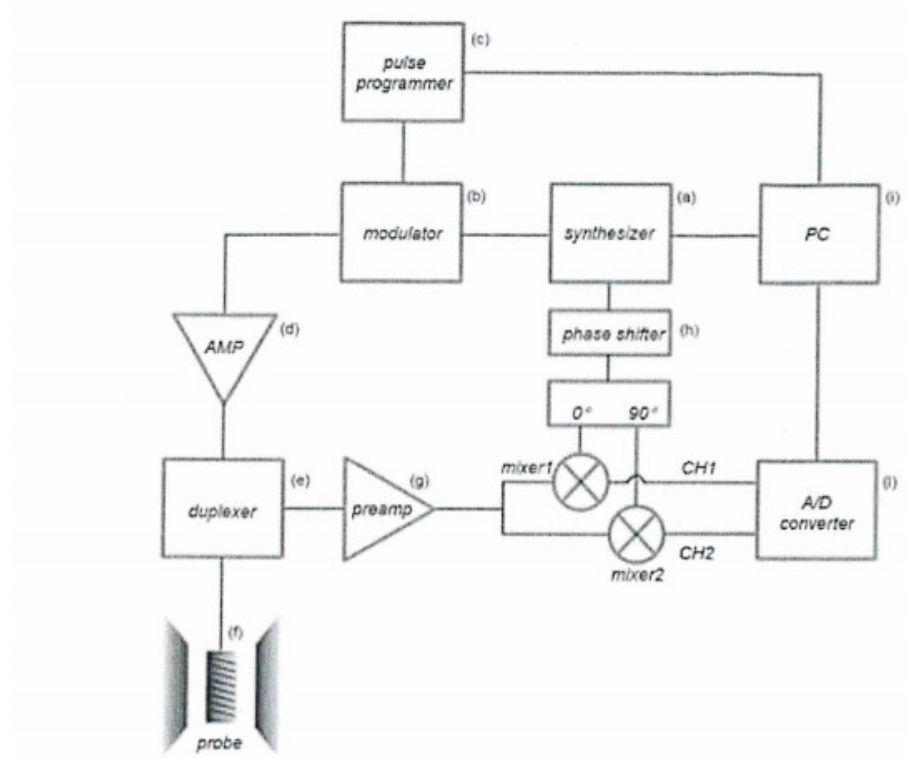


Figure 1.3: Scheme of the components of a Nuclear Magnetic Resonance (NMR) Experiment.

The NMR experiment purpose is to measure the relaxation times for the evaluation of the magnetization recovery. To realize an NMR experiment, it is required a static magnetic field and a transmit coil to generate the radiofrequency (rf) pulse.

The sample is located inside the coil that is in resonance condition at the Larmor frequency. The static magnetic field B_0 is generated by an electromagnet and the magnetization precesses around the B_0 direction. To have the magnetization rotation in the transverse plane, the rf pulse B_1 is applied perpendicular to the B_0 direction for a short interval of time.

Once the radiofrequency pulse is set off, the longitudinal component of the magnetization returns at equilibrium in a time interval T_1 while the transverse component of the magnetization relaxes at zero in a time interval T_2 . At this point, the synthesizer receives the NMR signal produced by the sample and it generates the rf pulses at the correct resonance frequency.

Then, the signal goes through the modulator where it is split in four different channels dephased by 0 , $\pi/2$, π and $3\pi/2$ respectively.

The pulse generator, that controls the time duration of each signal, is connected to an external amplifier which is connected to a duplexer. The duplexer is a device that allows the signal split between sender and receiver.

The output signal is a Free Induction Decay (FID).

1.1.4 NMR Signal

During an NMR experiment, when nuclei are excited, it is possible to detect an NMR signal.

When the magnetization changes direction to return along the z-axis (precession of $\vec{M}$), it is induced an alternated electromotive force (emf) in the coil.

The signal equation (as described by the Faraday's law of induction) is

$$S(t) = emf = -\frac{d\Phi_M(t)}{dt} \quad (1.19)$$

where Φ_M is the magnetic flux of a magnetic induction B through the coil.

Using the Stoke's theorem, it is possible to rewrite the flux as

$$\Phi_M(t) = \int_{sample} d^3r \vec{B}^{received}(\vec{r}) \cdot \vec{M}(\vec{r}, t) \quad (1.20)$$

where $\vec{B}^{received}$ is the magnetic field per unit current that would be produced by the coil at the point $\vec{r}$.

Combining (1.19) and (1.20), the signal (usually few μV) can be written as

$$s(t) \propto \omega_0 e^{-\frac{t}{T_2}} \int d^3r M_{\perp}(\vec{r}, t) B_{\perp}(\vec{r}) e^{i((\Omega - \omega(\vec{r}))t + \phi_0(\vec{r}) - \theta_B(\vec{r}))} \quad (1.21)$$

where the receive field components are written in terms of the magnitude $B_{\perp}$.

The signal measured in the rotating frame is called demodulated signal and it is defined by the reference frequency $\Omega = \omega_0 + \delta\omega$, where $\delta\omega$ is the offset frequency. The original signal is demodulated into two components, one close to zero frequency and the other nearly twice the Larmor frequency.

The NMR signal, collected after a single $\pi/2$ - pulse, is also called Free Induction Decay (FID) and it can be written as

$$s(t) \propto \omega_0 \Lambda B_{\perp} \int d^3r M_{\perp}(\vec{r}, 0) e^{i(\Omega t + \phi(\vec{r}, t))} \quad (1.22)$$

where the demodulating frequency is $\Omega = \omega_0$, $B_{\perp}$ has been considered constant, $e^{-\frac{t}{T_2}} \rightarrow 1$ and Λ is a constant which includes the gain factors from the electronic detection system.

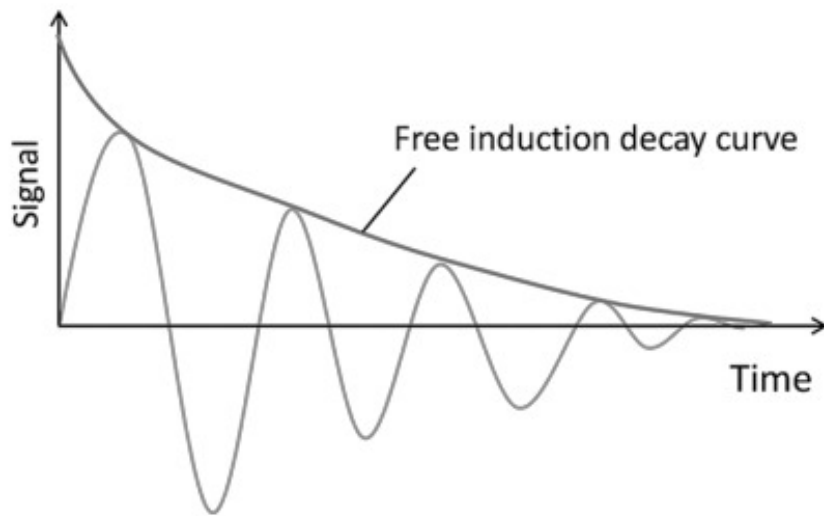


Figure 1.4: Free Induction Decay (FID) Curve. The y-axis represents the signal amplitude, reflecting the transverse magnetization immediately following the excitation pulse, while the x-axis denotes time elapsed in milliseconds.

The envelope of the signal in the laboratory frame decays exponentially with T_2^* .

1.1.5 NMR Sequences

Specific pulse sequences are used to excite the nuclear spins and to acquire signals that reflect the spin dynamics. Here below, the fundamental NMR sequences designed to measure the relaxation times T_1 and T_2 .

Spin Echo Sequence

The Spin Echo (SE) Sequence has been formulated to sample the signal due to the spin-spin interaction, allowing the T_2 measurement.

This sequence is composed by two pulses: a $\pi/2$ -pulse along the x'-axis and a π -pulse along the y'-axis. These pulses are divided by an interval time τ , called echo time. The spins rephase creating the echo signal at the echo time $TE = 2\tau$.

When in presence of diffusing nuclei, the transverse magnetization signal can be modeled considering also the diffusion effect

$$M_{xy} = M_0 e^{-\frac{2\tau}{T_2}} e^{-\frac{\gamma^2 G^2 D (2\tau^3)}{3}} \quad (1.23)$$

where D is the diffusion coefficient, G is the magnetic field gradient and γ is proton gyromagnetic ratio.

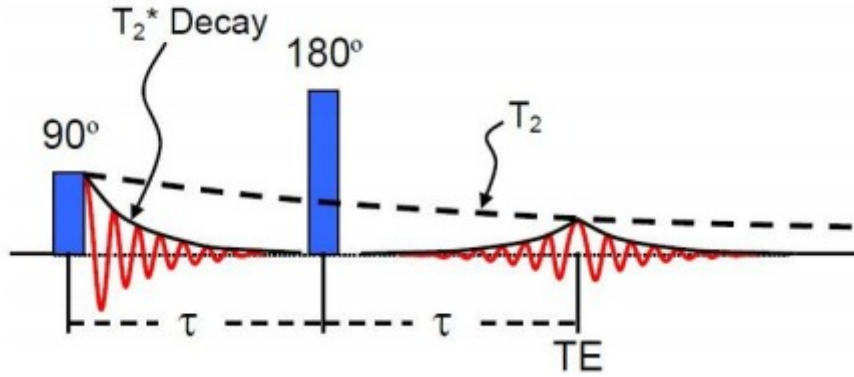


Figure 1.5: Schematic of a spin echo sequence showing signal evolution and relaxation effects. After an initial 90° excitation pulse, the transverse magnetization decays rapidly due to T_2^* relaxation and field inhomogeneities. A subsequent 180° refocusing pulse, applied at time τ , inverts the dephased spins and generates a spin echo at the echo time ($TE = 2\tau$).

Carr-Purcell-Meiboom-Gill (CPMG) Sequence

Considering the diffusion particularly important in non-solid samples (liquids, gels, gases, ...) affecting the SE signal, the most common method to suppress it is using the Carr-Purcell-Meiboom-Gill (CPMG) sequence. In CPMG, a series of π -pulses is applied to prevent the phase accumulation due to spin diffusion.

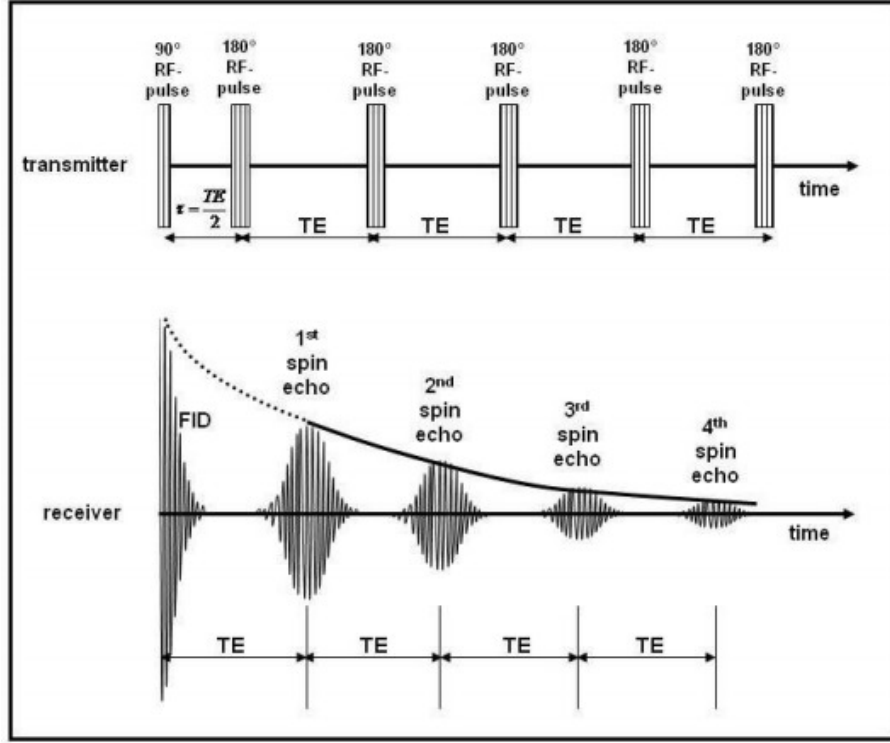


Figure 1.6: Carr-Purcell-Meiboom-Gill (CPMG) Sequence scheme. The top diagram shows the transmitter activity with an initial 90° RF pulse followed by a train of 180° refocusing pulses spaced by the echo time (TE). The bottom plot illustrates the corresponding received signal: the initial free induction decay (FID) is followed by a series of spin echoes, each formed at integer multiples of TE. [6]

In this case, the M_{xy} signal can be written as

$$M_{xy} = M_0 e^{-\frac{2\tau}{T_2}} e^{-\frac{\gamma^2 G^2 D (2\tau^2) t}{12n^2}} \quad (1.24)$$

where t is the time at which the signal is read and n is the number of the π -pulses applied.

Saturation Recovery Sequence

For the spin-lattice relaxation time (T_1) measurement, the most used sequence is the Saturation Recovery (SR). It consists of two $\pi/2$ -pulses that null the longitudinal component of M . After those pulses, the signal can be detected by means of FID or SE readout.

The reconstruction of the longitudinal magnetization relaxation curve is sampled at different times after the initial $\pi/2$ -pulses.

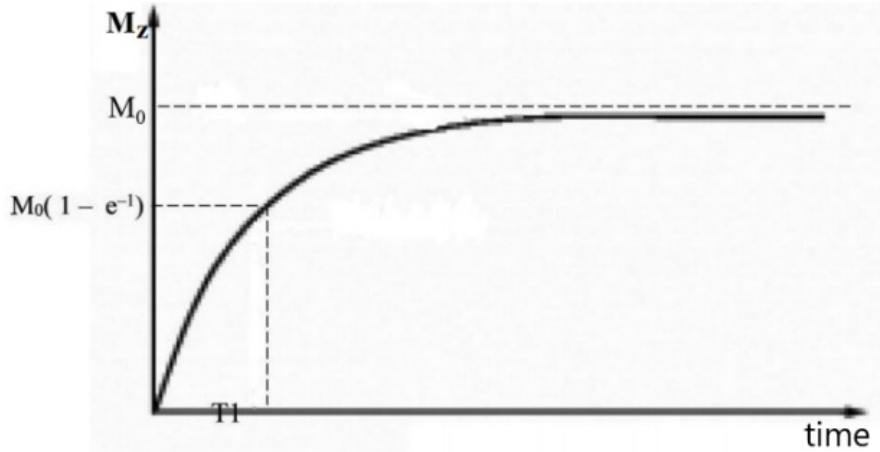


Figure 1.7: Saturation Recovery signal curve showing the longitudinal magnetization (M_z) as a function of time after a saturation pulse. The y-axis represents the recovery of the magnetization along the z-axis, indicating how the spins realign with the static magnetic field over time. The x-axis corresponds to the recovery time interval, typically measured in milliseconds.

Inversion Recovery Sequence

Another method to study the spin-lattice relaxation time is the Inversion Recovery (IR) Sequence.

This sequence starts with a π -pulse that reverses the longitudinal component of the magnetization from $+M_0$ to $-M_0$. Then, to read the signal, it is necessary to use a $\pi/2$ -pulse to put the magnetization along the xy-plane.

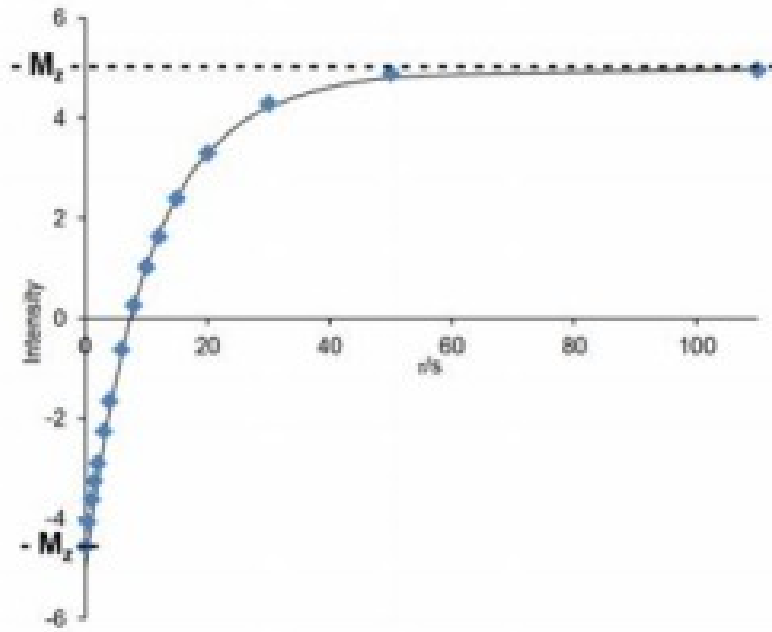


Figure 1.8: Inversion Recovery curve depicting the longitudinal magnetization (M_z) as a function of the inversion time (τ), divided by seconds on the x-axis. The y-axis shows the intensity of the magnetization, which initially inverts following a π pulse and then recovers towards equilibrium over time.

1.2 Magnetic Resonance Imaging (MRI)

The Magnetic Resonance Imaging (MRI) is a non-invasive technique that allows the detection of the spatial distribution of nuclei thanks to the presence of magnetic field gradients added to the static magnetic field.

1.2.1 Fundamentals of NMR and the Development of MRI

Further potentiality of the MRI in comparison to the NMR is the spatial localization of protons inside the sample and the conversion of the relaxation times in an image characterized by a gray scale depending on the T_1/T_2 values of protons in different positions and also on the proton density.

To generate an image of the internal structure of the body, it is necessary to develop a method to spatially differentiate the NMR signal. In the presence of a magnetic field with a gradient overlapped to the static magnetic field, the resonance frequency becomes site-dependent allowing the identification of the various position of nuclei. In MRI, the signal is collected from the entire excited volume and the spatial information is decoded to build the image.

1.2.2 Spatial Localization Principle and MRI Signal

The spatial localization principle is based on the Larmor equation

$$\omega(\vec{r}) = \gamma \vec{B}_0(\vec{r}) \quad (1.25)$$

To vary $\vec{B}_0$ in each voxel, linear magnetic field gradients along the three directions are used

$$G_x = \frac{\partial B_0}{\partial x} \quad (1.26)$$

$$G_y = \frac{\partial B_0}{\partial y} \quad (1.27)$$

$$G_z = \frac{\partial B_0}{\partial z} \quad (1.28)$$

After the application of a gradient, the magnetic field becomes

$$\vec{B}(\vec{r}, t) = \vec{B}_0(\vec{r}) + \vec{G}(t)\vec{r} \quad (1.29)$$

leading to a variation in the angular frequency of the spins that can be calculated as

$$\omega(\vec{r}, t) = \omega_0 + \omega_G(\vec{r}, t) \quad (1.30)$$

where the deviation from the Larmor Frequency is linear in both $\vec{r}$ and $\vec{G}$

$$\omega_G(\vec{r}, t) = \gamma \vec{G}(t)\vec{r} \quad (1.31)$$

In the 1D case, the MRI signal can be written as

$$s(t) = \int dz \rho(z) e^{i(\Omega t + \phi(z, t))} \quad (1.32)$$

The accumulated phase is

$$\phi_G(z, t) = - \int_0^t dt' \omega_G(z, t') = -\gamma z \int_0^t dt' G(t') \quad (1.33)$$

Assuming no offset ($\Omega = \omega_0$), the equation (1.32) becomes

$$s(t) = \int dz \rho(z) e^{i\phi_G(z, t)} \quad (1.34)$$

The signal reconstruction, voxel by voxel, is made through the spatial frequency k defined as

$$k(t) \equiv \frac{\gamma}{2\pi} \int_0^t dt' G(t') \quad (1.35)$$

For a constant gradient field, it becomes

$$k(t) = \frac{\gamma}{2\pi} Gt \quad (1.36)$$

Replacing (1.36) in the accumulated phase equation (1.33), it is obtained

$$\phi_G(z, t) = -2\pi z k(t) \quad (1.37)$$

The 1D imaging equation, in the k -space, is the Fourier Transform (FT) of the sample spin density

$$s(k) = \int dz \rho(z) e^{-2\pi i k z} \quad (1.38)$$

The effective sample spin density, instead, can be seen as the inverse FT of the signal

$$\rho(z) = \int dk s(k) e^{+2\pi i k z} \quad (1.39)$$

The signal $s(k)$ and the image $\rho(z)$ are a Fourier transform pair.

1.2.3 k-space Coverage

The k -space coverage is done in terms of image space (z) and data space (k) because of their relationship through the Fourier Transform. $\rho(z)$ can be reconstructed from $s(k)$ if the latter is collected over a sufficient set of k values: the integration in (1.39) requires the k -space coverage. k -space is an array of numbers that represent the spatial frequencies in a MR image, where k -space cells are shown on a rectangular grid with k_x and k_y as principal axes.

To collect a uniform distribution of points in the k -space, it is enough to sample the signal at a constant rate in the presence of a constant gradient G . The sampling of both positive and negative values of k can be obtained by changing the sign of G .

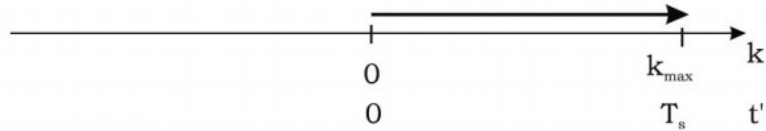


Figure 1.9: One-dimensional k -space coverage plotted along the x -axis, where both the spatial frequency variable k and the corresponding acquisition time are indicated. [7]

In the case of a 2D representation, for a planar object or for a 3D object thin slice, only k_x and k_y values need to be sampled.

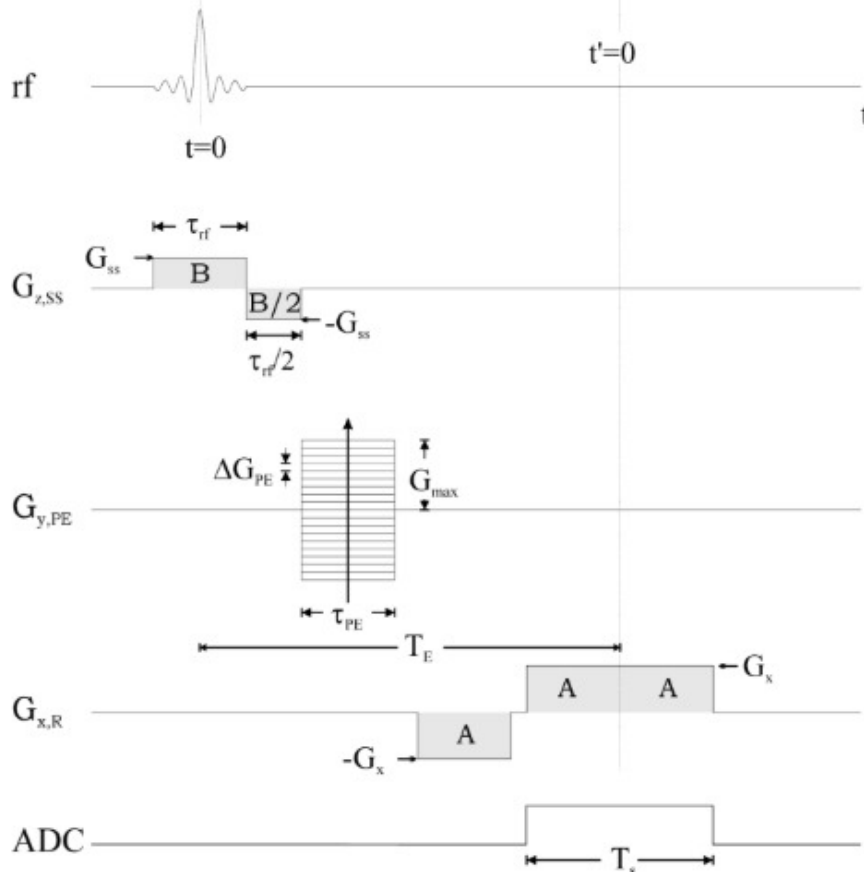


Figure 1.10: Diagram of a 2D Gradient Echo pulse sequence, showing the timing and coordination between the RF excitation pulse, slice selection gradient G_{SS} , phase encoding gradient G_{PE} , and frequency encoding (readout) gradient G_R . The sequence includes gradient rewinding and prephasing lobes to ensure proper echo formation at the echo time TE , followed by data acquisition (ADC). This scheme illustrates the interplay between gradient axes to achieve spatial encoding in both frequency and phase dimensions for image reconstruction. [8]

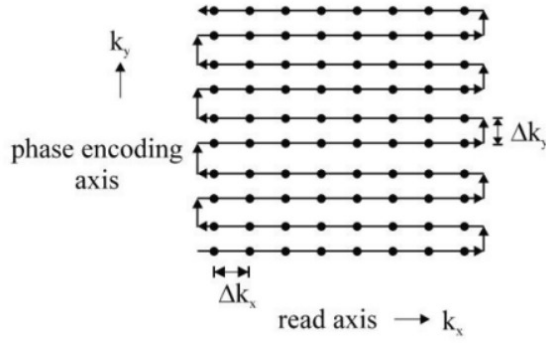


Figure 1.11: Representation of a 2D k-space sampling trajectory. The horizontal axis k_x corresponds to the readout (frequency encoding) direction, while the vertical axis k_y corresponds to the phase encoding direction. Each horizontal line represents a single acquisition with a specific phase encoding value, and the sequence of lines fills k-space line-by-line with spacing Δk_y .

The sequence scheme shown in figure (1.10) uses one single rf pulse to recover the 2D k-space, with a discrete set of parallel lines sampled as shown in figure (1.11). The standard 2D k-space coverage consists of alternatively turning G_x and G_y : the vertical spacing is obtained by the step size

$$\Delta k_y = \frac{\gamma}{2\pi} G_y \tau_y \quad (1.40)$$

while the horizontal spacing is obtained by the step size

$$\Delta k_x = \frac{\gamma}{2\pi} G_x \Delta t \quad (1.41)$$

The data are not collected during the short vertical step. The total time needed for a 2D imaging is

$$T_{acq} = N_y T_R \quad (1.42)$$

where N_y is the number of discrete points sampled and T_R is the repetition time.

The 2D k-space coverage can be generalized in the 3D dimension considering a series of planes leading to a three-dimensional set of parallel lines: the set can be obtained by sampling both in k_y and k_z directions. The imaging obtained by the use of two phase encoding gradients perpendicular to the read direction is called 3D imaging.

The read direction is always defined by G_x , while G_y and G_z are the phase encoding gradients. G_y and G_z are switched off during the read sampling.

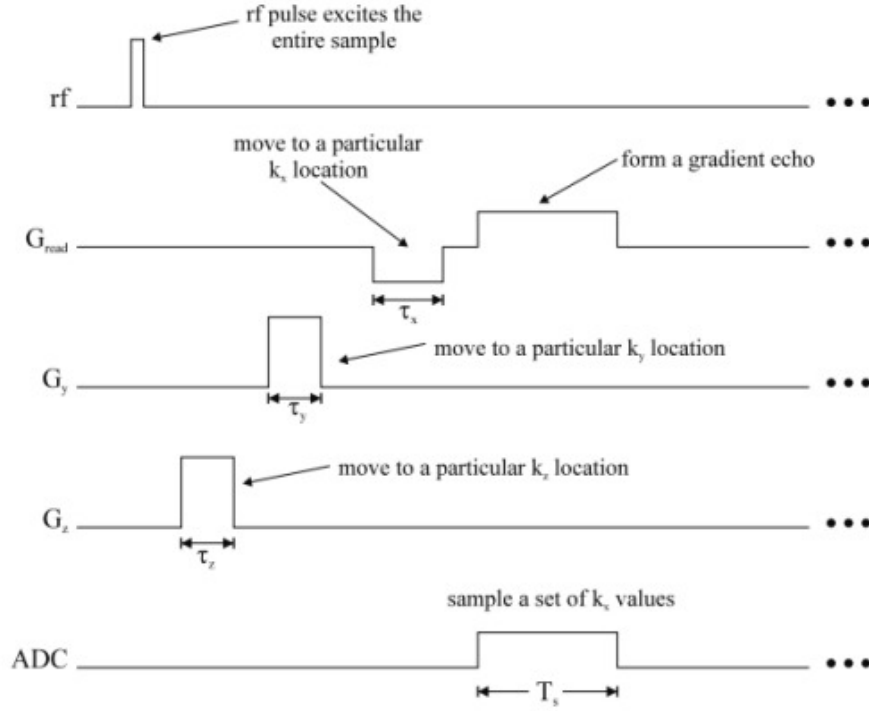


Figure 1.12: 3D Gradient Echo Pulse Sequence scheme. The top trace represents the radiofrequency pulse that excites the entire sample, initiating the acquisition process. The subsequent traces show the gradient waveforms applied along different spatial directions: readout gradient G_{read} , phase-encoding gradient G_y and slice-selection gradient G_z . The readout gradient waveform is used both to move to a specific k-space location and to form the gradient echo, while the phase-encoding gradients incrementally change the k-space position between acquisitions. τ indicates the time delay to move to the desired k-space location, and T_S corresponds to the sampling period during which a set of k-space values is acquired by the ADC.

The corresponding shift in the z-direction is

$$\Delta k_z = \frac{\gamma}{2\pi} G_z \tau_z \quad (1.43)$$

The total time needed for a 3D imaging is

$$T_{acq} = N_y N_z T_R \quad (1.44)$$

MR images are produced by exciting a single thin slice of the body by using a combination of gradient fields and spatially selective rf pulses.

1.2.4 Image Contrast

For a SE sequence, the signal as a function of the adjustable parameters in the MRI sequences and of the NMR time constants can be written as

$$s(t) = k\rho_0(1 - e^{-\frac{T_R}{T_1}})e^{-\frac{T_E}{T_2}} \quad (1.45)$$

where k is the system electronic factor and ρ_0 is the nuclei density.

T_E and T_R are, respectively, the echo time and the repetition time. These latter are the operator adjustable parameters. The image contrast is given by the differences among T_1 , T_2 and ρ of the tissues.

There are three type of contrasts, which correspond to different values of the acquisition parameters (TE , TR):

Type of contrast	T_R	T_E
T_1 -weighted	$\leq T_1$	$\ll T_2$
ρ_0 -weighted	$\gg T_1$	$\ll T_2$
T_2 -weighted	$\gg T_1$	$\geq T_2$

Table 1.1: Type of contrast and corresponding T_R and T_E values for MRI acquisition.

- **T_1 -Weighting**

An image whose contrast is mainly due to the T_1 s differences among tissues is called T_1 -weighted image.

For this type of contrast, T_2 effects need to be minimized

$$T_E \ll T_2 \implies e^{-T_E/T_2} \rightarrow 1 \quad (1.46)$$

while T_R is chosen $\leq T_1$.

The signal equation becomes

$$s(t) \propto \rho_0(1 - e^{-\frac{T_R}{T_1}}) \quad (1.47)$$

- **ρ_0 -Weighting**

In order to obtain an image contrast based on the spin density, it is necessary to minimize the MR image dependence on both T_1 and T_2

$$T_E \ll T_2 \implies e^{-T_E/T_2} \rightarrow 1 \quad (1.48)$$

$$T_R \gg T_1 \implies e^{-T_R/T_1} \rightarrow 0 \quad (1.49)$$

giving an MRI signal determined by the spin density

$$s(t) \propto \rho_0 \quad (1.50)$$

- **T_2 -Weighting**

The last type of contrast is based on the differences in the T_2 relaxation time. This type of contrast is fundamental when there are local magnetic field susceptibility differences between tissues.

As for the previous contrasts, in this case it is necessary to minimize the T_1 contribute

$$T_R \gg T_1 \implies e^{-T_R/T_1} \rightarrow 0 \quad (1.51)$$

in order to have a signal only dependent on T_2

$$s(t) \propto \rho_0 e^{-\frac{T_E}{T_2}} \quad (1.52)$$

where $TE \leq T_2$.

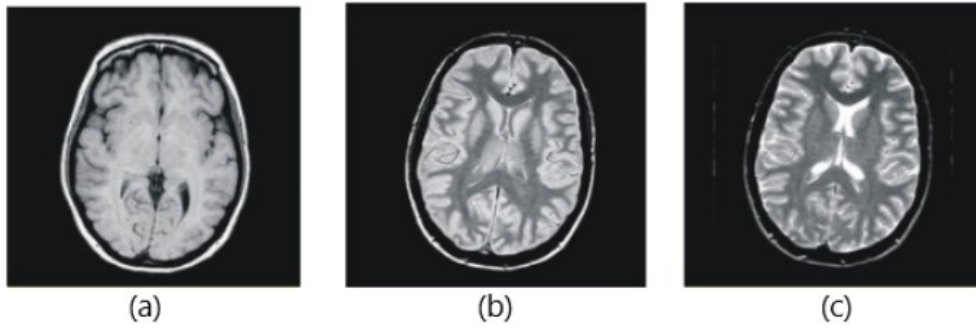


Figure 1.13: Different types of contrasts: (a) T_1 -weighted, (b) ρ_0 -weighted and (c) T_2 -weighted MRI brain images.

1.2.5 MRI Sequences

MRI sequences represent different acquisition protocols useful in medical diagnostics to visualize inside the human body. These sequences are characterized by different adjustable parameters (timing, radiofrequency pulses and gradients) to create different type of contrast obtaining different information.

- **Gradient Echo Sequence**

Gradient echo (GRE) sequences are widely used in MRI techniques thanks to the fast imaging acquisition. This sequence starts with a negative gradient lobe along the z-axis. The reconstruction of the echo signal is possible by changing the polarity of G.

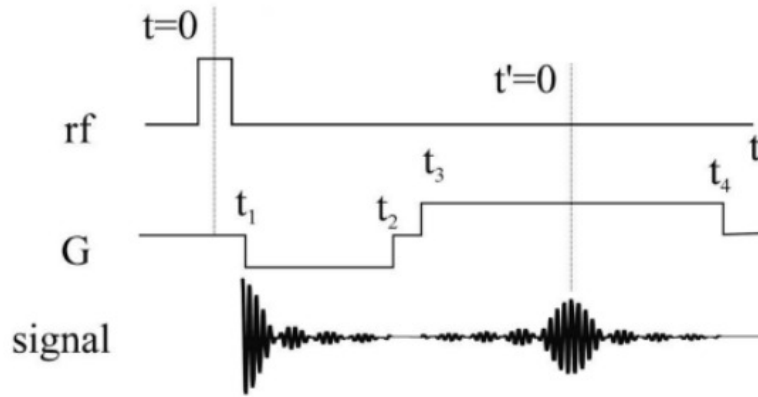


Figure 1.14: Gradient Echo (GRE) Sequence diagram. The signal trace at the bottom represents the gradient echo formed during readout.

The first lobe is the phase encoding gradient, and it is applied in time interval (t_1, t_2) sampling discrete points from 0 to $-k_{max}$. Once the negative gradient lobe is turned off, it is applied the read gradient with the same intensity but for an interval time (t_3, t_4) which is the double of (t_1, t_2) . This allows the sampling in the interval $(-k_{max}, +k_{max})$.

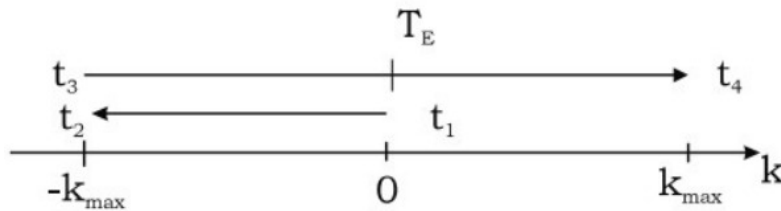


Figure 1.15: Gradient Echo (GRE) Sequence k-space coverage. This schematic representation links the timing diagram in Figure 1.14 to the spatial frequency domain coverage.

- **Echo Planar Imaging Sequence**

The Echo Planar Imaging (EPI) sequence is part of the GRE sequences and it allows a fast image acquisition. In this case, it is sampled the whole slice in a single T_R . This sequence is characterized by a set of echoes after a $\pi/2$ -pulse with a short phase encoding gradient between each echo.

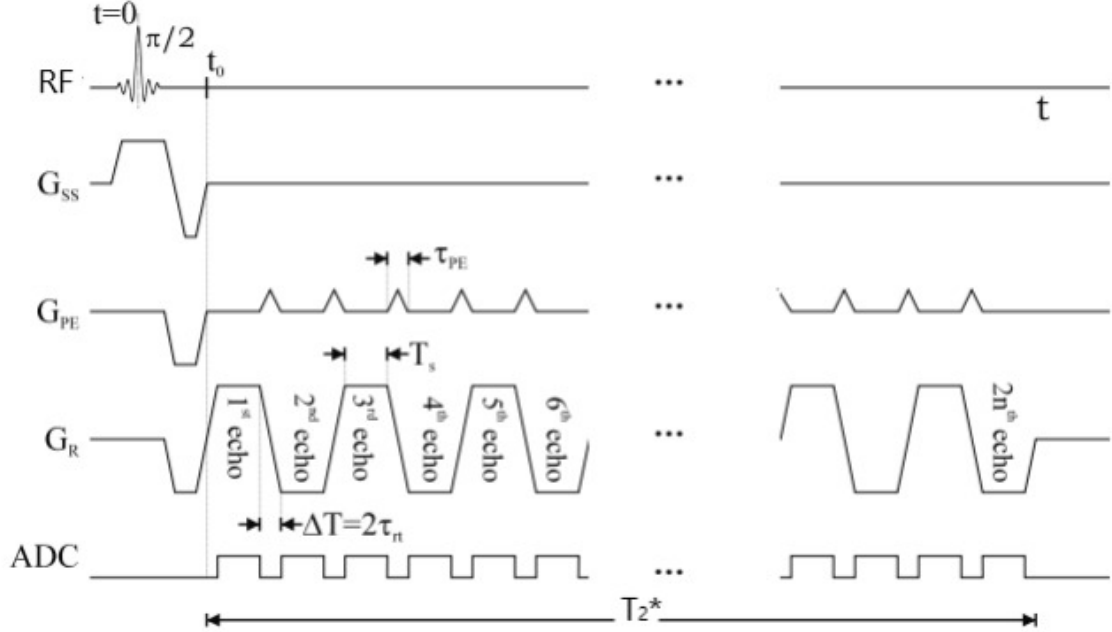


Figure 1.16: Echo Planar Imaging Sequence diagram. This fast imaging technique starts with a $\pi/2$ RF pulse applied at $t=0$ to excite the sample, followed by a series of oscillating readout gradients G_R and blipped phase-encoding gradients G_{PE} , combined with a constant slice-selection gradient G_{SS} for spatial localization. The acquisition window (ADC) captures the MR signal during each gradient lobe, enabling rapid traversal of k-space in a zig-zag pattern within a single shot. [4]

With this type of sequence, it is possible to acquire 15/30 images/sec.

- **Spin Echo Imaging Sequence**

The Spin Echo (SE) sequence allows the image reconstruction by using only positive gradients. The difference with respect a GRE sequence is the application of a π -pulse to refocus the magnetization.

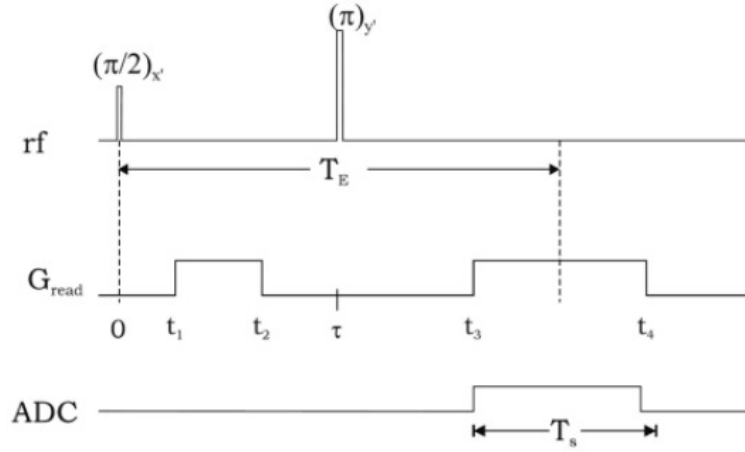


Figure 1.17: Spin Echo Sequence diagram (1° method). The sequence begins with a $\pi/2$ RF pulse to tip the magnetization into the transverse plane, followed by a phase accumulation period determined by the applied readout gradient. At time $T_E/2$, a π -pulse is applied to reverse the phase dispersion caused by magnetic field inhomogeneities, leading to the formation of a spin echo at echo time T_E . The readout gradient G_{read} is applied during the echo to sample k-space, with acquisition occurring over the interval T_s .

In the figure (1.17), it is shown the first method to do the SE sequence. In this case, the π -pulse is applied in between the two gradients to change the first gradient sign producing the same accumulated phase cancellation. The second method, instead, requires a bipolar gradient structure

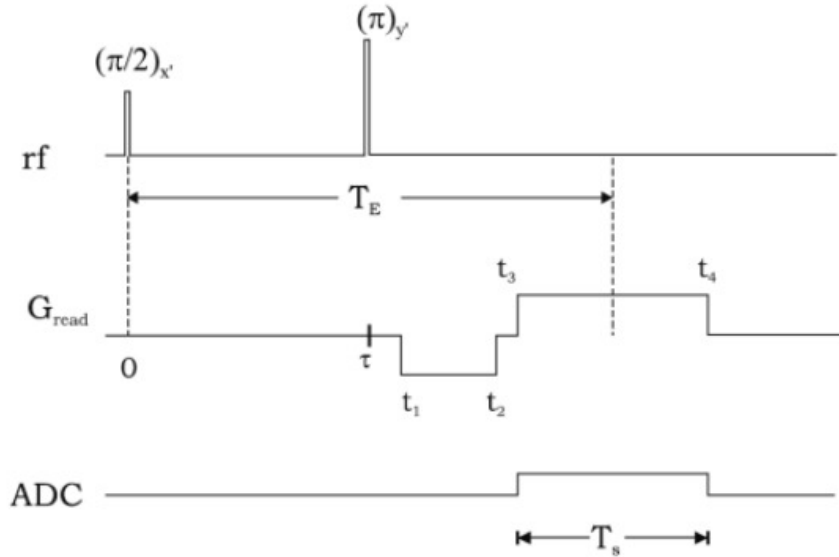


Figure 1.18: Spin Echo Sequence diagram (2° method). In this method, the π -pulse is applied before the two readout gradient lobes of opposite polarity, which are used to refocus the signal and form the spin echo at echo time T_E .

The polarity inversion of the readout gradients before and after the refocusing pulse helps to reduce motion-induced phase errors, improving image quality.

- **Spin Echo Multi Slice Sequence**

The Spin Echo Multi Slice (SEMS) sequence allows the acquisition of multiple slices in the same T_R (through a series of radiofrequency pulses) where all these slices are centered at different resonance frequencies.

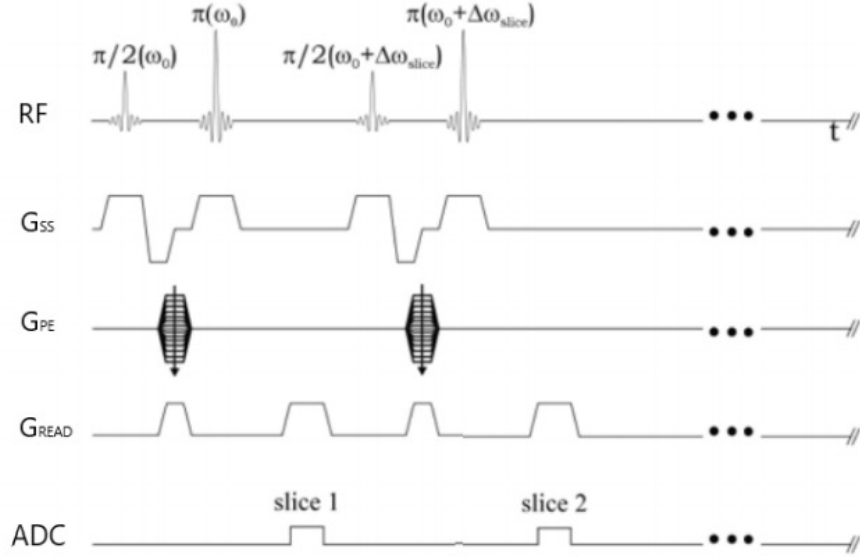


Figure 1.19: Spin Echo Multi Slice Sequence diagram. This method enables the acquisition of multiple slices within the same repetition time T_R by applying a series of RF excitation pulses, each tuned to a different resonance frequency corresponding to a specific slice position. Each slice undergoes its own spin echo formation through a $\pi/2$ excitation pulse, a π refocusing pulse, and the appropriate gradient combinations: slice selection G_{SS} , phase encoding G_{PE} and frequency encoding G_{read} . The ADC acquires data from each slice sequentially, while the gradient waveforms are synchronized to maintain spatial encoding.

To improve the signal to noise ratio (SNR), it is possible to repeat this sequence after a time T_R .

Chapter 2

Quantitative Susceptibility Mapping (QSM)

Quantitative susceptibility mapping (QSM) is an MRI technique that non-invasively measures magnetic susceptibility in vivo. QSM entails the solution of an ill-conditioned inverse problem that relates the acquired gradient echo (GRE) phase information to the susceptibility distribution, that is the cause of the local magnetic field inhomogeneities. [9]

This chapter aims to describe the susceptibility as a physical quantity and to introduce the QSM method along with its main applications.

2.1 Magnetic Susceptibility

The magnetic susceptibility χ is a physical quantity that allows to describe how materials behave when magnetized by an applied magnetic field. If the relationship between the magnetization $\vec{M}$ and the applied field $\vec{H}$ is linear, it is possible to write the magnetization as

$$\vec{M} = \overleftrightarrow{\chi} \cdot \vec{H} \quad (2.1)$$

When the material is homogeneous and isotropic, its magnetic response is uniform: χ has an isotropic behavior and it can be modeled as a scalar quantity. If the material is not homogeneous, the response varies with the field orientation: χ shows an anisotropic behavior and it is modeled as a tensor $\overleftrightarrow{\chi} = \overleftrightarrow{\chi}(\vec{H})$. $\vec{M}$ and $\vec{H}$ have the same physical dimensions, while χ is dimensionless.

The relationship between the magnetic susceptibility and the magnetic induction $\vec{B}$ is given by

$$\vec{B} = \mu_0(\vec{H} + \vec{M}) \quad (2.2)$$

where $\mu_0 = 4\pi \cdot 10^{-7} \text{ H/m}$ is the vacuum permeability constant.

Most materials exhibit zero magnetization ($\vec{M} = 0$) in absence of an external field and, consequently, $\vec{B} = 0$. When $\vec{B} \neq 0$, also $\vec{M} \neq 0$. In this case, some magnetization parameters (such as the intensity, the versus and the direction) are related to

the magnetic susceptibility and to the magnetic permeability μ

$$\vec{B} = \mu_0(\vec{H} + \chi\vec{H}) = \mu_0(1 + \chi)\vec{H} = \mu_r\mu_0\vec{H} \quad (2.3)$$

where μ_r is the relative permeability specific for each material.

All materials show some magnetic properties depending on their composition and state. According to these properties, materials can be classified into: diamagnetic ($\chi < 0$), paramagnetic ($\chi > 0$) and ferromagnetic ($\chi \gg 0$).

Diamagnetic materials, when placed in an external field, produce a weak induced magnetic field oriented in the opposite direction due to their characteristic of no unpaired orbital electrons. This leads to a small and negative magnetic susceptibility. Paramagnetic and ferromagnetic materials have unpaired orbital electrons: if no external field is applied, the magnetic moments of the particle are directed randomly. When an external field is applied, the material is magnetized. For paramagnetic materials, χ assumes small and positive value.

Ferromagnetic materials, instead, are characterized by a total magnetic moment: they became permanently magnetized even when the external field has been turned off.

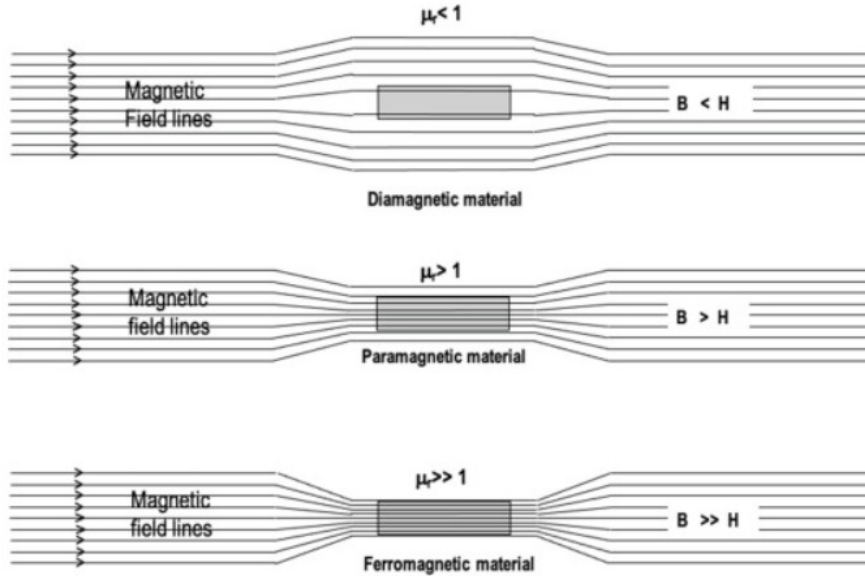


Figure 2.1: Magnetic Susceptibility Behavior for Different Materials. The diagrams illustrate how magnetic field lines interact with a sample depending on its magnetic susceptibility μ and magnetic response. [10]

Magnetic susceptibility is the key physical parameter quantified in QSM, allowing the generation of informative images that reveal internal structural and compositional details of materials or biological tissues.

2.2 Magnetic Susceptibility in Biological Tissues

In biological tissues, susceptibility values are typically very low, ranging between 10^{-6} and 10^{-5} , and are measured in parts per million (ppm).

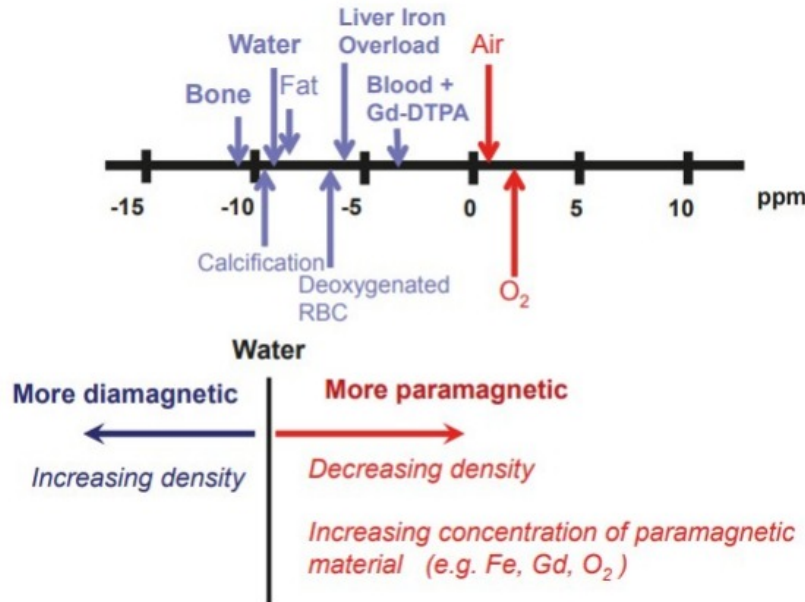


Figure 2.2: Magnetic susceptibility values for various tissues and substances in the human body, expressed in parts per million (ppm) relative to water.

Most biological tissues are weakly diamagnetic. [11]

Several factors can alter the magnetic susceptibility of biological tissues.

- Iron accumulation, as seen in conditions like neurodegeneration or microbleeds, can increase tissue susceptibility, making it more paramagnetic. [12]
There are around 3–5 g of iron in the human body (67% hemoglobin, 25% ferritin, 5% myoglobin, 2% intracellular iron, < 1% transferrin, enzymes, etc). The paramagnetic susceptibility values in the human brain is closely due to iron deposition. Iron is known to contribute to the synthesis of myelin, DNA, and neurotransmitters. Changes in iron homeostasis are related to the pathogenesis of several neurodegenerative diseases.
- Hemoglobin is a metalloprotein containing four iron atoms and is involved in the oxygen transport. The magnetic properties of blood change with oxygenation. Deoxygenated hemoglobin is paramagnetic while oxygenated hemoglobin is diamagnetic, affecting the susceptibility of blood-rich tissues. [13]
Hemoglobin plays a crucial role in functional Magnetic Resonance Imaging (fMRI), which is based on the Blood Oxygenation Level-Dependent (BOLD) effect. The BOLD contrast arises from differences in the magnetic properties of oxyhemoglobin and deoxyhemoglobin. Neural activity alters the local concentrations of these two forms of hemoglobin, enabling fMRI to map brain function and connectivity.

- Myelin is a spiralling sheath that wraps around axons, in the brain and in the spinal cord. Myelin is composed of approximately 50% lipids, 40% water, and 10% protein. Its high lipid content makes it slightly more diamagnetic than other tissues. Changes in myelin concentration or organization, such as in demyelinating diseases like multiple sclerosis, can be detected using QSM. [14]
- Calcium is stored in various structures of the human body and plays a fundamental role in bone formation and blood cell synthesis. Calcium deposits in tissue, such as those found in vascular calcifications or neurodegenerative conditions, exhibit strong diamagnetic susceptibility. Unlike iron, which increases susceptibility (paramagnetic), calcium decreases it. QSM and phase imaging can help differentiate calcifications from hemorrhages due to their opposite susceptibility signs. [15]

The magnetic susceptibility of brain tissue is primarily influenced by water content, iron concentration, and myelin. Water has a susceptibility of approximately -9.04 ppm relative to vacuum. In the brain, susceptibility typically ranges from -9.2 to -8.8 ppm relative to vacuum, varying between grey and white matter. [14] Heavily myelinated white matter is more diamagnetic, due to the presence of diamagnetic lipids in myelin.

2.3 QSM Processing

The QSM processing pipeline generally consists of 4 main stages: acquisition of a T_2^* -weighted image, phase unwrapping, background field removal, and local field inversion.

Phase unwrapping, as will be seen below, corrects for the discontinuities in the phase images caused by wrapping artifacts, that occur when a measured quantity's magnitude exceeds the instrument's unambiguous range, causing it to "wrap around" and appear as a different value, often seen in MRI when an object's size exceeds the field of view or in velocity encoding where velocity measurements exceed the defined range. This phenomenon, a type of aliasing, misrepresents the true value and can be mitigated by increasing the field of view, oversampling the signal, or using a saturation band. Background field removal isolates the local magnetic field induced by the tissue's susceptibility, eliminating contributions from sources outside the region of interest, such as air-tissue interfaces. The final stage, local field inversion solves an ill-posed inverse problem to reconstruct the spatial susceptibility distribution from the local field map. This step, often, uses regularization techniques to ensure stability and accuracy.

Each step in the pipeline plays a crucial role in determining the quality and reliability of the resulting susceptibility maps.

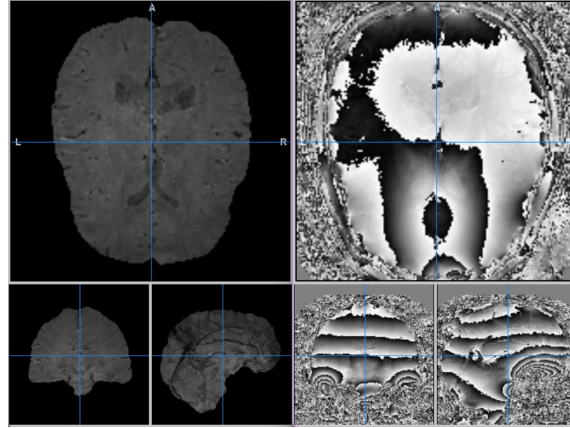


Figure 2.3: Phase and Magnitude components of the MRI signal from a brain scan. The left image shows the magnitude, representing the signal intensity and anatomical structure, while the right image displays the wrapped phase, which contains information about local magnetic field variations but includes phase discontinuities due to wrapping.

2.3.1 T_2^* -Weighted Images Acquisition

The first step of the QSM pipeline is the acquisition of a T_2^* -weighted image. The most commonly used sequence in χ -based imaging techniques is the three-dimensional Gradient Echo (3D GRE) sequence, which enables sampling of the T_2^* decay at multiple echo times (TE).

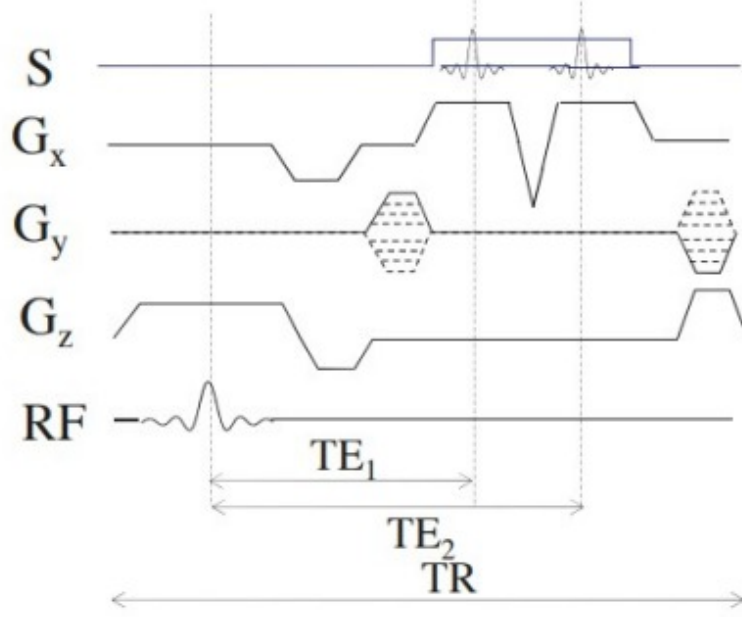


Figure 2.4: 3D Gradient Echo Sequence. The sequence begins with an RF excitation pulse that uniformly excites the entire imaging volume. Following excitation, a series of gradients is applied along the three spatial axes to achieve spatial encoding: the slice-selection gradient G_z is applied during the RF pulse to define the excited volume, the phase-encoding gradient G_y is applied in a stepwise fashion across successive repetitions to encode spatial information along the phase axis, and the frequency-encoding (readout) gradient G_x is applied during the signal acquisition window. The signal S is sampled at specific echo times (TE_1 and TE_2) within each repetition time (TR), allowing for the acquisition of multiple gradient echoes.

The resulting T_2^* -weighted images serve as the starting point for estimating magnetic susceptibility.

The MRI signal at the time TE is

$$s(TE) \propto e^{-\frac{TE}{T_2^*}} e^{i\phi} \quad (2.4)$$

where ϕ is the total accumulated phase, and it is proportional to the magnetic field change $\Delta\vec{B}(\vec{r})$

$$\phi(\vec{r}, TE) = -\gamma\Delta\vec{B}(\vec{r})TE + \phi_0(\vec{r}) \quad (2.5)$$

where $\phi_0(\vec{r})$ is a coil dependent phase offset related to the sequence adjustment.

The equation of the magnetic field change $\Delta\vec{B}(\vec{r})$ is

$$\Delta\vec{B}(\vec{r}) = \chi(\vec{r}) \otimes d(\vec{r}) \quad (2.6)$$

which is the convolution of the magnetic susceptibility and the dipole kernel.

Combining (2.5) and (2.6), the phase shift can be written as

$$\phi(\vec{r}, TE) = -\gamma TE[\chi(\vec{r}) \otimes d(\vec{r})] \quad (2.7)$$

In general, this first step is the most critical because the image suffers from a wrapped phase, an artifact related to the acquisition process.



Figure 2.5: T_2^* -Weighted Image of the human brain shown in three orthogonal views: coronal (left), axial (center), and sagittal (right).

2.3.2 Binary Mask Creation

The creation of a binary mask is a crucial step for isolating the region of interest (ROI) from the surrounding background in MRI data before starting with the QSM process. This mask is typically generated by applying a threshold to the magnitude image, where voxels representing tissue of interest (e.g., brain matter or specific structures) are distinguished from those outside the desired region. The resulting binary mask helps to define the boundaries of the tissue or structure being analyzed (see figure 2.6), ensuring accurate susceptibility measurements.

This mask is subsequently applied to the phase data to focus the QSM processing on the areas of interest, eliminating noise and artifacts from irrelevant regions.

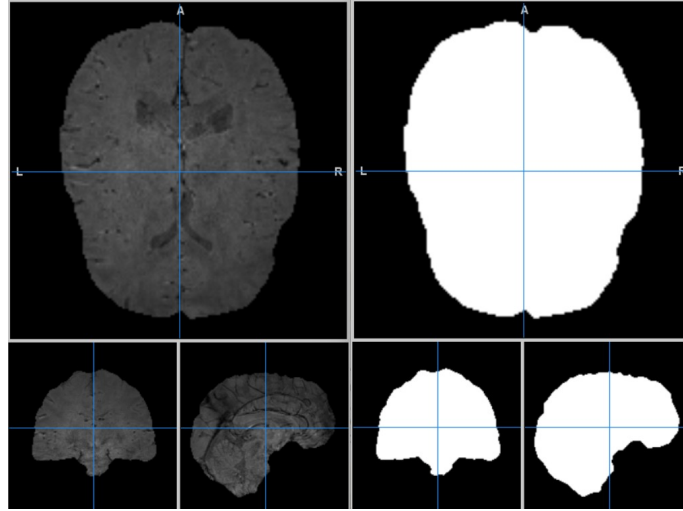


Figure 2.6: Magnitude image of the brain alongside the corresponding binary mask used for region-of-interest selection. The magnitude image (left) shows the anatomical details with signal intensity, while the binary mask (right) highlights the areas included in the analysis by distinguishing brain tissue from background and noise.

2.3.3 Phase Unwrapping

The second step of the QSM model is related to the cancellation of the phase artifacts generated by the image acquisition process.

The raw phase signal in MRI reflects the accumulated phase of the precessing spins due to local magnetic field variations. However, the MRI scanner records phase values within a limited range of $[-\pi, \pi]$ radians. When the true (unwrapped) phase exceeds this range, it is "wrapped" back into it by adding or subtracting multiples of 2π , creating discontinuities in the image. These artificial jumps are known as phase wraps and do not correspond to actual physical changes in the tissue. Phase wrapping typically occurs in regions with strong magnetic susceptibility gradients or large background field variations, such as near air-tissue interfaces or at high field strengths. To recover the true phase distribution, a phase unwrapping algorithm must be applied.

This is realized through phase unwrapping which can be performed both in time and in space domain.

The spatial phase unwrapping addresses discontinuities between adjacent voxels at the same echo time, due to high field perturbations. In this case, the phase unwrapping algorithms add multiples of 2π to each phase input, to restore the original value.

The temporal phase unwrapping happens when it exists a phase discontinuity in the same spatial position but at different echo times. To remove this artifact, it is sufficient to choose carefully the echo time variation (ΔTE).

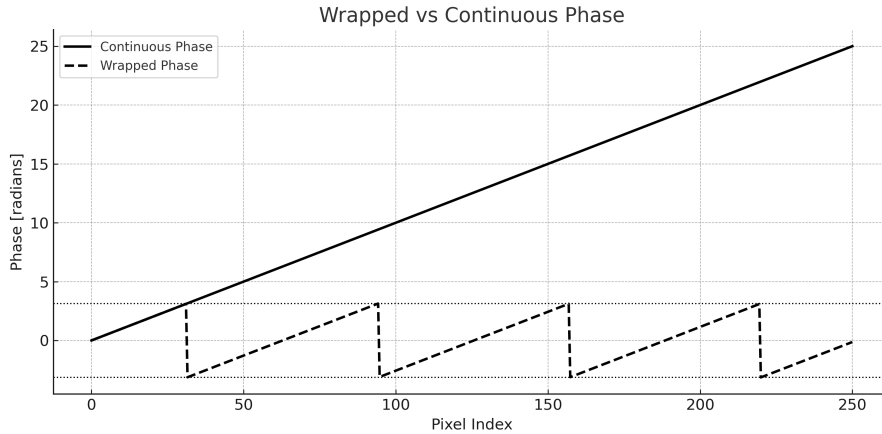


Figure 2.7: Example of spatial phase unwrapping, comparing the continuous phase (solid line) and the wrapped phase (dashed line) as a function of pixel index. In the wrapped phase, the measured phase values are restricted to a principal range (e.g., $-\pi$ to $+\pi$), resulting in abrupt 2π discontinuities whenever the true phase exceeds this range. The continuous phase, in contrast, represents the true underlying phase evolution without discontinuities.

If the phase data are correctly unwrapped, the resulting image should appear spatially smooth. The presence of discontinuities indicates a failure in the unwrapping process.

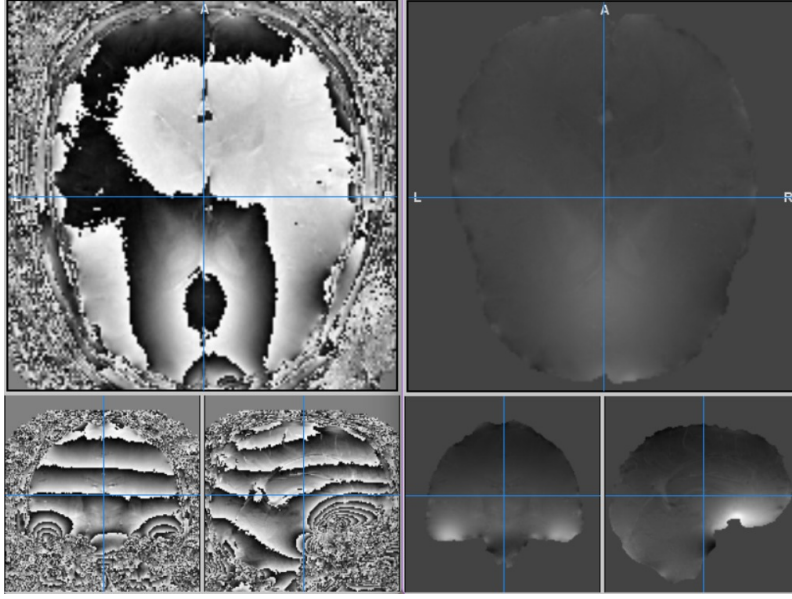


Figure 2.8: Wrapped and unwrapped phase images of a brain MRI. The left panel shows the wrapped phase containing discontinuities due to phase wrapping, while the right panel presents the unwrapped phase after correction of these discontinuities.

2.3.4 Background Field Removal

The background field is related to the susceptibility discontinuities at the air-tissues interfaces, to the shim fields or to the magnetic field inhomogeneities.

The third step of the QSM pipeline focuses on the elimination of its contribution to the field map. The field map is estimated by inverting the phase expression (2.8) respect to ΔB

$$\phi(r, TE) = -\gamma \Delta B(r) TE + \phi_0(\vec{r}) \quad (2.8)$$

Ignoring the phase offset $\phi_0(r)$, the total field map can be written as

$$\Delta B_z = -\frac{\phi}{B_0} \gamma TE \quad (2.9)$$

where the measured phase has been considered for a single echo.

In formula (2.9), the total field map can be seen as the sum of two contributes

$$\Delta B_z = \Delta B_L + \Delta B_B \quad (2.10)$$

where ΔB_L represents the biological tissue contribution and ΔB_B represents the background field one.

2.3.5 Ill-Posed Problem Resolution

Finally, the last step is the resolution of the inverse problem to estimate the tissue susceptibility distribution from the measured magnetic field. The magnetic field perturbation and the magnetic susceptibility distribution are related through the formula

$$\Delta B_z(\vec{r}) = B_0 \cdot d(\vec{r}) \otimes \chi(\vec{r}) \quad (2.11)$$

Due to challenges such as non-uniqueness, noise sensitivity, spatial susceptibility variations, limited data availability, and the necessity for regularization, the inverse problem in QSM is both ill-posed and ill-conditioned. Nevertheless, by transitioning to the Fourier domain (k-space), the problem becomes more tractable. In this domain, the use of the Fourier convolution theorem enables a reformulation of the inverse problem

$$\chi(\vec{k}) = \frac{\Delta B_z(\vec{k})}{B_0 \cdot d(\vec{k})} \quad (2.12)$$

Various strategies have been proposed in the literature to address these challenges, with the majority relying on the solution of a minimization problem such as

$$\chi(r) = \underset{\chi}{\operatorname{argmin}} \left(\|\Delta B_z - d \otimes \chi\|_2^2 + \lambda R(\chi) \right) \quad (2.13)$$

2.4 QSM Algorithms Overview

This section provides a detailed overview of the QSM methods used in this study, all implemented within SEPIA (Susceptibility Estimation for Phase Image Analysis). SEPIA is an open-source MATLAB toolbox designed to process multi-echo gradient echo MRI data and perform QSM. It provides a modular, GUI-based pipeline that integrates several key steps required for QSM, including phase unwrapping, background field removal and susceptibility inversion.

The selected algorithms are based on methods proposed in previously published studies [3] [17], and will be compared in the following chapters.

2.4.1 Phase Unwrapping Algorithms

SEPIA Toolbox works in both temporal and spatial domain providing the total phase unwrapping.

Optimum Weights Optimum weights method works on the temporal domain. A weighted combination of the phase differences between successive echoes is used, where the weights are inversely proportional to the noise variance in the field map. To each voxel is assigned a weight based on local phase measurements: a higher SNR indicates a more reliable signal, and therefore receives a higher weight, contributing more significantly to the phase unwrapping process.

Morphology Enabled Dipole Inversion (MEDI) nonlinear fit MEDI non-linear fit is the second method which works on the temporal domain. It incorporates anatomical information and a non-linear regularization approach to stabilize the inversion.

A T_1 or T_2 -weighted image is used to guide the reconstruction, under the assumption that magnetic susceptibility variations are spatially correlated with tissue morphology. This morphological prior introduces a regularization term that enforces consistency between the susceptibility distribution and the anatomical structures. The MEDI reconstruction problem is formulated as the following optimization:

$$\min_X \|W(D(X) - B_{loc})\|_2^2 + \lambda R(X) \quad (2.14)$$

where W is a weighting matrix that accounts for the SNR in the data, $R(\chi)$ is the regularization term derived from the anatomical image, and λ is a regularization parameter that balances data fidelity and prior information.

MEDI leverages the fact that anatomical boundaries typically coincide with changes in susceptibility. The optimization is non-linear due to the regularization term being dependent on the intensity image.

Laplacian (MEDI) Laplacian (MEDI) method considers the magnetic field continuous phase to follow the relationship

$$\nabla^2 \phi_{continuous} = \nabla^2 \phi_{wrapped} \quad (2.15)$$

where $\phi_{continuous}$ is the continuous phase that should be calculated and $\phi_{wrapped}$ is the phase that should be unwrapped. It is based on the local information given by the Laplacian operator to unwrap the phase, considering that the wrapping process happens due to the aliasing and not due to rapid physics variations.

It applies the Laplacian operator to the spatial wrapped phase, and then it solves a linear equations system to estimate the continuous phase.

Laplacian (STI Suite) Laplacian (STI Suite) method is commonly used to estimate the magnetic susceptibility tensor in tissues or materials with anisotropic magnetic properties.

The first step is the same described previously. After this procedure, it reconstructs the anisotropy susceptibility tensor. It requires MRI acquisitions in different B_0 orientations and the inverse problem solution to determine the susceptibility tensor elements. In this context, phase variations do not only depend on the spatial distribution, but also on the direction with respect to B_0 .

3D Best Path The 3D Best Path method analyzes the spatial wrapped phase to identify the phase residuals, which indicate the points where it exists a phase incoherence that requires the unwrapping procedure.

After the analysis of $\phi_{wrapped}$ to determine the phase residuals, it constructs a 3D grid on the data where each voxel is connected to the neighboring ones. Then, it is calculated a weight for each connection which represents the probability that this connection is the best path. The best path is determined minimizing a global

criterion, which takes into account phase variations and penalize discontinuities or noisy regions. Once the best path is determined, the phase jumps are corrected along it: the result is a continuous phase which respects data physics discontinuities and which minimizes the errors induced by the noise.

It is based on the assumption that physics signal phase variations are continuous and gradual if there are no real physics discontinuities. It assumes that phase residuals are caused by data errors and that the noise is distributed randomly.

Region Growing (MEDI) Region Growing (MEDI) is based on the initial region progressive expansion starting from data points reliable, correcting the spatial phase jumps each time that new points are added in that region.

The first step of the algorithm is to choose the starting voxel in which the wrapped phase is considered reliable and without important jumps. Then, it gradually adds voxels near the starting point, correcting the phase jumps between them.

This method uses a reliability criterion in which each new point is added in the region if the values are coherent with the already calculated ones. Additionally, it is possible to use thresholds to filter noisy data, avoiding points with low signal intensity or high noise. The process continues until all voxels are included and unwrapped, resulting in a continuous and coherent phase map.

GraphCut GraphCut method constructs a graph representing the spatial phase unwrapping problem, and it identifies the cut that minimizes a global cost function. Each voxel represents a node, and nodes' arcs represent the connection between adjacent voxels that are associated to a weight. This weight measures the probability that there is a phase jump ($\Delta\phi$) in between the connected voxels defined as

$$\Delta\phi = \phi_{wrapped}(i) - \phi_{wrapped}(j) \quad (2.16)$$

This process relies on an optimization algorithm to determine the optimum path (cut) that separates the graph regions. This method assumes that phase discontinuities are generated by the aliasing process.

Robust Estimation of the Magnetic field by using Optimal weights (ROMEO)

ROMEO combines both local and global strategies for phase unwrapping through a multi-step process.

First, the wrapped phase data undergo pre-processing using a regularized Laplacian filter, which improves the signal-to-noise ratio (SNR). Then, phase residuals are identified, highlighting regions affected by non-coherent 2π phase jumps, typically caused by noise or aliasing. Next, an iterative unwrapping procedure based on directed graph optimization is applied: the algorithm progressively corrects the wrapped phase by minimizing a global cost function that accounts for both local and global phase variations. This is followed by a multi-scale regularization step, which ensures both local continuity between adjacent voxels and global consistency across the volume.

Finally, a correction step is performed to eliminate residual artifacts, enforcing a continuous and physically coherent phase distribution throughout the dataset. This correction leverages the assumption that the true magnetic field phase varies smoothly and continuously, and that artificial 2π jumps induced by noise or aliasing must be resolved, in accordance with both local phase regularity and the global behavior of the magnetic field.

Speedy rEgion-Growing Unwrapping Engine (SEGUE) The SEGUE method combines gradient-based unwrapping techniques with an adaptive spatial regularization.

The first step is the calculation of $\phi_{wrapped}$ along each spatial directions (x,y,z). Discontinuities are identified in the gradient map, which represents the points where the phase jump is not coherent with the surrounding variations.

The spatial regularization applies an adaptive filter based on the gradient local characteristics and on the phase map, reducing the noise impact and preserving important data information. Then, the regularized gradient is integrated to obtain the continuous phase map, taking into account spatial variations. Optionally, it is possible to apply an iterative correction in noisy data to increase the solution accuracy, improving the continuous phase coherence.

This method exploits the real phase gradient properties, physically correlated to the local magnetic field, to distinguish in between real variations and the variations caused by the aliasing and by the noise.

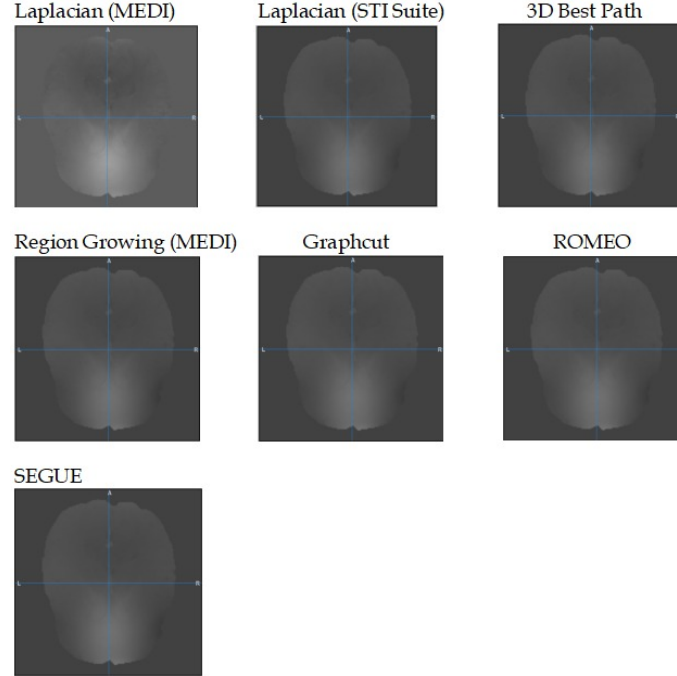


Figure 2.9: Phase Unwrapping Algorithms Comparison using Optimum Weights. From the left top: Laplacian (MEDI), Laplacian (STI Suite), 3D Best Path, Region Growing (MEDI), Graphcut, ROMEO and SEGUE.

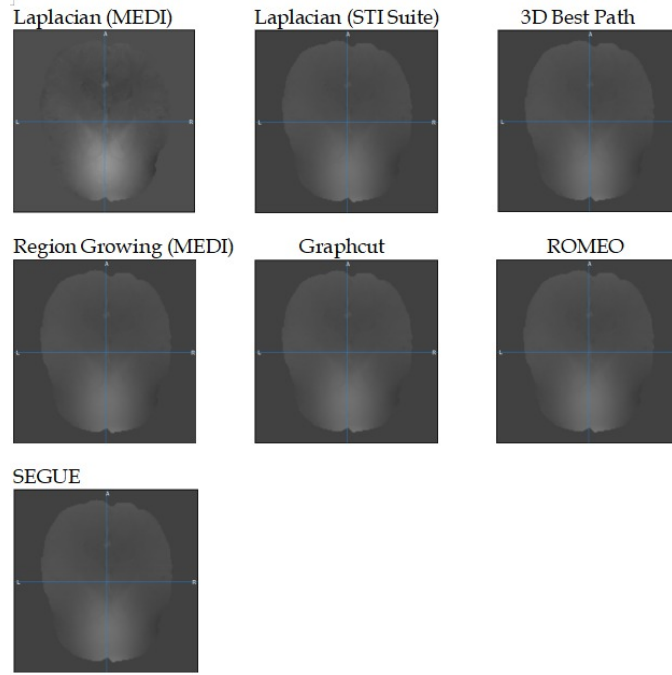


Figure 2.10: Phase Unwrapping Algorithms Comparison using MEDI nonlinear fit. From the left top: Laplacian (MEDI), Laplacian (STI Suite), 3D Best Path, Region Growing (MEDI), Graphcut, ROMEO and SEGUE.

2.4.2 Background Field Removal Algorithms

SEPIA performs background field removal by solving spatial-domain inverse problems.

Laplacian Boundary Value (LBV) LBV method is based on the Poisson equation resolution related to the local boundary conditions, using the local field and a binary mask that defines the volume of interest.

The first step is the definition of a binary mask to identify the internal voxels (tissue) and the external ones, allowing the distinction in between local fields and background fields. Then, it is solved the Poisson equation

$$\nabla^2 \phi = -\nabla v \quad (2.17)$$

where ϕ represents the local field scalar potential and v is a vector calculated by the estimation of the background field gradient.

Boundary conditions are defined locally on the mask surface, allowing the preservation of the local field continuity inside the masked volume. This method applies an iterative approach to calculate the local field, eliminating progressively the background contributes. At the end of the process, LBV gives a local magnetic field estimation inside the mask separated from the background contributes.

LBV considers the background contributions on long distances (global), while the local contributions directly derive from the mask internal variations.

Projection onto Dipole Fields (PDF) PDF method uses the projection principle in the k-space domain, exploiting the dipolar kernel properties.

The first step is the k-space calculation, where the residual field is transformed in the k-space using the Fourier transform. Then, the dipolar kernel is mathematically defined as

$$D(k) = \frac{1}{3} - \frac{k_z^2}{(k_x^2 + k_y^2 + k_z^2)} \quad (2.18)$$

where k_x, k_y, k_z are the k-space components.

The background field is modeled as the contribution projected along the k-space components directions, and the local field is calculated by eliminating this projection

$$B_{local} = \Delta B - B_{background} \quad (2.19)$$

Such as LBV, a binary mask is used to define the volume of interest, excluding the external regions that can contain unwanted contributes. After the separation, the local field is reported in the real space through the inverse Fourier transform.

Sophisticated Harmonic Artifact Reduction for Phase data (SHARP)

SHARP method relies on the assumption that the background field satisfies Laplace's equation $\nabla^2 B_{background} = 0$ and is, therefore, harmonic. Such the previous methods, the first step is the definition of a binary mask. Then, to filter the residual field, it uses an harmonic spherical convolution kernel

$$B_{background} = K_s \otimes B_{observed} \quad (2.20)$$

where K_s is the spherical kernel and $\otimes$ is the convolution operation.

The observed field is filtered through the kernel to isolate the background contribution, and the local field is obtained by subtracting the background field from the observed one

$$B_{local} = B_{observed} - B_{background} \quad (2.21)$$

One limitation of SHARP is that mask borders may not be fully processed due to the kernel request of valid voxels around the calculation point.

Regularization-Enabled SHARP (RESHARP)

RESHARP improves the SHARP method, adding a regularization to face SHARP limitations in noisy data and in mask boundaries.

The first step is the volume of interest masking and definition to exclude external regions. Then, to filter the residual field, it uses an harmonic spherical kernel defined in (2.18).

SHARP limitations are related to the possibility of adding errors in the mask boundary due to the lack of valid voxels. RESHARP regularization overcomes these limitations by formulating the process as a regularized optimization problem solving

$$\min \|B_{observed} - K_s \otimes B_{local}\|^2 + \lambda \|\nabla B_{local}\|^2 \quad (2.22)$$

where $\|B_{\text{observed}} - K_s \otimes B_{\text{local}}\|^2$ is the data fidelity term, $\|\nabla B_{\text{local}}\|^2$ is a regularization term to penalize rapid variations in the local field and λ is the regularization weight parameter.

This method uses iterative approaches to find a solution that balances the data fidelity and the local field continuity. At the end, the local field is returned without background contributions.

Variable kernel SHARP (VSHARP) VSHARP method introduces variable masks to better manage the discontinuities and the borders artifacts.

The first step is a multi-level approach based on variable masks, where nested masks are used to decrease the dimensions and create an inner area with improved stability for processing. For each level, equation (2.20) is iteratively applied, from the biggest mask to the smallest one, removing the background contribution. This procedure allows a borders artifacts progressive reduction, excluding gradually the most problematic voxels. Finally, the calculated local fields contributions are integrated to obtain the complete local field inside the volume of interest.

Variable kernel SHARP (VSHARP) STI Suite VSHARP (STI Suite) refines several steps of the general VSHARP algorithm by introducing specific optimizations.

First, the management of variable masks is improved through more accurate nested mask scaling, utilizing advanced techniques for border treatment and interpolation. Additionally, the harmonic kernel computation is optimized to reduce computational complexity, particularly in large volumes. Furthermore, the iterative processing pipeline is partially automated through the integration of predefined parameters, which significantly decrease the need for manual intervention.

With respect VSHARP, a quality control step is also included to automatically evaluate the convergence of the process and flags any errors detected in the computed field. This approach remains grounded in the same theoretical assumptions as the original SHARP method.

improved HARmonic Phase REMoval using LAPlacian (iHARPERELLA)

iHARPERELLA is based on an iterative approach that combines harmonic background field estimation with local refinement in order to enhance the separation between the local and background magnetic fields.

Initially, the background field is estimated under the assumption that it is harmonic, typically using a spherical harmonic kernel or a similar method. At each iteration, the local field is re-estimated to refine the background field through a local linear analysis, which progressively reduces the errors from the initial separation.

To minimize artifacts generated during the previous steps, the method applies an additional harmonic correction, further improving the distinction between the local field and the background field. Special attention is also given to the treatment of border voxels, which are often challenging due to the lack of sufficient neighboring information; advanced strategies are employed to ensure accurate processing in these regions.

The iterative process continues until a convergence criterion is satisfied or a predefined maximum number of iterations is reached. This approach is grounded in two main principles. First, it assumes that the background field originates from sources located outside the volume of interest and can therefore be modeled as a harmonic field satisfying the Laplace equation. Second, it relies on the linearity of the total magnetic field, which is considered to be a linear combination of the background and local components.

By leveraging this linearity, the method can iteratively isolate the local field while progressively refining the background estimation.

Background Field Removal network (BFRnet) BFRnet is based on deep learning networks designed for background field removal. It employs a convolutional neural network architecture optimized for 3D data, specifically engineered to capture the spatial characteristics of the magnetic field and to distinguish between harmonic contributions, representing the background field, and non-harmonic contributions, corresponding to the local field.

The network is trained on datasets (both simulated and real) where the background and local fields are known beforehand, often obtained using traditional methods such as SHARP or RESHARP. The loss function measures the difference between the expected background field and the network’s prediction, guiding the learning process.

The input to the network is the total magnetic field, while the output provides an estimate of the local field by subtracting the predicted background field. Once trained, the network can rapidly and accurately process new datasets to estimate the local field. To prevent overfitting and ensure physical plausibility, the model incorporates regularization constraints based on known physical principles. These include the separation of the total magnetic field into harmonic background and non-harmonic local components, learning to differentiate their characteristics, and enforcing that the estimated background field satisfies the Laplace equation.

2.4.3 QSM Algorithms

SEPIA provides different type of QSM algorithms: direct, numerical, iterative and deep-learning based.

Tikhonov Regularization with Kinetic Data (TKD) TKD method estimates the magnetic susceptibility distribution starting from the residual magnetic field generated by local sources. It uses an approach based on the Fourier transform. It calculates the magnetic susceptibility through the formula

$$\Delta B(k) = D(k) \cdot \chi(k) \quad (2.23)$$

where k represents the k-space coordinates, $\chi(k)$ is the magnetic susceptibility estimated in the k-space, $\Delta B(k)$ is the residual field Fourier transform and $D(k)$ is the dipolar kernel.

From (2.23), the susceptibility can be defined as

$$\chi(k) = \frac{\Delta B(k)}{D(k)} \quad (2.24)$$

$D(k)$ contains zeros or really small values in some directions, causing numerical instability in the direct inversion. To solve this problem, TKD truncates the problematical values under a threshold T :

if $|D(k)| > T$, formula (2.24) is valid
if $|D(k)| < T$, then $\chi(k) = 0$.

The result in the k -space is reported in the real space to obtain a susceptibility map. TKD is a direct QSM algorithm.

Closed-form solution Closed-form solution method is a direct technique for calculating magnetic susceptibility from the measured local field by solving the QSM forward model equation.

The method inverts this relationship in the Fourier domain, where the convolution operation becomes a multiplication. Initially, the local field is transformed into the Fourier domain, then the susceptibility is computed as the inverse Fourier transform of the ratio between the transformed local field and the dipole kernel $D(k)$. Since $D(k)$ takes very small values along the principal magnetic field axis ($\theta=0$), a direct inversion can amplify noise; therefore, various filters and regularization strategies are employed to mitigate this issue.

iterative Least Squares with Regularization (iLSQR) iLSQR method relies on an iterative optimization technique to minimize the quadratic error between the local field and the dipolar model predicted field. To solve the equation

$$B_{local} = D \cdot \chi \quad (2.25)$$

iLSQR formulates it as a minimization problem

$$\min_{\chi} \|D \cdot \chi - B_{local}\|^2 \quad (2.26)$$

This algorithm uses the Least Squares with Regularization (LSQR) method to calculate an approximated solution. The iteration number works as an implicit regularization. The process continues until reaching the convergence criterion defined by a specific tolerance or by a maximum iterations number. iLSQR is a numerical QSM approach.

STI Suite iLSQR STI Suite iLSQR method is primarily designed for tensorial susceptibility imaging analysis, which requires the calculation of anisotropic susceptibility tensors. It is also configured to handle more complex directional inversions when the measured magnetic fields necessitate them. Optimized for multi-orientation data acquisition, it enables the reconstruction of the full magnetic susceptibility tensor. Compared to other approaches, it offers greater flexibility in noise

handling.

While based on the same principles as iLSQR, the key difference lies in its configuration to process multiple orientation measurements, allowing for the estimation of anisotropic susceptibility tensors.

Fast Nonlinear Susceptibility Inversion (FANSI) FANSI is a non-linear method that combines an iterative optimization with advanced regularization techniques, focusing on the noise and artifacts reduction during the local magnetic field inversion. The iterative optimization relies on the Newton method or on accelerated gradient techniques. Advanced regularizations are included to preserve the structures borders while reducing the noise. The algorithm exploits Fourier transforms to fastly calculate convolutions and derivatives, increasing the computational efficiency.

To improve the convergence and the stability, pre-conditioning or iterative parameters dynamic adaptation are applied. The process stops when the convergence criterion is reached. FANSI belongs to the class of iterative regularized algorithms for QSM reconstruction.

starQSM starQSM is an advanced method based on an iterative optimization, with a structure adaptive regularization, informed by anatomical data. The adaptive regularization is applied on anatomical information from T_1 -weighted images. The magnetic susceptibility is iteratively solved, minimizing an objective cost function that combines fidelity and regularization

$$\min_{\chi} \|D \cdot \chi - B_{local}\|^2 + \lambda R_{adaptive}(\chi) \quad (2.27)$$

where $R_{adaptive}(\chi)$ is the regularization term that adapts locally to the detected anatomical structures in the image. The regularization is less intense in the borders to preserve the details, while it is stronger in homogeneous areas to reduce the noise. STAR algorithm builds a weighted map to influence the inversion process. starQSM is categorized among iterative inversion techniques incorporating regularization for susceptibility mapping.

Morphology Enabled Dipole Inversion (MEDI) MEDI algorithm incorporates morphological information to improve the magnetic susceptibility reconstruction quality. It includes a constraint that exploits the correlation between the susceptibility variations and the visible anatomical borders.

The ill-posed problem is solved by minimizing an objective function that includes two main components

$$\min_{\chi} \|D \cdot \chi - B_{local}\|^2 + \lambda R_{morphology}(\chi) \quad (2.28)$$

where the second term is a regularization constraint that penalizes the susceptibility variations not correlated to the detected anatomical borders. The susceptibility variation is strongly regularized in homogeneous regions and weakly regularized along the borders

$$R_{morphology}(\chi) = \sum \omega |\Delta\chi| \quad (2.29)$$

where ω is a weight-map derived from the magnitude image.

Such as FANSI and starQSM, MEDI belongs to the class of iterative regularized algorithms.

Local Polynomial Convolutional Neural Network (LPCNN) LPCNN method is based on convolutional neural networks designed for reconstructing susceptibility maps. The network architecture begins with an initial layer that applies a Laplacian filter, enhancing sensitivity to structural details and emphasizing the borders of susceptibility regions. Subsequent layers progressively refine the output to improve the quality of the QSM inversion. The inversion process itself is non-linear and aims to minimize the error between the observed magnetic field map and the field calculated through the dipolar transfer function D . The network is trained on datasets containing reference field maps and corresponding susceptibility maps, learning the complex relationship between input data and the desired output. Optimization of the network is performed using backpropagation algorithms with a loss function that measures the error between the predicted susceptibility map and the reference. This loss function can incorporate regularization terms to preserve anatomical details while reducing noise.

MRI Susceptibility Calculation This method reconstructs a quantitative susceptibility map by leveraging the relationship between the localized magnetic field and the underlying susceptibility distribution within the tissue.

The process begins with data acquisition, typically performed using MRI sequences such as Susceptibility Weighted Imaging (SWI) or low-field gradient-echo sequences. These datasets provide information about the total magnetic field, which includes contributions from both the external field and the susceptibility-induced internal field. The next step involves calculating the local magnetic field, which is related to the susceptibility distribution. This relationship can be exploited using various inversion strategies, including direct inversion, regularization-based minimization, or iterative methods such as SQUASH and MEDI. Inversion techniques often rely on Fourier domain solutions, and additional filters or regularization strategies (such as Laplace filters) are applied to improve solution stability. More advanced approaches, such as LPCNN, use deep neural networks to enhance the quality and resolution of the reconstructed susceptibility maps.

QSMnet+ QSMnet+ is an advanced approach based on the automatic deep-learning to calculate the magnetic susceptibility maps. It uses convolutional neural networks (CNNs) and other deep learning techniques to elaborate the MRI data. The network architecture can be made by different slices with different components, such as the use of 3D convolutions to elaborate 3D data volumes. Some models can include attention mechanisms to better focus on anatomically relevant features. The model is trained using a training dataset with known susceptibility maps to learn the relationship between the input data and the desired output. The training can also include augmentation data techniques to improve the robustness of the model. This algorithm is designed to elaborate the data very quickly with respect to the conventional inversion methods.

Nonlinear Dipole Inversion (NDI) NDI is based on the direct non-linear resolution of the magnetic dipole inverse problem. Unlike traditional approaches that rely on linear simplifications, it formulates the inversion as a non-linear optimization problem. The goal is to minimize a cost function that accounts for the discrepancy between the predicted magnetic field and the measured one, while incorporating regularization terms to suppress noise and preserve anatomical structure. The solution is obtained iteratively using optimization techniques, such as the conjugate gradient method, progressively updating the susceptibility map to reduce the mismatch between estimated and observed fields. Non-linear regularization strategies, tailored to the structural features of the input images, are applied to enhance stability and minimize the impact of noise.

The method avoids linear approximations and, instead, incorporates anatomically and physically informed regularization to ensure robustness and accuracy in the reconstructed susceptibility map.

xQSM xQSM uses an explicit model that combines anatomical and physical information to estimate the magnetic susceptibility distribution with higher precision. The anatomical data integration comes from T_1 - or T_2^* -weighted images. The inverse problem is solved iteratively and, during the iterations, the susceptibility estimations are updated to reduce the error between the observed field and the expected one. Constraints are included to maintain the morphological coherence between the estimated susceptibility map and the anatomical images.

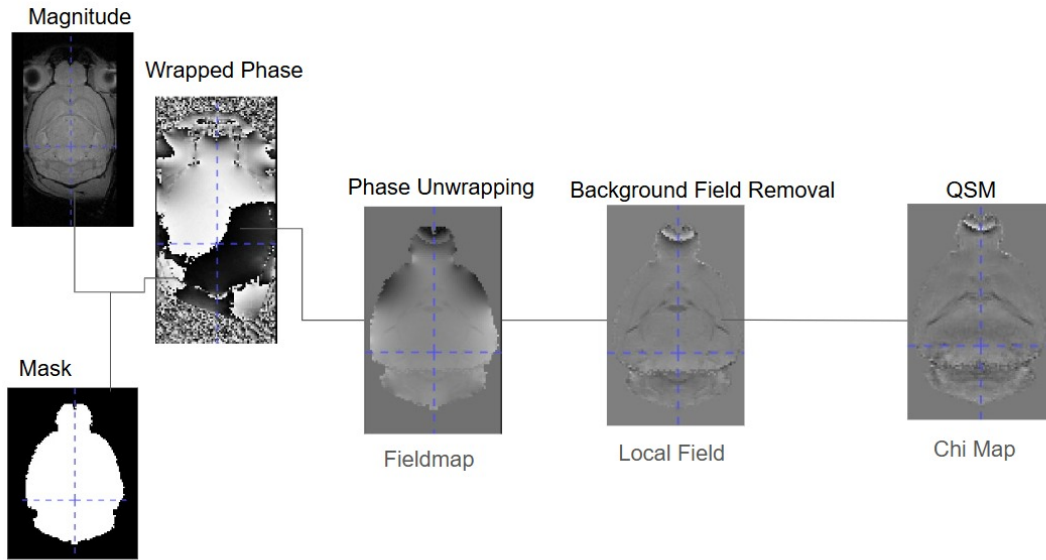


Figure 2.11: Overview of the QSM Preprocessing Pipeline, including Magnitude and Phase Image Acquisition, Mask, Phase Unwrapping, Background Field Removal and Susceptibility Map Reconstruction.

Here below, a list of the algorithms that have been used in this work.

- Phase unwrapping: Optimum Weights combined with GraphCut for the phantom, and MEDI nonlinear fit combined with Laplacian (MEDI) for mouse brain.
- Background Field Removal: LBV, PDF, RESHARP and SHARP for the phantom, and LBV, PDF and VSHARP for mouse brain.
- QSM: TKD, iLSQR, FANSI and MEDI for the phantom and the mouse brain.

Chapter 3

Experimentals and Methods

This chapter outlines the experimental procedures and methodological framework adopted to perform QSM analyses in both phantom and animal models.

The first part focuses on the design, acquisition and processing of QSM data from a phantom containing nine different concentrations of VivoTrax, while in the second part QSM was applied to mouse brain images in order to compare the behavior of different reconstruction algorithms. This allowed for assessing their robustness and accuracy in a biologically relevant setting.

VivoTrax is a superparamagnetic iron oxide nanoparticle (SPIO) contrast agent designed for use in preclinical magnetic resonance imaging (MRI). It is primarily used for cell tracking, perfusion imaging and molecular imaging studies. [19]

Unlike gadolinium-based contrast agents, which predominantly affect T_1 relaxation, VivoTrax produces negative contrast by shortening the T_2 and T_2^* relaxation times of surrounding water protons. As a result, regions containing VivoTrax appear darker on T_2 - and T_2^* -weighted images. [20] The iron oxide core of VivoTrax generates strong local magnetic field inhomogeneities, which accelerate the dephasing of transverse magnetization. This leads to a significant reduction in signal intensity on sequences sensitive to transverse relaxation, such as gradient-echo and spin-echo sequences.

3.0.1 Phantom

The phantom used in this study was inspired by a previously published protocol by Lu et al. [16], which served as the basis for its structural and compositional design. As shown in figure 3.1, each compartment is externally composed of 2% agar, while internally of nine wells there are various concentrations of VivoTrax (dextran coated iron oxide nanoparticle of 4nm) [18], and 1% gel. To minimize the long-term degradation, an additional 2% agar layer was applied over the compartments. The concentrations of VivoTrax used were: [0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.005, 0.0] mM.



Figure 3.1: Structural image of the phantom containing nine distinct compartments with different concentrations of VivoTrax contrast agent. Each region corresponds to a specific concentration, allowing for the assessment of signal variation and quantitative analysis across a range of known contrast levels.

The phantom was designed for stability and placement on the support structure as shown in figure 3.2.



Figure 3.2: Phantom Structure for Positioning. This orientation ensures consistent alignment with the magnetic field and coil setup, minimizing artifacts and allowing for uniform signal reception across all compartments.

3.0.2 Pre-Clinical Models

In-vivo imaging was conducted on twelve adult females C57Bl6/J mice. Mice were anaesthetised under 2% isoflurane in 100% O_2 and were immobilised by securing the head with a bite bar and ear bars. Subsequently, the anaesthesia was reduced to 1.5% isoflurane and maintained at this level throughout imaging. Mice were maintained at $\sim 37^\circ\text{C}$ using heated water tubing (SA instruments).

3.0.3 MRI Instrumentations and Protocols

- **7T Apparatus**

The MRI scanner used for the phantom acquisition is the Bruker PharmaScan [21]. It is equipped with a horizontal 7 Tesla magnet and a 16 cm bore. The system is based on Avance NEO electronics and Bruker BGA 9S HP gradients, with a maximum gradient strength of 760 mT/m. The scanner features four transmission and reception channels and is capable of acquiring not only proton signals, but also those of other X-nuclei.

The high magnetic field strength of the PharmaScan enhances the sensitivity of MRI studies and enables optimal resolution for the investigation of millimeter-scale structures.

For mice brain acquisitions, data were acquired with a 7T horizontal bore scanner (Bruker Biospec 70/30). A 72 mm inner diameter volume coil was used for RF transmission and signal was received using a two-channel head array.

Core temperature and respiration were monitored using a rectal probe and pressure pad (SA instruments).

- **MRI Sequences**

For QSM processing, two types of MRI sequences can be used: Multi Gradient Echo (MGE) and Multi-Slice Multi-Echo (MSME).

Multi Gradient Echo (MGE) The Multi Gradient Echo (MGE) sequence is an MRI technique that acquires multiple gradient echoes following a single radiofrequency (rf) excitation pulse. It is widely used in applications such as T_2^* relaxation mapping, QSM, and magnetic field inhomogeneity estimation. After the rf excitation pulse, the transverse magnetization begins to decay due to spin dephasing. The decay follows the time constant, which reflects both irreversible dephasing due to spin-spin interactions (T_2) and reversible dephasing caused by magnetic field inhomogeneities (ΔB_0).

Unlike spin echo sequences, MGE does not include a π rf refocusing pulse. Instead, a series of gradient refocusing lobes is used to acquire multiple gradient echoes at different echo times (TEs): this results in higher sensitivity to magnetic susceptibility effects. Each TR (repetition time) period of the MGE sequence includes: a single rf excitation pulse, slice selection, phase encoding, readout gradients and a series of gradient echoes at increasing echo times.

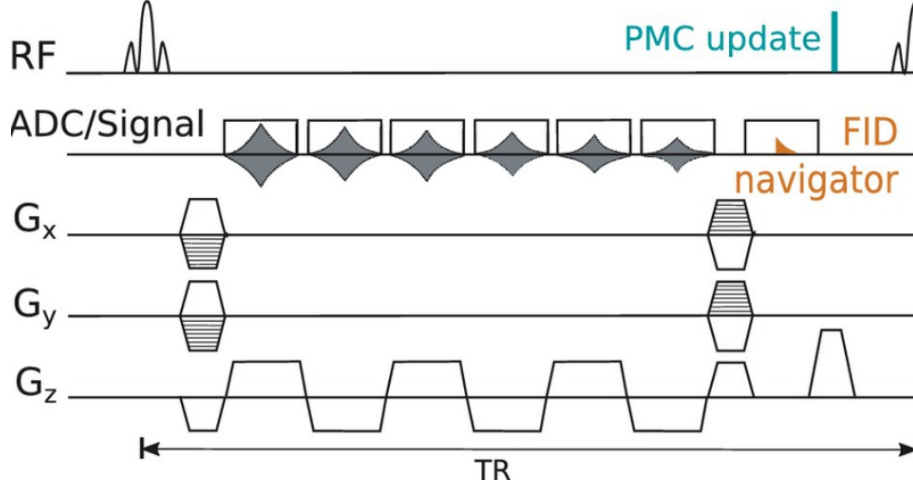


Figure 3.3: Multi Gradient Echo Diagram Sequence. Following the initial RF excitation pulse, multiple gradient echoes are acquired within a single repetition time TR by alternating the polarity of the readout gradient G_x while keeping the phase-encoding G_y and slice-selection G_z gradients constant for the duration of each echo train. The ADC captures the MR signal during each readout lobe, enabling the acquisition of multiple echo times within one excitation. An additional free induction decay (FID) navigator signal is acquired for motion or phase correction and a prospective motion correction (PMC) update is applied before the next excitation. [22]

The magnitude of the signal at each echo time TE_i is given by:

$$S(TE_i) = S_0 \cdot e^{-\frac{TE_i}{T_2^*}} \quad (3.1)$$

By acquiring signals at multiple echo times, it is possible to fit the signal decay and estimate voxel-wise T_2^* maps or extract phase data for further processing (e.g., QSM).

Multi-Slice Multi-Echo (MSME) The Multi-Slice Multi-Echo (MSME) sequence is an MRI technique that combines the acquisition of multiple spin echoes per slice with the imaging of multiple slices within a single repetition time (TR). This approach enhances imaging efficiency and enables comprehensive tissue characterization, particularly for T_2 relaxation mapping.

As shown in figure 3.4, a $\pi/2$ rf excitation pulse is applied to a specific slice, tipping the net magnetization into the transverse plane. Then, a series of π rf refocusing pulses follow, each generating a spin echo at a specific echo time (TE). This process is repeated for multiple slices, with each slice being excited and refocused in a temporally spaced manner within the same TR period. This type of acquisition allows for efficient use of the TR period, maximizing the number of slices and echoes acquired without extending the total scan time.

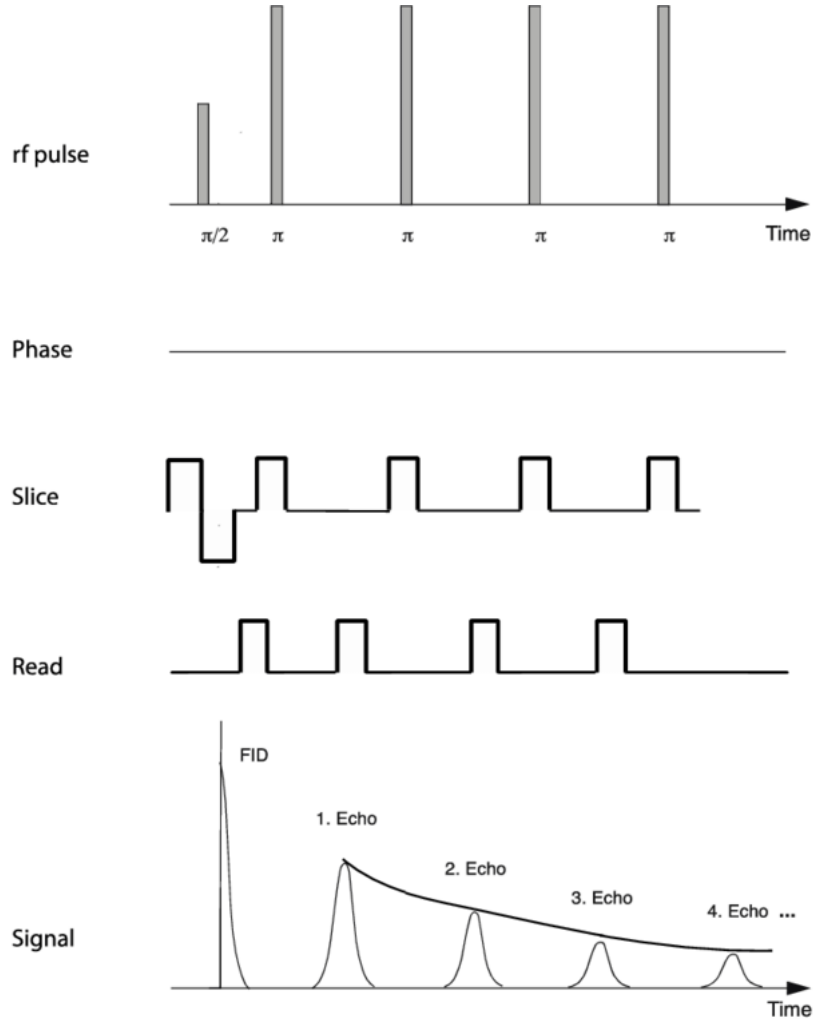


Figure 3.4: Diagram of a Multi-Slice Multi-Echo (MSME) sequence showing the RF pulse train, gradient activity, and signal evolution. The top row illustrates the series of RF pulses, starting with an initial $\pi/2$ excitation pulse followed by multiple π refocusing pulses to generate successive echoes within a single repetition time (TR). The phase, slice selection, and readout gradients ensure spatial encoding, with the slice-select gradient enabling sequential excitation of multiple slices. The bottom plot shows the resulting signal: an initial free induction decay (FID) followed by a train of spin echoes with amplitudes decreasing according to T_2 relaxation. [23]

The signal intensity $S(TE)$ for each echo time TE can be described as

$$S(TE) = S_0 \cdot e^{-\frac{TE}{T_2}} \quad (3.2)$$

By acquiring multiple echoes at different TEs, it is possible to fit this equation voxel-wise to generate quantitative T_2 maps.

- **Experimental Protocols**

Phantom The imaging data for the phantom were acquired using a MSME sequence with the following parameters: 10 echoes, $TR = 15000$ ms, $TE = [3.5, 8.5, 13.5, 18.5, 23.5, 28.5, 33.5, 38.5, 43.5, 48.5]$ ms, $\Delta TE = 5$ ms, $matrixSize = [256, 256, 10]$, $voxelSize = [0.3, 0.3, 1]$ mm^3 , flip angle = 180° . These settings were chosen to ensure optimal phase contrast for QSM processing.

Phantom acquisitions were made in the Magnetic Resonance Imaging and X-ray Microtomography Laboratory (Centro Grandi Strumenti CGS, University of Pavia).

Pre-Clinical Models For the mouse brain imaging, data were collected using a 3D spoiled gradient recalled multi-echo (GRE) sequence with the following parameters: 8 echoes, $TR = 80$ ms, $TE = [2.65, 7.38, 12.11, 16.84, 21.57, 26.3, 31.03, 35.76]$ ms, $\Delta TE = 4.7$ ms, $matrixSize = [72, 200, 54]$, $voxelSize = [0.15, 0.15, 0.15]$ mm^3 .

The mouse brain data used in this study were acquired by Mario Negri Pharmacological Research Institute, with all procedures approved by their institutional ethics committee and in accordance with applicable regulations for animal research.

3.1 QSM Analysis Data

- Methods of Phantom Data Analysis

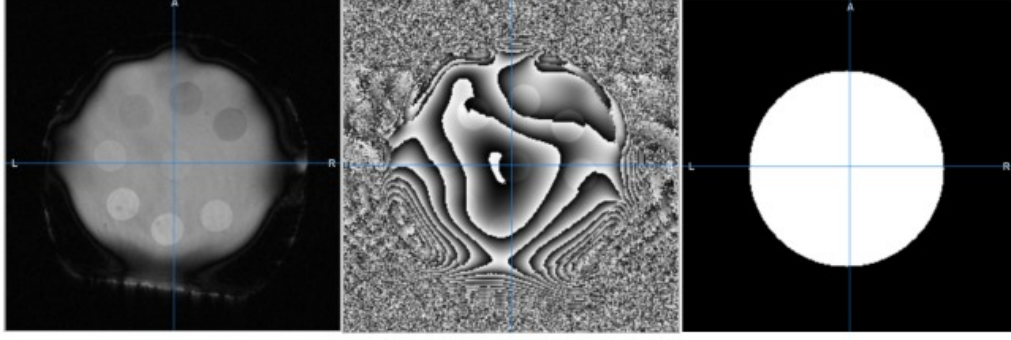


Figure 3.5: Magnitude, Phase and Mask Images of the Phantom used for the QSM Analysis. The magnitude image (left) displays the signal intensity and structural details of the phantom, the wrapped phase image (center) shows phase information with discontinuities due to wrapping and the binary mask (right) delineates the region of interest, isolating the phantom from the background for accurate analysis and processing.

Based on the study of reference [3], the QSM algorithms evaluated were TKD, iLSQR, FANSI and MEDI. For background field removal, the tested methods included are LBV, PDF, RESHARP and SHARP, while the phase unwrapping process was evaluated using both Optimum Weights and MEDI Nonlinear Fit as temporal phase unwrapping methods. In addition, all available algorithms were tested for spatial phase unwrapping, in order to determine the most effective combination of techniques.

To evaluate and compare the effectiveness of different algorithms combination, an analysis was conducted in MATLAB focusing on the susceptibility and susceptibility standard deviation behavior. The mask was created by including all nine wells together to evaluate its overall integrity, rather than generating separate masks for each individual well.

A preliminary analysis was conducted to assess the signal trend as a function of VivoTrax concentration. For each well with a different VivoTrax concentration, the center of the circular area was selected and a region of interest (ROI) was created around it. The mean signal intensity and the standard deviation within each ROI were then calculated through a MATLAB code.

Results are shown in the following figures.

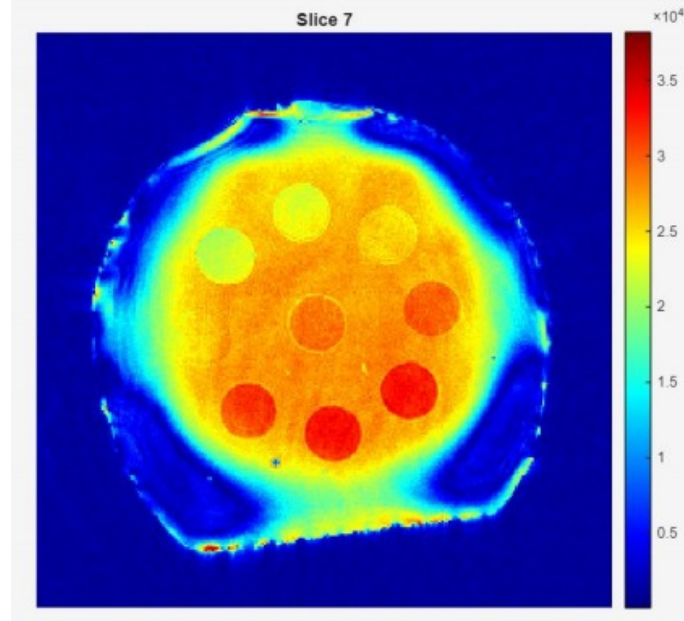


Figure 3.6: Signal Intensity map of the phantom displayed with a MATLAB colorbar. The color scale highlights variations in signal intensity across different regions, where the red and more intense areas correspond to regions with lower contrast agent concentration. This visualization aids in identifying spatial differences in signal response related to varying contrast levels within the phantom.

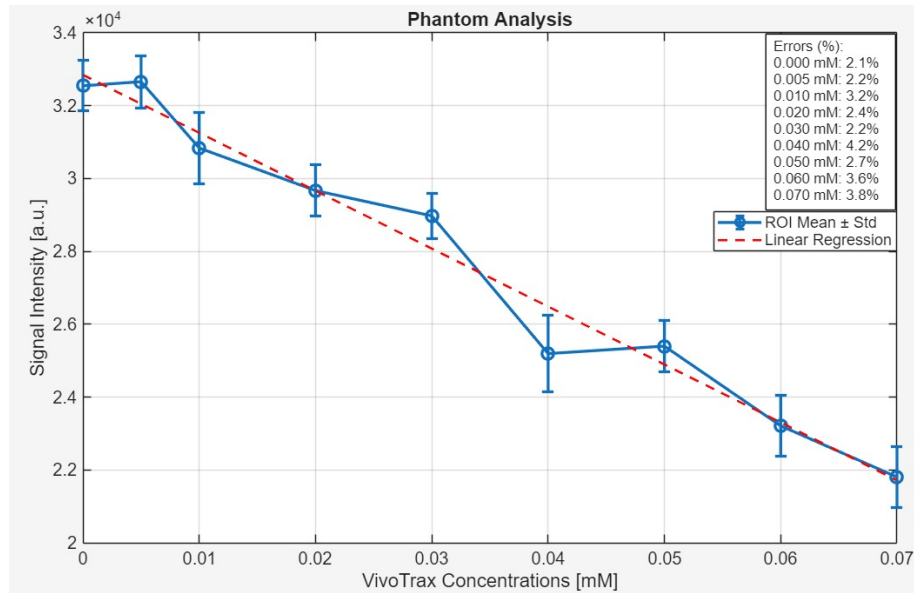


Figure 3.7: Signal Intensity as a Function of VivoTrax Concentrations. Signal intensity was evaluated by manually defining ROIs for each concentration and computing the mean value within each region.

Consistent with expectations, the signal intensity decreases as the VivoTrax concentration increases (Figure 3.7) due to enhanced magnetic susceptibility effects, which cause faster transverse relaxation and consequently reduce the MR signal. Concentrations 0.01, 0.04, 0.06 and 0.07 mM show an high signal standard deviation, possibly due to their position at the extremes of the phantom. The phantom itself had relatively large dimensions and was slightly compressed during the acquisitions to fit into the scanner, which positioned those regions very close to the RF coil. This proximity may have introduced some artifacts, potentially affecting the signal uniformity in those areas.

For the phase unwrapping process, the combination that yielded the best results was Optimum Weights for temporal unwrapping and GraphCut for spatial unwrapping (Figure 3.8). Figure 3.9 presents the background removal results, obtained using the most effective phase unwrapping method identified in the previous analysis. Consistent with the background removal results, the QSM images presented below were selected based on the best-performing phase unwrapping method.

The grayscale scale shown alongside the images was created through a MATLAB-based script.

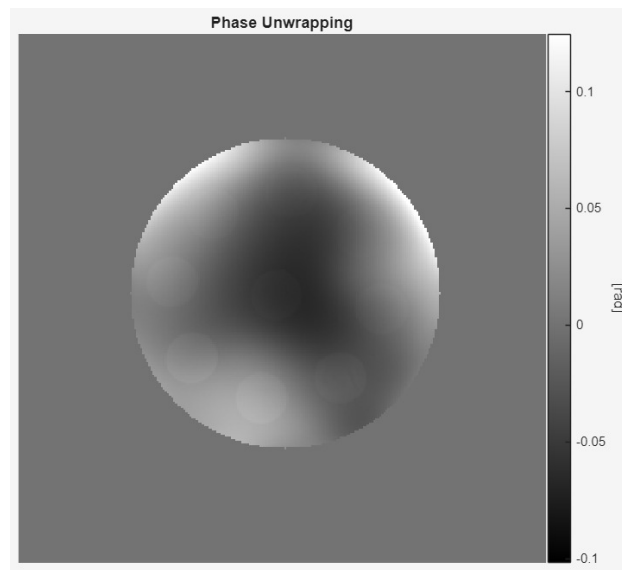


Figure 3.8: Result of Phase Unwrapping performed using the Optimum Weights method combined with the GraphCut algorithm.

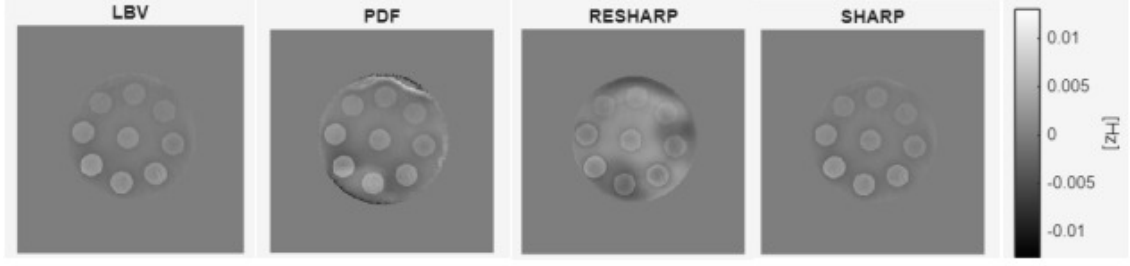


Figure 3.9: Comparison of Background Field Removal Results using four different algorithms: LBV, PDF, RESHARP and SHARP.

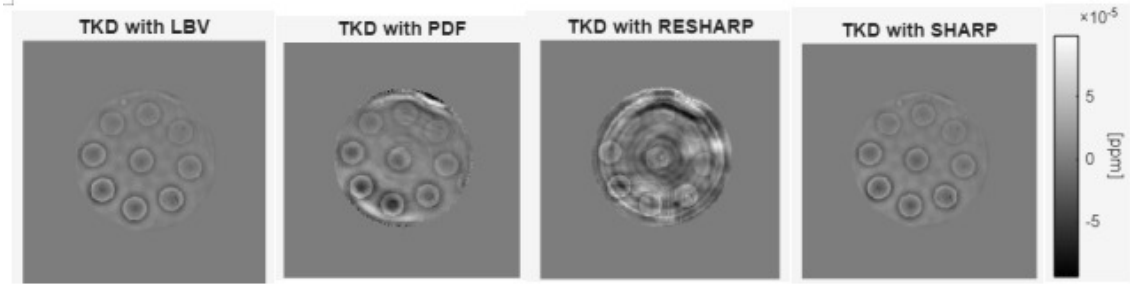


Figure 3.10: TKD Algorithm Results on the gel phantom, obtained using four different Background Field Removal Techniques. From the left to the right: LBV, PDF, RESHARP, and SHARP.

The figure 3.10 compares the effects of different background field removal algorithms on TKD-based QSM reconstruction. While LBV and SHARP provide smooth and artifact-free susceptibility maps, PDF introduces some edge artifacts and RESHARP shows residual background structures.

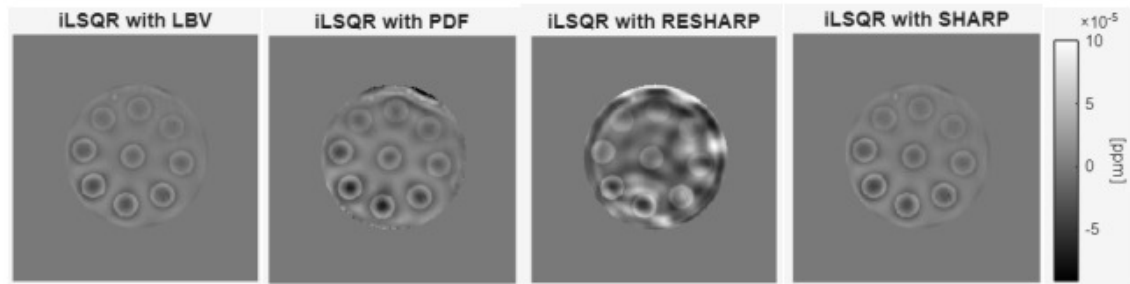


Figure 3.11: iLSQR Algorithm Results on the gel phantom, obtained using four different Background Field Removal Techniques. From the left to the right: LBV, PDF, RESHARP, and SHARP.

As already observed with TKD, both LBV and SHARP maintain the structural details of the inclusions with minimal artifacts, whereas PDF still produces edge-related distortions and RESHARP amplifies background noise patterns.

iLSQR tends to better preserve smooth susceptibility transitions compared to TKD, but remains sensitive to background removal inaccuracies.

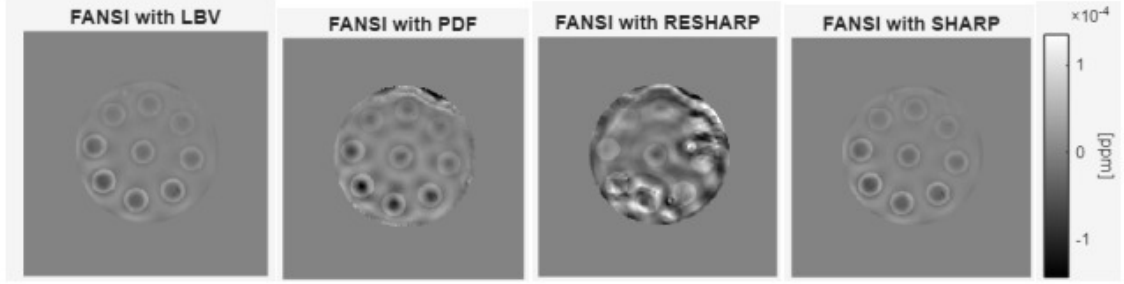


Figure 3.12: FANSI Algorithm Results on the gel phantom, obtained using four different Background Field Removal Techniques. From the left to the right: LBV, PDF, RESHARP, and SHARP.

For FANSI reconstructions, the influence of the background field removal method is again evident. LBV and SHARP produce smooth and consistent susceptibility maps, whereas PDF leads to visible edge distortions and RESHARP introduces strong residual background artifacts and noise. Compared to TKD and iLSQR, FANSI appears more sensitive to imperfections in background removal, particularly with RESHARP.

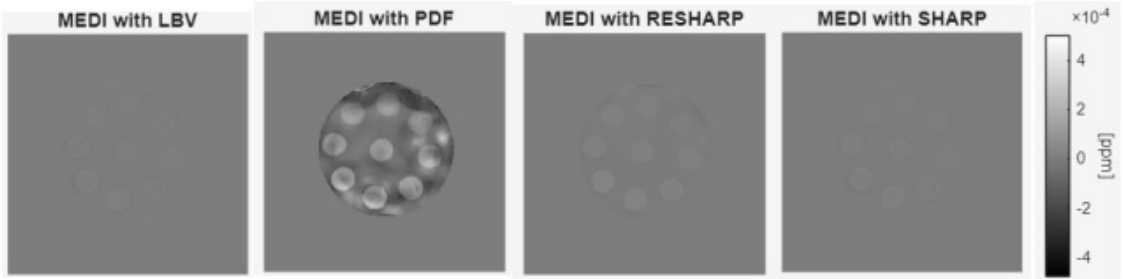


Figure 3.13: MEDI Algorithm Results on the gel phantom, obtained using four different Background Field Removal Techniques. From the left to the right: LBV, PDF, RESHARP, and SHARP.

In the MEDI reconstructions, the choice of background field removal method significantly affects the visibility of the phantom structures. LBV and SHARP yield smoother maps with reduced noise but lower contrast, while PDF preserves internal contrast but introduces peripheral distortions. RESHARP results in very low overall signal, making structures less distinguishable. Compared to the other inversion methods, MEDI appears to suppress noise more aggressively, but at the cost of reduced susceptibility contrast.

In medical imaging, ppm values represent the subtle voxel-wise or pixel-wise differences in magnetic susceptibility within biological tissue relative to a reference susceptibility baseline of that tissue. Since biological tissues have relatively low iron concentrations and susceptibility differences, the resulting ppm changes are inherently very small. Therefore, the low ppm values observed reflect the minimal but physiologically relevant variations in iron content that QSM is sensitive enough to quantify.

The very low susceptibility values measured in parts per million (ppm) in the QSM images of the phantom can be explained by several factors. First, the phantom is prepared using Vivotrax, which consists of diluted superparamagnetic iron oxide nanoparticles (SPIO). The possible high dilution of these nanoparticles leads to very low iron concentrations, resulting in correspondingly small susceptibility variations. Second, the magnetic response of such diluted SPIO nanoparticles is inherently low, further contributing to the small range of susceptibility values observed. Finally, the QSM reconstruction algorithms used are often conservative by design. They tend to suppress extreme susceptibility values to minimize reconstruction artifacts and noise, which can lead to an overall attenuation of the measured susceptibility range. Together, these factors explain why the susceptibility values in the phantom are significantly lower than those typically seen in clinical QSM brain images, where iron concentrations and magnetic susceptibility variations are higher.

- **Methods of Pre-Clinical Models Data Analysis**

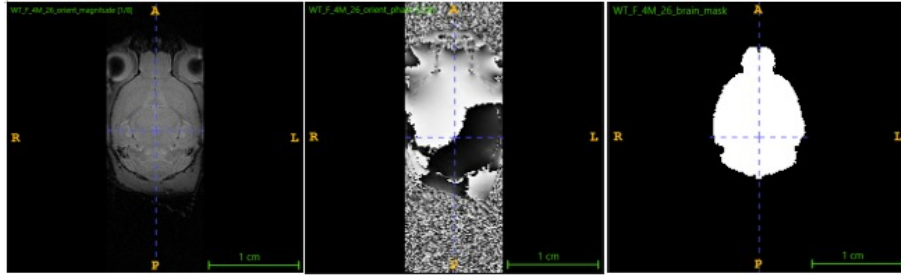


Figure 3.14: Magnitude image, wrapped phase, and binary mask of a mouse brain MRI. The magnitude image (left) displays the anatomical structure with signal intensity, the wrapped phase image (center) contains phase information including discontinuities caused by phase wrapping and the binary mask (right) delineates the brain region for focused analysis.

Based on studies [3] and [17], the QSM algorithms chosen to be compared are the same as for the phantom.

Phase unwrapping was carried out using Laplacian (MEDI) algorithm, while background field removal was performed with LBV, PDF and VSHARP methods. To compare the performance of background removal and QSM algorithms, a MATLAB-based analysis was performed focusing on three main artifacts: noise, streaking and unnaturalness.

Noise refers to random fluctuations in image intensity that do not correspond to underlying anatomical or physiological structures. In brain imaging, noise can obscure fine details and reduce the accuracy of quantitative analyses, especially in low-signal regions such as deep gray matter or near air-tissue interfaces.

Streaking artifacts are linear or curvilinear intensity distortions that typically radiate from high-susceptibility regions, such as veins or calcifications. They are often caused by imperfect inversion of the dipole kernel in QSM and are more pronounced in poorly regularized reconstructions.

Unnaturalness refers to image features that deviate from realistic anatomical appearance, such as overly smoothed regions, artificial contours or blocky textures. It often results from aggressive regularization, overfitting or algorithmic artifacts that distort the true underlying tissue structure.

To determine noise, a 3D Gaussian filter was applied to smooth the susceptibility map. The residual was then computed by subtracting the smoothed map from the original one, thereby isolating high-frequency components. [24] [25] These residuals represent the local noise. The standard deviation (std) of the residuals within the brain mask was calculated to quantify the level of noise.

For streaking, the 3D gradient of the susceptibility map was computed to detect sudden spatial variations. The magnitude of the gradient was used to identify regions with high values, which may indicate the presence of streaking artifacts (i.e., anomalous stripes or bands). [26] [27] [28] Edges were detected on the smoothed susceptibility map using a method such as Canny (edge3), to capture structural transitions. These edges were then dilated using a spherical structuring element to extend the region of interest. Only the voxels within the brain mask and close to these dilated borders were considered. The standard deviation of the gradient magnitude within these regions was calculated: high values reflect strong localized variations indicative of streaking artifacts. Finally, unnaturalness was assessed by evaluating two types of artifacts: the number of susceptibility values falling outside the physiologically plausible range (-0.15 to $+0.15$ ppm), which indicates unrealistic intensity levels, and the number of voxels with excessively high gradient magnitudes (above the 99th percentile), corresponding to abrupt spatial discontinuities. [30] [31] [41] The sum of these two components provided an overall metric reflecting the degree of unnatural appearance or the presence of visible artifacts in the susceptibility maps.

The results presented below refer to one representative case, as similar patterns and outcomes were observed across all twelve subjects.

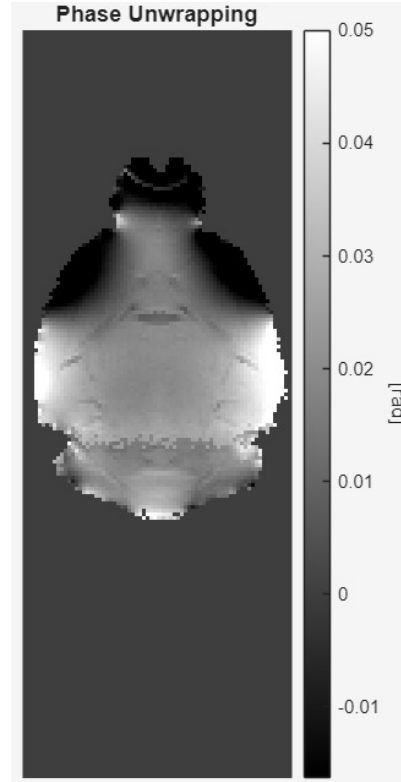


Figure 3.15: Phase Unwrapping Result using the MEDI nonlinear fit combined with the Laplacian (MEDI) Algorithm.

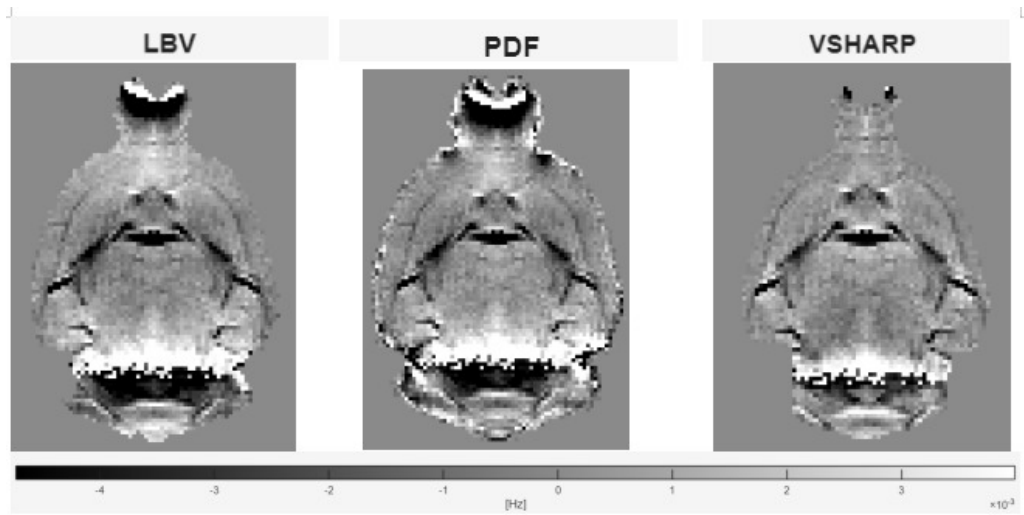


Figure 3.16: Background Field Removal Comparison using three different algorithms: LBV, PDF and VSHARP.

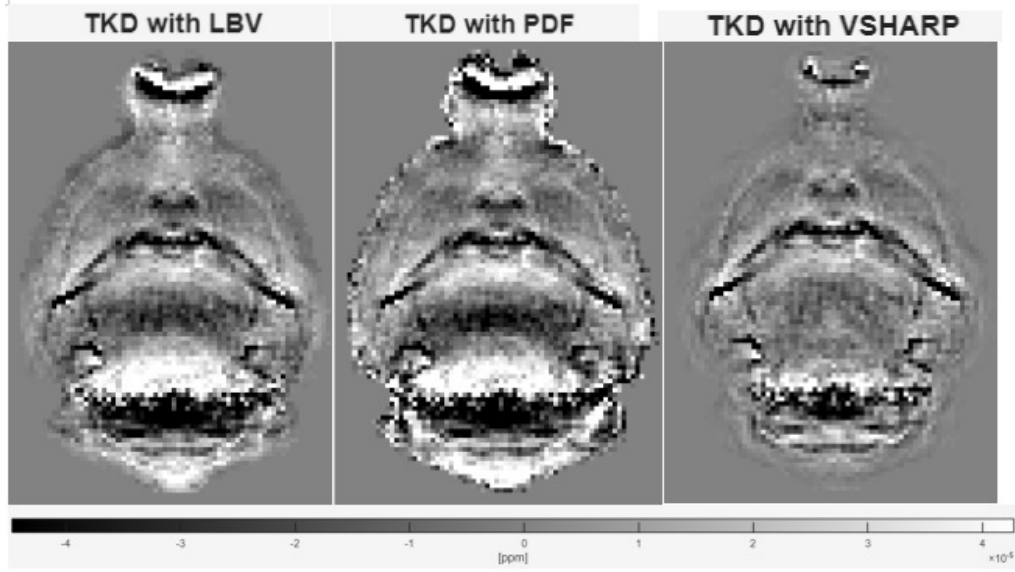


Figure 3.17: TKD Algorithm Results on the mouse brain, obtained using three different Background Field Removal Techniques. From the left to the right: LBV, PDF, and VSHARP.

For the mouse brain TKD reconstructions, differences between background field removal methods are clearly visible. LBV produces clean and homogeneous susceptibility maps with well-preserved anatomical structures, while PDF enhances structural details but also introduces noticeable ringing and edge artifacts. VSHARP yields smoother results but with a slight loss of fine structural contrast. Overall, the choice of background removal method has a substantial impact on the balance between contrast and artifact suppression.

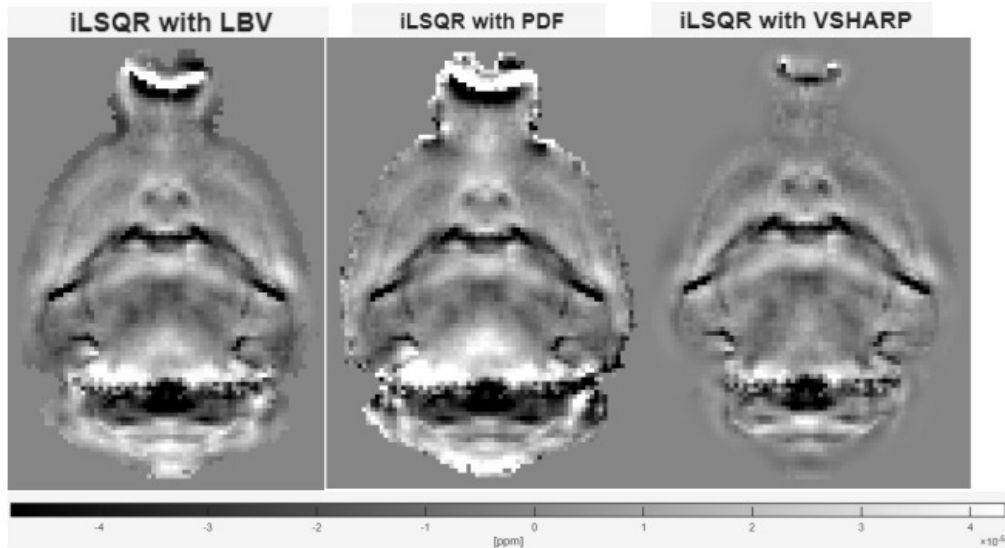


Figure 3.18: iLSQR Algorithm Results on the mouse brain, obtained using three different Background Field Removal Techniques. From the left to the right: LBV, PDF, and VSHARP.

The iLSQR results show a generally smooth and homogeneous contrast distribution across all background field removal techniques. LBV provides effective suppression of background noise, PDF introduces slightly more residual noise but enhances sharpness in certain internal structures and VSHARP yields the smoothest appearance, however with some loss of fine details.

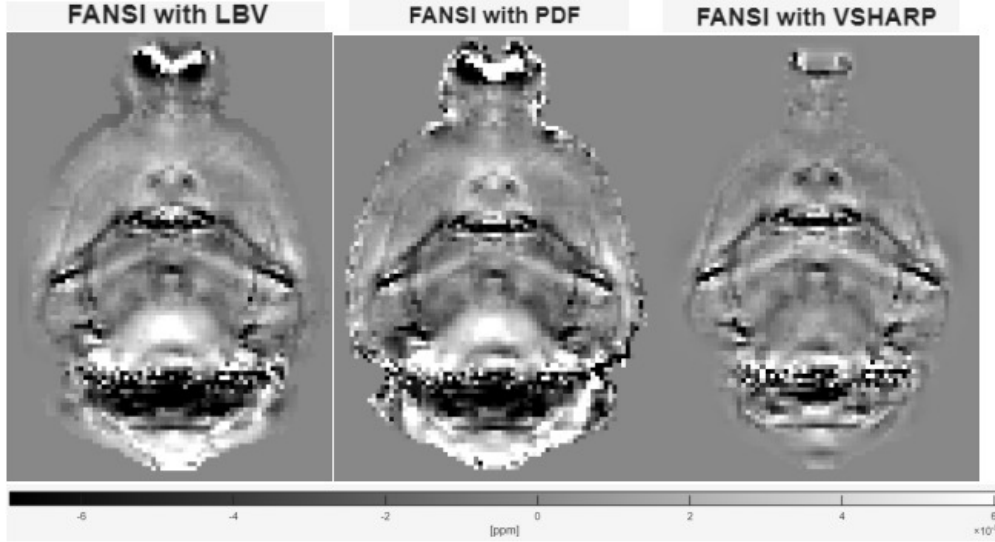


Figure 3.19: FANSI Algorithm Results on the mouse brain, obtained using three different Background Field Removal Techniques. From the left to the right: LBV, PDF, and VSHARP.

The FANSI results exhibit stronger preservation and enhancement of high-gradient structures compared to iLSQR. With LBV, the background is well suppressed while retaining local variations. PDF increases both detail visibility and residual noise, particularly in peripheral areas, whereas VSHARP produces visually smoother reconstructions but slightly reduces structural sharpness.

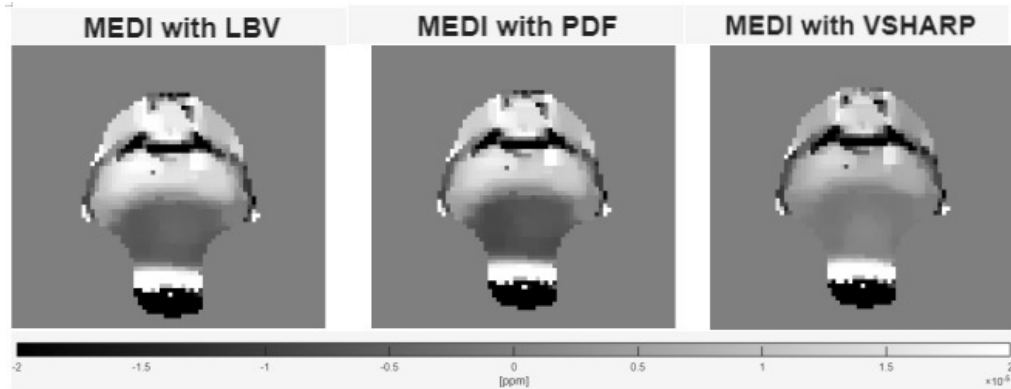


Figure 3.20: MEDI Algorithm Results on the mouse brain, obtained using three different Background Field Removal Techniques. From the left to the right: LBV, PDF, and VSHARP.

The MEDI reconstructions exhibit a noticeable loss of anatomical information, with some brain regions entirely absent across all background field removal techniques. This effect is likely due to the intrinsic regularization strategy of MEDI, which strongly enforces morphological consistency between the susceptibility map and the magnitude image. While this prior can improve robustness to noise, it may also suppress regions with low signal intensity in the magnitude image, leading to underestimation or complete removal of susceptibility values in those areas. Previous studies have reported similar findings, noting that MEDI tends to underestimate susceptibility in regions with poor magnitude contrast, such as near air–tissue interfaces or areas affected by signal dropout. [3] [29]

Chapter 4

Results and Discussion

4.0.1 Phantom

Concerning the outcomes related to phase unwrapping presented in section 3.1, a plausible reason why Optimum Weights outperforms MEDI Nonlinear Fit in phantom studies may lie in how each method handles noise and spatial variation.

Optimum Weights combines multi-echo phase information using weights inversely proportional to the noise variance. This weighted averaging improves SNR without imposing strong spatial priors, thereby preserving subtle variations in the measured field conditions often encountered in phantom setups. [32] MEDI Nonlinear Fit, in contrast, incorporates morphological priors and L_1 -regularization derived from anatomical magnitude images: this may inadvertently smooth out small-scale variations and low-contrast features typical of phantom data, limiting its sensitivity in low-susceptibility regions.

Concerning GraphCut, its superiority over other spatial phase unwrapping algorithms stems from its foundation in global energy minimization. Several studies have shown that GraphCut-based methods consistently achieve lower root-mean-square error (RMSE) under challenging conditions, such as in phantoms with large susceptibility gradients or in vivo where phase wraps are complex. For example, Bechler et al. [33] demonstrated that GraphCut delivered the most robust results and lowest deviation from ground truth when comparing phase unwrapping methods in a numerical abdomen phantom. This advantage is attributed to its ability to balance local continuity and global consistency, efficiently minimizing unwrapping errors even in rapid phase variations.

The superior performance of LBV and SHARP as background field removal algorithms in this study can be attributed to their distinct methodological advantages and compatibility with subsequent QSM inversion techniques. LBV provides a robust estimation of the background field by solving the Laplacian boundary value problem, which effectively preserves local field variations necessary for accurate morphology-enabled inversion methods like MEDI [34]. This synergy likely explains why LBV combined with MEDI produced high-quality susceptibility maps in the phantom. On the other hand, SHARP utilizes the spherical mean value property to isolate and remove background fields iteratively, excelling in eliminating large-scale field variations. This approach works particularly well when coupled with inversion

algorithms such as TKD and MEDI, which benefit from the smoother and more accurate local field estimation provided by SHARP. [35] Hence, the combination of SHARP with both TKD and MEDI resulted in improved QSM reconstruction in our experiments.

Moreover, while LBV's approach is robust against noise amplification, SHARP's filtering properties reduce background artifacts that might otherwise affect direct inversion methods like TKD. [36] [37] These factors together support the observed outcomes where LBV paired best with MEDI, and SHARP was optimal with both TKD and MEDI.

Based on the susceptibility trend with respect to the concentrations, only three combinations yielded results consistent with the expected behavior; these results are presented in the following figures 4.1 and 4.2.

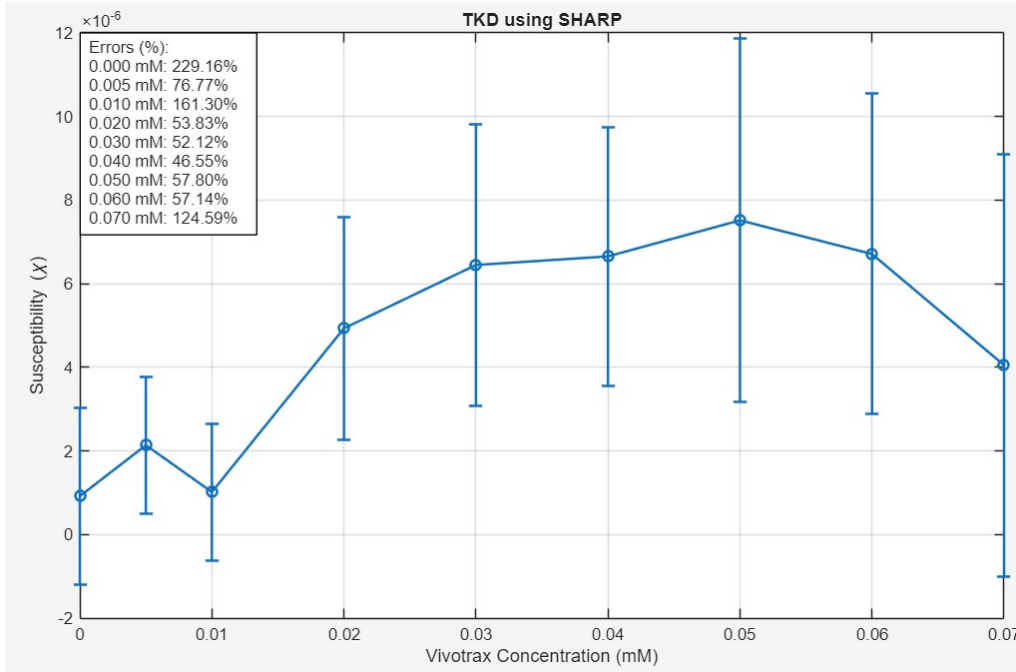


Figure 4.1: Susceptibility χ values (ppm) as a function of VivoTrax concentration (mM) obtained using the TKD reconstruction with SHARP background removal. Data points represent the mean susceptibility for each concentration and error bars indicate the standard deviation across repeated measurements. The percentage errors reported in the inset were calculated by dividing the χ standard deviation by its mean value and multiplying by 100 to express the result as a percentage, providing a relative measure of variability for each concentration.

The reconstruction performed using TKD exhibits substantial variability in susceptibility values, with particularly high standard deviations at intermediate VivoTrax concentrations. This behavior indicates a higher sensitivity of the method to noise and phase imperfections, which can reduce the reliability of the estimates under non-ideal experimental conditions.

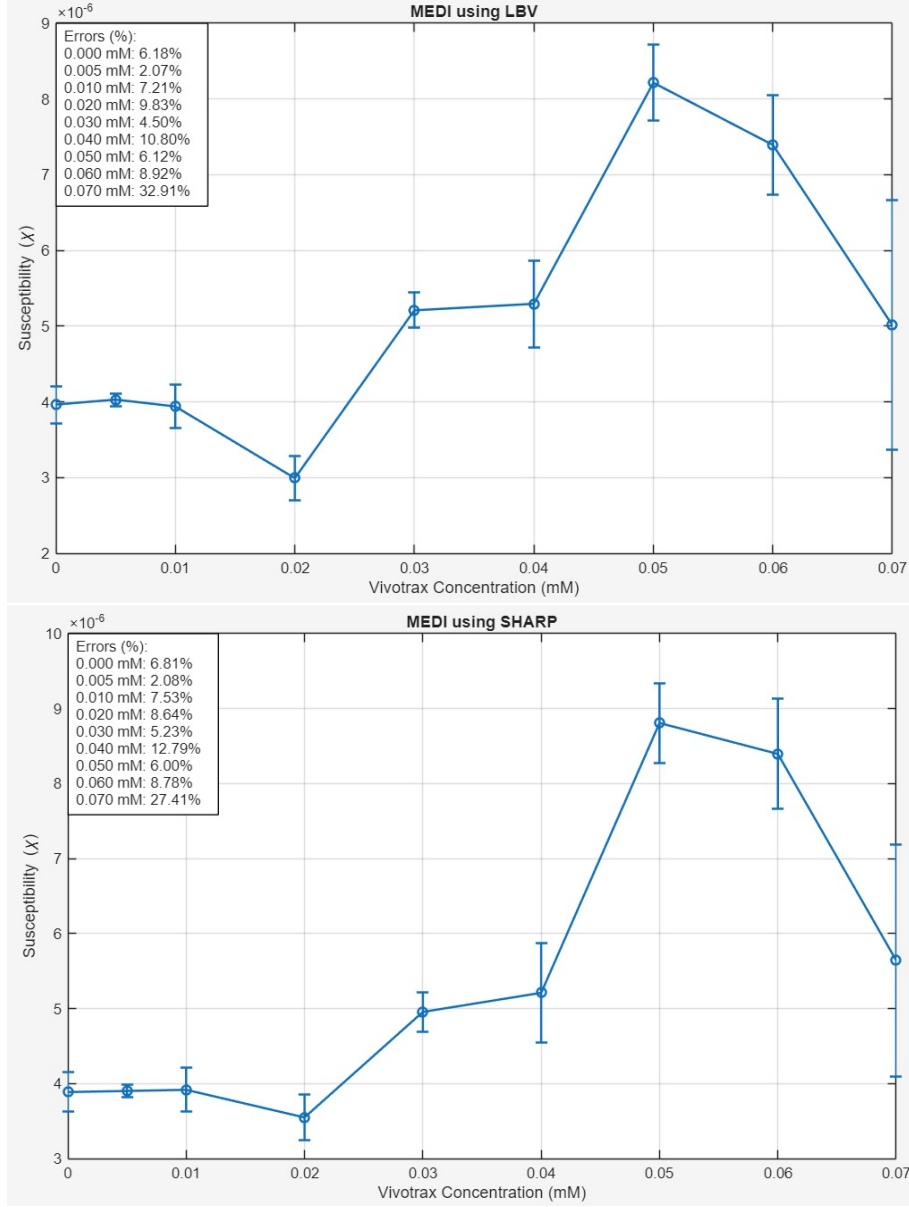


Figure 4.2: Susceptibility χ values (ppm) as a function of VivoTrax concentration (mM) obtained using the MEDI reconstruction with LBV and SHARP background removal. Data points represent the mean susceptibility for each concentration and error bars indicate the χ standard deviation across repeated measurements.

Compared to TKD, both MEDI reconstructions (LBV and SHARP) show considerably lower standard deviations at most concentrations. This suggests that MEDI provides more stable susceptibility estimates, less affected by noise. Furthermore, between the two MEDI variants, the LBV approach generally yields slightly lower variability than SHARP, particularly at the lowest concentrations.

In the first result, the concentrations 0.01, 0.06, and 0.07 mM show an high error. These correspond to three outermost wells, which were likely compressed by the coil due to the phantom’s dimensions.

In the second and third results, the concentrations that do not follow the expected susceptibility trend are 0.02, 0.06, and 0.07, again corresponding to peripheral positions. Moreover, concentrations 0, 0.005, and 0.01 are not clearly distinguishable from one another, unlike in the first case where they appear more clearly separated.

A possible explanation for MEDI’s reduced ability to distinguish lower concentrations, compared to TKD, lies in the intrinsic algorithmic characteristics of the two methods. TKD performs a direct inversion of the dipole kernel in the Fourier domain by truncating values near the zero-cone, allowing it to preserve subtle susceptibility variations (such as those from low concentrations), even at the cost of increased noise and potential streaking artifacts. [38] MEDI, on the other hand, applies an L_1 -based regularization guided by anatomical priors from magnitude images. This effectively suppresses noise and streaking artifacts, but can also smooth out low-contrast details. As a result, MEDI can underrepresent subtle susceptibility differences, particularly in regions with weak contrast.

Empirical comparisons confirm this behavior: one study found that TKD demonstrated the highest sensitivity to detect disease-related susceptibility variations, while MEDI showed the lowest sensitivity, likely due to its stronger regularization damping subtle changes. [39] Consistent with these findings, in these measurements TKD exhibited an extremely high relative error at 0.00 mM (229.16%), whereas MEDI yielded markedly lower errors at the same concentration (6.16% with LBV and 6.81% with SHARP), highlighting MEDI’s ability to suppress noise-driven fluctuations in regions of negligible true susceptibility.

In particular, studies on phantoms with varying susceptibility concentrations have shown that while the mean susceptibility increases linearly with the contrast agent concentration, the corresponding standard deviation tends to increase with susceptibility, particularly in reconstructions that lack strong regularization, such as TKD [40]. In these results, this trend was evident for TKD, although with an exceptionally high relative error at 0.00 mM, likely due to noise amplification in the absence of regularization. In contrast, MEDI reconstructions (LBV and SHARP) maintained consistently low standard deviations across all concentrations, indicating greater robustness to noise-driven variability.

This behavior can be explained by several factors:

- Noise amplification in QSM inversion: susceptibility mapping involves the inversion of the dipole kernel, a process that inherently amplifies noise, especially in regions with higher susceptibility. As the concentration increases, the induced local field becomes stronger, potentially increasing local phase variance and propagating the noise in the reconstructed susceptibility map. [36]
- Partial volume effects and non-linearity near boundaries: at higher concentrations, the susceptibility contrast between the well and the surrounding matrix becomes more pronounced. This can increase partial volume artifacts at the

edges of the wells, contributing to a higher standard deviation within those ROIs.

- Lower SNR in high-susceptibility regions: regions with higher susceptibility may suffer from local dephasing and signal loss due to field inhomogeneities. This drop in SNR can worsen the precision of phase measurements and lead to increased variability in the QSM reconstruction. [34]
- Residual background field contributions or phase unwrapping errors: even small inaccuracies in background field removal or unwrapping can be magnified in regions with higher phase shifts, potentially introducing artifacts or increasing variability in the estimated susceptibility.

While these factors generally contribute to the increase in standard deviation with susceptibility, their impact is strongly method-dependent. As shown before, TKD reconstructions exhibited clear manifestations of these effects, including the extreme relative error at 0.00 mM. In contrast, MEDI's regularization suppressed most of these sources of variability, resulting in consistently lower standard deviations across all concentrations.

Finally, to provide an overall view, the figure below illustrates the susceptibility trends for the three reconstruction approaches, highlighting that the lowest concentration at which susceptibility starts to noticeably increase is approximately 0.02 mM.

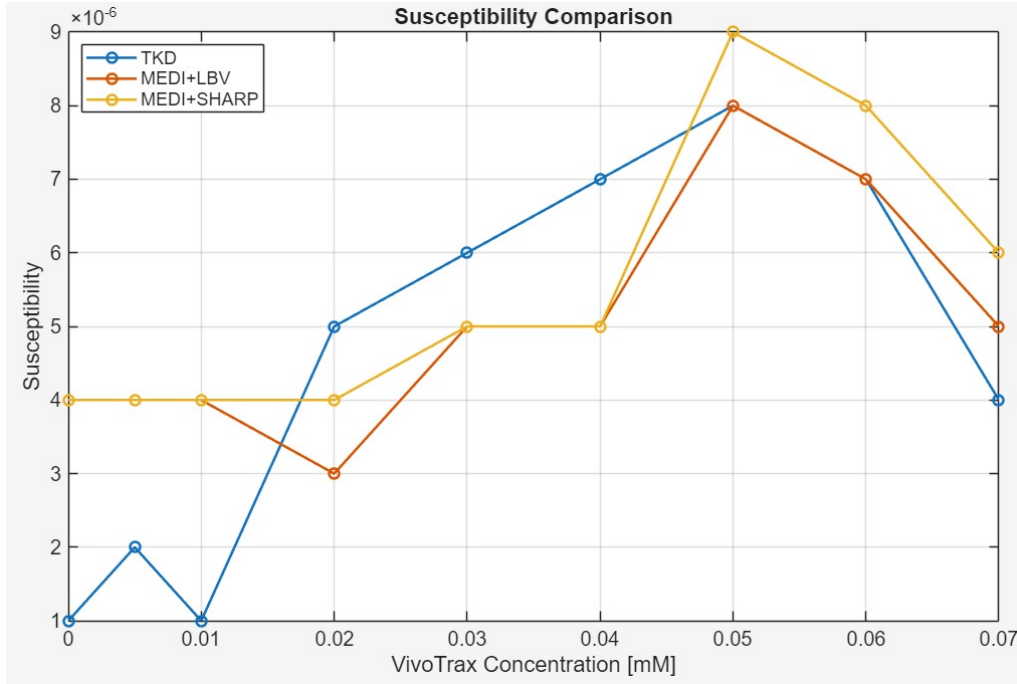


Figure 4.3: Comparison of the susceptibility values (ppm) across different VivoTrax concentrations obtained using TKD and MEDI with LBV background removal, and MEDI with SHARP background removal. The plot highlights differences in the reconstruction trends, with TKD showing larger variability at low concentrations, while MEDI-based methods exhibit smoother and more stable responses.

From the comparison, TKD shows the highest sensitivity to subtle susceptibility changes, detecting increases from concentrations as low as 0.02 mM. This is consistent with its direct inversion approach, which preserves fine variations but amplifies noise. Between the two MEDI variants, MEDI+SHARP tends to produce higher susceptibility values at intermediate-to-high concentrations, likely because SHARP background removal preserves more local field information compared to LBV. Physically, the overall increasing trend of χ with concentration reflects the proportionality between magnetic susceptibility and the amount of paramagnetic contrast agent in the sample. The deviations from perfect linearity at high concentrations (≥ 0.05 mM) can be attributed to partial volume effects, local field inhomogeneities and reduced SNR due to increased dephasing, which may cause underestimation in TKD or saturation-like effects in MEDI reconstructions.

4.0.2 Pre-Clinical Models

The result presented in the following figure refers to one representative brain and is consistent, on average, with the outcomes observed across the entire dataset.

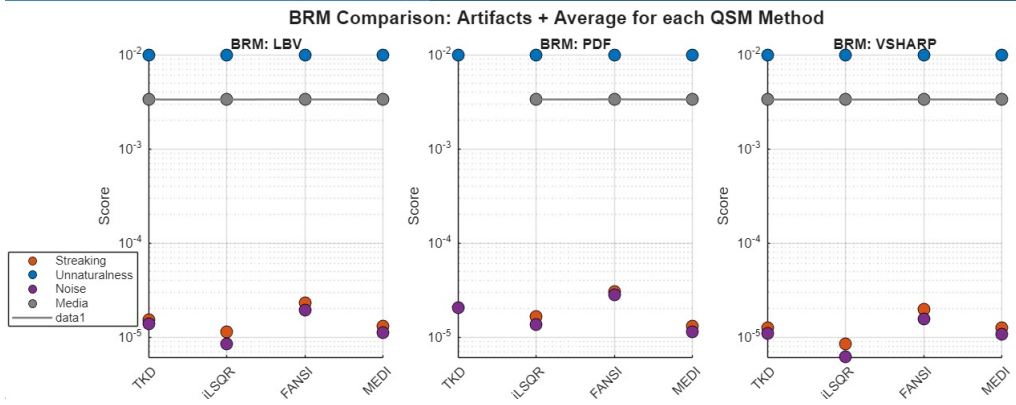


Figure 4.4: Comparison of artifacts in QSM reconstructions obtained using different reconstruction algorithms, with each panel corresponding to a different Background Removal Method (BRM): LBV, PDF, and VSHARP. The y-axis represents the artifact score on a logarithmic scale, while the x-axis lists the QSM reconstruction methods being compared. Colored markers indicate the contribution of different artifact types (streaking, underestimation, noise and bias) to the overall quality score, where data1 represents the average value of all artifacts. The results show both individual artifact scores and the average score for each method, allowing direct comparison of performance across BRM approaches.

Among the tested QSM reconstruction algorithms, iLSQR exhibited fewer artifacts, particularly streaking and noise amplification, compared to TKD, FANSI, and MEDI when applied to mouse brain data. This observation can be explained by the iterative nature of iLSQR, which solves the dipole inversion problem in a least-squares sense with Tikhonov regularization, allowing for better suppression of noise and streaking artifacts, especially in regions of low SNR or in the presence of strong susceptibility gradients. Unlike TKD, which applies a hard threshold in the dipole

kernel inversion and is prone to streaking artifacts near sharp susceptibility transitions [37], iLSQR maintains more stable reconstructions by iteratively refining the solution and incorporating prior information through regularization.

Moreover, while FANSI incorporates TV regularization, it may still suffer from noise sensitivity in small structures such as mouse brain tissue, and MEDI, although effective in larger-scale human data, can be overly influenced by morphological priors and may underperform when the structural contrast is weak or when the background field is not perfectly removed.

These results are consistent with previous findings that iLSQR performs well in high-resolution, small-animal imaging, balancing artifact reduction and quantitative accuracy [14] [41]. Comparing the present results with those described in [17], a general agreement is found: FANSI exhibits higher levels of artifacts and MEDI shows low noise. A discrepancy is observed related to iLSQR algorithm, which has been identified as high noise level algorithm in the literature. Regarding unnaturalness, all 12 analyzed brains display the same value, indicating consistent results across the dataset.

Among all background removal algorithms, VSHARP generally results in lower artifact values compared to LBV and PDF. PDF tends to produce the highest level of artifacts overall.

This finding aligns with mouse brain studies comparing PDF and VSHARP: for various spherical kernel sizes, VSHARP demonstrated lower phase variability within cortical and white matter ROIs and achieved higher tissue contrast-to-noise ratios (CNR). PDF, by comparison, resulted in significantly larger phase standard deviations and residual dipolar artifacts, especially near highly curved structures like the olfactory bulbs [42]. Additionally, comprehensive reviews note that methods based on spherical mean-value filters (like SHARP and its variants) are designed to effectively remove large-scale harmonic background contributions, offering better suppression of spatially smooth artifacts while preserving local field structures [43]. PDF, relying on a projection theorem, often struggles in regions of high curvature or near brain boundaries, resulting in shading or streaking artifacts [44].

To conclude the study, the best-performing combinations identified in the phantom analysis were tested on mouse brain data to assess their effectiveness in a biological context. These combinations consistently resulted in lower artifact levels, particularly in terms of noise and streaking, thus confirming their robustness and versatility.

Results are shown in the following figures, where VSHARP-based reconstructions were selected for comparison as they exhibited the lowest levels of artifacts across methods.

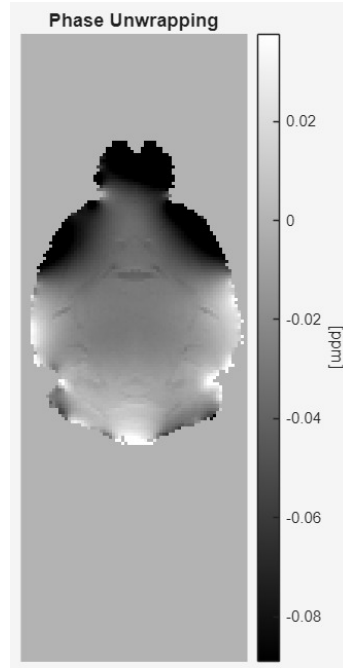


Figure 4.5: Phase Unwrapping Result on the mouse brain using Optimum Weights Combined with GraphCut Algorithm.

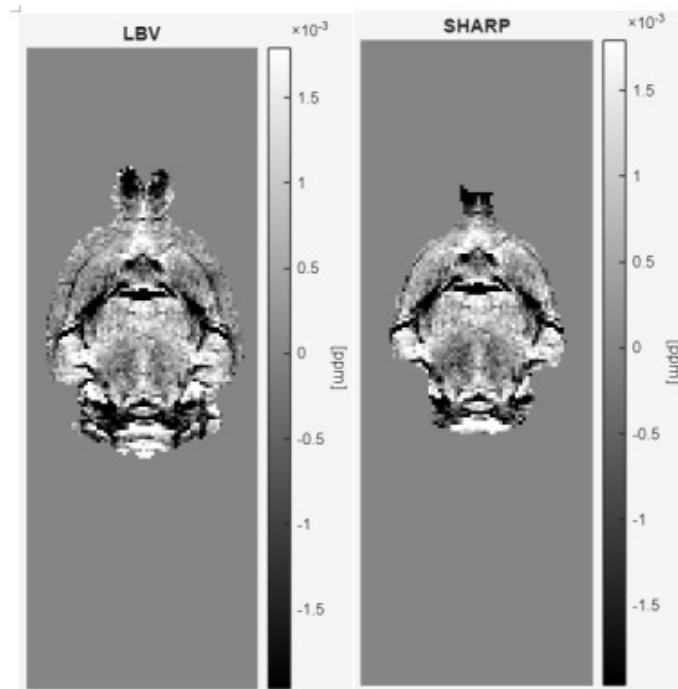


Figure 4.6: Background Field Removal Result on the mouse brain using LBV and SHARP Algorithms.

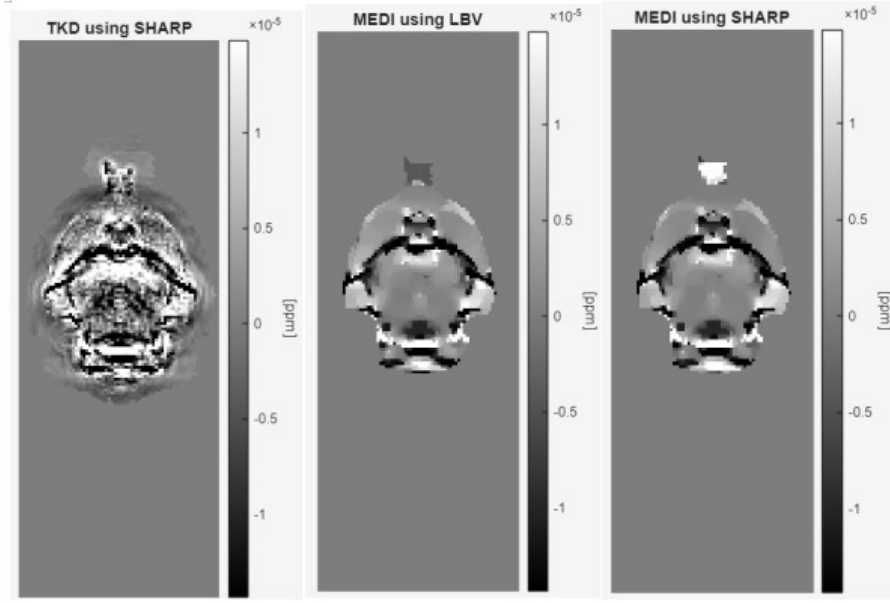


Figure 4.7: QSM Result on the mouse brain using TKD with SHARP, MEDI with LBV and MEDI with SHARP Algorithms.

Figure 4.7 illustrates the qualitative differences in susceptibility maps obtained using TKD with SHARP, MEDI with LBV, and MEDI with SHARP. The TKD reconstruction presents higher contrast in fine structures but is also affected by pronounced streaking artifacts, particularly visible in regions adjacent to strong susceptibility sources. In contrast, MEDI-based reconstructions (both with LBV and SHARP background field removal) yield cleaner images with reduced noise and streaking, improving the delineation of anatomical boundaries. Between the two MEDI variants, LBV shows slightly smoother background regions, while SHARP maintains a marginally higher definition of local susceptibility variations. These differences highlight the influence of both the inversion algorithm and the background field removal strategy on the visual quality and interpretability of QSM results.

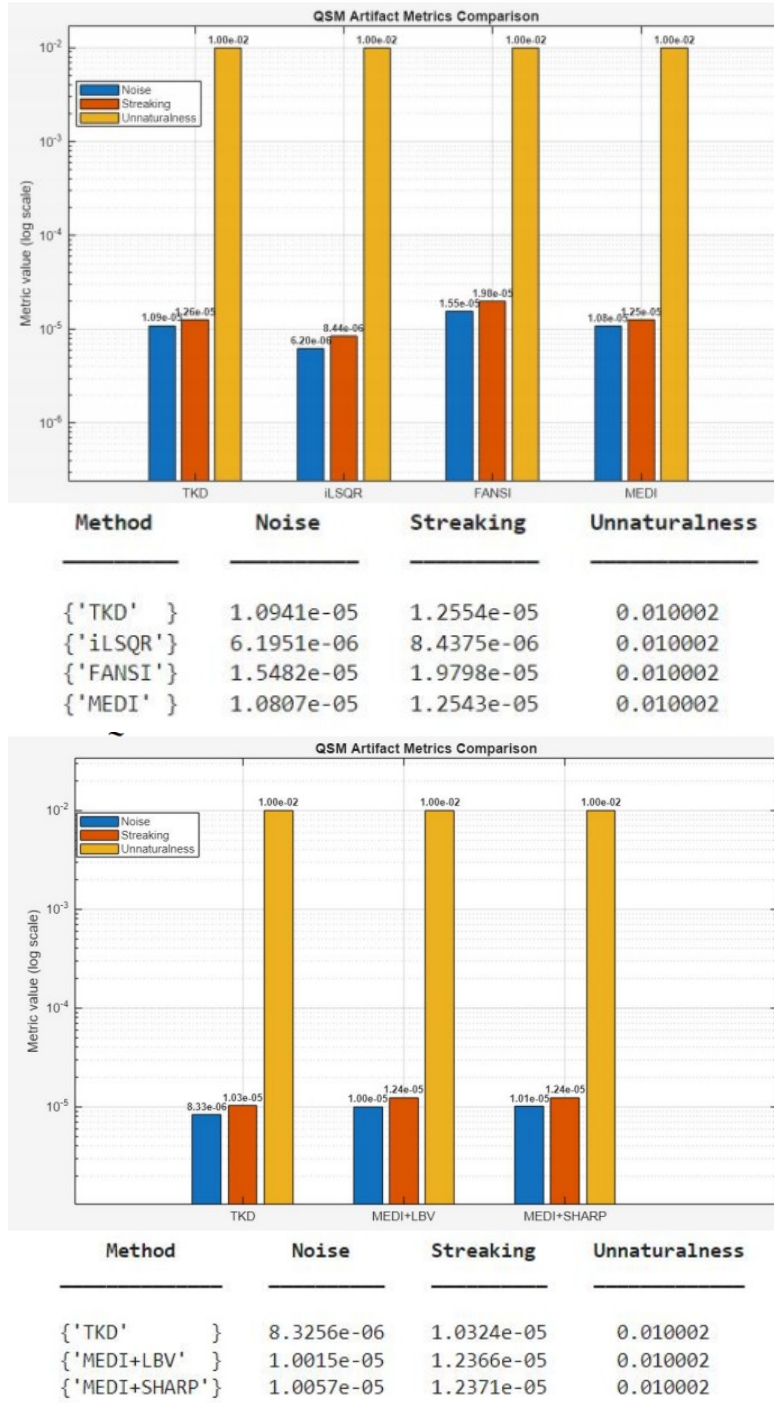


Figure 4.8: On the top: quantitative comparison of artifact metrics obtained from QSM reconstructions using four inversion methods (TKD, iLSQR, FANSI, and MEDI) in combination with the V-SHARP background field removal technique. On the bottom: analogous artifact analysis performed on the three best-performing reconstruction pipelines identified from the first comparison (TKD, MEDI+LBV, and MEDI+SHARP). The three evaluated metrics are Noise, Streaking, and Unnaturalness, expressed in logarithmic scale to emphasize differences across methods. The numerical tables below each plot provide the corresponding quantitative values for each metric.

These results confirm the trend observed in the previous sections: the use of optimum weights combined with a GraphCut background removal consistently reduces both noise and streaking artifacts, while preserving the overall natural appearance of the susceptibility maps. This improvement is evident when comparing TKD with MEDI-based reconstructions, where the latter show lower noise levels and reduced streaking despite slightly increased computational complexity.

Conclusions

This thesis was carried out within the framework of a research project aimed at advancing the imaging and therapeutic potential of extracellular vesicles (EVs) in neurodegenerative diseases. Among the various goals of the project, this work specifically addressed enhancing the sensitivity and precision of EV tracking using advanced magnetic resonance imaging (MRI) techniques, with a focus on Quantitative Susceptibility Mapping (QSM).

The research focused on validating and optimizing QSM for preclinical applications, particularly in controlled phantom experiments and in mouse brain samples. A custom-designed phantom with multiple wells containing different concentrations of Vivotrax was used to assess the sensitivity of QSM to varying magnetic susceptibilities. All QSM processing was performed using the SEPIA toolbox, which allowed testing and comparing different reconstruction algorithms. The study showed that TKD provided the highest sensitivity at low concentrations, with linear calibration curves down to approximately 0.1–0.25 mg/mL, while iLSQR and MEDI produced more stable reconstructions with reduced artifacts, lower RMSE values, and more uniform susceptibility maps in both phantom and mouse brain datasets. These experiments demonstrated that QSM is capable of detecting susceptibility differences correlated with Vivotrax concentration starting from T_2^* -weighted images, thus confirming its potential for quantitative tracking of iron-labeled EVs. QSM is characterized by its remarkable sensitivity, enabling the visualization and quantification of very low susceptibility values that are often undetectable with conventional MRI techniques. This high sensitivity is essential for studying subtle changes in tissue magnetic properties, which are critical in various biomedical applications. For example, a multisite reproducibility study found that QSM yielded within-site precision of about 0.005 ppm in regions like the caudate nucleus, putamen, and globus pallidus, compared to coarser precision (~ 0.01 ppm) in more challenging regions such as the substantia nigra and red nucleus. [45]

In parallel, the QSM technique was applied to ex vivo mouse brain scans without the use of contrast agents, in order to evaluate the intrinsic susceptibility contrast and the performance of QSM under physiological conditions. A systematic comparison of different combinations of QSM processing steps was conducted to identify the most accurate and robust reconstruction pipelines. The results revealed that the choice of processing pipeline significantly impacts the quality of the final susceptibility maps.

Moreover, even in neurodegenerative diseases, susceptibility values tend to remain relatively low, making their detection challenging. QSM can resolve these sub-

tle changes, as shown in Alzheimer’s disease (frontal gray matter ≈ 0.011 ppm vs -0.008 ppm in plaque-free tissue, globus pallidus 0.277 ppm vs -0.009 ppm in controls [46]) and Parkinson’s disease (substantia nigra 0.224 ppm vs 0.199 ppm, sensitivity 90%, specificity 86% [47]). QSM’s ability to detect these subtle susceptibility variations supports its potential utility in studying pathological changes associated with neurodegeneration. It is important to note that QSM calculates an average susceptibility value within each voxel, reflecting the mean magnetic properties over that volume. Consequently, in complex biological tissues where microstructural heterogeneity exists, the measured susceptibility differences may appear less pronounced. This intrinsic spatial averaging partly explains the modest differences observed in our results and highlights the ongoing need to improve QSM techniques including enhanced spatial resolution and advanced reconstruction methods.

From a methodological perspective, the phantom experiments indicated that the combination of MEDI with LBV background removal offered the best balance between noise suppression and preservation of susceptibility contrast, enabling reliable detection of Vivotrax concentrations as low as 0.02 mM. TKD demonstrated higher sensitivity to subtle susceptibility variations, being able to resolve susceptibility differences corresponding to the lowest Vivotrax concentrations tested (~ 0.1 – 0.25 mg/mL, i.e. variations of the order of 0.01 ppm), but was more affected by noise and streaking artifacts, confirming its role as a high-sensitivity but lower-specificity method. In preclinical mouse brain scans, MEDI-based reconstructions again proved optimal for minimizing artifacts while preserving anatomical fidelity, with noise levels around $1.0 \cdot 10^{-5}$ and streaking artifacts reduced by approximately 20–25% compared to TKD. These quantitative findings support the selection of MEDI pipelines for future in vivo studies where robust artifact suppression is critical. These results contribute directly to the achievement of providing a methodological foundation for reliable susceptibility mapping in preclinical models. The ability to quantitatively detect susceptibility changes in a controlled setting, as well as in unaltered biological tissue, supports the feasibility of applying QSM for in vivo tracking of EVs in future studies.

The study has some limitations: the experiments were performed on phantoms and ex vivo samples, which do not fully replicate the complexity of in vivo conditions, such as motion, physiological noise and perfusion. Moreover, the absence of contrast agents in the mouse brain scans limited the evaluation of QSM’s sensitivity for detecting EVs directly. Therefore, future work will focus on applying the validated QSM pipelines to in vivo datasets acquired from animal models injected with magnetically labeled EVs. Further optimization of acquisition parameters, together with the integration of QSM data with other imaging modalities such as MPI and CT, will also be considered to improve sensitivity and spatial resolution.

In conclusion, this thesis has demonstrated the feasibility and reliability of QSM for detecting and quantifying magnetic susceptibility variations in preclinical MRI settings. It has also highlighted the importance of careful selection of processing algorithms to ensure accurate and interpretable results. These contributions represent a crucial step toward the implementation of QSM as a sensitive and quantitative

imaging tool for tracking EVs in the context of neurodegenerative disease research.

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