Total Iron Overload Measurement in the Human Liver Region by the Susceptometer Magnetic Iron Detector (MID)

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Introduction

The present work is devoted to study the application of susceptometry for the non-invasive measurement of iron overload in the human body.

An accurate assessment of body iron accumulation is essential for the diagnosis and therapy of iron overload in diseases such as hereditary hemochromatosis, thalassemia and other forms of severe congenital or acquired anaemia. For example, in hereditary hemochromatosis, the subject adsorbs an excess of iron from the diet every day, and in thalassemia major, the iron overload is caused by the frequent blood transfusions administrated to the patient to contrast its anemia. Being toxic, the iron in excess must be removed by a tuned therapy: for this reason hemochromatosis patients are subjected to phlebotomy therapy while a chelation therapy is administrated to transfusion dependent patients.

The organs which are more frequently affected by iron overload are liver, heart and endocrine glands [1]. Liver Iron Concentration (LIC for short) is used as a reliable indicator of the overall body iron burden [2]. In normal subjects, LIC is between 0.1 and 0.6 mg of iron per gram of liver measured in wet weight of tissue (mg/gram ww). In severe iron overload states, the liver iron concentration can be one order of magnitude higher than the normal values [1]. LIC up to about 10 mg/gram ww was measured by the suscepectometer described herein and, before the introduction of chelation therapy, when the maximum estimated life was about 20 years, LIC up to 50 mg/gram ww were measured. Up to now, the liver biopsy is considered the gold standard to evaluate LIC [1]. Nevertheless, this measure is made problematic by the risks associated with the procedure itself and by the sampling error introduced by the small size of the sample. Quantitative methods to measure the amount and distribution of body iron stores that are noninvasive, safe, accurate, and readily available could improve the assessment and management of patients with thalassemia and transfusional iron overload.

The Superconducting Quantum Interference Device (SQUID) susceptometry and Magnetic Resonance Imaging (MRI) are the only validated non-invasive alternatives to liver needle biopsy for measuring LIC. The susceptometer Magnetic Iron Detector (MID for short) obtains the amount of iron in the liver region by measuring the susceptibility of the human body. A magnetic field $B$, applied to the human body, induces a magnetization of the tissues which generates, in the neighborhood of the body, a small change of the field ($\Delta B < 10^{-6}B$). To perform the measure, a low frequency magnetic field is applied to the body and
the small change of the magnetic field is measured by a pickup coil and a lock-in amplifier.

The iron overload is then obtained making the difference between the measured magnetization signal of the patient and its background signal. The latter is the magnetization signal that the patient would generate with a normal iron content. The evaluation of the background signal is performed under the hypothesis that the magnetization signal of a patient, with no iron overload, is the same as the one of a volunteer with the same anthropometric characteristics.

About 150 healthy volunteers have been measured and several models have been developed to solve the problem of calculating the background signal of patients from their anthropometric characteristics. The first model was developed essentially looking at the correlation coefficient between the measured magnetization signal and the anthropometric variables. Another approach, based on statistical learning technique, was applied later. This technique is an example of supervised learning, or learning from examples, and refers to systems that are trained with a set of input-output pairs [3]; in this context, training means synthesizing a function that best represents the relation between the inputs and the corresponding outputs.

As an alternative, the background signal can be also calculated directly, knowing the shape of the body, the contribution of a unitary volume of matter to the magnetization signal and the susceptibility distribution of the body. The specific contribution to the signal of MID was measured as a function of the space with a probe. Moreover, for each subject, the 3D body shape is measured with a system of lasers. A first model to calculate the magnetization signal of the patient was constructed assuming a uniform distribution of the susceptibility, equal to that of water (whence the name waterman). This model has then been updated, in order to account for the empty region inside the body (i.e. the lungs) and outside it (i.e. the cavity of the body that the laser system is unable to detect). This technique was tested on about 80 volunteers, who were measured both with MID and with the laser system.

The present MID sensitivity is about 1 g (1std) and the reproducibility of the iron overload measurement of the same patients is better than 0.5 g. For comparison, an healthy liver contains a mean of about 0.4 g of iron.

Between February 2005 and February 2010, the MID has been used by the Galliera Hospital of Genoa and about 750 patients have been measured avoiding biopsies to the patients. The MID measurements were correlated with the results of liver biopsies, SQUID susceptometer, MRI and blood serum ferritin concentration measurements, as well as with the results of phlebotomy therapy in hemochromatosis patients. As an example the follow-up of a hemochromatosis patient undergoing phlebotomy therapy is reported in the figure 1; the black lines of figure 1A refer to the measured signal and the gray one to the background signal.

The results and the descriptions of MID have been presented to a number of conferences both of physics and medicine. Moreover the apparatus was at-
Figure 1: Hemochromatosis patient. (A) Represents the measured magnetization signal (a) and the calculated background signal (b) of the hemochromatosis patient. The first measurement (i) was carried out on 7th March, 2005 and the last one (vii) on 26th October, 2007. (B) Represents the liver iron overload measured by the MID and the iron removal estimated from phlebotomy therapy.

tributed in 2008 with both US and European patent. Both patents are shared with Galliera Hospital of Genoa.

The description of the apparatus and of the method used to acquire the magnetization signal of the patient is the object of Chapter 1. The method used to obtain the iron overload and the clinical results will be presented respectively in Chapter 2 and 3. Finally, Chapter 4 is devoted to the analysis of the models based on statistical learning technique and on waterman.
The ability to accurately measure the body iron overload is essential for managing iron chelation therapy, both to prevent iron toxicity and to avoid the adverse effects of over-chelation. In addition, in hereditary and non-hereditary forms of hemochromatosis, the determination of body iron stores allows to identify those individuals that risk an iron-induced organ damage and would therefore benefit from phlebotomy therapy [4]. Perhaps, the simplest indirect method to measure body iron overload and to determine the adequacy of chelation therapy is the analysis of serum ferritin concentrations [5]. However, these simple biochemical measurements may be invalidated by certain clinical conditions, such as infections, inflammations and cancer [6].

The direct measurement of Liver Iron Concentration (LIC for short) by chemical analysis of needle biopsy samples is considered the ”gold standard” for the evaluation of the liver iron concentration [1]. Nevertheless, this measure is made problematic by the risks associated with the procedure itself and by the sampling error introduced by the small size of the sample. The latter can actually lead to large errors, due to the heterogeneous distribution of iron deposition in the liver [7, 8].

Over the past two decades, non-invasive methods of measurement of iron overload have been developed. As a result, LIC can be measured non-invasively both by Magnetic Resonance Imaging and biomagnetic liver susceptometry. The MRI relates LIC to the decreasing of the transverse relaxation time of the proton of hydrogen in the presence of iron. From a technical viewpoint, the more the iron is and the darkest the T2*-weighted images are [4]. The reciprocal of T2* increases with the iron concentration [4, 9]. On the other hand, magnetic liver susceptometry measures the variation of the magnetic field produced by the magnetization of both the body tissues (mainly diamagnetic) and of the iron deposits (paramagnetic). The apparatus discussed in this work is a susceptometer, named Magnetic Iron Detector (MID for short) [10, 11, 12, 13, 14]. MID was
constructed in the INFN laboratories of Genoa and has been measuring patients at Galliera Hospital of Genoa since February 2005. At present (February 2010), about 750 patients and 150 volunteers have been measured. The MID is the only apparatus capable of measuring the total iron overload of the whole liver, as opposed to the existing competing methods, that actually return the LIC measured over a limited region of the liver.

1.1 The working principle of magnetic susceptometry

A magnetic field $B$ applied to a human body induces a magnetization of its tissues (figure 1.2). This magnetization generates a small change $\Delta B$ of the field in the neighborhood of the body, depending on the magnetic properties of the body itself.

The variations of $\Delta B$ generated by the magnetization of the iron deposit in the tissues are the object of the measurement. We remind that the biological tissues have diamagnetic properties; their magnetic susceptibility is similar to that of water $\chi_{\text{water}} = -9 \cdot 10^{-6}$ (SI units).

As a matter of fact, iron deposits into two main storage proteins, ferritin and hemosiderin that exhibit paramagnetic behavior at the temperature of interest [15]. At 310 K, the positive contribution of a concentration $C_{\text{iron}}$ of iron atoms to the susceptibility is $\chi_{\text{iron}} \sim (1.5 \cdot 10^{-6} \text{cc/mg}) C_{\text{iron}}$. Because the mean iron concentration in a healthy liver is about 0.3 mg/cc [1], it gives a positive contribution to susceptibility which is, considering the absolute values, less than 1/10 of what water does.

The relative variation $\Delta B/B$ produced near the body by the magnetization of tissues can be shown to be of the same order as susceptibility itself. As a consequence, the instrumental sensitivity needs to be better than one part over $10^6$.

Figure 1.2: Magnetization of the body in a magnetic field.

The magnetization of body tissues produced a variation of the magnetic field $\Delta B$. As a consequence of the diamagnetic properties of tissues, a decreasing of the field is produced (i.e. $\Delta B$ is negative). The presence of iron overload makes this decrease less intensive; only in severe form of iron overload $\Delta B$ may assume positive sign.

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1 The numerical coefficient was calculated associating an effective magnetic number of 4 $\mu_B$ to each iron atom (see section 2.2).

2 For example, the calculation of the variation of the magnetic field produced by the magnetization of a sphere in an uniform magnetic field is $2/3 \chi$, on the surface of the sphere under the hypothesis that $\chi \ll 1$. 

1.1 The working principle of magnetic susceptometry

Figure 1.3: MID instrumentation (A): The magnet and the twin pickup coils are placed in a temperature-controlled environment. Two pickup coil sensors are placed symmetrically with respect to the magnet: the sum of their signals is zero in the absence of the body. The gray scale figure and the graphs report the specific contribution of the present magnet and pickup coil sensor arrangement to the magnetic signal. (B): The external view of MID and of the stretcher used to place the body into the measurement region. The stretcher can be moved in the X direction while the Y scan of the body can be performed placing the patient in different positions along the stretcher.

ten millions, in order to measure the variations of magnetic field of interest.

The schematic of the MID susceptometer is shown in figure 1.3. During the exam the patient is placed between the magnet and the pickup: this is called the measurement region. The opening of the MID, available for the human body and the stretcher, is 265 mm wide. Here, the amplitude of the AC field $B$ is lower than 190 Gauss and the frequency is 234 Hz ($\omega \sim 1500 \text{ s}^{-1}$). The 190 Gauss value of the field intensity is the limit imposed by the CEI norm to this working frequency [16]. This is the maximum value of the field in the measurement region: the mean magnetic field applied to the patient abdomen is about 20 Gauss. Two twin pickup coils are symmetrically located with respect to the magnet. Their electrical connections are such that the signal measured is zero when no patient is in the measurement region. The magnet is made of 128 turns of hollow copper conductor, through which cooling water flows, and has a mean diameter of 140 mm (figure 1.6 A). The current through the magnet is $39 \text{ A}_{RMS}$ at 234 Hz. Each pickup coil is made of 740 turns and has a mean diameter of 161 mm. The mean voltage induced by the AC field flux is about 3 V$_{RMS}$. The voltage variation corresponding to the expected $\Delta B \sim 10^{-6}$ Gauss, is $(\omega NS\Delta B)$ and it results to be a few $\mu V$. This signal is greater than both the Johnson noise of the pickup and the noise of the lock-in amplifier by means o
which the measurement is performed.

1.2 The apparatus description

The most relevant difference between the MID apparatus and other existing susceptometers is the disposition of the magnet and pickups with respect to the patient.

The SQUID susceptometer has been used to measure liver iron concentration for more than 20 years [2, 17, 18]. It uses the Superconducting Quantum Interference Device (SQUID) to measure the magnetic field variation produced by the magnetization of the body. The sensitivity of this cryogenic device is such that it can detect fractions of the magnetic flux quantum ($2.07 \cdot 10^{-15}$ Wb). In order to measure body iron overload, the sensitivity of the apparatus must be sufficient to detect the small (one part over ten million) change in the magnetic field produced by the iron contribution. Even in the presence of this very small relative modification of the magnetic field, the absolute change of the magnetic field flux, produced by the human body and threaded with the pickup, is equivalent to millions of magnetic flux quanta: so, SQUID is not necessary to detect it and a simpler, non-cryogenic pickup coil can be used. Freed from the constraint of using a cryogenic device, it is possible to design a susceptometer that is capable of measuring the iron overload in the whole liver region or in other body parts having equivalent dimensions. Placing the body between the magnet and pickup and making their diameter comparable with the dimension of the liver, it is possible to have an apparatus capable of measuring the magnetization signal generated by the whole liver (figure 1.3).

The magnet and pickup coils of SQUID susceptometer (respectively in a first and a second order gradiometer configuration) are placed on the same side with respect to the patient [19, 20, 21] (figure 1.4). SQUID susceptometry measures the iron content of tissues by comparing the signal of the region of the human body within its sensitivity region with a signal from an equivalent amount of water. As a consequence of this, the diameter of each coil is a few centimeters, to make the lung contribution to the signal negligible. Because the magnetic susceptibility of the lungs is
Figure 1.5: Sketch of the MID. The MID is composed by the magnet and pickup coil, the structure and the thermal shield. In the figure, two concentric magnets and two sets of concentric pickups are drawn. This configuration was designed to generate a total of four weight functions, in order to be capable of weighting the tissues of the body in four different ways.

about one half that of water, the comparison of their signal with that generated by the water would be detected erroneously as iron overload. As a result, such an assembly measures the signal generated from the superficial region of the abdomen of the patient: this is why the measurement performed by this instrument is called ”magnetic biopsy” [2]. Moreover, other groups developed room temperature susceptometers which possess a geometrical configuration of the magnet and the sensors that is very similar to the SQUID one [22, 23]. None of them are recognized as validated techniques.

The gray scale picture in figure 1.3 reports the weight function for the magnetic configuration of the MID. For comparison, figure 1.4 reports, in arbitrary units, the weight function of SQUID configuration.

According to [24], the magnetization signal of a body, with the magnetic susceptibility distribution $\chi(\vec{r})$, is

$$\phi = \int_V g(\vec{r}) \chi(\vec{r}) \, d\vec{r}$$

where $V$ is the volume of the body. The weight function $g(\vec{r})$ represents the contribution of a unitary volume of matter, having a unitary magnetic susceptibility, to the signal generated by the magnetization of the body. The value of $g(\vec{r})$ is proportional to the number of turns of both the magnet and the pickup coil and to the frequency and amplitude of the current flowing in the magnet. Its maximum value is $850 \, V_{RMS}/m^3$. A way to calculate the function $g(\vec{r})$ is to make the scalar product between the magnetic field generated by the magnet and the one that would be generated by the pickup, supposing a unitary current flowing into it (Appendix A). The value of the weight function was calculated using the Vector Fields software for electromagnetic design Opera and experimentally verified using water samples. In figure 1.5, the sketch of the system is reported; it is composed by a magnet, two pickup coils, a support structure
The Magnetic Iron Detector description

Figure 1.6: MID pictures. A: Magnet (left) and pickup (right): the pickup assembling (top) and the single printed board (bottom) which the pickup is realized with. On the board, two concentric magnets and two sets of concentric pickups are present. This configuration was designed to generate a total of four weight functions, each weighting in a different way the tissues of the body. Only the inner magnet and the inner pickup are used. B: MID structure. A fiber-reinforced resin structure holds the susceptometer magnets and pickups. C: MID thermal shield.

Figure 1.7: The thermal drift induced by a warm body. The magnetization signal of a rat measured with a smaller susceptometer. In the left part of the graph the shield was enabled; in right one it was disabled.

and a thermal shield. The data presented in this work refers to the configuration with the inner magnet and the inner pickup. This configuration was chosen making a trade-off between reducing the spatial spread of the weight function and allowing a significant contribution to the signal by the inner part of the body (i.e. the whole liver).

A fiber-reinforced resin structure holds the magnets and the pickups of the susceptometer into position (figure 1.6B). A thermal shield wraps this structure without touching it (figure 1.6C). The temperature of the whole susceptometer is controlled by water flowing through a large number of adjacent channels that are embedded within all the walls and the roof of the thermal shield. The temperature of the magnets is kept stable within a few milli-Kelvin by temperature controlled water, flowing through their hollow copper conductors.
1.2.1 The control of temperature

In order to measure variations of the magnetic field lower than 1 ppm, the thermal expansions of the system must be controlled. A variation of the pickup surface or a displacement of the pickup coil in the gradient of the magnetic field may produce a variation of the signal that would not make the measurement possible. The relative variations of the voltage of the pickup need to be lower than the susceptibility of the body in order to detect the variation of the magnetic field of interest (section 1.1): \( \frac{\Delta V}{V} \sim 10^{-7} \). Being \( \frac{\Delta V}{V} \) of the same order of magnitude as \( \frac{\Delta l}{l} \), and being \( \Delta l \) connected with the thermal expansion of the system (i.e. \( \sim \alpha \Delta T l \), with \( \alpha \sim 10^{-4}K^{-1} \), the thermal expansion coefficient, and \( l \) of the order of the MID dimensions), the temperature must to be stable on the scale of milli-Kelvin. Two requirements must be satisfied by the construction specifications of MID: a control of the temperature on the scale of milli-Kelvin, which makes the structure stable, and a mechanical protection, which prevents the contact with the structure itself. Both requirements are satisfied by the thermal shield which wraps the structure without touching it (figure 1.6C).

Example. Let us explicitly consider the example of the displacement of the pickup with respect to the magnet: the variation \( \Delta z \) of the distance between the magnet and one pickup coil produced by thermal expansion must give rise to a variation \( \Delta V \) lower than the one we want to detect (i.e. lower than \( 10^{-6}V \)). The variation of the voltage produced by a displacement of the pickup is equal to \( \frac{\partial V}{\partial z} \sim 20 V/m \). If we suppose that the displacement \( \Delta z \) of the pickup with respect to the magnet is produced by the thermal expansion alone, we can ascribe it to a temperature variation \( \Delta T \) of

\[
\Delta T \sim \frac{\Delta z}{\alpha z_0} \sim \frac{1}{\alpha z_0} \frac{\Delta V}{\partial z} \sim 10^{-3}K
\]

where \( z_0 \) is the distance between magnet and pickup (\( \sim 0.5m \)) and \( \alpha \sim 10^{-4}K^{-1} \) is an estimation of the thermal expansion coefficient of the structure and it takes into account both the material and the assembling of the structure.

In order to show how thermal drift can create difficulties, a measurement performed with the small version of a small version of susceptometer, used for rats, is reported in figure 1.7. The first part of the graph reports the signal produced by the insertion and the extraction of the living rat from the measurement region. In the second part the shield was disabled and a sudden appearance of a drift of the signal can be observed.

1.2.2 The zero procedure

As previously discussed, the voltage induced in the pickup, is 1 million times greater than the signal of interest. The voltage induced at the edge of the pickup coil is about 3 V\textsubscript{RMS}. To reject the common mode signal, the pickup assembling is differential: two pickup coils are symmetrically located with respect to the magnet and their electrical series is connected to the lock-in amplifier. The symmetry of the mechanical assembling is such that the signal is zero within some tens of mV. This residual signal is compensated connecting a small coil,
threading the necessary amount of magnetic field flux, in electrical series with the pickup (figure 1.8). This coil is composed by two parts (A and B) allowing to perform the zero procedure in two steps. In the first one, a subset of the coils of pickup A is selected and connected to the main pickup: this procedure makes the signal to be a few tens of µV. In the second, a fine regulation is performed, varying the flux concatenated with the coil B, by means of another field which is phase-locked with the principal magnetic field.

**NOTE.** A printed board, assembled with the main pickup, contains the coils among which the subset, which composes the pickup A, is selected. Each coil was designed in such a way that the flux threaded with it is a definite fraction of the flux threaded with the main pickup coil. The flux of magnetic field threaded with the bigger coil is 1/16 (i.e. $2^{-4}$) of the one threaded with the main pickup coil, and the others respectively $2^{-5}$, $2^{-6}$, $2^{-7}$ ... $2^{-15}$. With this technique, a limited number of coils (12) can correct every magnetic field flux between 1/10 and $10^5$ of the flux threaded with the main pickup. At present, after the start up of the magnet is performed, an automatic procedure select the coils, producing a zeroing of a part over $10^5$. The last stage of the zeroing procedure is performed varying the current in the little magnet near the pickup with B in figure 1.8. A 12 bit DAC system pilots its current, phased locked with the AC magnetic field. A dipole disposition of the pickup B coils makes the flux of the magnetic field generated by the principal magnet negligible. Before every measurement, an automatic procedure adjusts the current, making the signal measured by the lock-in amplifier lower than 1 µV.

### 1.3 The Magnetization and the Eddy Current Signal

The presence of the patient in the measurement region makes the difference between the voltage induced in each pickup coil different from zero. This is the *magnetization signal* of the subject. For example, the magnetization signal generated by a healthy volunteer is reported in figure 1.9. The patient is put inside the measurement region by means of a stretcher (B in figure 1.3).

Beside the magnetization signal, the oscillating magnetic field also induces *eddy currents* in the human body. Considering the electrical resistivity of tissues
§1.3 The Magnetization and the Eddy Current Signal

Figure 1.9: The magnetization signal of a body. The magnetization signal measured by the lock-in amplifier during the insertion and extraction of a patient is reported in function of time.

Figure 1.10: Eddy currents. At the low frequency (234 Hz) of the oscillating magnetic field, the magnetic moment of the iron atoms oscillates with the same phase as the applied magnetic field and the phase lag of the eddy currents within the human body, relative to the induced electric field, is negligible. So, the magnetic signal of the oscillating iron atoms and the one of the induced eddy currents are out of phase by one fourth of period.
and the inductance of the average current loop within the body, it is possible to show that the phase lag of the eddy currents, relative to the induced electric field, is negligible. Therefore, at this low frequency, the magnetization of tissues oscillates with the same phase as the applied magnetic field. So, the phase difference between the magnetization and the eddy current signals is one fourth of period, i.e. when the first one reaches its maximum value the other one is zero (figure 1.10). This was also verified measuring the magnetic and eddy currents signals of a phantom, having the dimensions of a human trunk, filled with aqueous solutions of NaCl. The two signals are $\pi/2$ phase lagged with respect to each other and thus the digital lock-in amplifier (EG&G 7265) can perform the synchronous detection of both simultaneously. Only the magnetization signal is affected by iron overload.

### 1.3.1 The measurement procedure

The aim of this section is to describe the procedure applied to measure the magnetization signal when the signal is affected by a residual drift. Figure 1.11 reports the signal measured by the lock-in amplifier in function of time.

The magnetization signal, when the sample is inserted one time in the measurement region, is the difference between the signal measured when the patient is IN and the one registered when it is OUT. The drift makes the baseline to change with the time; so, the baseline that would be measured at the time in which the patient is IN is different from the one measured with the patient OUT. Some elaboration is required to evaluate the correct magnetization signal. The procedure requires the acquisition of three sets of measurements, each time putting the patient OUT, IN and OUT of the measurement region. The first and the last set of measurements (both with the patient OUT) are used to extrapolate the baseline of the signal at the time of the acquisition when the patient is IN the measurement region. The evaluated baseline is subtracted to the mean of the measured signal with the patient IN. Moreover, in order to account for

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**Figure 1.11: Signal elaboration.** The variation of the signal, measured by the lock-in amplifier, generated by the magnetization of the body is reported in function of time. At the time "OUT" the patient is out of the measurement region and at the time "IN" the patient is inserted in it. The linear fit is used to extrapolate the value that the magnetization signal, registered when no patient is present in the measurement region, during the insertion of the patient in it.
§1.3.1 The measurement procedure

Figure 1.12: Water and stretcher signal distributions. Left: distribution of the magnetization signal of a box filled with 6 liters of demineralized water. The signal is obtained subtracting the signal of the stretcher to the measured signal of water and stretcher together. The mean is $(-12.5 \pm 0.1) \mu V$. The true value of the mean is 0.1 $\mu V$ lower because the stretcher is measured together with the clothes typically worn by the patient. This contribution is not present during the measurement of water. Right: The distribution of the magnetization signal of the stretcher as a function of the position of its center relative to the magnetic field axis. The data reported (260) have been collected since June 2008, after the introduction of the automatic movement of the stretcher. The mean of the magnetization signal in X=-16 is $(0.61 \pm 0.05)$, in X=0 is $(0.77 \pm 0.05)$ and in X=+16 is $(0.88 \pm 0.05)$.

The changes of the environment magnetic properties (arrival or departure of a car from the nearby parking lot, shifting of a metallic furniture in the nearby rooms,...), the above described procedure is repeated a few times.

The stretcher, made by a honeycomb structure of electrical insulating fibers, is automatically translated on rails. Its small magnetic signature, with the coat worn by the patient, is always checked before each measurement. The distribution of the magnetization signal of the stretcher is reported in figure 1.12 (right) as a function of the position of the magnetic field axis relative to its center (figure 1.3): in the position 0 the center of the stretcher is in correspondence with the magnetic field axis. Of course, there are no eddy currents. The apparatus calibration is checked every day measuring the signal of the stretcher bearing a plastic box filled with water (rectangular base 16 cm 21 cm and containing 6.0 liters of deionized water) positioned in its center (figure 1.12 - left). The mean magnetization signal of the water sample is $(-12.5 \pm 0.1) \mu V$. In the right part of the figure, the signal of the stretcher is reported: it may be noted that it is about 1/10 of the one generated by the human body (figure 1.9).

In vitro calibration of MID was performed using water samples and was verified using aqueous solutions of iron chloride FeCl$_3 \cdot 6$H$_2$O. The measured magnetic moment for Fe$^{3+}$...
Figure 1.13: Tool used to measure the shape of the patient. This object is used both to perform the positioning of the patient on the stretcher (left) and register its shape (right).

matches the value of 5.9 $\mu_B$ reported by literature [25]. In addition, in order to verify that the magnetization signal of iron does not change, the magnetization signal of three solutions containing the same number of Fe$^{3+}$ ions in low conductivity water (LCW), 25% acetone + 75% LCW and 50% acetone + 50% LCW was measured. For each solvent composition, the iron-ion contribution to the signal was calculated as the difference between the signal of the solution with iron chloride and the one without it. The result was always identical and, given the number of iron atoms in solution, it was also possible to measure the Fe$^{3+}$ magnetic moment, which agrees with literature. According to [26], acetone can be used as an alternative to fat, because it is soluble in water.

1.3.2 In vivo measurement on patient

During the measurement, the patient is put inside the measurement region by means of the stretcher reported in figure 1.3. The patient’s position along the stretcher is such that the Y axis, positively oriented towards the head, is placed along the longitudinal symmetry axis of the body. The Y coordinate of the center of mass of liver is negative. The centre of the patient’s trunk falls on the X axis origin and the abscissa of the liver center of mass is negative. As the stretcher moves along the rails, the vertical axis of the magnetic field slides along the X axis. This allows the scanning of the whole liver region.

Figure 1.13 (left) shows a patient positioned on the stretcher: the tool placed over the patient is used to place him in the correct position and has been used to measure the anthropometric characteristics of some parts of the patients’ body until June 2008; figure 1.13 reports, in its right, the measured shape of the body in correspondence of the liver. In June 2008, a laser system was assembled, in order to perform this measurement more accurately (figures 1.14(a), 1.14(b)) [27, 28, 29].

As the stretcher moves along the rails, the vertical axis of the magnetic field
(a) Up: The system is composed by 6 lasers, which can be automatically moved along X and Y axis. **Down:** projection of the lasers on the stretcher surface.

(b) Example of the acquisition of the shape of a body of a volunteer. The dots in the right draft represent the measurement positions. The units in the scales are cm.

**Figure 1.14: Laser system.** The system is composed by 6 lasers whom can move in the XY plane. The automatized movement is performed by means of stepping motors.
Figure 1.15: The iron-overload contribution to the magnetic signal. (A) Anthropomorphic plastic phantom with different doses of paramagnetic powder. (B) The magnetization signal of a patient with liver iron overload and of a volunteer with similar anthropometric data. The abscissa $X$ (cm) is the position of the magnetic field axis relative to the center of the trunk.

The magnetic scan slides along the X axis and allows scanning the whole liver region. Each scan of the liver region is taken placing the centre of the patient’s trunk in several positions relative to the magnetic field axis (figure 1.3): the abscissa $X$ (cm) is the position of the magnetic field axis relative to the centre of the patient’s trunk. Figure 1.15 reports the magnetization signals of an anthropomorphic plastic phantom dosed with paramagnetic powder, equivalent to 6.5 g and 30 g of iron with a magnetic moment of $4 \mu_B$, evenly distributed throughout the liver region. Each point reported is calculated by the procedure described in subsection 1.3.1. The $\sim 100$ nV instrumental error (comparable with the point dimensions) means that the minimum quantity of detectable iron inside the entire liver region of the phantom is $\sim 270$ mg of iron. Figure 1.15 compares the signal from a patient, having an iron overload of about 9 g in the liver, with that of a healthy volunteer having similar anthropometric data.
Chapter 2

The iron overload measurement

The aim of this chapter is to describe the method used to evaluate iron overload in patients. This is calculated making the difference between the magnetization signal of the patient and its background signal, which is the magnetic signal that would be generated by the patient if ideally depleted of its overload (i.e. with a normal amount of iron in the body). The background signal is calculated by means of a statistical model, based on the anthropometric characteristics of the patient. The model, described in section 2.3, was developed using the magnetization signal of healthy volunteers and their anthropometric characteristics. At present, more than 1200 medical reports have been given using this procedure.

2.1 The procedure to calculate liver iron overload

As previously discussed, the magnetization signal of a body, having a magnetic susceptibility distribution \( \chi(\vec{r}) \), is

\[
\phi(\vec{R}) = \int_{V} g(\vec{r} + \vec{R}) \chi(\vec{r}) \, d\vec{r}
\]  

(2.1)

where \( V \) is the volume of the body, the vector \( \vec{r} \) joins the origin of the reference frame of the patient \( F_{body} \) to the points inside its volume. Finally, the vector \( \vec{R} \) is the position of the reference frame \( F_{body} \) with respect to the one of the MID magnetic system \( F_{MID} \) (figure 2.1). Because the scanning of the whole liver region is performed moving the stretcher along the X axis alone, the coordinate X may be used in place of the vector \( \vec{R} \). The patient may be potentially moved in the Y direction. The dimension of the opening of the MID make a displacement of the patient along the Z direction not available. The weight function of MID is reported in figure 1.3: its maximum value is 850 V/m³.

For a patient with iron overload, the magnetic susceptibility \( \chi(\vec{r}) \) is the sum of the iron-overload-free magnetic susceptibility \( \chi_{Tissue} \) and of the iron-overload
Figure 2.1: Definition of the reference frames $\mathcal{F}_{body}$ and $\mathcal{F}_{MID}$. The origin of the frame $\mathcal{F}_{body}$ is in correspondence with the center of the stretcher where the patient is lied out. The origin of the frame $\mathcal{F}_{body}$ is in correspondence with the center of the stretcher. The $Z$ axis of the frame $\mathcal{F}_{MID}$ coincides with the magnetic field axis. The two frames coincide when the vector $\vec{R}$ is null.

Figure 2.2: MID weight function $g(\vec{r})$. The gray scale picture represents the weight function $g(\vec{r})$. It gives the contribution of a unitary volume of matter, having a unitary magnetic susceptibility to the magnetization signal. This is an enlargement of figure 1.3.
magnetic susceptibility $\chi_{Fe}$. Equation (2.1) becomes:

$$\phi(\vec{R}) = \int_V g(\vec{r} + \vec{R}) \chi_{\text{issue}}(\vec{r} + \vec{R}) d\vec{r} + \int_{V_{\text{OVL}}} g(\vec{r} + \vec{R}) \chi_{\text{OVL}}(\vec{r} + \vec{R}) d\vec{r},$$  \hspace{1cm} (2.2)

where $V_{\text{OVL}}$ is the volume where the iron overload is present.

The first addendum of equation (2.2) will be denoted by $\phi_0(\vec{r})$. It is the background signal of the patient, the one that would be generated by the patient if ideally depleted of its overload. The statistical model used to calculate this signal will be described in section 2.3, while other alternative models will be presented in chapter 4. At the moment, let us assume that the background signal is known. The second addendum of equation (2.2) represents the contribution of the iron overload alone to the signal. If the liver center of mass is positioned in correspondence with the magnetic field axis ($\vec{R}_{CM}$), the whole liver falls in the region where the weight function $g(\vec{r})$ has positive sign (figure 2.2). In this case, the magnetic signal is mainly generated by the magnetization of the liver and the iron overload refers to the liver only. Equation (2.2) becomes:

$$\phi(\vec{R}_{CM}) \sim \phi_0(\vec{R}_{CM}) + \chi_{\text{OVL}} \int_{V_{\text{Liver}}} g(\vec{r}) d\vec{r},$$  \hspace{1cm} (2.3)

where $\bar{\chi}_{\text{OVL}}$ represents the mean susceptibility of iron overload in the liver and $\vec{R}_{CM}$ the position of the reference frame $\mathcal{F}_{\text{body}}$ when the center of mass of the liver is in correspondence with the magnetic field axis.

It may be noted that the scan of the patient makes the detection of iron overload in other region of the body possible. For example, figure 2.3 shows a patient having iron overload in the spleen; after the splenectomy the iron overload disappeared. The signal of the excess of iron was detected by the MID in the left side of the body and the difference after the splenectomy was clearly measured [11]. Moreover, figure 2.3B compares the background signal calculated for the patient before the splenectomy to the magnetization signal measured afterwards: after the splenectomy the magnetization signal superimposes (within the error of the model) with the one predicted by the model.

At 310 K, the iron atoms stored in the deposit proteins have a paramagnetic behavior [15, 18, 30, 31]. From the Curie law, the contribution of $N$ atoms in the volume $V$ to the susceptibility is

$$\bar{\chi}_{\text{OVL}} = \frac{N \mu_0^2 \mu_{Fe}^2}{V 3kT},$$

where $\mu_0 = (4\pi \cdot 10^7 \text{ Henry/m})$ is the vacuum magnetic permeability, $k = (1.38 \cdot 10^{-23} \text{ J/K})$ is the Boltzmann constant, $T$ is the absolute temperature, $N$ is the number of atoms per unit of volume and $\mu_{Fe}$ is the effective magnetic moment of iron. From (2.3), introducing explicitly $\bar{\chi}_{\text{OVL}}$, the iron overload $Q$ in the liver can be calculated as:

$$Q = \frac{\phi - \phi_0}{(1.45 \cdot 10^{-6} m^3)(R_g / V_{\text{Liver}})} \left( \frac{1}{V_{\text{Liver}}} \int_{V_{\text{Liver}}} g(\vec{r}) d\vec{r} \right)$$  \hspace{1cm} (2.4)
The iron overload measurement

**Figure 2.3: Patient with iron in the spleen.** The X coordinate of the spleen is positive. **A:** the magnetization signal measured before splenectomy (a) is compared with the one measured after (b). **B:** the background signal (c) calculated before the splenectomy is compared with the magnetization signal measured after it (b).

where $\phi$ and $\phi_0$ are respectively measured and calculated in the position where the axis of the magnetic field passes through the center of mass of liver. The numerical coefficient was calculated assigning an effective magnetic moment of $4 \mu_B$ to the iron atoms (see section 2.2). The second term in the denominator is the mean value of the weight function $g(\vec{r})$ over the liver volume. In order to calculate this contribution, a standard shape is ascribed to the liver of the patient and its volume is assigned as a function of the body weight; the formula is valid for western adults with no hepatic abnormality and is obtained from the literature [32]. As an example, the liver volume, corresponding to a body weight of 70 Kg, is about 1500 cc. The formula to obtain the liver volume was also tested measuring the liver shape of a few volunteers using MRI. For some healthy volunteers and patients, the volume calculated is compared to the one measured by means of MRI images: the results are reported in table 2.1. In the case considered, the results agrees for volunteers and underestimates the liver volume in patients. In some patients with hepatomegalies, the liver volume can even reach the value of 2000 cc. The mean value of the weight function $g(\vec{r})$ depends poorly on the dimensions of liver; for instance, the error on the iron overload $Q$, corresponding to an increase of the liver volume of about 30%, is lower than 10%. This systematic error may be comparable with the one made by the models for the calculation of the background signal only in the most severe iron overloaded states; otherwise it is negligible. Figure 2.4 reports, for different liver volumes, the contribution to the magnetic signal of 1 g of iron, uniformly distributed over the liver: the maximum of the signal is reached in correspondence of the center of mass of the liver. Although the liver volume triplicates, the maximum of the magnetization signal changes of about 25 %.

The MID susceptometer measures the total iron overload within the liver region. The aim of the therapy is to reduce the iron overload. Since other non invasive methods provide liver iron concentration, in order to make a comparison
2.1 The procedure to calculate liver iron overload

with the MID, the measurement of the latter is divided by the weight of the liver
and then the mean basal iron concentration (0.3 mg/g [1]) of the healthy liver
is added. However, it may be noted that, this procedure over-estimates the LIC
in patients with a liver enlargement. This is because the method attributes a
normal liver to a patient with an enlargement of the organ.

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<th>MRI evaluation [cc]</th>
<th>% Error</th>
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<td>1356</td>
<td>3%</td>
</tr>
<tr>
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<td>1599</td>
<td>1687</td>
<td>5%</td>
</tr>
<tr>
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<td>1597</td>
</tr>
<tr>
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<td>Pat. 3</td>
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<td>1062</td>
<td>1863</td>
</tr>
</tbody>
</table>

Table 2.1: Liver volume estimation. Comparison between the liver volume calculated by [32] \( V_{\text{Liver}} = 191.80 \text{cc} + 18.51 \text{cc/Kg} \cdot W \) where \( W \) is the body weight and the one calculated from the MRI images. For the two volunteers the agreement is within 5%, for patient with hepatomegalies the agreement is between 30 ÷ 40%.

Figure 2.4: Magnetization signal of 1 g of iron in the liver. (A) Contribution to the magnetization signal of 1 g of iron uniformly distributed in the liver. Each liver has a standard shape and a volume attributed from the body weight (20 Kg, 40 Kg, 60 Kg and 80 Kg). The position X=0 coincides with center of mass of each liver. (B) Decreasing of the maximum contribution to the signal of the liver in function of its volume (or body weight).
2.2 Physical properties of iron deposits in tissues

The aim of the present section is to give an overview of the magnetic properties of iron in biological tissues. This is necessary to justify that the effective magnetic moment of the iron stored in tissues is 4 $\mu_B$.

As previously cited, the iron deposits are predominantly in the form of ferritin and hemosiderin [31]. The iron storage protein named ferritin consists of a spherical shell composed of 24 protein sub-units surrounding an anti-ferromagnetic iron core [33], each core containing typically between 2000 and 2500 Fe$^{3+}$ ions [34, 35]. As a result of the partial compensation between spins, the protein is endowed with a net magnetic moment [35, 36, 37].

The magnetization curve of an assembly of single domain particles can be described by the Langevin function for paramagnetic substances

$$M\left(\frac{\mu H}{kT}\right) = \coth\left(\frac{\mu H}{kT}\right) - \frac{\mu H}{kT}$$

where $\mu$ is the magnetic moment, $H$ is the magnetic field, $k$ is the Boltzman constant and $T$ the absolute temperature. In the literature, the behavior of such a system is known as superparamagnetic [38]. A superparamagnetic system can be described as a paramagnetic one but the magnetic moment involved is bigger than that of the single ion: in the case of ferritin the magnetic moment is about 200 $\mu_B$ [35, 36].

In the case of anti-ferromagnetic iron core of ferritin, the uncompensated magnetic moment is found to be well described by the magnetic moment of Fe$^{3+}$ ion (5.9 $\mu_B$) multiplied by the square root of the number of ions in the core [35]. However, the effective magnetic moment of iron in ferritin cannot be directly deduced from it. In fact, a linear contribution, presumably associated with the bulk anti-ferromagnetic susceptibility, must be added to the superparamagnetic component to explain the experimental behavior [35, 39]. Figure 2.5 [40] reports the magnetization curve measured for ferritin samples; the Langevin function alone cannot explain the experimental behavior because of the lack of saturation, in the high field region. A deep analysis of the curves shows that, at low temperature, the magnetic linear contribution is negligible while, at about 310K, the two contributions are of the same order of magnitude. The hemosiderin is the other molecule in which iron deposit may appear. It is a degraded form of ferritin in which the iron deposit is irreversible [41, 42, 43]. For this molecule, both paramagnetic and super-paramagnetic behaviors have been observed [31].

Despite these facts, in the low field region and at the temperature of the human body, the susceptibility is found to follow Curie’s law [15, 18, 30, 31] both for ferritin and hemosiderin; so an effective magnetic moment can be associated with each iron atom. Literature values for the iron atoms bounded in ferritin molecules range between 3.7 and 4.2 and between 3.3 and 4.7 for hemosiderin [30, 44, 45]. Another work obtained an effective iron magnetic moment of 4.2 [46], measuring the magnetic susceptibility of a
§2.2.1 The measurement of the effective magnetic moment of iron in the liver of rats

The effective magnetic moment of iron in the liver of rats was measured with a smaller version of susceptometer, that was constructed to measure the magnetic properties of living rats [10, 13]. Similarly to MID, the susceptometer was composed of two pickup coils (only one is shown in figure 2.6) that were symmetrically located with respect to the AC magnetic source. The susceptometer was placed inside a thermal insulating container (figure 2.7), having weak magnetic properties (polystyrene foam), and the internal instrument temperature was controlled in the milli-Kelvin range. The sample was inserted into the measurement region through a thermally insulating tunnel. During the measurement, the sample was inserted and withdrawn through the tunnel, in a direction perpendicular to the magnetic field axis. This instrument was capable of measuring susceptibility variations with an intrinsic instrumental sensitivity corresponding to 35 µg/cc of iron [13].

Once again, the magnetic signal of a body is given by equation (2.1). The weight function $g(\vec{r})$ of the smaller apparatus is reported in figure 2.6; about 1/3 of the body of the rat falls in the measurement region of the instrument. The
Figure 2.6: The MID weight function $g(r)$. Two pickup coil sensors (only one is shown) are placed symmetrically with respect to the magnet: the sum of their signals is zero in the absence of the sample.

Figure 2.7: The sketch of the MID susceptometer. Top: The magnet and the pickup coil are placed in a temperature-controlled environment. Down: The measurement region is accessible by means of a tunnel.
evaluation of the magnetic moment was performed measuring the magnetization signal of the excised liver taken from twelve rats, injected with sub-toxic doses of iron dextran. The iron contribution $\chi_{OV L} = \gamma C$ ($C$ is the iron concentration) to liver susceptibility was obtained making the difference between the magnetic signal of a box containing the excised liver immersed in water and the same box filled with an equivalent total weight of water. The magnetic signal of the box is:

$$\phi = (\chi_{\text{Water}} + \gamma C) \int_V g(\vec{r}) d\vec{r}$$  \hfill (2.5)

where $\chi_{\text{Water}} = -9 \times 10^{-6}$ is the susceptibility of water and $\gamma C$ is the susceptibility of the iron atoms. The integral in equation is calculated knowing the spatial distribution of $g(\vec{r})$ and the sample geometry. Figure 2.8 reports the linear fit of $\gamma C$ plotted as a function of the iron concentration $C$; the latter was measured by chemical analysis. From the slope $\gamma$ and the Curie law one obtains that the effective magnetic moment of these iron atoms was $(3.6 \pm 0.1) \mu_B$.

![Figure 2.8: The magnetic moment of iron in rat tissues.](image)

The iron contribution $\gamma C$ to liver susceptibility was obtained making the difference between the magnetic signals of a box containing the excised liver immersed in water and those of the same box filled with water only and having the same total weight. The liver iron concentration is obtained by chemical analysis.

2.3 The background signal calculation: a parametric model

Let us now describe the model used to calculate the background signal ($\phi_0$ in equation (2.3)) of the patient. We remind that the magnetization signal of a patient has two possible sources: the overall diamagnetic background level of the body (background signal) and the excess iron in the liver region. The magnetic signal of the iron overload is obtained making the difference between the measured magnetization signal and the background signal of the patient.

The evaluation of the background signal is performed under the hypothesis that the magnetization signal of a patient, with no iron overload, is the same as
The iron overload measurement

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Figure 2.9: The ratio between the eddy current and the magnetization signal. A: the magnetization and the eddy current signal of a medium-built person (age=31; height=1.7 m; weight=65 Kg). In our measurements, there is no experimental evidence of an eddy current signal with a skew shape similar to the one of the magnetization signal of patients with iron overload. B: the ratio \( R(x) \) between the eddy current signal and the magnetization signal is evaluated in positions from \( X=-8 \) cm to \( X=+8 \) cm; the center of mass of liver always falls in this range.

The eddy current and magnetization signals are both proportional to the magnetic field amplitude. When they are generated by a healthy body, they have a similar dependence on the body size and shape. This can be noticed observing figure 2.9A, which reports the magnetization and eddy current signals of a person having a medium build. The curve reported in figure 2.9B represents the ratio between the two components, calculated in the central part of the X axis: the dependence on X of this ratio is simpler than that of the measured signal. The statistical model estimates the ratio \( R(X) \) between the two signals measured by the MID. Only the positions between \( X=-8 \) cm and \( X=+8 \) cm are considered; the center of mass of all patient’s liver always falls between -8 cm and 0 cm.

The model was trained on a data set of 84 healthy volunteers (whose specifications are reported in table 2.3). For each volunteer, the five ratios \( R(X) \), calculated in the positions \( X = -8, -4, 0, +4, +8 \) cm, are fitted with a parabola. Three multiple linear regressions (one for each parabola coefficients) as a function of a set of selected features are performed. The features are the independent variables that the model identifies as significant for predicting the ratios.
variables of the model: the anthropometric characteristics of the body and the eddy current signals. The selection of the features was made using a forward stepwise regression analysis using a cut off of \( p=0.001 \) in order to include a variable. This means that a variable is included in the model if the probability that the associated regression coefficient is zero is lower than 0.001 [48].

It may be noted that this statistical model is a parametric one, because it depends on some fixed parameters, chosen a priori by the operator. In particular, the operator can choose the degree of the polynomial, by means of which \( R(X) \) is fitted, and the significativity of the regression coefficient for including the variables in the model. Every change in the parameters would produce a different model. For example, a cut off of \( p=0.05 \) in the forward stepwise regression analysis would introduce more features in the model.

Let us now consider the error by which the model is affected to. The generalization error is the error made by the model on the evaluation of the background signal of a new healthy volunteer. Ideally, this may be evaluated using another set of healthy volunteers (the test set). Because of the limited number of elements in the training set, the generalization error is estimated applying the technique named Leave One Out Cross Validation (LOO) [49]. Consider the data set of 84 volunteers: each example is composed by the 3 parabola coefficients of the ratio \( R(X) \) and by the features measured for the subject. This technique calculates a model using the data of 83 volunteers, removing one from the original data set. The model is subsequently used to calculate the background signal of the removed volunteer. The procedure is repeated 84 times, each time leaving a different volunteer out. It may be noted that this technique underestimates the true error; this is because the feature selection is performed on the same data before the error estimation and because the data set is small [50].

Figure 2.10 reports the distribution of the differences between the measured magnetization and the background signals (converted in iron overload following the procedure described in section 2.1) for patients examined by MID. The Gaus-
The iron overload measurement

The Gaussian distribution has the same standard deviation of 0.8 g evaluated, by LOO technique, for the volunteers. The MID sensitivity may be improved because the error coming from the instrumental noise (∼100 nV) is lower than 0.4 g and the reproducibility error is lower than 0.5 g. The latter is the reproducibility of the iron overload for the same patient: it was assessed measuring the same patients within a lapse of time of one or two weeks.\footnote{In this lapse of time the therapy effect is negligible.} Please note that the Gaussian distribution with the standard deviation 0.8 g is wider than the distribution of the basal iron of normal subjects which has a mean value of about 0.4 g [1]. The iron overload evaluations reported were performed in the 658 patients enrolled between February 2005 and November 2009. Most of the patients underwent several measurements within a time interval ranging from 1 to 32 months depending on the therapy regimen, and a total of 1260 valid measurements was performed. The iron overload was greater than 3 g in 162 measurements out of 1260, it was between 1 g and 3 g and lower than 1 g respectively in 458 and in 640 measurements. Some highly negative iron overload evaluations were obtained both for patients and volunteers, and can be ascribed to the error in the background signal calculation.

The model previously described was developed using the data of 84 healthy volunteers. The data used to train the model was updated introducing 58 new examples and the generalization error was evaluated again by means of LOO technique. Table 2.3 reports the error (1 std) made by the model on the evaluation of the magnetization signals in positions $X = -8$, -4, 0, +4, +8 cm. The errors get worse of about 30\% (from 300 to 400 nV) and this can be converted into an error on iron overload evaluation growing from 0.8 g to 1.1 g. The most likely explanation to this fact is that this model was tuned on the first 84 examples and the possibilities of generalizing it are not good enough. This model was used to produce the results discussed in chapter 3. The MID sensitivity is equivalent to about 1 g of iron overload: it is capable of detecting moderate and severe iron overloads. Mild overloads are indistinguishable from healthy volunteers population.

Other methods have been developed in order to obtain the background signal
§2.3 The background signal calculation: a parametric model

<table>
<thead>
<tr>
<th>Training set</th>
<th>Measurement Position</th>
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<td>340 nV</td>
</tr>
<tr>
<td>n=142</td>
<td>400 nV</td>
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</table>

Table 2.3: Errors made by parametric model on two groups of volunteers. The errors, evaluated by means of LOO technique, of the parametric model trained with \( n=84 \) and \( n=142 \) (i.e. \( 84+58 \)) volunteers.

of the patient and are the object of the chapter 4. In particular, in section 4.1.2 a model based on statistical learning technique will be presented; in this case the model seems to be more stable with the growth of the number of patients.

Appendix B contains a synthesis of all available models to calculate the background signal of the patient. Herein the reader can find table B.3 which reports the list of all variables measured for the volunteers, and the table B.4 which reports the details of the model explained in this chapter.
Chapter 3

Clinical results

The present chapter is devoted to present the results on about 700 patients obtained applying the methods to calculate the iron overload presented in the previous chapter. The data has been collected in the period between February 2005 and November 2009. We then correlated MID measurements with the results of liver biopsies, blood serum ferritin concentration measurements and with the results obtained from other non invasive methods. Currently, the only two validated non-invasive methods for measuring liver iron concentrations are the Superconducting Quantum Interference Device (SQUID) susceptometry [2, 17, 18, 19, 20, 21, 51, 52] and Magnetic Resonance Imaging (MRI) [4, 9, 53, 54].

3.1 Patients’ Specification

Between February 2005 and November 2009, 658 patients (62% males and 38% females, 18-88 years old) were enrolled, ranging from 18 to 89 years of age. These included patients with hereditary hemochromatosis, several forms of congenital or acquired anemias, such as thalassemia major and thalassemia intermedia, drepanocytosis and microdrepanocytosis, Blackfan-Diamond anemia and other diseases (CDA, MDS, NASH, Post-BMT, Aplasia Midollare...). Patients with hyperferritinemia (i.e. abnormal value of blood serum ferritin), in whom iron overload had not previously been detected, were also included. Table 3.1 reports the median of the anthropometric characteristics of all measured patients: the square brackets contain the interquartile range (IQR). In descriptive statistics, the IQR is the interval, around the median, which contains \( \pm 25\% \) of the data.

Exclusion criteria included pregnancy (a pregnancy test was performed in all female patients of child bearing potential) and the presence of metallic medical and surgical aids, hip replacements, pace makers etc., which might interfere with the magnetic field and making the measurements non valid. As an example of disturbance, figure 3.1 reports the magnetization signal of a patient measured several times with MID. The spurious magnetization signal came from a hairpin forgotten in the hair of the patient. The third measure (plot A) is very different.
<table>
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<th>Weight [Kg]</th>
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<th>BLS [µg/L]</th>
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Table 3.1: Clinical and iron overload data of patients measured with MID between February 2005 and November 2009. The median values and interquartile range (IQR) are reported (square brackets). The BMI is the Body Mass Index defined as body weight divided by the square of height. The LIO is the Liver Iron Overload measured by MID. We remind that the error on the LIO measurement is 1 g (1std); the negative values means that iron overload is not detectable by the MID.
Clinical results

Figure 3.1: An example of magnetization signal with a spurious contribution of an hairpin. The magnetization signal contaminated by the signal of a hairpin forgotten in the hair of the patients. The eddy current signals (not reported) did not change.

from the others; it shows a non realistic diminution of iron overload. After the removal of the hair pin, the patient was measured again and its magnetization signal was in range with the previous ones (plot B). The magnetization signal seems more diamagnetic because the hairpin was positioned in the head of the patient and it generates a magnetic field which flux passes through the surface of the pickup from the back (i.e. it falls in the region where the function \( g(\vec{r}) \) assumes negative value - figure 2.2).

3.2 Follow-up of hemochromatosis patients

In hereditary hemochromatosis, iron overload is generated by an increased absorption of the dietary iron. The therapy of iron removal is usually performed by periodically removing blood, namely, phlebotomy. Figure 3.2 shows the follow-up of a hemochromatosis patient undergoing phlebotomy therapy; the black line refers to the magnetization signal and the grey one to the background signal. The decrease of iron overload for this patient was confirmed both by a biopsy (from 24 mg/g\(_{\text{dw}}\)-calculated as dry weight of tissue - to no iron overload) and by blood serum ferritin evaluations (from 3600 \( \mu \text{g/L} \) to 33 \( \mu \text{g/L} \)). Figure 3.2B reports the liver iron overload, measured by MID and the iron removal, estimated from phlebotomy therapy. The measured iron reduction is smaller than its assessment by the phlebotomy therapy.

The same behavior was found in other hemochromatosis patients during phlebotomy therapy. In figure 3.3A, the iron removal, estimated from phlebotomy therapy, is compared with the measured iron reduction measured by MID for 15 hemochromatosis patients who were measured more than once (R=0.89). The results show that the measured iron reduction is smaller than its assessment by the phlebotomy therapy. Figure 3.3B shows the iron absorption by diet as it results from the difference between the iron removal estimated from phlebotomy
3.2 Follow-up of hemochromatosis patients

Figure 3.2: Hemochromatosis patient (male, age 36, weight 90 Kg). (A) Represents the measured magnetization signal (a) and the calculated background signal (b) of the hemochromatosis patient. The first measurement (i) was carried out on 7th March, 2005 and the last one (vii) on 26th October, 2007. (B) Represents the liver iron overload measured by the MID and the iron removal estimated from phlebotomy therapy.

Figure 3.3: Data of 15 hemochromatosis patients. (A) The iron removal estimated from phlebotomy therapy is compared to the iron reduction measured by MID for 15 hemochromatosis patients who were measured more than once (41 data). The fit is relative to all patients (R= 0.89). The slope of the linear fit is 0.49 with a statistical error of 0.04. The measured iron reduction is smaller than its assessment by the phlebotomy therapy. (B) Iron absorption from the diet during the course of phlebotomies is calculated making the difference between the iron removal estimated from phlebotomy therapy and the measured reduction by MID. The net iron absorption is 4.9 mg/day with a statistical error of 0.3 mg/day (R=0.90).
therapy and the measured reduction by MID as a function of time: the rate of absorption of iron in these fifteen patients was found to be 5 mg/day with a statistical error of 0.3 mg/day ($R=0.90$).

The increased iron absorption from the diet during the course of phlebotomy is the most likely explanation [55] for the greater than normal measured value which is about 1mg/day [1]. We remind that the absorption rate was calculated assuming an effective magnetic moment of iron atoms of 4 $\mu_B$ (section 2.2).

The value calculated for the absorption rate cannot be ascribed completely to the absorption of dietary iron because of the lack of knowledge of the magnetic moment of the iron in the in vivo liver. Assuming that these patients receive only the minimum physiological amount of necessary iron from their diets and that the variation of the magnetic susceptibility measured is to be attributed to all the iron removed by phlebotomy, one will obtain a magnetic moment of 3.0 $\mu_B$.

The pedice $ww$ was used in the unit of measurement of LIC to indicate the concentration evaluated in the wet weight of liver tissue. This notation is necessary to distinguish from biopsy, whose measurement gives the value of the LIC evaluated in the dry tissue of liver (in this case the pedice $dw$ is used).

### 3.3 Follow-up of thalassemia major patient

In thalassemia major patients, the iron overload is induced by the transfusional therapy and is then removed by administrating drugs to them (DFO and L1). Figure 3.4 shows the follow-up, over a period of more than four years, of a patient suffering from thalassemia major, undergoing an intensive chelation therapy regimen. The black line refers to the magnetization signal and the one to the background signal. The background signal is calculated according to the anthropometric data of the patient at the time of measurement.

The enlargement of the liver explains the non-asymmetric shape of the magnetization signal. By comparison, the liver of the patient in figure 3.4 is placed mainly in the right part of the abdomen. The patient was well-compliant to the therapy and the iron overload decreased from 8 g to less than 2 g when the therapy (DFO) was dismissed. A correlation can be noted between the change in the liver iron overload shape and the therapy administration (figure 3.4 B and C respectively). The reduction of iron overload was also supported by the variation of serum-ferritin (from 6100 $\mu g$/L to less than 200 $\mu g$/L) and by MRI measurements. Figure 3.5B reports both the cardiac R2* ($=1/T2^*$) measurements both for liver and heart during the treatment.

The liver iron concentration of 2.9 mg/$g_{ww}$ was measured with the SQUID susceptometer at the time of the fourth MID measurement. At that time, the iron overload was 4.6 g. An evaluation of LIC equal to 4.1 mg/$g_{ww}$ is obtained by way of the procedure described in section 2.1, that attributes a normal liver to the patient (1200 g). These LIC values, however, may be over-estimated due to the pathological enlargement of the organ.
§3.3 Follow-up of thalassemia major patient

Figure 3.4: Thalassemia patient (female, age 26, weight 55) under therapy with L1+DFO. (A) The measured magnetization (a) and the calculated background signal (b) of the thalassemia patient. The first measurement (i) was on 22th February, 2005 and the last one (xi) on 16th October, 2009. (B) The liver iron overload is calculated making the difference between the signals (a) and (b) at the liver center of mass. The background signal is calculated according to the anthropometric data of the patient at the measurement time. (C) Therapy administration.

Figure 3.5: Variation of iron overload measured with MRI and with MID. A: variation of liver iron overload measured with MID. B: variation of the hepatic and cardiac R2* measured with MRI for the thalassemic patient of figure 3.4. Both the measures decrease with the time but the value of the hepatic R2* is at least a factor 4 greater that the cardia one.
3.4 Comparison with other techniques

3.4.1 Comparison with needle biopsy

The biopsy measurement gives the iron concentration in dry weight liver tissue that is typically expressed in milligrams of iron per gram of dry weight liver (mg/g\textsubscript{dw}). The MID measures the liver iron overload; by means of the procedure described in section 2.1, the LIC, evaluated in wet tissue of liver, may be obtained (units mg/g\textsubscript{ww}).

The factor of conversion from wet to dry weight is not well known: literature reports that it ranges from 3.3 to 5.8 [4, 20, 56, 57], depending on the sample treatment. In order to avoid a systematic error, the LIC in wet tissue obtained by MID is compared directly with the one in dry tissue obtained by biopsy in figure 3.6. The angular coefficient of the fit is (4 ± 1) and is a measure of the mean value of the wet to dry weight conversion factor for the 26 samples.
3.4.2 Comparison with SQUID susceptometer

The SQUID susceptometer measures the LIC in wet tissue and is expressed in milligrams of iron per gram of wet weight of liver (mg/g ww). The LIC of 50 patients who were measured by SQUID and by MID are reported in figure 3.7 (R = 0.79). The number of reported measurements (64) is higher than the number of patients because 11 of them were measured more than once with the two susceptometers, after a lapse of time between two consecutive measurements greater than 4 months.

3.4.3 Comparison with Blood Serum Ferritin

Figure 3.8 reports the comparison of the LIC measurements of 509 patients with their blood serum ferritin (BSF) concentration (R = 0.76). The BSF test and the MID measurement have been conducted less than 30 days apart for each patient. The BSF is considered by physicians to be an indirect measurement of the body’s iron burden [5], but it can be influenced by many other clinical conditions other than that of iron overload [6]. It should be noted that more than one hundred patients underwent the two tests more than once (with a gap of more than 2 months between consecutive measurements), giving a total of 830 tests.

3.4.4 Comparison with MRI

The use of Magnetic Resonance Imaging (MRI) to estimate liver iron concentration has been studied for nearly twenty years [9, 58, 59]. The MRI measures the decreasing of proton-transverse relaxation in the presence of iron which changes the local magnetic field and than the Larmor frequency of proton of water. The decreasing time is named transverse relaxation time T2*. The more iron there is, the darker the T2*-weighted images are [4]. The reciprocals of T2* (the transverse relaxation rate R2*) increase with the iron concentration [4, 9].
Figure 3.9: S. Pierre calibration of MRI [4]. **Left:** calibration with solutions of Mn\(^{2+}\) ions. **Right:** calibration with biopsy.

Figure 3.9 reports the calibration of MRI with solutions and with biopsy performed by S. Pierre [4, 9]. It may be noted that the same value of R2* is generated by an in vivo iron concentration greater than that in solutions of more than one order of magnitude \(^1\).

The MRI performs an indirect measurement of the iron overload and it needs the biopsy to be calibrated [4, 9, 53, 54, 59, 60]. As it will be clear with the next simple example, the magnetic moment of the atom is not the only parameter that influences the MRI signal [4, 9, 59]. The following example compares the signal of aqueous solutions of Fe\(^{3+}\) and Mn\(^{2+}\) ions. It shows that two atoms with the same magnetic moment produce different MRI signals.

The MRI calibration with solutions was reproduced by means of the 1.5 T magnetic resonance installed at Galliera Hospital and the relaxation times of solutions with different concentrations of Fe\(^{3+}\) and Mn\(^{2+}\) ions were measured (figure 3.10). Looking at figure 3.10, it can be noted that, even if the two ions have equal magnetic moment, the relaxation process is different in solution having the same concentration of Mn\(^{2+}\) and Fe\(^{3+}\) ions. More specifically, Mn\(^{2+}\) ions produce a T2* lower than Fe\(^{3+}\) ions. The most likely explanation of this behavior is the different electrical charge of the ions. The water molecule has a dipolar electric moment and each positive ion (Fe\(^{3+}\) or Mn\(^{2+}\)) attracts the water molecules which are positioned with the oxygen in the direction of the ion. The ion Fe\(^{3+}\) attracts more water molecules than the Mn\(^{2+}\) ion, so the Fe\(^{3+}\) ions is like a sphere composed by molecules of water having the ion in the center and a radius bigger than the one of the Mn\(^{2+}\) ion.

The MRI signal decays because of the de-phasing process of the resonance

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\(^1\)The magnetic moment of Mn\(^{2+}\) is the same of Fe\(^{3+}\) which is 5.9 \(\mu_B\) [25]; the atomic weight of Fe\(^{3+}\) ion is 55.85 g/mole and that of Mn\(^{2+}\) is 54.96 g/mole.
of protons of the hydrogen of water. A hydrogen proton which passes near the ion, because of the diffusive process, acquires a difference of phase with respect to other protons. That is because the Larmor frequency changes in the local field of the ions (Mn$^{2+}$ or Fe$^{3+}$). The molecules of water attracted by the ions diffuse less than the others, so that the MRI signal comes mainly from the water molecules outside the spheres. We remind that, in our picture, the sphere around the Fe$^{3+}$ ion is greater than that around the ion Mn$^{2+}$. The consequence of this is that the perturbation to the local magnetic field produced by the Fe$^{3+}$ ion is less intensive than the one produced by the Mn$^{2+}$ (because the distance from the ion is higher). The final results of this process is the shortening of the relaxation time in the case of Mn$^{2+}$ ions.

Moreover, two aqueous solutions with the concentration 0.2 mol/liter of Mn$^{2+}$ and 0.2 mol/liter of Fe$^{3+}$ were measured by MID: the magnetization signals of the solutions are the same. The contribution to the magnetic susceptibility of each ions was obtained comparing the magnetization signal of the solution with the one generated by the water only. The calculated magnetic moments are, within an error of about 5 %, the same reported in literature [25].

We remind that the MRI measures the transverse relaxation time T2*, whose reciprocal (R2*) is proportional to the liver iron concentration [4, 9], at least for those patients who are not heavily iron overloaded. The R2* is converted in LIC (evaluated in dry weight of liver tissue) by means of the calibration curve obtained from biopsies [9]. On the other hand, the MID gives the measure of the total iron overload in the liver. The procedure to convert it in LIC was described in section 2.1.

Figure 3.11A compares the total quantity of iron with R2* in 34 patients measured by MRI (R =0.88): the measurements were performed in three different centers - Pisa, London and Turin. The total iron quantity is then obtained adding the basal iron amount to the overload of iron measured by the MID. The quantity of basal iron, approximately 0.4 g, is calculated multiplying the

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**Figure 3.10:** In vitro MRI measurements. R2* obtained from MRI for solutions with different concentrations of Fe$^{3+}$ and Mn$^{2+}$ ions.
Clinical results

Figure 3.11: Comparison between MID and MRI. (A) Comparison between the total iron amount in the liver measured by the MID and the $R^*_2$ value measured by MRI on the same patients. (B) Comparison between the LIC obtained from the MID and MRI data.

attributed volume of the patients liver for the average basal concentration (0.3 mg/g$_{ww}$). The number of reported measurements is 41 owing to 6 patients having been measured more than once with the two instruments after a greater than 12 months time-lapse. In addition, figure 3.11B compares the LIC in dry tissue obtained from MRI with the LIC in wet tissue calculated by MID measurements (R =0.88) in the same patients. The relation “$LIC = 0.202 \text{mg/g}_{dw} + (0.0254 \text{ s mg/g}_{dw})R^*_2$” is used to obtain LIC from the $R^*_2$ measurement: this relation is obtained by [9] comparing the $R^*_2$ obtained by MRI with biopsy. For simplicity of the reading, in the plots the error bars are not displayed. The error of MRI, within the confidence interval of 95%, is about 50%; the error of MID, within the same confidence interval, is about 2 g.
Chapter 4

Alternative models to calculate the background signal of the patient

In the previous chapters it was explained that the iron overload is obtained from the difference between the measured magnetization signal of the patient and its background signal. The latter is the magnetization signal that patient with a normal iron content would generate. The evaluation of the background signal is performed under the hypothesis that the magnetization signal of a patient, with no iron overload, is the same as the one of a volunteer with the same anthropometric characteristics.

Since February 2005, about 150 volunteers have been measured by MID and some models have been developed to calculate the magnetization signal of a healthy person from its anthropometric characteristics. One model was described in section 2.3; it was used to produce the results described in chapter 3.

The aim of this chapter is to describe two different models: the first one is based on the statistical learning technique and the second is a direct calculation of the background signal, starting from the shape of the body (measured by a laser system), the contribution of a unitary volume of matter to the magnetization signal and a supposed susceptibility distribution of the healthy body. The two approaches will be respectively described in section 4.1 and in section 4.2.

4.1 Statistical learning model

The theoretical point of view of statistical learning technique is widely described in [3, 61, 62, 63]. In the following, a brief description of the algorithm, leading to the model that best represents and generalizes the experimental data, will be presented. Some examples will help to understand the technique.
4.1.1 The algorithm of statistical learning

The aim of the statistical learning technique is to find, from a set of \( m \) examples \((\vec{x}_i, y_i)_{i=1}^{m} (X \subset \mathbb{R}^p, Y \subset \mathbb{R})\), the function that best reproduces the relation between the input \( \vec{x}_i \) and the corresponding output \( y_i \) [3]. The input \( \vec{x} \in X \) is the independent variable of the model and the output \( y \in Y \) is the dependent one. In the particular case of modelling the background signal, each component of \( \vec{x} \) is an anthropometric variable and \( y \) is the magnetization signal in only one measurement position. In principle, the operator may fit the same set of examples with different functions (linear fit, parabola ...); the aim of the learning technique is to learn from the data what function is most capable of generalization, among the many functions fitting the example equally well. For example, in section 2.3 the ratio between the eddy current and the magnetization signal was described with a parabola and each coefficient was fitted with a multiple linear regression as a function of the anthropometric data. A priori, this choice may not be the best one, because the operator may be inclined to choose a law adapted to one particular set of examples.

For each input \( \vec{x} \in X \) and output \( y \in Y \), the quantity \([y - f(x)]^2\) represents the error suffered from the function \( f \) used to model the process which generates \( y \) from the given \( \vec{x} \). The noise, by which the experimental data are affected, may be represented introducing a probability distribution \( \rho(x, y) = \rho(x)\rho(y|x) \), defined on the space \( X \times Y \). The samples are supposed to be independent, identically distributed and generated according to some (unknown) probability distribution. This probability distribution governs the sampling and is not known in advance; it is not a goal of the method to reveal it. The mean square error is

\[
\epsilon(f) = \int_{X \times Y} [y - f(x)]^2 \rho(x, y) \, dx \, dy. \tag{4.1}
\]

The problem is to find the function \( f \) which minimizes 4.1. Let us define the function \( f_\rho \), which is named regression function, as

\[
f_\rho(x) = \int_{Y} y \rho(y|x) \, dy, \tag{4.2}
\]

representing the average of the \( y \) coordinate on \( \{x\} \times Y \). The function \( f_\rho \) can be though as the true input-output function reflecting the environment which produce the data. Unfortunately, the function \( f \), which best approximates \( f_\rho \), cannot be directly determined because of the sampling process.

Starting from the examples, one is only able to minimize the quantity named empirical error defined as

\[
\epsilon_z(f) = \frac{1}{m} \sum_{i=1}^{m} [y_i - f(x_i)]^2. \tag{4.3}
\]

The subscript "\( z \)" reminds that this error depends on the particular set of examples. The minimization of this error produces a function \( f_z : X \times Y \) which
best approximates the regression function and can be regarded as the empirical optimum for the problem.

The error, due to the use of \( f_z \) in place of \( f_\rho \), can be decomposed into two parts: the first one, named the sample error, depends on the use of the function \( f_z \) instead of \( f_\rho \) and the second one, named approximation error, on the use of \( f \) in place of \( f_\rho \). The theory establishes limits to this errors [63].

The minimization of the empirical error 4.3 cannot be done without imposing constrictions on the function \( f_z \): in fact, without any hypothesis, the algorithm would select the function that makes the error null. Obviously, this function would overfit the data points because it passes through all experimental data and the noise would be fitted too. To avoid this problem, a different approach is used: the hypothesis space (i.e. the space which the function belongs to) is restricted, taking advantage, if possible, of some a priori knowledge of the function. For example one may impose a linear fit. The approach adopted here is called regularization: the algorithm looks for the function in a \( \infty \)-dim space \( \mathcal{H} \) and, during the minimizing process, a penalty term is added, to avoid the selection of the functions having too many oscillations, which may overfit the example.

For instance, in the regularized least square (RLS for short) [62], the functional

\[
\epsilon^2_z(f) = \frac{1}{N} \sum_{i=1}^{N} [y_i - f(x_i)]^2 + \lambda \|f\|_H^2,
\]

is minimized; in the method named L1L2 the quantity

\[
\epsilon^2_{\lambda_1,\lambda_2}(f) = \frac{1}{N} \sum_{i=1}^{N} [y_i - f(x_i)]^2 + \lambda_1 \|f\|_2^2 + \lambda_2 \|f\|_1;
\]

is minimized, where \( \|f\|_H^2 \) is the norm defined in the \( \infty \)-dim space \( \mathcal{H} \), \( \|f\|_2^2 \) is the norm defined in \( L_2 \) and \( \|f\|_1 \) the one in \( L_1 \).

The function \( f \) is supposed to belong to a Reproducing Kernel Hilbert Space (RKHS) [63] and it is represented as the superimposition of some positive-definite functions \( K : X \times X \rightarrow \mathbb{R} \) named Reproducing kernels. Each function \( K \) satisfies the following properties:

1. \( K(\cdot, x) \in \mathcal{H} \quad \forall x \in X \);
2. \( f(x) = \langle f, K(\cdot, x) \rangle_{\mathcal{H}} = \langle f, K_x \rangle_{\mathcal{H}} \quad \forall x \in X \), \( K(\cdot, x) \in \mathcal{H} \) (reproducing property).

Some examples of such functions are provided by the linear kernel \( K(x, x') = x \cdot x' \), the polynomial kernel \( K(x, x') = (x \cdot x' + 1)^d \) and the gaussian kernel \( K(x, x') = \exp \left( -\frac{\|x_i - x_j\|^2}{2\sigma^2} \right) \).

In the case of regularized least squares in the class of RKHS functions, the solution \( f \) is proved to be unique, and is determined minimizing the quantity:

\[
\min_{f} \epsilon^2_z(f) = \min_{f} \left( \frac{1}{m} \sum_{i=1}^{m} [y_i - f(x_i)]^2 + \lambda \|f\|_{\mathcal{H}}^2 \right)
\]
In particular $f \in \text{span}[K_{x_i}] \subset \mathcal{H}$ and can be written as superimposition of kernel functions evaluated on the examples:

$$f(x) = \sum_{i=1}^{m} \alpha_i K(x_i, x). \quad (4.7)$$

Following this formulation, the problem can be written as

$$(K + \lambda I)\vec{\alpha} = Y \Rightarrow \vec{\alpha} = (K + \lambda I)^{-1}Y$$

where $K$ is the kernel matrix, calculated on the examples ($K_{ij} = K(x_i, x_j)$). The estimated value for another example will be:

$$f(\vec{x}_{\text{new}}) = \sum_{i} \alpha_i K(\vec{x}_{\text{new}}, \vec{x}_i).$$

The regularization process is very important, since it avoids the overfitting of examples. To understand the meaning of the regularization parameter properly, let us consider equation (4.7) and a gaussian kernel. In this case, the equation approximates the unknown function by a weighted superposition of gaussian blobs, each centered at the location $x_i$ of the corresponding example. The weight $\alpha_i$ of each gaussian is such to minimize a regularized empirical error, that is, the error on the training set. The $\sigma$ of the gaussian, together with the parameter $\lambda$, control the degree of smoothing, of noise tolerance, and of generalization. Notice that, for the gaussian with $\sigma = 0$, the representation of equation (4.7) effectively becomes a look-up table that cannot be generalized (it provides the correct $y = y_i$ only when $x = x_i$ and otherwise outputs 0) [3].

In practice, in order to choose the best value of the parameter $\lambda$, the procedure provides different estimators $f_{\lambda}^z$ for different values of the parameter. The best value of the regularization parameter is the one that minimizes the error on a validation set that differs from the training set used to train the model. Being the number of data typically small, a technique named cross validation is adopted for estimating the regularization parameter [49, 64]. A k-fold cross-validation consists in dividing the examples in k different subsets. The model is trained k times, each time leaving out one of the subsets from training, and using the omitted subset to compute the prediction error of the model. The leave one out technique, introduced in section 2.3, is the extreme limit of this technique where each subset is composed by one volunteer. This technique is used both to choose the regularization parameter and to estimate the generalization error of the model.

### 4.1.2 Modelling the background signal

To obtain the background signal of the patients, the RLS technique, described in the previous section, was applied 5 times in order to produce 5 rules by which to calculate the background signal in the 5 positions $X = -8, -4, 0, +4, +8$ cm.
With this choice, the existing correlation between the measurements in adjacent sites is lost (i.e. for example the similarity of the measure in positions -4 and 0). The function $f$ was supposed to be linear; introducing the linear kernel in (4.7), it may be written as

$$f(\vec{x}) = \beta \cdot \vec{x} = \sum_{i=1}^{N} \beta_i \vec{x_i} \tag{4.8}$$

where $N$ is the number of measured features (the list of features used by this model is reported in table B.3 of appendix B). Because of the low amount of data, the LOO technique was used to estimate both the best value of $\lambda$ and the model error.

Figure 4.1 compares the performance of the parametric and the statistical learning model for the original training set of 84 volunteers and the updated one of 142. The comparison is performed by means of box-plots: a horizontal bar is drawn at the height of the median, the dimension of the box is equal to the interquartile range (IQR) which is the interval, around the median, which contains $\pm$ 25% of the data. Finally, the whiskers indicate the range of the data distribution and the extreme limits are computed on the basis of the IQR. The anomalous values of the distribution are displayed as outliers values: these values have a low probability to belong to the distribution (considering a Gaussian distribution, this probability is lower than 0.4%).

The first two boxes report the distributions of the difference between the measured and the background signal, evaluated with the parametric model, for the two groups of volunteers. Boxes three and four report the same quantities in the case of statistical learning model. The distributions refer to the error, evaluated by means of LOO technique, made in the only position $X=-4$ cm. In table 4.1.2 the standard deviation of the LOO error distributions is reported for positions between $X=-8$ cm and $X=+8$ cm. For completeness, the bottom of the table
Alternative models to calculate the background signal of the patient

<table>
<thead>
<tr>
<th>Model</th>
<th>Training set</th>
<th>Measurement Position</th>
</tr>
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<td>X=-8 cm</td>
<td>X=-4 cm</td>
</tr>
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<td>Statistical</td>
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<td>340 nV</td>
</tr>
<tr>
<td>Learning</td>
<td>n=142</td>
<td>370 nV</td>
</tr>
<tr>
<td>Parametric</td>
<td>n=84</td>
<td>340 nV</td>
</tr>
<tr>
<td>Model</td>
<td>n=142</td>
<td>400 nV</td>
</tr>
</tbody>
</table>

| Rough data |
|------------|-------------|
| X=-8 cm    | X=-4 cm     | X=0 cm  | X=+4 cm | X=+8 cm |
| Standard   | n=84        | 610 nV | 400 nV | 420 nV | 450 nV | 690 nV |
| deviation  | n=142       | 670 nV | 470 nV | 490 nV | 510 nV | 740 nV |
| Mean       | n=84        | -3.9 μV| -4.3 μV| -4.6 μV| -4.0 μV| -2.4 μV|
|           | n=142       | -2.9 μV| -4.2 μV| -4.5 μV| -3.9 μV| -2.4 μV|

Table 4.1: Comparison between errors made by statistical learning and parametric model. The standard deviation of the distribution of the errors, evaluated by means of LOO technique, of the statistical learning and parametric model trained with n=84 and n=142 (i.e. 84+58) volunteers, is reported. The bottom of the table reports the standard deviations (in nV) and the means (in μV) of signals’ distribution.

reports the standard deviation and the mean of the distribution of the rough data. The performance of both models are equivalent on the first group of volunteers (84). For the parametric model the error, increases from 0.8 g to 1.1 g and for the statistical learning from 0.7 g to 0.9 g. The error is the mean of the standard deviations of the distribution in the positions of the liver center of mass (i.e. X= 0, -4, -8 cm). Moreover, the box-plots show that the number of anomalous cases of the parametric model is higher than that of statistical learning. From this analysis, it seems that the statistical learning model is less affected by the sample variation and so it is more capable of generalizing than the parametric model originally developed.

The choice of using a linear kernel was done after some experiences in which both gaussian and polynomial kernel were used and the $L1L2$ regularization was used in the place of RLS. The performances of all methods were equivalent, so the choice fell on the simplest one. Moreover, a different model was constructed to take the correlation between measurements in adjacent sites into account [65, 66]. A vector-valued regression technique was used with the aim of finding the predictions for the 5 considered measurements sites all at once: the error is again comparable with the previous models.
4.2 The direct calculation of the magnetization signal of the body.

The magnetization signal of a body having a magnetic susceptibility distribution \( \chi(\vec{r}) \) can be calculated by means of equation (2.1), which is rewritten here for convenience:

\[
\phi(\vec{R}) = \int_{V} g(\vec{r} + \vec{R}) \chi(\vec{r} + \vec{R}) d\vec{r}
\]

We remind that \( V \) is the volume of the body, the vector \( \vec{r} \) joins the origin of the reference frame to the points inside its volume and \( g(\vec{r}) \) is the weight function of MID.

A direct calculation of the background signal by means of equation (2.1) can be performed if the function \( g(\vec{r}) \), the geometry of the body and a reliable susceptibility distribution of a healthy body are all known in advance.

4.2.1 The weight function \( g(\vec{r}) \)

The weight function \( g(\vec{r}) \) gives the contribution of a unitary volume of matter, having a unitary magnetic susceptibility, to the magnetization signal. It depends on the magnet and pickup coil geometry and its intensity is proportional to the number of turns of both magnet and pickup coils, to the frequency and to the amplitude of the magnet’s current. The theoretical values of \( g(\vec{r}) \) is calculated by means of a software for electromagnetic design, knowing the magnet and pickup coils geometry, the magnet’s current and the frequency. For example, the weight function reported in figures 1.3 and 2.2 was calculated using the Vector Fields software for electromagnetic design Opera. Because of the non ideality of the system, the values of \( g(\vec{r}) \) were measured in the region of space immediately between the magnet and pickup coil. The probe was a ferromagnetic sphere with the diameter of about 1.5 cm and the sampling step was 4 cm. It was verified that the signal generated by the probe had no dependence with its orientation in the magnetic field.

A more reliable estimation of the weight function, in the whole measurement region, was obtained by using both the measured and the theoretical data. A comparison between the two sets of data allowed to find an affine transformation by which the theoretical data points were mapped in the measured ones. The transformation consists in the composition of a translation, a scale and a shear. The final distribution of the function \( g(\vec{r}) \) was constructed by matching the measured data with the transformed ones, where no sampling was performed. Finally the data were interpolated at steps of 1 cm.

The quality of this weight function was verified with water samples. The magnetization signal of a cylindric sample of water (about 1.2 liters) was measured with MID in different positions with respect to the magnetic field axis. These measured signals were compared with the ones calculated through the integral (2.1); the calculation was performed by means of the The MathWorks’s
Alternative models to calculate the background signal of the patient

Figure 4.2: Comparisons between the measured magnetic signal of a sample of water with the calculated one (Matlab). (A): sampling points over the grid in the XY plane. (B): Signals with the Y coordinate of the center of the sample equal to zero. (C): Signals with the Y coordinate of the center of the sample respectively equal to -16, -8, +8, +16 cm.

software Matlab. Figure 4.2A reports the sampling grid and the section of the water sample. Each time, a measurement was acquired with the center of the basis of the cylindrical sample positioned in correspondence with a different dot of the grid. In the part B of the figure we report the comparison between the measured and calculated signal when the Y coordinate of the center of the cylinder is null and in part C we report the same comparison in the remaining positions. The distribution of all the differences between the measured and the calculated signals is reported in figure 4.3. In the central positions (figure 4.2 part A), where the signal is about 4 µV the error is lower than 0.2 µV; in some cases (figure 4.2 part C, plot Y=+8 cm) the error is still about 0.3 µV and the signal is less than 2 µV.

In order to further improve the knowledge of $g(\mathbf{r})$, an automatized system, to measure its value in the whole space accessible to the patient, was developed. The system, whose pictures are reported in figure 4.4, consists in a rod with low magnetic properties and a probe positioned at its edge (A). The rod is assembled on XYZ movement system. The YZ movement is performed by means of stepping motors (C); the displacement along the X direction is performed by means of
4.2.2 Measuring the 3D shape of the body

In order to perform the calculation (2.1), the geometry of the body under exam must be known. As introduced in section 1.3.2, a system, composed by six lasers, was constructed to perform this measurement; the sensitivity of the laser is better than 0.5 mm. The human body 3D shape is obtained measuring the region of space having a length of 60 cm in the X-direction and 100 cm in the Y one. A sampling step of 1 cm in the X direction and 2 cm in the Y direction

The calibration of this system is in progress. Because in some positions the contribution of the metallic structure to the signal is comparable with the one of the probe, the not-negligible contribution of the structure will be subtracted to the signal, to obtain the signal of the probe alone. In order to compensate the drift which may affect these measurements, the same technique described in section 1.3.1 will be adopted. This procedure is expected to produce a measurement of the \( g(\vec{r}) \) with an error of about a part over \( 10^5 \).

### Figure 4.3: Hystogram of errors

Distribution of the differences between the measured and the calculated signal of the cylindrical sample of water.

### Figure 4.4: Measurement of the function \( g(\vec{r}) \)

Experimental apparatus to measuring the distribution of the function \( g(\vec{r}) \) in the whole space accessible to the patient. (A): the probe assembled on the rod. (C) and (D) the system which made the movement of the rod possible.
Figure 4.5: Laser measurements. A: human body shape sampled with laser (X-sampling: 1 cm; Y-sampling: 2 cm). B: frustum of cone.

is used: the X-step is smaller in order to take the more marked curvature of the body in this direction into account. The time required by the system to perform the acquisition of the human shape is about 12 minutes and is comparable with the time required by the magnetic acquisition. Figure 4.5 reports an example of acquisition.

The error made by the system in measuring the geometry was tested using objects having a simple geometry. The geometrical evaluation of the volume was compared with the one obtained from the laser acquisition. For example, the evaluated volume of the frustum of cone, reported in figure 4.5B, is 300 cc lower than the measured one (5200 cc). This error comes both from the sampling and the calculation process.

4.2.3 Modelling the susceptibility distribution of the body

Let us proceed with the calculus of the magnetization signal of the body by means of equation (2.1). The first calculation was performed under the hypothesis that the susceptibility distribution is uniform and equal to that of water. Since this signal is generated by the body, supposing it is totally filled of water, we will call this model *waterman*.

The 3D body shape of 84 volunteers was measured with the laser system in the period between between February 2008 and June 2009 and the magnetization signal of each of them has been compared with the waterman calculation. Figure 4.6 reports this comparison for four volunteers with different body shapes: the body weight ranges between 28 kg (case A) and 88 kg (case D).

In all cases it can be noted that, in the positions around X=0, the measured signal is more intensive (in absolute value) than the one calculated by the waterman. Whereas, the opposite observation can be done for the border positions. This is true quite in general as it is shown in figure 4.7, which reports the spatial dependence of the differences between the waterman and the measured signal for each of 84 volunteers. For each position X, 84 differences are reported.

The most likely explanation for the error is that the model fills with water some empty regions hidden under the body, especially at its edge where the trunk has its greatest curvature. The validity of this hypothesis can be verified calculating the magnetic signal that would be generated by the empty regions
4.2.3 Modelling the susceptibility distribution of the body

Figure 4.6: Waterman signal A: thin-built volunteer (28 kg); B: little-built volunteer (53 kg); C: normal-built volunteer (75 kg); D: strong-built volunteer (88 kg).

of the body, located at the lateral edge of the trunk, if filled with water. These regions have been simulated with 2 prisms with triangular basis filled with water (left part of figure 4.8). The plot of figure 4.8 reports the magnetization signal of the prisms compared with the mean difference between the waterman and the magnetization signal of the volunteers (i.e. the best fit of figure 4.7)\(^1\). It may be noted that the shape and the intensity of the two curves is quite similar. The introduction of this correction may contribute to reduce the error and this could

\[ \phi_{\text{wat}} = \int_{V_{\text{prisms}}} + \int_{V_{\text{body}}} \]

Making the difference between the waterman and the measured signal, one obtains:

\[ \phi_{\text{wat}} - \phi_{\text{meas}} = \int_{V_{\text{prisms}}} - (\phi_{\text{meas}} - \int_{V_{\text{body}}}) \]

Assuming that the difference in parenthesis is null, the difference in figure 4.7 is the magnetization signal generated by the prisms artificially filled with water.

\(^1\)The calculation of waterman, which results from the acquired 3D body shape, is the sum of the contribution of the body and the spurious contribution of the prisms:
be obtained by developing a dedicated software, that modifies the 3D shape of the body of each subject.

The fit of the data of figure 4.7 represents the mean signal generated by the empty regions that the calculus fills with water. These regions are both outside the body (prisms) and inside it (susceptibility distribution of the body). A new model may be developed combining the waterman calculation and a rough statistical approach. In the following, we will call it hybrid model. The direct calculation of the magnetization signal may be performed using the waterman; then the mean of the errors (figure 4.7), made by the waterman in the calculation of the measured signal of volunteers, may be subtracted as an offset. Figure 4.9 reports the background signal calculated by the hybrid model for the four volunteers of figure 4.6. Once again, the LOO technique was used: the mean subtracted to each waterman was obtained using the errors of 83 data.

The prediction errors of the three models illustrated in this work, are reported in table 4.2.3 as functions of X position and as box plots in figure 4.10. The mean error$^2$ of the hybrid model is 360 nV and is comparable with the one obtained from the parametric model (410 nV) and from the statistical learning model (340 nV). These errors are all equivalent to about 1 g of iron. Table 4.2.3 reports the errors of the three models for the calculation of the background signal of volunteers.

The sensitivity of the measurement increases if the standard deviation of the Gaussian distribution reported in figure 2.10 is reduced. A way to narrow the distribution of the errors reported in figure 4.7 is to correct the waterman considering for each volunteer the empty region of the body and a proper susceptibility distribution. Please note that a different correction may be introduced for each volunteer; in fact, we are attributing the distribution of the errors, which spreads out of few hundreds of nano-Volts, to a different position of the empty regions in and out of the body.

Let us consider a different susceptibility distribution inside the body. A big

---

$^2$Once again the mean error is the mean of the standard deviation in positions X=0, -4 and -8 cm.
§4.2.3 Modelling the susceptibility distribution of the body

Figure 4.8: Simulation of empty regions filled with water. Left: the example of body shape of a subject and prisms with triangular basis used to simulate the empty region which are invisible to the laser system (the volume of each rod is 600 cc). Right: the mean difference (figure 4.7) between the waterman and the measured signal is compared with the signal of generated by the prisms with triangular basis.

Figure 4.9: Calculation of the background signal by means of the Hybrid model:
A: thin-built volunteer (28 kg); B: little-built volunteer (53 kg); C: normal-built volunteer (75 kg); D: strong-built volunteer (88 kg).
Figure 4.10: Distribution of errors of the parametric model, the statistical learning model and the hybrid model. The box-plot reports the distribution of errors in the position X=-4 cm. The error is defined as the difference between the measured magnetization signal and the background signal of the volunteer. Each error was obtained by means of LOO technique.

Figure 4.11: Contribution to the signal of the lung. Variation of the lung signal positioned differently in respect to the Y coordinate.
§4.2.3 Modelling the susceptibility distribution of the body

<table>
<thead>
<tr>
<th>Model</th>
<th>Training set</th>
<th>Measurement Position</th>
</tr>
</thead>
<tbody>
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</tr>
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</tr>
<tr>
<td><strong>Parametric Model</strong></td>
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</tr>
<tr>
<td><strong>Hybrid Model</strong></td>
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**Rough data**

<table>
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<th>X=0 cm</th>
<th>X=+4 cm</th>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
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<td>-4.1 µV</td>
<td>-4.4 µV</td>
<td>-3.8 µV</td>
</tr>
</tbody>
</table>

Table 4.2: Comparison of the errors made by the statistical learning, the parametric model and the hybrid model. The errors are evaluated by means of LOO technique. The bottom of the table reports the standard deviations (in nV) and the means (in µV) of the distributions of the signals of the two groups of patients. Please note that this group of 84 subjects is different from the one described in table 4.1.2.

Approximation of considering the human body completely filled with water is certainly due to the susceptibility of the lungs. Though the magnetization signal generated by the lung tissues is very similar to that generated by an equivalent weight of water, the lung density is lower. The mean magnetic susceptibility of lungs is \( \sim -4 \cdot 10^{-6} \) (SI) [67] which is about one half of that of water \( (-9 \cdot 10^{-6})\).

A first model of lungs has been created and the correction to the magnetization signal generated by this organ calculated. The organ is composed by few slices, created by looking at the MRI images of two healthy volunteers examined by MRI. The right and left lobes of the lung differ because of the presence of hearth; the model gives also the possibility of changing the dimensions of the organ with the dimension of the chest.

Figure 4.11 reports the correction that may be added to the waterman signal when a lung of fixed geometry is positioned differently with respect to the magnetic field axis. The function \( g(\vec{r}) \) is reproduced in gray scale color, limited to its positive values (circle in figure 2.2). The corrections were calculated supposing a magnetic susceptibility of \( -4 \cdot 10^{-6} \) for the lungs. We remind that, during the exam, the person is positioned on the stretcher in such a way that the center of mass of the liver falls in correspondence with the magnetic field axis when the subject is in the measurement region of the MID. Because the liver dimension may change between subjects, the Y coordinate of the beginning of the lungs...
increases with the liver dimensions. In the most realistic case, excluding E and D, the corrections spans between -500 nV and +500 nV. It follows that, after positioning the patient on the stretcher, the registration of the beginning of the lungs may be useful in the correction of the waterman signal.

Another correction that may be introduced in our model is the basal iron content of the normal liver. Figure 2.4 reports the magnetization signal generated by 1 g of iron evenly distributed in the liver. Because the mean concentration of iron in healthy subjects is about 300 \( \mu \text{g/cc} \), the iron content of a normal liver may be between 0.2 g (for a baby with a body weight of 20 Kg) and 0.5 g (for an adult with a body weight of 80 Kg). The corresponding correction to the signal is positive and it ranges between 100 and 200 nV in the X positions around the center of mass of the liver.

A question may rise at this point. Why none of the three models is capable of giving rise to a prediction with an error lower than 300 nV, which is equivalent to about 1 g of iron? What do we have to do in order to pass over this point? A first approach was described with the model of the lungs and the liver. The same approach may be upgraded introducing the stomach, the spleen and other organs in the body.

In order to take into account the magnetic susceptibility distribution of the organs of a normal body, at present, a fourth method is under test. The idea is to adapt a standard distribution of organs to the body of each volunteer (figure 4.12). The organ distribution of the whole trunk is obtained using a phantom (named Zubal phantom) obtained from several sets of segmented images of two living human males\(^3\). This phantom was developed by a staff of Yale University.

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\(^3\)Segmentation refers to the process of partitioning a image into multiple segments (sets of pixels) to which a label is assigned. This process allows to easy locate objects and boundaries.
The idea here is to construct an algorithm by which adapt the skin of the Zubal phantom to the skin of the patient measured by the lasers system. The final result will be a phantom with the external shape measured by the laser system and the distribution of organs obtained by the Zubal phantom. Being the phantom segmented, it will be simple to ascribe a susceptibility value to each organ in the patient abdomen and subsequently to calculate the magnetization of the body. This approach would also be useful for the study of the conductibility distribution of the eddy current signal (section 4.3).

At present the MID sensitivity is equivalent to 1 g of iron. It may be reduced to 0.5 g, that is the reproducibility error of the instrument. We plan to apply the technique described before to a set of volunteers that will be examined with a new version of the susceptometer, named MID2, presently under construction.

This new susceptometer, named MID2, will be attributed with CE mark. MID2 will be constructed in the INFN’s laboratories of Genoa during 2010 and subsequently installed at the Galliera Hospital of Genoa. Moreover, the new susceptometer will be capable of measuring strongly built persons that, because of the dimensions of the apparatus, the present susceptometer is not capable of measuring.

### 4.3 Modelling the eddy current signal

The final part of this work is devoted to describe a potentially new application of MID. This is the study of the electrical conductibility of human tissues which is another field of interest for physicians. For example, in patients affected by steatosis (i.e. fatty liver), the electrical conductibility of liver tissues is smaller than in other tissues [69]. On the other hand, an increase in the electrical conductibility may be found in patients affected by severe fibrosis or cirrhosis. Once again, the liver biopsy seems to be the gold standard to perform this kind of analysis. As previously explained (section 1.3), the MID magnetic field is an AC one; the pickup coil detects the variations of the field produced both by the magnetization of the human tissues and by the eddy currents. Beside magnetization, the oscillating magnetic field induces eddy currents in the human body. The intensity and the distribution of the eddy current depends on the tissue electrical conductibility and on the geometry of the body under exam.

Preliminary tests conducted on phantoms showed that the MID has enough sensitivity for detecting the variations of signal generated by different distributions of electrical conductibility. For example, figure 4.3 compares the eddy current signal generated by a box filled with about 7 liters of physiological solution (A) and the one generated by the same box with a low conductivity region (B). The magnetization signal (black curves) is the same because the quantity of water was unchanged. Another example is reported in figure 4.3. The phantom in images.
Figure 4.13: Example 1. Left: at the top is reported the picture of the phantom used to perform the experiment (box - ~30 cm x 30 cm - filled with 7 liters of physiological solution. The low conductibility region is a bottle with 0.5 liters of low conductive water. The drafts A and B refer to the sample configurations. Right: the eddy current signal of configurations A and B are reported; the black line refers to the magnetization signal: 2 different magnetization curves are reported.

Figure 4.14: Example 2. Left: at the top is reported the picture of the phantom used to perform the experiment (box - ~30 cm x 30 cm - filled with 7 liters of physiological solution, and backer - ~ 12 cm of diameter filled with 1 liter of solution with a conductibility 10% higher than physiological). The drafts A and B refer to the sample configurations. Right: the eddy current signal of configurations A and B are reported; curve C refers to the magnetization signal (2 different curves are reported).
§4.3 Modelling the eddy current signal

<table>
<thead>
<tr>
<th>Model</th>
<th>Training set</th>
<th>Measurement Position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X=-8 cm</td>
</tr>
<tr>
<td><strong>Statistical Learning</strong></td>
<td>n=142</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>390 nV</td>
</tr>
<tr>
<td><strong>Rough data</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>840 nV</td>
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</table>

Table 4.3: Errors made by the model for the calculation of the eddy current signal. The comparison of the errors, evaluated by means of LOO technique (142 volunteers), of the statistical learning model for the eddy currents signal, is reported. The bottom of the table reports the standard deviations of the eddy current signals measured for this group of volunteers.

was constituted by the same box of figure 4.3 filled with about 7 liters of physiological solution and a beaker with about 1 liter of solution having a 10% higher conductivity than the one of physiological solution. The right part of the figure reports the eddy current and the magnetization signals for the two configurations of the phantom. Once again the distribution of the currents changes and the MID reveals it. On the contrary, the magnetization signal does not change (black lines).

In order to reveal the changes of the tissues conductivity, the eddy currents signal of the patient must be compared with the one expected by the same subject supposed with a normal distribution of electrical conductivity. The statistical learning technique was applied to the study of the eddy current signal using the data of volunteers. The model is equivalent to the one chosen in the study of the magnetization signal and it was trained using the signals of 142 control subjects. The features used are the same reported in table B.3 with the obvious exclusion of the eddy currents signals that are now the five outputs of the model. In table 4.3 the LOO errors are compared with the standard deviation of the distribution of the rough data. It may be noted in advance that the performance of this model might not be the best one, because we are not sure that the volunteers used as control subjects were not affected by steatosis.

The figure 4.15 reports the magnetic signal of a cirrhotic patient compared with the magnetic signal predicted by the statistical learning model: the two signals are clearly different. Please note that if the mean conductivity of tissues is higher than normal, the eddy current signal is more intense. As a consequence, for augmented values of the conductivity, the difference between the signal measured and calculated is negative: this might be the case of fibrotic/chirrotic patients. On the contrary, if the mean conductivity of tissues is lower than
normal, this difference is positive.

The analysis of 11 patients affected by cirrhosis or severe form of fibrosis was performed. The results of MID has been compared with the one obtained by the apparatus named Fibroscan which returns the measurements of the stiffness of the liver tissues [70, 71, 72]. Figure 4.16 reports the correlations of the differences, between the measured and predicted eddy current signal, with the measurements obtained by Fibroscan. In normal subjects the values of stiffness are within 6 kPa: all the patients examined are out of this range. For 50% of the patients, the measured eddy current signal is more intensive than the calculated one, indicating an augmented value of the conductivity of tissues. The two horizontal lines around zero represent the limits of the distribution of the error made in the evaluation of the signal of volunteers (i.e. the 90% of the errors are within this range).

Moreover, 21 patients with an hepatic configuration that suggests the presence of steatosis were examined. This indication came from the physician after the ecographic exam and we underline that the presence of steatosis is not sure
but is only possible. The 21 subjects were classified into three different classes, depending on the steatosis score evaluated by the physician. The first group (score I) contains 3 subjects, the second group (score II) 10 and the last one 9 (score III). The preliminary results show no evidence of differences between the signal of patients and that of volunteers used to train the model.

These preliminary results show that in the MID can detect variations of electrical conductivity in humans. If the sensitivity is good enough need to be investigated. Once again, the model to calculate the background signal generated by the eddy current need to be improved. This will be performed with the volunteers that will be examined with MID2.
Appendix A

Explicit calculation of the weight function $g(\vec{r})$

In the next we report the explicit calculation of the weight function $g(\vec{r})$ which is obtained from [24].

Consider a body with the magnetic susceptibility $\chi(\vec{r})$ and immersed in an external magnetic field $\vec{H}(\vec{r})$. The magnetization of the body is then

$$\vec{M}(\vec{r}) = \chi(\vec{r}) \vec{H}(\vec{r})$$

and the field $\vec{B}(\vec{r})$

$$\vec{B}(\vec{r}) = \mu_0 \left( \vec{M}(\vec{r}) + \vec{H}(\vec{r}) \right) = \mu_0 \left( 1 + \frac{\chi(\vec{r})}{\chi(\vec{r})} \right) \vec{M}(\vec{r}).$$

For biological tissues, the magnetic susceptibility is similar to that of water, which is $-9 \cdot 10^{-6}$ (SI). So, the last relation can be approximated as:

$$\vec{B}(\vec{r}) \sim \frac{\mu_0}{\chi(\vec{r})} \vec{M}(\vec{r}).$$

This means that there are no differences between the field $\vec{B}$ in and out of the material.

Every magnetized element of volume generates a variation of the magnetic field whose intensity and sign depends on the local magnetic susceptibility. In the magnetic field the elementary volume $dV$ possesses the magnetic moment:

$$d\vec{m}(\vec{r'}) = \vec{M}(\vec{r'}) dV \sim \frac{\chi(\vec{r'})}{\mu_0} \vec{B}(\vec{r'}) dV,$$

where $\vec{r'}$ is the position of the elemental volume $dV$ in the reference frame of the laboratory. The field generated by the magnet dipole $d\vec{m}$ in the position $\vec{r}$, which is the position where the field variation is measured (figure A.1), is:

$$d\vec{B}(\vec{r}) = \frac{\mu_0}{4\pi} \nabla \wedge \left( \frac{d\vec{m}(\vec{r'}) \wedge (\vec{r} - \vec{r'})}{|\vec{r} - \vec{r'}|^3} \right)$$
Figure A.1: Magnetization signal generated by the human body.

Every magnetic dipole generates a magnetic field in the position of the pickup coil.

Performing the integral over the body volume, it is possible to calculate the variation of the field in the position of the sensor. Being the sensor a pickup coil, we measure the flux $\Phi$ of the field generated by the magnetization of the body.

$$\Phi(S) = \int_V \int_S dS \cdot dB(\vec{r}) = \frac{\mu_0}{4\pi} \int_V \int_S dS \cdot \nabla \wedge \left( \frac{dm(\vec{r}') \wedge (\vec{r} - \vec{r}')}{|\vec{r} - \vec{r}'|^3} \right),$$

where $S$ is the surface of the pickup.

Stokes theorem assures that it is possible to pass from the integral over the surface of the pickup to the integral over its contour $l$. Moreover the scalar triple product properties make possible to rewrite the last expression as

$$\Phi(S) = \frac{\mu_0}{4\pi} \int_V dm(\vec{r}') \cdot b(\vec{r}') = \frac{1}{\mu_0} \int_V dV \chi(\vec{r}') \cdot \vec{B}(\vec{r}') \cdot \vec{b}(\vec{r}')$$

Looking at this expression one recognizes that the term in square parenthesis is the magnetic field that would be generated by the pickup coil if a unitary current flowed through it (Biot-Savart law). So this relation may be written as:

$$\Phi(S) = \int_V dm(\vec{r}') \cdot \vec{b}(\vec{r}') = \frac{1}{\mu_0} \int_V dV \chi(\vec{r}') \cdot \vec{B}(\vec{r}') \cdot \vec{b}(\vec{r}')$$

where $\vec{B}$ is the main field and $\vec{b}$ is the one that would be generated by the pickup. Both fields are evaluated in the position $\vec{r}'$ of the volume $dV$.

Finally, because the magnetic field generated by the MID magnet is an AC one (with pulsation $\omega$), the temporal variation of the field flux through the pickup surface generated by the magnetization of the body alone is

$$\frac{\partial \Phi}{\partial t} = \omega \frac{1}{\mu_0} \int_V dV \chi(\vec{r}') \cdot \vec{B}(\vec{r}') \cdot \vec{b}(\vec{r}') = \int_V dV \chi(\vec{r}') \cdot \vec{g}(\vec{r}')$$
The calculation of the function $g(\vec{r})$ may be performed in the vacuum introducing only the conductors (i.e. the magnet and the pickup coils) in the simulation with the electromagnetic field software. Moreover, being the magnetic field sinusoidal, the simulation may be performed assuming in the magnet a constant value of the current equal to the root mean square of the real one.

The theoretical calculation of the weight function $g(\vec{r})$, used in the calculation of the magnetization signal of water samples and human body, has been performed by means of this technique.
Appendix B

Model’s data sheet

This appendix is dedicated to the lists of all available models for the calculation of the background signal of the patient.

These models were trained using the data of three different groups of volunteers. Group 1 refers to 84 volunteers measured during the 2005. Group 2 refers to 142 volunteers measured during the 2008 and 2005; there are some common volunteers in groups 1 and 2. Finally, Group 3 refers to the 84 volunteers with the measurement of the 3D body shape: they are 63 out of 65 volunteers measured in 2008 plus 21 new volunteers measured during 2009. Table B.1 reports the standard deviations and the means of the distribution of the signals measured (magnetization and eddy current) for each group of volunteers.

B.1 Modelling the magnetization and the eddy current signal

At present a total of 4 models for the prediction of the background signal are available. All the models predict the magnetization signal in the positions $X = -8, -4, 0, +4, +8$ cm. Moreover, as described in section 4.3, a model has been developed to obtain the eddy current signal of patients. The complete list of the available models is:

I Parametric model. This model predicts the ratio between the Eddy Current and the Magnetization signal. The description of this model can be found in section 2.3. The features used to train this model are a combination of the measured ones.

II Stat-learn-A. It is developed using the RLS technique using all measured anthropometric variable as input of the model. Its description can be found in section 4.1.

III Stat-learn-B. It is the same model as the previous one but the Eddy Current signal has been excluded from the features. This because the prelim-
inary study of the eddy current signal indicates that it may change with the pathology of the patient (fibrotic, cirrhotic and steatotic subjects).

IV Hybrid-model. It was described in section 4.2. It is hybrid in the sense that the waterman calculation is corrected with the mean value of the difference between the measured signal and the waterman calculated for the population of healthy volunteers. Table B.5 reports the offset that must be subtracted to the waterman signal to obtain the basal signal of the patient.

V Eddy Current. It is developed using the RLS technique and predicts the eddy current signal in the positions X= -8, -4, 0, +4, +8 cm.

The performance of each model has been tested comparing the standard deviation of the distribution of the residuals obtained by the LOO technique. The residual is the difference between the measured and the predicted signal of the excluded volunteer. Table B.2 reports these comparisons.

Finally, the complete list of the features is reported in table B.3. Because of the Parametric Model (I) uses a combination of the variables the detail of the model, trained with Group 2, is reported in table B.4.

B.2 Creating the medical report

The results presented in chapter 3 have been created using the parametric model described in section 2.3. At present, the medical reports delivered to the patients from the Galliera Hospital has been created by means of this model. From February 2005 more than 1200 medical reports have been created. An example of such a report is reported in figure B.1.

From the analysis presented in chapter 4, it was gathered than it may be replaced by the new models developed with statistical learning technique or with waterman. The procedure that will be soon adopted to create the medical report is described in the following.

Exam Acquisition of the magnetic signal of the patient. It consists in the acquisition of the magnetization signal generated by the patient in the positions X=-16, -12, -8, -4, 0, +4, +8, +12, +16 cm. The weighed mean of the repeated measurements will be considered.

First evaluation of the background signal. The background signals in the positions X=-8, -4, 0, +4, +8 cm will be evaluated by means of the model Stat-learn-B. We choice this model instead of Stat-learn-A because it does not use the eddy current signals as features of the model and so it can be applied to the whole population.

First evaluation of the background signal. The background signals in the whole measurement positions will be evaluated by means of the hybrid model.
Final evaluation of the background signal. The weighted mean of the two model will be calculated to obtain the final evaluation of the background signal of the patient. The weights used to calculate the means will be the one reported in table B.2. The results of this mean will be reported in the medical report and indicated as basal signal of the patient.

Evaluation of the magnetization signal of the iron. This will be calculated as the difference between the measured and the background signal.

Conversion in iron overload. The magnetization signal of the iron will be converted in iron overload by dividing it for the signal generated by 1 g of iron uniformly distributed over the liver associated to the patient on the basis of its body weight.

Iron overload. The iron overload reported on the medical report will be the one evaluated in the position of the liver center of mass. That is because, in this position, the magnetization signal of the hepatic iron deposits is maximum.

LIC evaluation. An indicative value of the liver iron concentration will be evaluated dividing the iron overload by the liver weight and adding the basal iron concentration of an healthy liver (0.3 mg/g). The weight of the liver is evaluated from the body weight and assuming a density of the tissues equal to that of water.

Border line subjects. A border line subject is defined as a person which anthropometric parameters has a probability major than 95% to be different from the population used to train the model. This subject might appear as false-positive. A manual analysis of the magnetization signal will be performed comparing the magnetization signal of the patient with the one generated by others with similar anthropometric characteristics. The medical report will be composed by the baseline of the patient but no numerical number will be attributed the iron overload and LIC. A comment will reports the final results of the analysis.
E.O. Ospedali Galliera di Genova
Ospedale di rilievo nazionale di alta specializzazione
Centro della Microcitemia e delle Anemie Congenite
Riferimento Regionale per le Anemie Congenite e le Alterazioni del Metabolismo del Ferro
Responsabile: Dott. Gian Luca Forni

MISURA DEL SOVRACCARICO DI FERRO NELL’INTERA REGIONE EPATICA
TRAMITE MAGNETIC IRON DETECTOR

REF PAZIENTE: PXXX
PAZIENTE:

DIAGNOSI: Thalassemia Major
PROVENIENZA: Dott. Forni - Centro della Microcitemia, Genova

REF ESAME: 5

DATA ESAME: xx-xx-xxx
DATA DI NASCITA:

Ferritina [μg/ml]: 4439
DATA Ferritina: 16-06-2005
PESO [Kg]: 56,2
ALTEZZA [m]: 1,51
BMI [Kg/m^2]: 24,5

RISULTATI
Sovraccarico di Ferro [g]: 5,8
LIC (ww) [μg/g]: 5200

<1 g: Non rilevabile
1-3 g: Sovraccarico Moderato
>3 g: Sovraccarico Grave

L’errore sulla misura del sovraccarico di ferro è 1g (1std), quello sulla LIC (ww) è 500 μg/g (1std).

COMMENTI: sovraccarico grave

Il medico referrante: Dott. xxxxxx
Il medico responsabile: Dott. xxxxxx

Il calcolo della LIC nel tessuto umido (Liver Iron Concentration - wet weight) è eseguito dividendo il sovraccarico di ferro per il peso del fegato della persona ed aggiungendo la concentrazione di ferro basale della popolazione normale (500 μg/g). Il volume del fegato è calcolato a partire dal peso corporeo, la densità dell’organo è considerata pari a 1g/cc.

Via Volta 10 16128 Genova, Tel +39-0105634557, Fax +39-0105634556, microcitemia@galliera.it

Figure B.1: Example of medical report. This report refers to a thalassemia major patient. It was created using the parametric model. For the privacy all the sensible data was deleted.
### MAGNETIZATION SIGNAL [\(\mu V\)]

<table>
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<th>X=(-4) cm</th>
<th>X=0 cm</th>
<th>X=(+4) cm</th>
<th>X=(+8) cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=84)</td>
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<td>-4.58</td>
<td>-3.97</td>
</tr>
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<tr>
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</table>

### EDDY CURRENT SIGNAL [\(\mu V\)]

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<th>X=0 cm</th>
<th>X=(+4) cm</th>
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</thead>
<tbody>
<tr>
<td>Group 1 (n=84)</td>
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**Table B.1: MID measurements statistic.** The descriptive statistic of the magnetization and eddy current signals of the three groups of volunteers is reported.
Table B.2: Errors made by the models in the evaluation of the background signal. The standard deviation of the differences between the measured and calculated background signals is reported for the positions between X=-8 cm and X=+8 cm. The LOO technique was used to calculate the error.

<table>
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<tr>
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<th>Magnetization</th>
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<td></td>
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<td>I</td>
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<tr>
<td>EC(-16)</td>
<td>Eddy current signal measured in position X=-16 cm (µV)</td>
<td>x</td>
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<td>Eddy current signal measured in position X=-12 cm (µV)</td>
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<td>Eddy current signal measured in position X=+16 cm (µV)</td>
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<td>EC(min)</td>
<td>The minimum value of the eddy current signal (µV)</td>
<td></td>
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<tr>
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<td>Area of the torso cross section at the liver level (cm²)</td>
<td>x</td>
</tr>
<tr>
<td>A₆</td>
<td>Area of the torso cross section at the shoulders level (cm²)</td>
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<tr>
<td>A₉</td>
<td>Area of the torso cross section at the hips level (cm²)</td>
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<tr>
<td>H₀</td>
<td>Torso mean mean thickness at liver level (cm)</td>
<td>x</td>
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<tr>
<td>H₆</td>
<td>Torso mean mean thickness at shoulders level (cm)</td>
<td>x</td>
</tr>
<tr>
<td>H₉</td>
<td>Torso mean mean thickness at hips level (cm)</td>
<td>x</td>
</tr>
<tr>
<td>Y</td>
<td>Age</td>
<td>x</td>
</tr>
<tr>
<td>W</td>
<td>Body weight (Kg)</td>
<td>x</td>
</tr>
<tr>
<td>H</td>
<td>Body height (m)</td>
<td>x</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index: $W / H^2$ (Kg/m²)</td>
<td>x</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area (evaluated from $W$ and $H$) (m²)</td>
<td>x</td>
</tr>
<tr>
<td>L₁</td>
<td>Circumference of thorax (cm)</td>
<td>x</td>
</tr>
<tr>
<td>L₂</td>
<td>Circumference of the body at subcostal arc level (cm)</td>
<td>x</td>
</tr>
<tr>
<td>3D-shape</td>
<td>Shape of the body measured with the laser system</td>
<td>x</td>
</tr>
</tbody>
</table>

Table B.3: Features. The list of the anthropometric variables measured is reported. The symbol x in the column of the model indicates that the variable is used as feature for the calculation of the background signal. I: Parametric model; II: Stat-Learn-A; III: Stat-Learn-B; IV: Hybrid model; V: Eddy current model.
§B.2 Creating the medical report

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_0$</td>
<td>Torso mean mean thickness at liver level (cm)</td>
</tr>
<tr>
<td>$W$</td>
<td>Body weight (Kg)</td>
</tr>
<tr>
<td>$H$</td>
<td>Body height (m)</td>
</tr>
<tr>
<td>$BMI$</td>
<td>Body mass index: $W / H^2$ (Kg/m$^2$)</td>
</tr>
<tr>
<td>$A_0$</td>
<td>Area of the torso cross section at the liver level (cm$^2$)</td>
</tr>
<tr>
<td>$EC(min)$</td>
<td>The minimum value of the eddy current signal ($\mu$V)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parabola coefficient</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>$-7.4 \cdot 10^{-2} \cdot H_0 + 4.8 \cdot 10^{-4} \cdot BMI \cdot W \cdot A_0 - 0.21 \cdot EC(min) + 1.03$</td>
</tr>
<tr>
<td>$C_1$</td>
<td>$-2.3 \cdot 10^{-3} \cdot H_0 + 4.9 \cdot 10^{-2}$</td>
</tr>
<tr>
<td>$C_2$</td>
<td>$5.9 \cdot 10^{-9} \cdot BMI \cdot W \cdot A_0 + 5.1 \cdot 10^{-3}$</td>
</tr>
</tbody>
</table>

Table B.4: **Parametric model (details).** Features used and explicit form of the parametric model.

<table>
<thead>
<tr>
<th>Measurement Position</th>
<th>Mean [(\mu)V]</th>
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</thead>
<tbody>
<tr>
<td>X [cm]</td>
<td></td>
</tr>
<tr>
<td>-16</td>
<td>-0.54</td>
</tr>
<tr>
<td>12</td>
<td>-0.56</td>
</tr>
<tr>
<td>-8</td>
<td>-0.09</td>
</tr>
<tr>
<td>-4</td>
<td>+0.42</td>
</tr>
<tr>
<td>0</td>
<td>+0.48</td>
</tr>
<tr>
<td>+4</td>
<td>-0.03</td>
</tr>
<tr>
<td>+8</td>
<td>-0.88</td>
</tr>
<tr>
<td>+12</td>
<td>-1.30</td>
</tr>
<tr>
<td>+16</td>
<td>-1.06</td>
</tr>
</tbody>
</table>

Table B.5: **Hybrid model.** The table reports the mean value of the difference between the waterman signal and the magnetization signal measured for 84 volunteers (Group 3) in each position between X=-16 cm and X=+16 cm. This values must be subtracted to the waterman signal to obtain the background signal calculated with the Hybrid model.
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1. **Hemochromatosis patient.** (A) Represents the measured magnetization signal (a) and the calculated background signal (b) of the hemochromatosis patient. The first measurement (i) was carried out on 7th March, 2005 and the last one (vii) on 26th October, 2007. (B) Represents the liver iron overload measured by the MID and the iron removal estimated from phlebotomy therapy.

1.2 **Magnetization of the body in a magnetic field.** The magnetization of body tissues produced a variation of the magnetic field $\Delta B$. As a consequence of the diamagnetic properties of tissues, a decreasing of the field is produced (i.e. $\Delta B$ is negative). The presence of iron overload makes this decrease less intensive; only in severe form of iron overload $\Delta B$ may assume positive sign.

1.3 **MID instrumentation (A):** The magnet and the twin pickup coils are placed in a temperature-controlled environment. Two pickup coil sensors are placed symmetrically with respect to the magnet: the sum of their signals is zero in the absence of the body. The gray scale figure and the graphs report the specific contribution of the present magnet and pickup coil sensor arrangement to the magnetic signal. (B): The external view of MID and of the stretcher used to place the body into the measurement region. The stretcher can be moved in the X direction while the Y scan of the body can be performed placing the patient in different positions along the stretcher.

1.4 **Weight function of assembling of SQUID susceptometer.** The magnetic field coils is a first order gradiometer (radius 3.6 cm) and the cryogenic sensor is a second order gradiometer (radius 3.4 cm) [19]. The calculation of the specific weight function was performed with Opera 3D.

1.5 **Sketch of the MID.** The MID is composed by the magnet and pickup coil, the structure and the thermal shield. In the figure, two concentric magnets and two sets of concentric pickups are drawn. This configuration was designed to generate a total of four weight functions, in order to be capable of weighting the tissues of the body in four different ways.
1.6 **MID pictures. A: Magnet (left) and pickup (right):** the pickup assembling (top) and the single printed board (bottom) which the pickup is realized with. On the board, two concentric magnets and two sets of concentric pickups are present. This configuration was designed to generate a total of four weight functions, each weighting in a different way the tissues of the body. Only the inner magnet and the inner pickup are used. **B: MID structure.** A fiber-reinforced resin structure holds the susceptometer magnets and pickups. **C: MID thermal shield.**

1.7 **The thermal drift induced by a warm body.** The magnetization signal of a rat measured with a smaller susceptometer. In the left part of the graph the shield was enabled; in right one it was disabled.

1.8 **Draft of the connections between the pickup coil (indicated with UP and DOWN) and the zeroing coils (A and B).** Coil A refers to the first stage of zeroing in which a series of coils, assembled on the pickup, is selected. Coil B refers to the second stage of zeroing. A magnetic field flux, phased locked with the main field, is concatenated with this coil.

1.9 **The magnetization signal of a body.** The magnetization signal measured by the lock-in amplifier during the insertion and extraction of a patient is reported in function of time.

1.10 **Eddy currents.** At the low frequency (234 Hz) of the oscillating magnetic field, the magnetic moment of the iron atoms oscillates with the same phase as the applied magnetic field and the phase lag of the eddy currents within the human body, relative to the induced electric field, is negligible. So, the magnetic signal of the oscillating iron atoms and the one of the induced eddy currents are out of phase by one fourth of period.

1.11 **Signal elaboration.** The variation of the signal, measured by the lock-in amplifier, generated by the magnetization of the body is reported in function of time. At the time "OUT" the patient is out of the measurement region and at the time "IN" the patient is inserted in it. The linear fit is used to extrapolate the value that the magnetization signal, registered when no patient is present in the measurement region, during the insertion of the patient in it.

1.12 **Water and stretcher signal distributions.** Left: distribution of the magnetization signal of a box filled with 6 liters of demineralized water. The signal is obtained subtracting the signal of the stretcher to the measured signal of water and stretcher together. The mean is $(-12.5 \pm 0.1) \mu V$. The true value of the mean is $0.1 \mu V$ lower because the stretcher is measured together with the clothes typically worn by the patient. This contribution is not present during the measurement of water. **Right:** The distribution of the magnetization signal of the stretcher as a function of the position of its center relative to the magnetic field axis. The data reported (260) have been collected since June 2008, after the introduction of the automatic movement of the stretcher. The mean of the magnetization signal in X=-16 is $(0.61 \pm 0.05)$, in X=0 is $(0.77 \pm 0.05)$ and in X=+16 is $(0.88 \pm 0.05)$.

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4.13 Example 1. Left: at the top is reported the picture of the phantom used to perform the experiment (box - ~30 cm x 30 cm - filled with 7 liters of physiological solution. The low conductivity region is a bottle with 0.5 liters of low conductive water. The drafts A and B refer to the sample configurations. Right: the eddy current signal of configurations A and B are reported; the black line refers to the magnetization signal: 2 different magnetization curves are reported.

4.14 Example 2. Left: at the top is reported the picture of the phantom used to perform the experiment (box - ~30 cm x 30 cm - filled with 7 liters of physiological solution, and backer - ~ 12 cm of diameter filled with 1 liter of solution with a conductibility 10% higher than physiological). The drafts A and B refer to the sample configurations. Right: the eddy current signal of configurations A and B are reported; curve C refers to the magnetization signal (2 different curves are reported).

4.15 Eddy current signal of a patient with high degree of fibrosis. Comparisons between the eddy current signal of a patients (liver stiffness 75 KPa) with the one obtained from the statistical learning model.

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A.1 Magnetization signal generated by the human body. Every magnetic dipole generates a magnetic field in the position of the pickup coil.

B.1 Example of medical report. This report refers to a thalassemia major patient. It was created using the parametric model. For the privacy all the sensible data was deleted.
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2.1 Liver volume estimation. Comparison between the liver volume calculated by [32] \( V_{\text{Liver}} = 191.80cc + 18.51cc/Kg \cdot W \) where \( W \) is the body weight and the one calculated from the MRI images. For the two volunteers the agreement is within 5%, for patient with hepatomegaly the agreement is between 30 \( \div \) 40%.

2.2 Characterization of volunteers used to train the statistical model. The BMI is the Body Mass Index defined as body weight divided by the square of height. The median values and interquartile range (IQR) are reported (square brackets). In descriptive statistics, the IQR is the interval, around the median, which contains \( \pm 25\% \) of the data.

2.3 Errors made by parametric model on two groups of volunteers. The errors, evaluated by means of LOO technique, of the parametric model trained with \( n=84 \) and \( n=142 \) (i.e. 84+58) volunteers.

3.1 Clinical and iron overload data of patients measured with MID between February 2005 and November 2009. The median values and interquartile range (IQR) are reported (square brackets). The BMI is the Body Mass Index defined as body weight divided by the square of height. The LIO is the Liver Iron Overload measured by MID. We remind that the error on the LIO measurement is 1 g (1std): the negative values means that iron overload is not detectable by the MID.

4.1 Comparison between errors made by statistical learning and parametric model. The standard deviation of the distribution of the errors, evaluated by means of LOO technique, of the statistical learning and parametric model trained with \( n=84 \) and \( n=142 \) (i.e. 84+58) volunteers, is reported. The bottom of the table reports the standard deviations (in nV) and the means (in \( \mu \)V) of signals’ distribution.

4.2 Comparison of the errors made by the statistical learning, the parametric model and the hybrid model. The errors are evaluated by means of LOO technique. The bottom of the table reports the standard deviations (in nV) and the means (in \( \mu \)V) of the distributions of the signals of the two groups of patients. Please note that this group of 84 subjects is different from the one described in table 4.1.2.
4.3 **Errors made by the model for the calculation of the eddy current signal.** The comparison of the errors, evaluated by means of LOO technique (142 volunteers), of the statistical learning model for the eddy currents signal, is reported. The bottom of the table reports the standard deviations of the eddy current signals measured for this group of volunteers.

B.1 **MID measurements statistic.** The descriptive statistic of the magnetization and eddy current signals of the three groups of volunteers is reported.

B.2 **Errors made by the models in the evaluation of the background signal.** The standard deviation of the differences between the measured and calculated background signals is reported for the positions between $X=-8$ cm and $X=+8$ cm. The LOO technique was used to calculate the error.

B.3 **Features.** The list of the anthropometric variables measured is reported. The symbol $\times$ in the column of the model indicates that the variable is used as feature for the calculation of the background signal. I: Parametric model; II: Stat-Learn-A; III: Stat-Learn-B; IV: Hybrid model; V: Eddy current model.

B.4 **Parametric model (details).** Features used and explicit form of the parametric model.

B.5 **Hybrid model.** The table reports the mean value of the difference between the waterman signal and the magnetization signal measured for 84 volunteers (Group 3) in each position between $X=-16$ cm and $X=+16$ cm. This values must be subtracted to the waterman signal to obtain the background signal calculated with the Hybrid model.
Bibliography


