Characterization of open issues in low-frequency computational dosimetry

Marco Soldati
Characterization of open issues in low-frequency computational dosimetry

Marco Soldati
Supervising professor
Prof. Ilkka Laakso, Aalto University, Finland

Thesis advisor
Prof. Ilkka Laakso, Aalto University, Finland

Preliminary examiners
Dr. Esra Neufeld, IT’IS Foundation, Switzerland
Dr. Marta Parazzini, Istituto di Elettronica e di Ingegneria dell'Informazione e delle Telecomunicazioni (IEIIT), Consiglio Nazionale delle Ricerche, Italy

Opponent
Prof. Theodoros Samaras, Aristotle University of Thessaloniki, Greece
**Abstract**

Two international organizations, namely the International Commission on Non-Ionizing Radiation Protection (ICNIRP) and the Institute of Electrical and Electronics Engineers International Committee on Electromagnetic Safety (IEEE ICES), have established exposure criteria and safety limits for human protection to electromagnetic fields.

In the low-frequency range, both organizations recognize that the main adverse health effects are represented by the induction of retinal phosphenes, the alteration of synaptic activity and the stimulation of nerves. On this basis, the exposure limits were derived from threshold data of internal electric fields with the purpose of avoiding such adverse effects. Since direct measurement of the induced electric field is not feasible, both the standard and guidelines have introduced limits for external electric and magnetic field strengths. In this context, computational dosimetry was used to relate the internal induced quantities with the external field strengths. However, low-frequency dosimetry suffers from various sources of error and uncertainty.

The main aim of the present thesis is to lessen such uncertainty, as well as further characterize computational artifacts in the evaluation of the induced electric fields. Investigations were carried out using state-of-the-art methods based on physiological measurements, high-resolution realistic anatomical models, individualized electric field computations and biological axon models. Several open issues affecting low-frequency dosimetry have been characterized with the aim of producing quantitative data useful for the harmonization and revision of current exposure standard and guidelines.

Our findings showed a large margin of safety in the current exposure limits established by both international organizations. In this regard, the obtained results represent a solid basis for deriving safety levels that offer acceptable protection for the human population without being overly conservative. In addition, the present work improves the reliability of human exposure assessment at low frequencies.

**Keywords** non-invasive brain stimulation, transcranial magnetic stimulation, international safety standard/guidelines, low-frequency dosimetry, individualized models, electrostimulation models, finite element method

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>978-952-64-0392-2</td>
<td>978-952-64-0393-9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location of publisher</th>
<th>Location of printing</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helsinki</td>
<td>Helsinki</td>
<td>2021</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pages</th>
<th>urn</th>
</tr>
</thead>
</table>
Preface

This thesis has been carried out in the Department of Electrical Engineering and Automation at Aalto University. The research has been funded by the Aalto ELEC doctoral school, to whom I am very grateful for the financial support. Most of the research activities were conducted at the Health Technology House (HTH), and in the Advanced Magnetic Imaging (AMI) centre, where magnetic resonance imaging acquisition and transcranial magnetic stimulation experiments were performed. I would like to thank the personnel of the AMI centre, with particular regard to Marita Kattelus, Toni Auranen and Mikko Nyrhinen for their valuable support.

Most of all, I would like to express my sincere gratitude and deep appreciation to Prof. Ilkka Laakso, who gave me the opportunity to pursue the Doctoral degree after being outside the academia for a while. Returning to university after several experiences of working in industry was rather challenging, especially at the beginning, but this gave me the possibility to further develop goal-oriented and team-working skills. I am proud of myself for being able to eventually become a Doctor. Thanks Ilkka for your trust and careful supervision, as well as for being available to help and support at any time. I wish to also thank the other members who are or have been part of our team, Dr. Marko Mikkonen, Dr. Juhani Kataja and Noora Matilainen, for their valid suggestions and insightful comments.

A special thanks to Zheng Zhao, a trusted friend whom I started this adventure with, and the other colleagues I had the good fortune of meeting during the last years: Prof. Ivan Vujaklija, Prof. Simo Särkkä, Dr. Lauri Palva, Dr. Toni Karvonen, Dr. Filip Tronarp, Rui Gao, Dennis Yeng and Wendy Lam. Thank you all for making this experience special.

Some of the studies were conducted with the collaboration of relevant experts in the field. An important contribution was given by Prof. Akimasa Hirata, who I would like to thank for his valuable support and useful discussions on the topics of this research. No less, I would like to thank Prof. Takenobu Murakami, Prof. Yoshikazu Ugawa and Dr. Jose Gomez-Tames for the fruitful work done together. I wish to express also my thankfulness to Dr. Esra Neufeld and Dr. Marta Parazzini who revised the
present thesis.
Above all, I would like to express heartfelt gratitude to my family and friends for their constant support. Without their help and understanding I could never have achieved this important result.

Otaniemi, May 19, 2021,

Marco Soldati
Contents

Preface 1
Contents 3
List of Publications 5
Author’s Contribution 7
Abbreviations 9
Symbols 11

1. Introduction 13

2. Background 17
   2.1 Exposure limits at low frequencies 17
   2.1.1 Basic restrictions and dosimetric reference limits 17
   2.1.2 Exposure reference levels 19
   2.2 Open issues in low-frequency computational dosimetry 20
   2.2.1 Exposure at intermediate frequencies 21
   2.2.2 Computational artifacts 21
   2.2.3 Variability between anatomical models 23
   2.2.4 Tissue conductivity uncertainty 23
   2.3 Transcranial magnetic stimulation 25
   2.4 Electrical stimulation of neurons 27

3. Materials and methods 31
   3.1 Realistic anatomical models 31
   3.1.1 Magnetic resonance imaging 32
   3.1.2 Voxelized head model 33
   3.1.3 Tetrahedral head models 35
   3.1.4 Volume conductor models 36
   3.2 Electric field modeling 37
3.2.1 Governing equations .................. 37
3.2.2 Modeling the source ................... 39
3.2.3 Finite element method ................. 41
3.3 Biological axon models .................. 45
  3.3.1 Action potential ...................... 45
  3.3.2 Circuit representation of myelinated fiber 45
3.4 Statistical methods ...................... 48
3.5 TMS experimental setup .................. 49

4. Summary of publications .................. 51

5. Discussion ................................. 59

6. Conclusion ................................ 67

References .................................. 69

Errata ...................................... 87

Publications ................................. 89
This thesis consists of an overview and of the following publications which are referred to in the text by their Roman numerals.

I Marco Soldati, Marko Mikkonen, Ilkka Laakso, Takenobu Murakami, Yoshikazu Ugawa and Akimasa Hirata. A multi-scale computational approach based on TMS experiments for the assessment of electro-stimulation thresholds of the brain at intermediate frequencies. Accepted for publication in *Physics in Medicine & Biology*, 63 (22), 225006, November 2018.

II Marco Soldati and Ilkka Laakso. Computational errors of the induced electric field in voxelized and tetrahedral anatomical head models exposed to spatially uniform and localized magnetic fields. Accepted for publication in *Physics in Medicine & Biology*, 65 (1), 015001, January 2020.

III Marco Soldati, Takenobu Murakami and Ilkka Laakso. Inter-individual variations in electric fields induced in the brain by exposure to uniform magnetic fields at 50 Hz. Accepted for publication in *Physics in Medicine & Biology*, 65 (21), 215006, October 2020.

IV Marco Soldati and Ilkka Laakso. Effect of electrical conductivity uncertainty in the assessment of the electric fields induced in the brain by exposure to uniform magnetic fields at 50 Hz. Accepted for publication in *IEEE Access*, 8, 222297–222309, December 2020.
Author’s Contribution

Publication I: “A multi-scale computational approach based on TMS experiments for the assessment of electro-stimulation thresholds of the brain at intermediate frequencies”

The author designed and developed the study based on an idea of Prof. Laakso. The author wrote the computational codes, the manuscript and derived the results. The co-authors contributed to improve the paper with their comments.

Publication II: “Computational errors of the induced electric field in voxelized and tetrahedral anatomical head models exposed to spatially uniform and localized magnetic fields”

The author designed the study, performed the modeling and numerical computations, derived the results and wrote the manuscript. The idea of the paper was based on discussions with Prof. Laakso, who assisted in the coding work.

Publication III: “Inter-individual variations in electric fields induced in the brain by exposure to uniform magnetic fields at 50 Hz”

The author designed the study, performed the modeling and numerical computations, derived the results and wrote the manuscript. The idea of the paper was based on discussions with Prof. Laakso.
Author's Contribution

Publication IV: “Effect of electrical conductivity uncertainty in the assessment of the electric fields induced in the brain by exposure to uniform magnetic fields at 50 Hz”

The author designed the study, performed the modeling and numerical computations, derived the results and wrote the manuscript. The idea of the paper was based on discussions with Prof. Laakso.

Language check

The language of my dissertation has been checked by William Martin. I have personally examined and accepted/rejected the results of the language check one by one. This has not affected the scientific content of this dissertation.
Abbreviations

AMT  Active Motor Threshold
BEM  Boundary Element Method
BR   Basic Restriction
CNS  Central Nervous System
CSF  Cerebrospinal Fluid
CT   Computer Tomography
DRL  Dosimetric Reference Limit
DTI  Diffusion Tensor Imaging
EIT  Electrical Impedance Tomography
ELF  Extremely Low Frequency
EMF  Electromagnetic Field
EMG  Electromyography
ERL  Exposure Reference Level
FDI  First Dorsal Interosseous
FEM  Finite Element Method
FID  Free Induction Decay
GM   Grey Matter
HS   Hotspot
ICES International Committee on Electromagnetic Safety
ICNIRP International Commission on Non-Ionizing Radiation Protection
Abbreviations

IEEE  Institute of Electrical and Electronics Engineers
IF    Intermediate Frequency
LF    Low Frequency
MEP   Motor Evoked Potential
MR    Magnetic Resonance
MREIT Magnetic Resonance Electrical Impedance Tomography
MRI   Magnetic Resonance Imaging
MSO   Maximum Stimulator Output
MT    Motor Threshold
PNS   Peripheral Nervous System
RF    Radio Frequency
RL    Reference Level
RMT   Resting Motor Threshold
rTMS  Repetitive Transcranial Magnetic Stimulation
SENN  Spatially Extended Non Linear Node
SPFD  Scalar-Potential Finite-Difference
TMS   Transcranial Magnetic Stimulation
WHO   World Health Organization
WM    White Matter
Symbols

A  Magnetic Vector Potential, [V m⁻¹]
B  Magnetic Flux Density, [T]
E  Electric Field, [V m⁻¹]
h  Planck Constant, [J s]
I  Electric Current, [A]
J  Current Density, [A m⁻²]
m  Magnetic Moment, [A m²]
sp  Angular Momentum or Spin
S  FEM Matrix
v  Test Function
γ  Magnetogyratic Ratio, [rad s⁻¹ T⁻¹]
ε  Permittivity, [F m⁻¹]
σ  Electrical Conductivity, [S m⁻¹]
ϕ  Electric Scalar Potential, [V]
ω  Angular Frequency, [rad s⁻¹]
ψ  Basis Functions
Ω  Domain of the Solution
∂Ω  Boundary of Ω
∇  Differential Operator
1. Introduction

Human exposure to electromagnetic fields (EMFs) has always been the subject of public concern due to their possible adverse health effects. For this reason, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [1] and the Institute of Electrical and Electronics Engineers International Committee on Electromagnetic Safety (IEEE ICES) [2, 3] have defined exposure limits for human protection. The IEEE standard refers to low frequency (LF) as time-varying electric and magnetic fields up to 5 MHz [2, 3], whereas in the ICNIRP guidelines the upper limit of the LF interval was set to be 10 MHz [1]. Based on the World Health Organization (WHO) [4], the LF range can be further divided into the extremely low frequency (ELF) range from 1 Hz to 300 Hz, and the intermediate frequency (IF) range from 300 Hz to 10 MHz.

In the ELF range, most of the studies consider frequencies of 50 Hz or 60 Hz [5, 6, 7, 9], as they correspond to the power-line frequencies used worldwide. On the other hand, investigations on the IF range are still scarce. However, an increasing number of devices using those fields has been observed, such as coupled wireless power transmission devices [10], electronic article surveillance systems [11], induction heating cookers and various medical devices. Hence the need for more studies investigating the effect of the electromagnetic field at intermediate frequencies.

The exposure to externally applied electric and magnetic fields at LFs produces an induced electric field in the body according to Faraday’s induction law. The induced electric field generates eddy currents, and it might cause alterations to the membrane potential of neurons. At ELF, the dominant adverse effect recognized by both the standard and the guidelines is the alteration of synaptic activity in the central nervous system (CNS), with the possible induction of retinal phosphenes and the modification of the brain function [1, 2, 3, 12]. On the other hand, excitation of nerve cells are reported to be the main established adverse health effects for human exposure to electromagnetic fields at IF [1, 2, 3, 12].

To prevent such unwanted health effects, the ICNIRP guidelines and IEEE standard introduced the basic restrictions [1] or dosimetric reference...
Introduction

limits [2, 3], which are expressed in terms of induced electric field strength. However, direct measurements of the induced electric fields in the human body are not feasible. Therefore, the standard/guidelines define additional metrics that are easier to measure, namely the reference levels [1] or exposure reference levels [3]. These represent the external electric/magnetic field strength capable of producing induced electric fields equal to the basic restrictions. In this context, computational dosimetry makes it possible to relate the external field with the induced electric field, and is therefore used for the development of exposure criteria and limits.

Several numerical methods have been developed to compute the induced electric field, such as the finite element method (FEM) [13, 14, 15], the scalar-potential finite-difference method (SPFD) [16], the boundary element method (BEM) [17] and the impedance method [18]. Among these numerical techniques, the FEM became widely adopted due to its capability of making accurate calculations by handling complex and irregular geometrical anatomies, anisotropy, and because it easily incorporates the boundary conditions [13]. Discrepancies produced by different numerical methods have been estimated to be in the order of 10% [19], and most recently around 5% [27], indicating a good agreement and reproducibility in the results.

High-resolution realistic anatomical models are needed in computational dosimetry for the accurate calculation of the induced electric fields. These models are mostly obtained through the segmentation of structural magnetic resonance (MR) images to obtain three-dimensional tetrahedral or voxelized anatomical models. This means that the body, or parts of it, is divided into many tiny elements, i.e. voxels or tetrahedra, to which a tissue electrical conductivity is assigned for creating a so-called volume conductor model. After modeling the source of the external electromagnetic field, numerical methods are employed to finally calculate the induced electric field in each element of the volume conductor model.

Computational dosimetry at LFs is affected by several sources of error and uncertainty, most of which have already been characterized [7, 8, 9, 19, 20, 21, 22, 23, 24]. Others still remain an open issue [12, 28]. This assumes particular importance considering that both the IEEE standard and ICNIRP guidelines have used computational dosimetry to developed safety limits for the human exposure to electromagnetic fields. Solving these issues is important for establishing acceptable limits that are not overly conservative for the human population. In this context, the aim of this thesis was to investigate the main issues that still affect LF dosimetry, in order to reduce computational uncertainty in the estimation of electric field strengths.

The present thesis consists of four peer-reviewed journal articles. Publication I focused on the IF electromagnetic fields, for which there is a lack of scientific data concerning human exposure. This study combined
for the first time physiological measurements from transcranial magnetic stimulation (TMS) experiments, electromagnetic dosimetry and biological axon models with the purpose of investigating the thresholds of stimulation of brain tissue at IFs. The use of biological axon models allowed the extrapolation of threshold-frequency curves, which were compared with the basic restrictions defined by the international standard/guidelines.

Publication II extensively investigated the uncertainty in LF dosimetry due to numerical errors that affect both voxelized and tetrahedral anatomical models. Several post-processing methods were analyzed for reducing these computational artifacts, with the aim of increasing the reliability in the evaluation of the electric field strengths for human exposure at LFs.

Publication III studied the variability in the induced electric fields between anatomical models. This assumes particular importance considering that the international standard/guidelines established exposure limits to electromagnetic fields based on a limited number of anatomical models (ICNIRIP) or simplified geometrical shapes (IEEE). Here we provided quantitative results on the actual variability of the peak electric fields induced in the brain of 118 individuals exposed to uniform magnetic fields at 50 Hz. For the first time, we also studied how anatomical differences and individual characteristics affect the induced electric field strengths.

Lastly, publication IV examined the uncertainty in LF dosimetry due to the variability in the electrical properties of the tissues. Here we showed that new estimations of brain tissue conductivity values gave significantly lower electric field strengths than those obtained with commonly used values in LF dosimetry. This has a major impact when considering that ICNIRP derived the reference levels from the basic restrictions using realistic anatomical models. In this context, the use of larger conductivity values produced a significantly lower conversion factor for the CSN tissues [29]. The sensitivity of electric field strengths due to variations in tissue conductivity was also investigated.

The results derived in this thesis are intended to provide quantitative data useful for the revision and harmonization of the current standard/guidelines for human protection to electromagnetic fields at LF. This data might be used for the evaluation and selection of appropriate safety factors in order to derive exposure limits that would offer sufficient protection for most people.
2. Background

2.1 Exposure limits at low frequencies

Two international organizations, the ICNIRP [1] and the IEEE ICES [3], have established safety limits to prevent adverse health effects when humans are exposed to electromagnetic fields at LFs. In this context, the ICNIRP guidelines [1] define exposure limits for two classes of people, namely the general public (i.e., individuals of all ages and of varying health status) and occupational exposure (i.e., adults exposed under known conditions and aware/trained to potential risk). The IEEE standard [3] refers to people in unrestricted environments, such as living quarters, public areas, and workplaces, or in restricted environments, if the adverse effects under specific circumstances can be mitigated with appropriate precautionary measures (personal protective equipment, awareness programs and so on). The limits are more conservative for general public exposure and for people in unrestricted environments.

However, there are discrepancies between the ICNIRP guidelines and the IEEE standard due to the fact that they used different thresholds of adverse reactions, reduction factors and magnetic induction models to derive the exposure limits [28, 30].

2.1.1 Basic restrictions and dosimetric reference limits

In the IEEE standard [3], the dosimetric reference limits (DRLs) were established for several tissues such as brain, heart, limbs and peripheral nerves (Figure 2.1). At frequencies below ~ 2.4 kHz, the lowest DRLs were set for the brain and derived from a threshold value of 75 mV m\(^{-1}\) (53 mV m\(^{-1}\) rms), which was obtained from magnetophosphene data (8.14 mT rms at 20 Hz) [31, 32] using an ellipsoidal dose model. For higher frequencies (>~ 2.4 kHz), the lowest DRLs were set for the peripheral nervous system (PNS), which were derived from an excitation threshold of
6.15 V m⁻¹ estimated for a 20 μm fiber using the spatially extended non-linear node (SENN) model [33]. Note that to account for uncertainties, several reduction factors were applied to the above thresholds to obtain the DRLs [3].

The ICNIRP specifies the basic restrictions (BRs) for the CNS tissues of the head, and for all tissues of head and body. Between 10 and 25 Hz, the BRs established for the CNS tissues of the head were derived from a threshold value of 50 mV m⁻¹ to avoid the induction of retinal phosphenes [34]. This value represent the actual limit in the case of occupational exposure, whereas an additional reduction factor of 5 is applied for general public exposure, giving a BR of 10 mV m⁻¹. Above and below the interval of 10 – 25 Hz, the BRs increase when the frequency increases (Figure 2.1). In addition, for frequencies higher than 400 Hz (occupational exposure) and 1 kHz (general public exposure), the BRs for the CNS are based on a threshold value of 4 V m⁻¹ to avoid the stimulation of peripheral nerves. This value was derived from a study carried out by So et al. [36], who computed the electric fields in subcutaneous fat and skin produced by a rheobase time rate of change of magnetic field equal to 18.8 ± 0.6 T/s, which was found to cause peripheral nerve stimulation using gradient-switching MR equipment [35]. Numerical calculations were performed on three human body models using the SPFD method. Results showed that the derived thresholds for PNS stimulation in terms of induced electric field ranged between 4 and 6 V m⁻¹. The minimum value of 4 V m⁻¹ was considered in the ICNIRP guidelines, to which a reduction factor of 5 and 10 is applied to obtain the BRs of the CNS tissues at IFs for occupational and general public exposures, respectively. These restrictions increase proportionally with frequency above 3 kHz. Note that the limits for all tissues of head and body correspond to those aimed at preventing peripheral nerve stimulation [1].

Figure 2.1. Comparison between the BRs and DRLs established by the international standard/guidelines. Limits defined in terms of induced electric field strengths (V m⁻¹) for (a) people in restricted environments/occupational exposure, and for (b) people in unrestricted environments/general public exposure.
When comparing the exposure with the BRs or DRLs, the induced electric field has to be spatially averaged over a contiguous tissue volume of $2 \times 2 \times 2 \text{mm}^3$ [1], or an arbitrarily oriented segment of 5 mm length [2, 3]. In addition, the ICNIRP recommends removing numerical artefacts (i.e., staircasing approximation errors) by determining the 99th percentile value of the averaged electric field for a specific tissue [1]. A recent study showed that both averaging schemes provide comparable results [37].

### 2.1.2 Exposure reference levels

Direct measurements of the induced electric fields in human tissues are not feasible. For this reason, both standard/guidelines have introduced measurable limits for the external magnetic field strength, which are termed reference levels (RLs) in the ICNIRP guidelines, and exposure reference levels (ERLs) in the IEEE standard [3]. The RLs/ERLs were determined as the minimum external magnetic field that produced an induced electric field strength corresponding to the BRs/DRLs. In particular, these limits were obtained from the BRs/DRLs using ellipsoidal [2, 3] or complex anatomical body [1] models. Compliance with RLs/ERLs should guarantee that the BRs/DRLs are not exceeded.

For all environments, the ERLs defined in the IEEE guidelines for head and torso are intended to protect individuals against adverse effects in the brain for frequencies $< 750 \text{ Hz}$, and against adverse PNS effects for higher frequencies (Figure 2.2). In particular, the ERLs can be easily calculated from the DRLs with the following formula:

$$ E = -B_\omega \left| \frac{a^2 u a_v - b^2 v a_u}{a^2 + b^2} \right|, $$

where $a_v$ and $a_u$ are unit vectors along the minor and major axes, $a$ and $b$ the semi-major and semi-minor axes, $u$ and $v$ is the location within the exposure area, $B_\omega$ the time rate of change of the magnetic flux density and $E$ the induced electric field strength. The geometrical parameters of the ellipses representing the different body parts are provided in the IEEE standard [2, 3].

The RLs established by the ICNIRP standard provide protection against adverse brain effects for frequencies lower than 300 Hz (occupational exposure) and 400 Hz (general public exposure). Above these frequencies, the limits should protect against peripheral nerve stimulation. As mentioned, the RLs were obtained from the BRs through dosimetry modeling by means of realistic anatomical models [38, 39]. At 50 Hz, the CNS induction factor used to convert the BR to the RL is equal to 33.0 mV m$^{-1}$, which corresponds to the maximum induced electric field strength found in the brain for a spatially uniform magnetic field of 1 mT directed along the lateral direction [38]. The latter value was set as the RL for occupa-
Figure 2.2. Comparison between the RLs and ERLs established by the ICNIRP standard and IEEE guidelines.

...tional exposure at 50 Hz, as it would produce the corresponding BR of $3 \times 33.0 \text{ mV m}^{-1} = 100.0 \text{ mV m}^{-1}$, where 3 represents an additional reduction factor that accounts for dosimetric uncertainty. Since there was no PNS conversion factor available, the skin was chosen to be the worst-case target tissues as it contains peripheral nerve endings. Therefore, the PNS induction factor at 50 Hz was derived from [38] based on the maximum induced electric field strength of 60.0 mV m$^{-1}$ that was found in the skin under an external magnetic field of 1 mT [28, 124].

2.2 Open issues in low-frequency computational dosimetry

In 2016, the IEEE ICES published a research agenda that provided a list of unresolved issues related to LF electrical dosimetry [28]. Several research projects followed with the purpose of resolving various sources of uncertainty and providing data for harmonizing the human exposure standard/guidelines. Some of these issues were resolved, while some additional issues were raised. More recently, an ICNIRP knowledge gap document [12] was released to further address the importance of undertaking studies aimed at reducing uncertainty in LF dosimetry for the development of acceptable and non-overly conservative safety limits. In this context, the main objective of this thesis was to investigate/resolve some of the open issues which will be described in the next paragraphs. Our purpose was to characterize uncertainty in computational dosimetry at LFs and provide quantitative data useful for the revision of the international standard/guidelines.
2.2.1 Exposure at intermediate frequencies

At IFs, the ICNIRP derived the BRs for the CNS tissues based on thresholds for the stimulation of peripheral nerve fibers [1]. In this range of frequencies, the IEEE obtained the DRLs for the brain from published data on stimulation thresholds of retinal phosphenes [2, 3]. Therefore, when deriving those limits, stimulation thresholds of the axons in the brain were not considered. In this context, there is still a lack of scientific data in literature concerning human exposure of the brain at the IFs, where the main health adverse effect is related to the electro-stimulation of axons. Producing such data is relevant for human protection to EMF, considering that an increasing number of devices working at IFs has been observed [6].

Setting up experiments for measuring the excitation thresholds of brain tissues at IFs has represented a rather challenging task. However, the development of non-invasive brain stimulation techniques together with high resolution head models based on MR imaging (MRI) has offered the possibility to directly relate physiological responses to the induced electric fields [40, 41]. The inclusion of biological models in a multi-scale computational approach allows estimation of the stimulation thresholds of brain tissue. For this reason, the IEEE ICES [28] highlighted the importance of relating computational dosimetry and biological models to investigate neuron stimulation thresholds.

In this context, TMS represents an effective technique in producing such data as it offers the possibility of non-invasively studying the excitability of the axons. On this basis, publication I represents the first attempt to combine physiological responses to TMS, individualized MRI-based computer simulations and biological axon models to estimate the stimulation thresholds of the brain at IFs. The use of biological axon models allowed the extrapolation of threshold-frequency curves, which revealed a large margin of safety in comparison with the basic restrictions/dosimetric reference limits. Following our investigation, Gomez-Tames et al. [42] employed a rather similar approach to investigate the stimulation thresholds of the brain for different scenarios of magnetic field exposures. In addition to the basic restrictions, the authors observed a large margin of conservatism even when comparing their results with the limits for the external magnetic field strengths [42].

2.2.2 Computational artifacts

Human exposure to LF electromagnetic fields has been mainly investigated using anatomical models discretized with regular grids of voxels [20, 21, 22, 24, 38, 43, 44]. The main advantage of employing cubic grids is represented by the relative ease in generating anatomical models from the segmentation of MR images, as well as their efficiency in deriving
Background

dependent numerical solutions, which makes the solver easier to implement and better performing [14]. However, cubical elements are quite inefficient in approximating curved surfaces, resulting in the staircase approximation error that introduces numerical artefacts in the calculation of the induced electric field. These errors make the maximum values unreliable especially at boundaries between tissues with high electrical conductivity contrast. The accuracy of the numerical solution also depends on the discretization error resulting from the resolution of the voxels. Decreasing the side length of the cubic elements improves the accuracy, but also increases the computational costs.

To lessen the effect of the staircase approximation error in the evaluation of the maximum induced electric field, the ICNIRP recommends determining the 99th percentile value of the averaged electric field over a contiguous tissue volume of $2 \times 2 \times 2 \text{ mm}^3$ [1]. The use of the 99th percentile value as a measure for obtaining reliable estimations of the peak electric field strengths was suggested for the first time by Dawson *et al.* [45]. In this study, the authors compared the numerical and analytical electric fields induced in homogeneous and layered spheres of different resolutions which were exposed to uniform magnetic and electric fields. The results showed large discrepancies between the numerical and analytical values of the maximum induced electric fields due to the staircase approximation error. However, ignoring the highest 1% of electric field values was effective in providing a satisfactory estimate of the maximum field, as the 99th percentile value of the analytical and numerical electric fields were in a good agreement. Additional studies confirmed the effectiveness of the 99th percentile filtering, which was shown to be invariant with the voxel resolution in both simple geometries [43, 45] and anatomical models [43, 44]. For this reason, the ICNIRP adopted the 99th percentile filtering for a specific tissue to lessen the effects of numerical artifacts deriving from the staircase approximation error.

Unlike voxelized models, tetrahedral meshes do not suffer from the staircase approximation error, as they conform better to complex geometries, allowing a more accurate representation of curved surfaces through the triangular faces of the tetrahedra elements. Therefore, they could be more suitable for computational modeling. In addition, they require a fewer number of elements than the voxelized models. However, the generation and refinement of high-quality meshes for complex three-dimensional domains constitutes a rather challenging task. This complicates the electromagnetic modeling workflow in comparison to that using voxels of the segmented images, which could be directly used as input in computational methods [14]. Nonetheless, tetrahedral meshes may suffer from artefacts originated by poorly shaped elements that cause the numerical solution to be less accurate.

In this context, publication II represented the first attempt to compare
computational results obtained using tetrahedral and voxel-based models in order to characterize numerical errors affecting the induced electric field at LFs. This is particularly important considering that an analytic solution for anatomical models does not exist. The effect of the discretization error on the induced electric field was analyzed by considering several meshes/grids with different resolutions. In addition, the authors investigated the effect of the 99th – or higher – percentile filtering on the induced electric fields for both uniform and localized exposure scenarios. This was highlighted as an open point to be further investigated by both the ICNIRP knowledge gap document [12] and the IEEE ICES [28], especially in the case of non-uniform scenarios where the 99th percentile value was found to significantly underestimate the peak electric field strengths [23, 46].

2.2.3 Variability between anatomical models

In the international standard/guidelines, the (exposure) reference levels were obtained as the minimum external magnetic field that induced an electric field strength corresponding to the basic restrictions/dosimetric reference limits. When deriving these limits, only a limited number of anatomical [1] and elliptical [2, 3] models were considered. As a consequence, the ICNIRP knowledge gap document [12] addressed the importance of reducing this source of uncertainty by undertaking inter-comparison studies that consider a larger number of anatomical models with the purpose of determining more reliable conversion factors. For this reason, publication III investigated the variability of the induced electric field strengths in a population consisting of 118 individuals, whose heads were exposed to uniform magnetic fields at 50 Hz from different directions. Using a linear mixed-effects model, the effect of different factors (i.e., age, gender, magnetic field direction and skull volume) on the electric field strengths induced in the brain was investigated.

2.2.4 Tissue conductivity uncertainty

Biological tissues have dielectric properties, therefore they are able to both conduct electric currents and/or being polarized. In this context, the electrical conductivity \( \sigma \) (S/m) measures the ability to conduct electricity, whereas the permittivity \( \epsilon \) (F/m) quantifies the electric polarizability of dielectric materials. However, at LFs tissues can be considered purely resistive as \( \sigma \gg \omega \epsilon \) [13], where \( \omega \) is the angular frequency. One of the earliest review papers to publish data about the LF conductivity of biological tissues was the work by Geddes and Baker [48]. Later on, more research on the tissue dielectric properties followed [49, 50, 51]. After a series of investigations dated 1996 [52, 53, 54], Gabriel provided a list of conductivity values that became the most commonly used in LF dosimetric studies. The first paper
to be published was an extensive review of the dielectric properties of the biological tissues [52]. This work showed a large variability between data from different investigations, and also gaps for several tissues and/or frequencies. On this basis, it followed an additional work where advanced techniques for that time were used to measure the dielectric properties of biological tissues [53]. These measurements were conducted in the range of frequency from 10 Hz to 20 GHz using fresh excised animal tissues, human autopsy materials and in vivo human skin/tongue tissues [53]. In a later study [54], the obtained experimental data together with additional values from literature were used together in a 4-Cole-Cole dispersion model to derive the tissue conductivities as a function of frequency in the range from 10 Hz to 100 GHz. As a result, a final technical report was released [55], which contained the fitted data for high frequencies, as well as a separate list of conductivity values for frequencies below 100 Hz. The latter set of values was employed by Dimbylow in two studies [38, 39] that were used as a basis for developing the ICNIRP guidelines.

The Gabriel database suffered from two main limitations [56]. The first one was represented by the fact that conductivity values at frequencies below 1 MHz were rather scarce. Secondly, most of the measurements were carried out on post-mortem tissues, which undergo substantial biochemical and biophysical modifications after death. As a result, those changes might affect significantly the electrical conductivity estimation [57, 58]. Measurements on live samples are preferable, but for a long time they have required invasive procedures to be performed on certain tissues. In this context, in vivo measurements at 50 kHz during brain surgery showed values of 0.28 S/m for grey matter (GM) and 0.25 S/m for white matter (WM) [59], which were relatively higher than those reported by Gabriel (0.12 S/m for GM and 0.08 S/m for WM) [54]. Later on, Logothetis et al. [60] carried out invasive measurements on the visual cortex of monkeys, finding a GM conductivity of 0.404 S/m for frequencies between 1 Hz and 10 kHz. However, only the development of advanced imaging techniques, such as electrical impedance tomography (EIT) and magnetic resonance EIT (MREIT), have allowed to perform in vivo conductivity measurements of brain tissues non-invasively. Within this framework, a recently published work [61] provided an extensive review of human head electrical conductivity values based on five data acquisition techniques, including MREIT, EIT, directly applied current (DAS), diffusion tensor imaging (DTI), and electromagnetic data recorded from magnetoencephalography (E/MEG). In particular, this study seem to confirm higher conductivity values for brain tissues (0.47 S/m for GM, and 0.22 S/m for WM) [61], and the skin (0.41 S/m). From a total number of 3121 publications initially considered, 56 investigations were selected for data extraction. Furthermore, a robust analysis for evaluating the systematic and random errors introduced in each study was performed. Notably, to assess the systematic errors in each
Background

Investigation, the Cochrane Collaboration recommended Quality Assessment of Diagnostic Accuracy Studies (QUADAS) checklist was used [62], which led to the determination of a Quality Assessment Score (QAS), whose maximum value was set to one. In addition, to account for random errors due to unpredictable variation in methodology, the authors employed a meta-analysis weighting [64, 63] that provided the means to determine confidence interval values (with a maximum of one) for each conductivity measurement. The QAS of each study and the confidence values of each conductivity value were then multiplied together to provide a final weight. The higher the weight, the more reliable the conductivity value. Finally, a weighted multiple regression analysis was performed and the weighted average conductivity values of the main head tissue types were calculated. Based on the large number of the considered studies and the robust quality analysis performed, the research carried out by McCann [61] represents a solid work on the latest head conductivity values in literature.

A large variability in the LF conductivity values of other tissues is also observed, for instance fat [54, 61, 65, 66, 67], skin [54, 61, 68, 69], muscle [54, 61, 66, 69, 70] and skull [54, 58, 61, 71]. Several reasons contribute to produce this uncertainty, such as the different techniques used to measure the conductivity, as well as differing samples (i.e., in vivo, ex vivo, in vitro) from various animals. On the contrary, the conductivity values of body fluids, for example blood and cerebrospinal fluid (CSF), are quite consistent among investigations since they can be more easily extracted. In particular, the conductivity of CSF is rather high, ranging between 1.79 S/m [72] and 2 S/m [54], as it mainly consists of water. The conductivity of blood is reported to vary between 0.57 S/m [61] and 0.7 S/m [54].

As highlighted in [12], variability in the tissue electrical conductivity represents a major source of uncertainty in the assessment of safety exposure limits. Within this framework, publication IV focused on evaluating the effect of this uncertainty on the electric fields induced in the brain. In addition, the sensitivity of electric field strengths due to variations in tissue conductivity was investigated.

2.3 Transcranial magnetic stimulation

TMS is a non-invasive technique capable of producing neural activation through a strong and brief time-varying magnetic field generated by a coil placed over the scalp [73, 74]. In current devices, the magnetic field is in the order of 2–3 T and derives from the rapid discharge (∼100–200 µs) of a large capacitor through the coil, which is fed with an intense current pulse (4–8 kA) [75]. According to Faraday’s induction law, the time-varying magnetic field induces an electric field in the brain that might alter the membrane potential of neurons. If the induced electric field exceeds a
certain threshold, the axonal membrane is depolarized leading to the generation of an action potential, and thereby neuronal excitation. In this context, the majority of TMS studies have targeted the motor cortex for the relative ease in measuring the produced neural responses through electromyography (EMG) [40, 76, 77, 78, 79, 80].

The stimulation above the threshold of the motor cortex gives rise to action potentials that propagate towards the contralateral targeted muscles which react to the stimulus by contracting. From these muscles, electrical signals in response to the stimulation, the so-called motor evoked potentials (MEPs), can be measured through EMG. As a consequence, TMS represents an effective tool for evaluating the motor thresholds (MTs) of the nervous system, i.e., the lowest stimulation intensities required to produce a muscle response. MTs are typically expressed in terms of the percentage of the maximum stimulator output (%-MSO). In publication I, the active (AMTs) and resting (RMTs) motor thresholds were determined in accordance to Rossini criterion [81]. Based on this, the RMT was measured as the lowest stimulation intensity which elicited a minimum MEP of 50 μV in at least 5 out 10 consecutive trials in the relaxed muscle. Similarly, the AMT was measured as the minimum intensity required to produce an MEP of 200 μV in at least 5 out 10 consecutive trials during a slight contraction of the targeted muscle. During the measurements, the stimulus intensity was increased in steps of 5 % until TMS consistently produced MEPs of 50 μV (RMT) or 200 μV (AMT). Then, the stimulus intensity was decreased in steps of 1 % until there were at least 5 positive responses out of 10 trials [81]. This stimulus intensity plus 1 gave the motor thresholds [81].

For the TMS experiments conducted in publications I and II, a figure-of-eight coil was used, which consists of two adjacent round wings where the current flows in opposite directions, summing up at the junction point. As a result, the induced electric field is maximum at the point of intersection between the two round coils [82, 83]. For this reason, figure-of-eight coils produce a more focal stimulation than simple round coils [74].

Two kinds of waveforms are typically used in clinical applications, namely the monophasic and biphasic pulses. In our investigations, monophasic current pulses were always employed, which commonly consist of a strong initial current flow that reaches a maximum in about 50 μs after the pulse onset. A second phase follows where the current is dampened with a power resistor and a shunting diode. In this context, only the first phase of the stimulus is capable of eliciting action potentials, as the dampened phase produces no brain stimulation [84]. On the other hand, the biphasic pulse consists of an initial peak followed by a current reversal below zero, which then rises again to zero. In this case, both phases are effective in producing a neuronal response, with the second phase having the largest amplitude and the longest duration [84]. Therefore, the second reversal phase is
more effective for cortical excitation. Because of the higher energy efficacy, biphasic pulses are commonly used in repetitive TMS (rTMS), whereas monophasic waveforms are mainly employed in single or paired pulses [85].

The orientation and position of the stimulation coil on the scalp significantly affect the responses elicited by TMS [86]. First, the coil has to be placed right above the targeted cortical region in order to produce the desired response. In addition, its orientation determines both the strength and depth of penetration of the electric field [86]. In this context, it has been shown that the motor cortex is particularly sensitive when the coil is angled so that the induced electric field is perpendicular to the central sulcus [15, 86, 152].

In publication I, TMS was used for the first time as an effective method to produce data about the CNS stimulation thresholds at IFs. This study was based on two hypotheses: (i) TMS directly activates axons rather than cell bodies [81, 87, 88]; (ii) the induced electric fields are uniform at the neuronal level [149], except at the axon endings and bends. In publication II, TMS was employed to characterize numerical errors in LF computational dosimetry for localized exposure, as it represents a useful non-invasive technique used to locally apply magnetic fields via a strong pulse of electric current feeding the coil. The purpose was to show that in the case of non-uniform scenarios the 99th percentile filtering might produce a significant underestimation of the peak electric field strengths.

2.4 Electrical stimulation of neurons

The nervous system mainly consists of electrically excitable cells, most commonly known as neurons, that are able to communicate with other cells through specific connections called synapses. The nervous system is divided in two parts: the CNS, which comprises the brain, retina, and spinal cord, and the PNS, which contains all the nerves that lie outside of the CNS.

Briefly, neurons consist of a cell body or soma, containing DNA and other organelles, and neurites (i.e., axons and dendrites). The neuronal membrane separates the inside from the outside of the neuron, and contains numerous proteins that regulate the flow of ions and nutrients inside the cell. The cell body originates an axon [89], which is the neuronal element that carries the electrical information of the neuron, the so-called action potential. Dendrites, typically branching profusely from the soma, receive this signal from the axons, or more rarely the dendrites, of other neurons. The signal is then transferred to the cell body and continues along the axon as an electric impulse. In particular, at rest the intracellular fluid of the axon has a negative charge compared to the extracellular fluid. The action
Background

potential originates from a brief reversal of this condition, i.e., the inside of the membrane becomes positively charged in relation to the outside. This depolarization is caused by the influx of sodium through the opening of hundreds of voltage-gated sodium channels. Action potentials are actually triggered when the depolarization reaches a critical level, which is defined as the excitation threshold. Shortly after, the voltage-gated sodium channels close and the voltage-gated potassium channels open [89]. Since the membrane is strongly depolarized, potassium flows out causing the restoration of the resting membrane potential. Please note that the activation of potassium channels strongly contribute to restore the resting state in amphibian nerve fibers [91, 92], whereas the repolarization of the membrane is mainly due the inactivation of sodium channels in mammalian axons [95, 96]. The presence of a myelin sheath insulates the membrane to reduce the possibility of ions leaking out from the axon. This sheath is interrupted regularly, leaving the axonal membrane exposed to form the nodes of Ranvier, through which the ions flow in and out of the membrane. By acting as an electrical insulator, myelin greatly speeds up the action potential conduction.

At the neuronal level, the effect of an external electric field is to force the displacement of ions possibly yielding to an alteration of the membrane potential. If the applied external stimulus is sufficiently strong to cause the exceeding of the excitation threshold, then an action potential is triggered. Therefore, an externally applied electric field can be used to stimulate the nervous system. To study the electrical behaviour of nerve fibers, a technical breakthrough was represented by the invention of the voltage clamp technique, developed by the American physiologist Kenneth C. Cole [90], that allowed to measure the time course of ion currents across the cell membrane. This device enabled the investigators to set a certain membrane potential, that caused ion channels to open and/or close, leading to changes in the ion current. These variations were compensated by the voltage clamp apparatus with a negative feedback that maintained the desired holding potential. Thus, the current produced by the clamp circuit to keep the membrane potential constant represented the ionic current. This technique was further improved by Hodgkin and Huxley, allowing them to publish in 1952 the first mathematical model describing the variations in membrane conductance at different membrane potentials by measuring the currents that flowed across the membrane [91]. This study earned them the Nobel Prize in Physiology in 1963. Based on experiments performed using the giant axon of a squid, the authors modeled each portion of an excitable cell as an electrical circuit consisting of a capacitance, representing the membrane, in parallel with potassium and sodium voltage gated-ion channels. In particular, each of the ion channel was modeled as a conductance in series with a potential source. A leakage ion channel was also included in the model to account for chloride and other ion channels.
The derived mathematical formulation from the equivalent electric circuit well described the initiation and propagation of action potentials in neurons. Later on, Frankenhaeuser and Huxley reformulated the classical Hodgkin-Huxley equations by extending the model to myelinated axons [92]. For the experiments, myelinated fibers of toad were used. Although these models were able to accurately describe the propagation of action potentials along the axon, they were inadequate to model an extracellular stimulation. The first computational model of this kind was developed by McNeal in 1976 [93], who used an electrical network for the representation of a myelinated nerve fiber. The myelin layer was treated as a perfect insulator (zero conductance). In addition, the model assumed that the axon was infinitely long with nodes of Ranvier equally spaced. Each node was modeled using the Frankenhaeuser-Huxley formulation derived from the toad fiber. However, due to the computational limits at the time, only a single node was modeled using the non-linear Frankenhaeuser-Huxley conductance [94]. The McNeal model represented a milestone as it was the first computational approach that made it possible to compute the threshold of a nerve fiber in response to a stimulus originated by electrodes that were not in direct contact with the fiber [93].

Later on, several studies followed with the purpose of extending the McNeal formulation by including additional features to the model, such as the conductance and capacitance of the myelin layer [95], the characterization of the equations for mammalian axons [95, 97, 96] and the inclusion of non-linearities at all nodes [33, 98]. Myelinated axons were found to be more easily exited than the unmyelinated ones, larger fibers having lower excitation thresholds [33, 99, 100]. In addition, much evidence showed that the stimulation occurs at the termination and at the bend when electric [101] and magnetic [102, 103] fields were applied, assuming the induced electric field being uniform at the neuronal level. Particularly, axon locations where the uniform electric field is directed out of the termination/bend are likely to be activated [102]. Thus, the induced electric field might stimulate different sets of axons depending on its direction [105].

In publication I, TMS experiments were carried out to investigate the excitability of axons in the brain by combining physiological responses, MRI-based realistic anatomical models, electric field calculations and biological axon models. This multi-computational approach made it possible for the first time to assess the excitation thresholds of the brain tissues at IFs.
3. Materials and methods

This chapter includes the research material and methodology used in the investigations which are part of the present thesis. Briefly, segmentation of structural MR images is used to generate three-dimensional tetrahedral or voxelized volume models of the head. As a next step, the volume conductor models are created from the segmented images by assigning the electrical conductivities of the tissues to each element of the computation domain. After exposing the volume conductor models to localized (TMS) and/or uniform magnetic fields, the induced electric field strengths are calculated using a numerical method based on the FEM. The combination of individual electric field modeling with biological axon models offers the possibility to investigate the stimulation thresholds of brain tissues.

3.1 Realistic anatomical models

Realistic anatomical models are key components in computational dosimetry, as they allow determination of the distribution of the induced electric field through numerical methods. Most of them are obtained using computer tomography (CT) or MR images. In this context, MRI offers a better contrast of soft-tissues which makes this technique preferable for segmentation of such kinds of tissue [106]. Segmentation consists of partitioning an image into regions with similar properties, such as contrast [107, 108]. As a result, the images are subdivided into compartments that correspond to different tissues. Once the segmented images are obtained, they are converted into a volume mesh consisting of voxel or tetrahedral elements. In particular, our segmentation pipeline uses cubic voxel of 0.5 mm size to generate anatomical models [109]. Finally, the tissue conductivity is assigned to each element of the domain to create a volume conductor model, which is then used for computing the induced electric field.
3.1.1 Magnetic resonance imaging

MRI is a medical tomographic technique that makes use of powerful magnets, radio waves, and computational reconstruction algorithms to generate detailed images of the human body and/or its parts [111]. In particular, MRI is based on the property of certain atomic nuclei to absorb radio frequency (RF) energy when exposed to a strong uniform magnetic field. Briefly, even mass nuclei consisting of an even number of protons and neutrons (i.e., $^{12}$C or $^{16}$C) do not have a resulting intrinsic angular momentum or spin ($s_p = 0$). However, odd mass nuclei with an odd number of protons and neutrons (i.e., $^1$H or $^{13}$C) are characterized by fractional spins ($s_p = 1/2, 3/2...$). Additionally, even mass nuclei consisting of odd numbers of both protons and neutrons have integral spins ($s_p = 1, 2...$). Only atoms with a non-zero spin can be detected using MRI, as they produce a magnetic moment $m$ equal to:

$$m = \frac{\gamma s_p \hbar}{2\pi}, \quad (3.1)$$

where $\gamma$ is the magnetogyric ratio ($\gamma = 42.58 \text{ MHz} \text{ T}^{-1}$ for the hydrogen nucleus) and $\hbar$ is the Planck constant. Among all the nuclei with a non-zero spin, a hydrogen nucleus is used for the MRI because of its abundance in the body, as it represents one of the main components of water and fat. Furthermore, a hydrogen nucleus has a higher magnetic moment which contributes to produce a relatively stronger signal compared to that of other nuclei.

In the absence of a magnetic field, the magnetic moments of the hydrogen nuclei are randomly oriented in space, resulting in a null magnetization of the tissues. However, when a strong external magnetic field $B_0$ is applied, the hydrogen nuclei tend to orient in a parallel or antiparallel direction to it. When aligned to these two directions, the magnetic spins continue to oscillate, or rather to say precess, around the axis of the magnetic field at the Larmor frequency $f_0$:

$$f_0 = \frac{\gamma B_0}{2\pi}. \quad (3.2)$$

As the parallel orientation corresponds to the lowest energy level, there will be more magnetic dipoles oriented in the parallel direction. This produces a longitudinal magnetization in the direction of the external magnetic field, which is typically in the order of 1–3 T. The higher the strength of $B_0$, the more the magnetic dipoles became aligned parallel to it.

To generate an MR signal, the magnetic dipoles must be precessing in a coherent manner in the traversal plane. Therefore, a 90-degree RF pulse at the Larmor frequency is applied to tilt the magnetization vector into a plane perpendicular to the static magnetic field $B_0$. If the RF pulse does not oscillate precisely at the Larmor frequency, then its effect
on the magnetization is weakened. Once the magnetization is flipped 90-degree, the transverse component of the magnetization generates an oscillating signal that can be detected in the receiver coil based on the Faraday-Lenz law. This signal, called free induction decay (FID), is maximized when the nuclear spins are deflected in the transverse plane as the receiver coil is positioned perpendicular to the static magnetic field. After the 90-degree RF pulse, the tilted vector tends to return to its state of equilibrium. Therefore, the longitudinal component of the magnetization increases whereas its transverse component decreases. As a consequence, the FID decays in amplitude with the relaxation time T2, due to local spin-spin interactions that lead to a dephasing of the nuclear spins. The relaxation time T1 is instead a measure of how quickly the longitudinal magnetization recovers to its initial steady state. As shown in Figure 3.1, these relaxation times differ among tissues, T1 and T2 being longer for pure liquids (i.e., CSF), and shorter for dense tissues (i.e., fat).

![Figure 3.1. Time courses of the longitudinal (M_z) and transverse (M_{xy}) magnetization for relaxation parameters T1 and T2 (ms) [112] for different tissues. The relaxation time T1 represents the time for M_z to reach 63% of its maximum value. On the other hand, the relaxation time T2 measures the time required for M_{xy} to fall to approximately 37% of its initial value.](image)

The signals emitted by the nuclear spins can be spatially localized by applying different magnetic field gradients. This makes it possible to create three-dimensional T1- and T2-weighted images characterized by a different contrast. As shown in Figure 3.2, tissues with a high water content (i.e., CSF and GM) appear to be darker in T1-weighted images, whereas dense tissues (i.e., fat) look brighter. Fat quickly realigns its longitudinal magnetization with B_0 (lower T1), and therefore appears brighter (stronger signal). On the contrary, CSF has a much slower longitudinal magnetization realignment resulting in a weaker signal and appears dark. The opposite occurs in T2-weighted images (Figure 3.2).

### 3.1.2 Voxelized head model

Our segmentation pipeline processes T1- and T2-weighted structural images to obtain voxelized anatomical models [109], and it consists of various...
Materials and methods

![Figure 3.2](image.png)

**Figure 3.2.** Example of (a) T1- and (b) T2-weighted magnetic resonance images of the head.

steps shown in Figure 3.3. Further details can be found in [109, 110].

Firstly, T1- and T2-weighted MR images are registered together using BRAINSFit [113]. Then, by interpolating the original images with an uniform grid of 0.5 mm, the T1- and T2-weighted MR images are unsampled to a resolution of 0.5 mm × 0.5 mm × 0.5 mm (Figure 3.3–(a)). This follows the reconstruction of the eyes (Figure 3.3–(b)). In particular, a first approximation of the location of the eyes is obtained from the Montreal Neurological Institute (MNI) ICBM2009a nonlinear asymmetric template [114, 115], and subsequently they are segmented through region growing [110]. FreeSurfer [117] is then employed to segment the brain tissues (Figure 3.3–(c)), which uses T1-weighted images to produce polygonal surfaces of the GM and WM, as well as a volumetric voxelization of 1 mm resolution. Since the polygonal surfaces provide a more accurate representation of the brain, those are directly voxelized in a uniform grid of 0.5 mm. In particular, the boundaries of the GM and WM are found by seeking the voxels intersecting the above polygonal surfaces. Thus, the voxels enclosed in these boundaries are assigned to be part of the GM and WM. The volumetric voxelization is also interpolated in the previous uniform grid of 0.5 mm, and used as an initial guess for the segmentation of the sub-cortical structures (Figure 3.3–(d)), which is then optimized with morphological image processing methods using T2-weighted data [110].

Vestibules are segmented from a high quality custom model as an initial guess, which is then improved by thresholding the T1- and T2- intensities (Figure 3.3–(e)). On the other hand, vertebrae, intervertebral disks, and the mandible are derived directly from the BodyParts3D model [116], as their reconstruction from MR images is not reliable. As a next step, the CSF is segmented through the T1- and T2- 2D histograms (Figure 3.3–(f)). Inside the skull, voxels which have not been classified yet as CSF, nervous tissue or blood are assigned to a compartment consisting of a mixture of CSF, dura mater and small blood vessels. The layer of this compartment
Figure 3.3. Overview of our in-house segmentation pipeline, which consists of the following steps: (a) alignment and unsampling of the T1- and T2-weighted images; (b) reconstruction of the eyes (black); (c) segmentation of the WM (purple) and GM (dark green); (d) reconstruction of brainstem (dark red), WM cerebellar (light blue), GM cerebellar (dark blue), hippocampus (yellow), caudate nucleus (violet) and ventricles (light green); (e) segmentation of vestibules (orange), vertebrae, intervertebral disks, and mandible; (f) classification of tissues as CSF (cyan), skull (dark grey), blood (red) and skin (pink).

is ensured to be continuously distributed under the inner surface of the skull by adding, if needed, at least one voxel. Additionally, the skull is segmented into cortical and cancellous bones using T2-weighted images. The darkest voxels in the T2-weighted images are assigned to cortical bone, whereas the brighter voxels are assigned to cancellous bone. Superior sagittal, lateral, sigmoid and straight sinuses together with other blood vessels are also segmented. Outside the skull, tissues are classified as muscle (including tendons and ligaments), fat and scalp (including skin and smaller blood vessels).

Lastly, all the above segmented head tissues are included together in a final voxelized model with a resolution of 0.5 mm.

3.1.3 Tetrahedral head models

In publication II, voxelized head models obtained through our segmentation pipeline were used by a mesh generation toolbox [119], namely iso2mesh, to create high quality three-dimensional tetrahedral meshes. Briefly, triangular surface meshes are reconstructed for each boundary between tissues in the input volumetric model. For this purpose, iso2mesh employs the CGAL Surface Mesh Generation library [120], which is based on Delaunay triangulation (i.e., no vertices can be contained within the circumcircle of any triangle in the domain). These surface meshes are
then repaired of topological deficiencies (i.e., duplicated triangles, isolated vertices and multiple triangles sharing the same vertices) and eventually smoothed [119]. Finally, three-dimensional meshes are constructed by filling the spaces between the previously generated surfaces with tetrahedra using TetGen [121], an open-source meshing utility which is based on a constrained Delaunay tetrahedralization. One of the main advantage of TetGen is the possibility to control the mesh density, and therefore to generate tetrahedral head model with different resolutions [118].

### 3.1.4 Volume conductor models

Volume conductor models are generated by assigning a tissue electrical conductivity to each tetrahedral or voxel element constituting the previously created anatomical grids/meshes. The tissue conductivities are assumed to be linear and isotropic.

In the case of TMS [6, 118], the GM and WM conductivities were derived from *in vivo* human measurements at 50 kHz (0.26 and 0.17 S/m, respectively) [122]. As the conductivity of the tissue increases with the frequency, those values cannot be directly used for modeling TMS. For this reason, a Cole-Cole parametric model [52] was used to extrapolate the corresponding conductivities at the frequency of the magnetic stimulator (3 kHz), obtaining the final values of 0.215 S/m (GM) and 0.142 S/m (WM). The conductivity of the CSF was set to be equal to 1.79 S/m based on human measurements performed at body temperature [72]. The other tissue conductivities were assigned as follows: blood, 0.7 S/m [53]; muscle and dura mater, 0.18 S/m [56]; skin, 0.43 S/m [123]; fat, 0.15 S/m [123]. The conductivities of compact and spongy bone were obtained from measurements performed at room temperature [58]. However, the conductivity of the tissues increases with the temperature. Therefore, to extrapolate the values at body temperature, we referred to a comparison analysis performed in [126], where the authors characterized the discrepancy in the tissue conductivities values measured at room and body temperature. Based on their results, the conductivities were increased 30%, obtaining the final values of 0.009 S/m (compact bone) and 0.034 S/m (spongy bone) [80].

For uniform magnetic field exposure, publications II and III used the conductivity values previously employed by Dimbilow [38, 39]. An additional two data sets were considered in publication IV, namely Gabriel [54] and McCann [61], to study the effect of uncertainty in the tissue conductivities on the electric fields induced by uniform magnetic field exposure at 50 Hz. Table 3.1 summarizes the tissue electrical conductivities employed in all the publications presented in this thesis, both for TMS and uniform magnetic field exposure at 50 Hz.
Table 3.1. Electrical conductivities (S/m) of the tissues employed for modeling TMS [6, 118], and for modeling uniform magnetic field exposure at 50 Hz [118, 127, 29].

<table>
<thead>
<tr>
<th>Tissues</th>
<th>TMS Gabriel[54]</th>
<th>Uniform exposure Dimbylow[38]</th>
<th>McCann[61]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM</td>
<td>0.215</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>WM</td>
<td>0.142</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Cerebellar GM</td>
<td>0.215</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Cerebellar WM</td>
<td>0.142</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>CSF</td>
<td>1.79</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.142</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Compact bone</td>
<td>0.009</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Spongy bone</td>
<td>0.034</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Fat</td>
<td>0.15</td>
<td>0.045</td>
<td>0.04</td>
</tr>
<tr>
<td>Skin</td>
<td>0.43</td>
<td>4.5 x 10^{-4}</td>
<td>0.10</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.18</td>
<td>0.24</td>
<td>0.35</td>
</tr>
<tr>
<td>Dura</td>
<td>0.18</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Blood</td>
<td>0.7</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>

3.2 Electric field modeling

Numerical methods are used to compute the induced electric field in realistic anatomical models. Such methods firstly require the spatial discretization of the domain into small elements (i.e., voxels or tetrahedra), whose vertices are shared between many neighbors, the so-called nodes. After obtaining the volume conductor models by assigning the tissue conductivity to each element of the mesh, the electric field can be computed in the domain. This section is focused on the discussion of the equations governing the induced electric field at LFs, as well as its numerical computation through an FEM.

3.2.1 Governing equations

In the LF range (up to 5 MHz in IEEE [2, 3] and 10 MHz in ICNRIPI [1]), the electromagnetic wavelength is much larger than the dimension of the human head (60 m at 5 MHz, 30 m at 10 MHz). Therefore, the quasi-static approximation holds, meaning that the time for an applied magnetic field to propagate through the conductive body is negligible [13]. As a consequence, the temporal variation of the induced electric field follows an identical time course in each point of the brain. For TMS, this means that the shape of the pulse does not need to be modeled, as it is possible
to derive the induced electric field in time by scaling the solution with the
derivative of the coil current. Another approximation is represented by the
fact that the displacement currents can be neglected, as the conductivity of
biological tissues exceeds the permittivity by several orders of magnitude
[13]. Under these assumptions, we can decoupled the electromagnetic
problem into the magneto-quasistatic and electro-quasistatic formulations.
For the magneto-quasistatic problem, let us consider the Maxwell–Faraday
equation, which relates the spatially variation of the total induced electric
field ($E$) to the externally applied time-varying magnetic flux density ($B$):

$$\nabla \times E = -j\omega B = -j\omega \nabla \times A, \quad (3.3)$$

where $A$ is the magnetic vector potential. From equation 3.3, one can easily
obtain:

$$\nabla \times (E + j\omega A) = 0. \quad (3.4)$$

As the term in parentheses is irrotational, it can be expressed as the
gradient of the scalar function $\phi$, namely the electric scalar potential:

$$E + j\omega A = -\nabla \phi. \quad (3.5)$$

Therefore, the total induced electric field $E$ is the sum of a primary ($E_1$)
and a secondary ($E_2$) electric field:

$$E = E_1 + E_2 = -j\omega A - \nabla \phi. \quad (3.6)$$

Additionally, from Ohm’s law, one can derive that:

$$J = \sigma E = -\sigma(j\omega A + \nabla \phi), \quad (3.7)$$

where $J$ is the current density. Please note that in the frequency domain,
the electrical conductivity of biological tissues is a complex quantity given
by $\sigma^* = \sigma + j\omega\varepsilon$, where $\sigma$ and $\varepsilon$ are the electrical conductivity and permit-
tivity of the tissues. However, as already mentioned, human tissues can be
assumed to be purely resistive in the LF range ($j\omega\varepsilon \ll \sigma$ [13]). In addition,
given the low conductivity values of the biological tissues ($\sigma \sim 1$ S/m),
we can assume that the magnetic field produced by the induced currents in the
body is negligible. Hence, the incident magnetic field is not significantly
modified by the induced currents in the human body, meaning that the
magnetic vector potential $A$ in 3.6 corresponds to that in free space. As a
consequence, the primary field $E_1 = -j\omega A$ only depends on the change in
the incident magnetic flux density $B$.

In the quasi-static limit, the divergence of the induced current density is
zero, therefore by applying $\nabla \cdot J = 0$ to 3.7, one can find:

$$\nabla \cdot \sigma \nabla \phi = -\nabla \cdot \sigma j\omega A, \quad \text{in } \Omega \quad (3.8)$$
where \( \Omega \) is the domain of the solution. In addition, at the boundary \( \partial \Omega \) of the domain \( \Omega \), the normal component of \( J \) is zero, as the current cannot spread out from the domain:

\[
\mathbf{n} \cdot (j\omega \mathbf{A} + \nabla \phi) = 0, \quad \text{in} \ \partial \Omega \tag{3.9}
\]

As a result, the boundary condition becomes:

\[
\frac{\partial \phi}{\partial n} = -j\omega A \cdot \mathbf{n}, \quad \text{in} \ \partial \Omega \tag{3.10}
\]

Equation 3.8 holds everywhere inside the volume \( \Omega \). Please also note that at the interface between two inner regions \( \Omega_1 \) and \( \Omega_2 \), the normal component of the current density \( J \) is continuous. Therefore, besides the boundary condition 3.10, the following continuity condition at the interface between two tissues holds [128, 129]:

\[
(J_1 - J_2) \cdot \mathbf{n} = 0. \tag{3.11}
\]

In particular, the primary induced electric field \( E_1 = -j\omega A \) produces a flow of current that generates charges accumulating at the boundaries separating tissues with different electrical conductivities [130], resulting in the electric scalar potential \( \phi \), and therefore the secondary field \( E_2 = -\nabla \phi \). As a result, the induced electric field is increased in the low conductivity tissue, and lowered in the high conductivity region [131]. Tissue heterogeneity can therefore strongly affect the induced electric field strengths.

### 3.2.2 Modeling the source

In Publication I, the TMS experiments were carried out using the Magstim 70 mm figure-of-eight coil, which consists of two wings with the current flowing in the opposite direction. For the numerical computations, this coil was modeled using thin-wire approximation by discretizing each wing with a series of nine circular current loops. Each concentric loop consisted of a finite number of 15 segments equally spaced (Figure 3.4–(a)). The outermost and innermost diameters of the loops were 9.7 cm and 7.2 cm, respectively. The time derivative of the magnetic vector potential \( A \) at any point \( \mathbf{r} \) is computed analytically using the Biot-Savart law:

\[
\begin{align*}
 j\omega A(r) &= \frac{\mu_0 j\omega I}{4\pi} \oint_C \frac{d\mathbf{r}'}{|\mathbf{r} - \mathbf{r}'|}, \\
\end{align*} \tag{3.12}
\]

where \( j\omega I \) is the time derivative of current feeding the coil and \( \mu_0 \) is the permeability of free space \( (4\pi \times 10^{-7} \text{ H/m}) \). The integration is performed along the wire element \( d\mathbf{r}' \) of the coil windings \( C \), and \( \mathbf{r} - \mathbf{r}' \) represents the displacement vector from the wire element \( d\mathbf{r}' \) at the point \( \mathbf{r}' \) to the point \( \mathbf{r} \) at which \( A \) is computed. By discretizing the coil with linear segments, the total magnetic vector potential in 3.12 can be obtained by summing
up the contributions from all these short segments. For the simulations, we used a peak time derivative of the current corresponding to 174 A/μs, which was previously measured for the maximum stimulation output of the Magstim 200\textsuperscript{2} stimulator [132]. Therefore, physiological responses can be related with the electric field calculations by simply multiplying the measured AMTs and RMTs by the peak time derivative of the current coil.

In publication II, the same coil was modeled as a series of infinitesimally short magnetic dipoles. This allowed comparison of the results obtained through our solver with the ones derived using SimNIBS, an open-source software tool for field calculations employing tetrahedral meshes [133]. In this context, SimNIBS uses magnetic dipoles to model the Magstim coil. Please note that our solver is based on regular voxel grids to discretize the geometry, whereas SimNIBS uses mesh consisting of tetrahedral elements. Therefore, the above two solvers were used to study the discrepancies in the electric fields induced in tetrahedral meshes and staircase grids at LFs [118]. In particular, the two wings of the coil were modeled as three circular disks shifted 4.2, 6.5, and 8.8 mm. Each circular disk was further divided into sub-regions consisting of sixteen rings [134, 135]. Each ring contained a certain number of magnetic dipoles (Figure 3.4–(b)), which were weighted by the area of the sub-regions and the coil current:

\[ \mathbf{m} = ID\hat{e}_n, \quad (3.13) \]

where \( \mathbf{m} \) is the magnetic dipole, \( I \) the current, \( \hat{e}_n \) the surface normal and \( D \) the area of the part of the surface modeled by the dipole. A single magnetic dipole generates a corresponding magnetic vector potential equal to [15]:

\[ A_{\text{dip}}(\mathbf{r}) = \frac{\mu_0 \mathbf{m}}{4\pi} \times \frac{\mathbf{r} - \mathbf{r}'}{|\mathbf{r} - \mathbf{r}'|^3}, \quad (3.14) \]

where \( \mathbf{r} \) is the position at which \( A_{\text{dip}} \) is calculated, and \( \mathbf{r}' \) is the position of the magnetic dipole. Therefore, the total magnetic vector potential generated by the coil can be determined as the superimposition of the contributions from all the \( k \) magnetic dipoles:

\[ A(\mathbf{r}) = \sum_{i=1}^{k} A_{\text{dip}}(\mathbf{r}). \quad (3.15) \]

Further details of how the TMS coil was modeled using magnetic dipoles can be found in [15, 134, 135]. In addition to localized exposure due to TMS, publication II also examined exposure to spatially uniform magnetic fields. In this case, the magnetic vector potential \( A \) was produced by a small and strong magnetic dipole placed very far away from the head, and oriented in three different orthogonal directions. Again, the use of a magnetic dipole made it possible to easily compute the induced electric fields in the voxelized and tetrahedral anatomical models.
Materials and methods

Figure 3.4. Coil modeled using using (a) thin-wire approximation or (b) a series of magnetic dipoles.

In publications III and IV, human exposure to spatially uniform magnetic fields was also considered. However, this time the induced electric field strengths were computed solely with our FEM solver. This allowed us to analytically determine the time derivative of the magnetic vector potential $j\omega A$ through the following equation:

$$j\omega A(r) = \frac{j\omega}{2} B_0 \times r,$$

where $B_0$ is the given and known incident uniform magnetic flux density.

### 3.2.3 Finite element method

Equations 3.8, 3.9 and 3.11 represent the strong formulation of the LF electromagnetic problem, which can be solved numerically by restating these differential equations into the so-called weak form. The latter allows to discretize the second order equation 3.8 using once-differentiable test functions. In particular, the weak form can be obtained by multiplying each member of 3.8 with a test function $v$ and integrating over the domain $\Omega$:

$$\int_{\Omega} v \nabla \cdot (\sigma \nabla \phi) d\Omega = -\int_{\Omega} v \nabla \cdot (\sigma j\omega A) d\Omega, \quad \text{in } \Omega$$

(3.17)
By applying the identity \(^1\) to both sides of equation 3.17, one can obtain:

\[
\int_{\Omega} \nabla \cdot (\nu \sigma \nabla \phi) d\Omega - \int_{\Omega} \nabla v \cdot \sigma \nabla \phi d\Omega = \]

\[
- \int_{\Omega} \nabla \cdot (\nu j \omega \mathbf{A}) d\Omega + \int_{\Omega} \nabla v \cdot (\sigma j \omega \mathbf{A}) d\Omega,
\]

and therefore:

\[
\int_{\Omega} \nabla \cdot (v[\sigma \nabla \phi + \sigma j \omega \mathbf{A}]) d\Omega - \int_{\Omega} \nabla v \cdot \sigma \nabla \phi d\Omega
\]

\[
= \int_{\Omega} \nabla v \cdot (\sigma j \omega \mathbf{A}) d\Omega.
\]

(3.18)

We now apply the Gauss theorem \(^2\) to the first term of equation 3.19:

\[
\int_{\partial \Omega} \mathbf{n} \cdot \left( v[\sigma \nabla \phi + \sigma j \omega \mathbf{A}] \right) dS - \int_{\Omega} \nabla v \cdot \sigma \nabla \phi d\Omega
\]

\[
= \int_{\Omega} \nabla v \cdot (\sigma j \omega \mathbf{A}) d\Omega.
\]

(3.19)

As per the boundary condition 3.9, the surface integral is equal to zero. Therefore, one can obtain the following weak form:

\[
\int_{\Omega} \nabla v \cdot \sigma \nabla \phi d\Omega = - \int_{\Omega} \nabla v \cdot (\sigma j \omega \mathbf{A}) d\Omega.
\]

(3.20)

In order to solve 3.21 numerically, \(\phi\) is discretized as a linear combination of basis functions \(\psi\):

\[
\phi \approx \sum_{i=1}^{N} \phi_i \psi_i,
\]

(3.22)

where \(N\) is the number of nodes of the domain \(\Omega\), \(\phi_i\) is the value of \(\phi\) at the discrete node \(i\), and \(\psi_i\) represents a piece-wise trilinear function that is equal to 1 at the node \(i\), and decays linearly reaching a value of 0 at all nodes \(j\) surrounding \(i\). Therefore, the basis function \(\psi_i\) is zero for each point of the domain \(\Omega\) except for the node \(i\). In our FEM solver [14], the Galerkin method was used, i.e., the test function \(v\) is expressed as a linear combination of the same basis functions \(\psi\). Thus, the discretized weak form of equation 3.21 becomes:

\[
\sum_{i=1}^{N} \int_{\Omega} \nabla \psi_j \cdot \nabla \psi_i \sigma d\Omega \phi_i = - \int_{\Omega} \nabla \psi_j \cdot (\sigma j \omega \mathbf{A}) d\Omega,
\]

(3.23)

\(^1\) \(\nabla \cdot (\nu \mathbf{F}) = \nabla \cdot \mathbf{F} + \nu \nabla \cdot \mathbf{F},\)

\(\nabla \cdot (\nu \sigma \nabla \phi) = \nabla \cdot \sigma \nabla \phi + \nu \nabla \cdot (\sigma \nabla \phi),\)

\(\nabla \cdot (\nu \sigma j \omega \mathbf{A}) = \nabla \cdot (\sigma j \omega \mathbf{A}) + \nu \nabla \cdot (\sigma j \omega \mathbf{A}),\)

\(^2\) \(\int_{\Omega} \nabla \cdot [ \ ] d\Omega = \int_{\partial \Omega} \hat{n} \cdot [ \ ] dS,\)
where $j$ spans from 1 to $N$. Equation 3.23 represents a system of $N$ equations, which can be written in the matrix form as:

$$\mathbf{Sx} = \mathbf{y}, \quad (3.24)$$

where $\mathbf{x}$ represents the vector of the unknown electric scalar potential $\phi_i$ and $\mathbf{S}$ is the FEM system matrix. When a regular grid consisting of voxels of the same side length $l$ is employed, the left-hand side of each row in equation 3.24, corresponding to one node in the grid, can be solved analytically from:

$$\frac{1}{l^2} \left( \frac{8}{3} \sigma_{12345678} \phi_i - \frac{1}{6} \left( \sigma_{12} \phi_{12} + ... + \sigma_{78} \phi_{78} \right) - \frac{1}{12} \left( \sigma_1 \phi_1 + ... + \sigma_8 \phi_8 \right) \right) = b_i, \quad (3.25)$$

where $\phi_i$ is the electric scalar potential solved at the node $i$, the sub-indexed $\phi$ represent the potential at the neighboring nodes $j$ (Figure 3.5), and the sub-indexed $\sigma$ is the average of the conductivity over the specified voxels (Figure 3.5). The integrals in the right-hand side of equation 3.24 are instead numerically solved using the first-order Gaussian quadrature rule at the center of each voxel.

Figure 3.5. Representation of a generic node $i$ surrounded by a total number of height adjacent voxels.

Our FEM solver employs the geometric multigrid method with successive over-relaxation to solve the matrix system equation 3.24 iteratively [14]. Multiple coarse grids are used to accelerate the convergence of the numerical solution, which are constructed from the fine-grid conductivity obtained from the segmentation of anatomical images. The iteration is continued until a certain error (i.e., relative residual norm) is reached. Please note that all the publications in this thesis made use of a relative residual norm.
equal to $10^{-6}$, with the only exception of publication II where a lower value of $10^{-10}$ was chosen to minimise potential differences between SimNIBS and our solver. Both the FEM methods employ piece-wise linear basis functions, and none of them use time-integration schemes. The convergence analysis in Figure 3.6 shows the relative error in the electric field computed with our solver as a function of the relative residual norm, when a representative head model with different resolutions was stimulated by TMS at the HS. In particular, the relative error was calculated as follows:

$$\text{relative error (\%)} = \frac{\sqrt{\sum_{n \in S} |E^i_n - E^0_n|^2}}{\sqrt{\sum_{n \in S} |E^0_n|^2}} \cdot 100, \quad (3.26)$$

where $S$ is the set of all the voxels over the whole head, $E^i_n$ is the magnitude electric field in the node $n$ for a certain relative residual norm $i$, and $E^0_n$ is the magnitude electric field of the reference solution. The latter was calculated from the potential converged to the machine precision. As shown in Figure 3.6–(a), a relative residual norm of $10^{-6}$ produces a relative error in the assessment of the electric field approximately equal to 0.005%, whereas it is in order of $10^{-7}\%$ when a relative residual norm of $10^{-10}$ is chosen. For completeness, Figure 3.6–(b) provides the relative error calculated using the additional metric:

$$\text{relative error (\%)} = \frac{\max(|E^i_n - E^0_n|)}{\max(|E^0_n|)} \cdot 100. \quad (3.27)$$

Further technicalities concerning the geometric multigrid method for solving the FEM problem can be found in [14].

![Figure 3.6](image)

**Figure 3.6.** Relative error of the induced electric field as a function of the relative residual norm, calculated through the metrics in eq. 3.26 (a) and eq. 3.27 (b). Each curve represents a different resolution of a representative voxelized head model.
3.3 Biological axon models

Biological axon models can be used to investigate the excitation thresholds of nerves when exposed to electromagnetic fields [94]. In publication I, computational dosimetry was coupled with biological axon models with the purpose of extrapolating brain threshold data to sinusoidally varying electric fields at IFs.

3.3.1 Action potential

In the resting axon, the intracellular fluid has a negative potential compared to the extracellular fluid [89]. This difference in electrical voltage, guaranteed by the sodium-potassium pump, is called the resting membrane potential (Figure 3.7). The action potential is a brief reversal of this condition, and for an instant, the inside of the axon membrane becomes positively charged with respect to the outside. Therefore, during the action potential the membrane potential becomes briefly positive.

At rest, the axonal membrane of the neuron is mainly permeable to potassium ions (K⁺). It follows that the conductance of potassium (gK) is greater than the conductance of sodium (gNa). The electrical conductance is indeed proportional to the number of opened ion channels. When the membrane is depolarized to the threshold, there is a transient increase in gNa (gNa ≫ gK). The voltage-gated sodium channels open and the sodium ions (Na⁺) enter in the neuron under a strong driving force caused by the membrane potential being negative. This part of the action potential is called the rising phase [89] (Figure 3.7). The influx of sodium depolarizes the neuron, and the membrane potential goes to a positive value close to the sodium equilibrium potential. The part of the action potential where the inside of the neuron is positively charged with respect to the outside is called the overshoot [89] (Figure 3.7).

At this point, the voltage-gated sodium channels quickly close and the voltage-gated potassium channels open, so that the dominant membrane permeability switches back from Na⁺ to K⁺ (gK ≫ gNa). This part of the action potential is called the falling phase [89] (Figure 3.7). The potassium gates open 1 ms after depolarization, and they speed the restoration of the membrane potential. This time, there is a strong driving force on K⁺ because the membrane potential is positive. The K⁺ ions leave the cell causing a rapid repolarization of the membrane until it reaches the resting potential (Figure 3.7).

3.3.2 Circuit representation of myelinated fiber

Based on the McNeal formulation [93], a myelinated nerve fiber can be represented as the equivalent electrical network shown in Figure 3.8. In
Materials and methods

Figure 3.7. Estimated membrane action potentials using the biological axon models that were employed in Publication I, i.e., the SENN and NIT model.

In particular, the axon membrane is modeled as a series of compartments representing the nodes of Ranvier, separated by a perfect insulator layer of myelin. In each node, the membrane current $I_i(n)$ flows either into the membrane capacitance ($C_m$), or into the ions channels, which are modeled as a conductance and a potential source in series (Figure 3.8). The potential source represents the concentration difference of a specific ion between the inside and the outside of the axon. The nodes are linked together through the resistance $r_a$ of the axoplasmic fluid, whose inverse is the internodal conductance $g_a$.

![Electrical circuit representation of myelinated fiber](image)

**Figure 3.8.** Electrical circuit representation of myelinated fiber, where $g_K$, $g_{Na}$, $g_L$, and $g_P$ stand for the conductances of potassium, sodium, leakage and nonspecific delayed ion channels, respectively. The potential source $E_K$, $E_{Na}$, $E_L$ and $E_P$ represent the Nernst potential of the correspondent ions. In addition, $V_o$ and $V_i$ are the external and internal nodal voltages of the membrane. The nodes are interconnected by the internodal conductance $g_a$.

By introducing the transmembrane potential as $V(n) = V_i(n) - V_o(n)$, where $V_o$ corresponds to the integral of the electric field along the axon of length $L$,
i.e., \( V_e(x) = -\int_0^x E_e(x)dx \), one can derive the following equation at the node \( n \):

\[
\frac{dV(n)}{dt} = \frac{1}{C_m} (g_a[V(n-1) - 2V(n) + V(n+1) + V_e(n-1)] - 2V_e(n) + V_e(n+1)) - I_i(n)).
\]

Note that the term \( V_e(n-1) - 2V_e(n) + V_e(n+1) \) is defined as the *activating function*. In particular, the activating function describes how the transmembrane potential is affected by the externally applied electric field. In this context, fibers will be depolarized if the activating function is positive and large enough. For a straight axon in a uniform electric field, the activating function is zero along the fiber (i.e., no activation) except at its terminations or bends. Since the the activating function corresponds to the second spatial derivative of the extracellular potential along the axon (i.e., negative derivative of the electric field), its termination/bend causes a strong variation in the transmembrane potential, which might lead to stimulation. For example, if the uniform electric field is directed *out* of the bend, the axon will be depolarized and therefore an action potential might be generated. On the other hand, if the uniform electric field is directed *into* the bend, the axon will not be activated.

In the McNeal formulation, the membrane current \( I_i(n) \) reduces to the linear form below the threshold [93]:

\[
I_i(n) = g_K(V(n) - E_K) + g_{Na}(V(n) - E_{Na}) + g_L(V(n) - E_L) + g_P(V(n) - E_P),
\]

and to a non-linear form near the threshold [93]:

\[
I_i(n) = \pi d n_g (J_K + J_{Na} + J_L + J_P),
\]

where \( d \) is the axon diameter, \( n_g \) is the nodal gap width and the \( J \) terms are the transmembrane ionic current densities specified by Frankenhaeuser-Huxley [92]. Please note that for computational reasons, the original formulation by McNeal [93] only modeled the central node of the fiber in a non-linear form [33, 94].

In publication I, two different axon models were employed, namely the SENN [33] model and an in-house (NIT) model [136]. Both are based on the ion channel dynamics described by McNeal [93]. However, the SENN model includes the Frankenhaeuser-Huxley non-linearities at each node of Ranvier [33], as well as the possibility to model the neuronal terminus and bends [33]. On the other hand, the NIT model is based on Basser-Roth investigation [98], which uses the same parameters obtained from rabbit myelinated axons [95]. The Basser-Roth approach treats all nodes as non-linear, and makes it possible to model the neuronal terminus. However, it does not consider potassium ion channels, as they were found negligible.
Materials and methods

for rabbit myelinated axons [95]. The SENN and NIT models allow us to represent axons of different size and geometry, as well as to compute the stimulation thresholds when the fibers are excited by sinusoidally varying electric field at various frequencies. An excited state is reached if at least three contiguous nodes are depolarized to the threshold.

3.4 Statistical methods

Most of the statistical analyses were performed using the open-source programming language R (version 3.6.2). Statistical methods mainly included widely adopted measures such as mean, median, standard deviation, and statistical hypothesis testing. In addition, a linear mixed-effects model [137] was used in publication III to investigate the effect of different predictors on the maximum electric field strengths induced in the brain by externally applied uniform magnetic fields [127]. For this purpose, the R package lme4 [138] was used. Briefly, linear mixed-effects models are a kind of regression models that combine fixed and random effects [137]. The fixed effects are the so-called explanatory or predictor variables in a standard linear regression, i.e., the parameters describing the entire population that are expected to have an effect on the dependent variable. On the other hand, the random effects characterize the differences between clusters (for example, among the subjects in a study). Please note that in a linear mixed-effects model the observations between different clusters are independent, but observations within each cluster can be considered dependent. As a result, the linear mixed-effects models offer the possibility to estimate different intercepts and slopes for each cluster, leading to more accurate results than a simple linear regression model. For this reason, mixed-effects models are becoming increasingly popular in medical research.

Let’s give a practical example: suppose that we are interested in inferring the relationship between the increase in the height in four species of plants after being treated with two fertilizers. Each species counts a total number of 10 plants, 5 treated with the fertilizer A and the remaining with the fertilizer B. Species and fertilizer are categorical variables, that represent the fixed effects of our model:

\[
\text{height} \sim \text{species} + \text{fertilizer} + e_r
\]

where \(e_r\) is the deviation from our prediction. Thus, we have multiple measurements of height per plant. Different species can be characterized by different heights. In this context, there could be individual variations within the species groups, meaning that some plants might be taller or shorter than the others belonging to the same species. To characterize these individual variations, linear mixed-effects models allow us to determine
a different intercept for each plant. Based on that, the additional term 
(1 | plant) is included in the model:

$$\text{height} \sim \text{species} + \text{fertilizer} + (1 | \text{plant}) + \epsilon_r. \quad (3.32)$$

However, the effect of the fertilizers can be different for different plants. 
In this context, the linear mixed-effects models enable us to estimate, in 
addition to the intercepts, different slopes for plants based on the effect of 
fertilizer:

$$\text{height} \sim \text{species} + \text{fertilizer} + (\text{fertilizer} | \text{plant}) + \epsilon_r. \quad (3.33)$$

As in the standard linear regression, inter-dependence between fixed ef-
fects, the so-called interaction, can be included in the model. For example, 
it might be of interest to determine whether the effect of fertilizer on height 
depends on the species:

$$\text{height} \sim \text{species} \times \text{fertilizer} + (\text{fertilizer} | \text{plant}) + \epsilon_r, \quad (3.34)$$

where the symbol $\times$ represent the interaction term.

The linear mixed-effects model developed in publication III included all 
the two-way interaction terms among the different fixed effects (i.e., age, 
gender, skull volume and incident magnetic field direction). In addition, as 
random effects, we estimated the slopes and intercepts depending on the 
relationship between participants and incident magnetic field directions. 
The parameters of the model were estimated using the maximum likelihood 
method.

### 3.5 TMS experimental setup

Publications I is based on TMS experiments previously performed in [80], 
where monophasic pulses were delivered with a Magstim 200\textsuperscript{2} stimulator 
(Magstim Co. Ltd., Whitland, UK) connected to a figure-of-eight coil 
(Magstim, part number 3271-00). In each individual participating in the 
study, the center of the coil was roughly placed above the hand knob of 
the motor cortex, in the left side of the scalp approximately 5 cm lateral to 
the vertex of the head, tangentially to the skull and at a 45-degree angle 
with respect to the anterior-posterior axis. The coil was then moved in 
little steps of about 1 cm to find the hotspot (HS), i.e., the location that 
elicited the maximum MEP responses in the right first dorsal interosseous 
(FDI) muscle. The MEPs were recorded from the right FDI muscle through 
surface EMG electrodes applied to the skin. Once the HS was localized, the 
stimulus intensity was varied to determine the AMT and RMT based on 
the Rossini criterion [81]. An additional four coil locations were targeted 
by TMS at a distance of 2 cm from the HS, and for each of them the AMT
and RMT were measured. By combining these physiological responses with individualized MRI-based computational analysis that make use of realistic anatomical models, the induced electric fields produced by TMS were then computed for each individual.
4. Summary of publications

(I) Publication I combined individualized MRI-based anatomical models, physiological response measurements and computational dosimetry to estimate the electro-stimulation threshold of brain tissues, i.e., the minimum electric fields needed to produce a cortical excitation. The experimental design of this study is illustrated in Figure 4.1.

As shown, high-resolution head models were reconstructed from T1- and T2-weighted images of nineteen subjects, who underwent TMS experiments. During the measurements, the active and resting motor thresholds were measured for each participant at the hotspot (HS) – the optimal location for the activation of the right FDI – and at four additional coil locations. For each combination of subject and coil location, the electric fields were calculated on a surface located...
at a depth of 2 mm below the surface of the grey matter to avoid averaging the electric field on a surface of discontinuity between two tissues (grey matter/CSF), where the electrical conductivity assumes different values. Please note that a displacement of 1 mm, 2.5 mm and 5 mm around the center of the coil placed at the HS caused an error in the calculation of the induced electric fields ranging between 1%–3%, 3%–7% and 5%–14% respectively. The highest errors were obtained when the displacement occurred along the vertical axis passing to the center of the coil. In [80], the error related to the uncertainty in the coil direction deviating $\pm 5^\circ$ from $45^\circ$ was estimated to vary between 3.8 and 7.6%. The obtained electric field distributions were then mapped to a standard (MNI) brain model, where an approximate location of the activation site ([X,Y,Z]=[−43,−11,60]$_{MNI}$) was previously determined [80]. However, the cortical activation site might vary slightly among the individual brains. In order to take account of this potential variation, we arbitrarily considered a region of 5 mm radius in the hand knob area centered in the activation site. For each individual/coil location, the electric fields computed at the vertices of the ROI were then averaged. Since previous findings showed that the above common activation site does not depend on the coil location [80], the additional averaging across the different coil locations was performed to get a reasonable estimate of the individual electro-stimulation thresholds. Based on physiological measurements, computational dosimetry was therefore used to estimate the distribution of the active and resting electro-stimulation thresholds among the individuals (Figure 4.2). Two outliers for active and one for resting electro-stimulation thresholds were excluded due to errors in the measurement of the motor thresholds. The median values derived from these distributions were employed in established biological axon models (SENN and NIT) to model straight and 90-degree bent axons, which were then used to extrapolate thresholds for sinusoidally varying electromagnetic fields.

**Figure 4.2.** Lognormal distributions of the obtained active and resting electro-stimulation thresholds.
The obtained threshold-frequency curves were eventually compared with the safety limits defined by both the ICNIRP [1] and IEEE [2, 3] (Figure 4.3). Results showed that the allowable induced electric fields defined by both standard/guidelines were significantly lower than the thresholds needed for the stimulation of the CNS. At frequencies around 0.5 kHz, our results differed from the limits by a factor of approximately equal to 30 (ICNIRP) and 50 (IEEE). This large margin of conservatism is mainly due to the fact that the basic restrictions for the brain tissues at IFs were derived based on thresholds for the retinal phosphene [2, 3], and peripheral nervous stimulation [1]. These stimulation thresholds are significantly lower than the ones obtained here for brain stimulation. Moreover, the additional reduction factors applied to the thresholds for the activation of phosphenes/peripheral nerves make this difference even more significant. So far, the human exposure to the intermediate frequency range has not been adequately and extensively investigated. Therefore, the obtained data constitute a basis for future development of the international safety standard/guidelines.

![Figure 4.3](image-url)

**Figure 4.3.** Strength-threshold curves of electrical brain stimulation derived in the intermediate frequency band, which are compared with the basic restrictions established by the ICNIRP guidelines and IEEE standard.

(II) In Publication II, the authors compared for the first time the electric field strengths induced at LFs in voxelized and tetrahedral anatomical head models when exposed to localized (TMS) and uniform magnetic fields. For this purpose, our in-house segmentation pipeline was firstly employed to generate voxelized grids of 0.5 mm resolution consisting of six compartments: skull, eyes, cerebrospinal fluid (CSF), other tissues (e.g. skin, muscle and nerves), grey matter (GM)
and white matter (WM). Although our semi-automatic segmentation pipeline makes it possible to obtain much detailed head models, this simplification was needed to perform numerical simulations in the tetrahedral meshes [25]. The obtained voxelized models were then used to generate tetrahedral meshes with different resolutions through a freely available toolbox [119]. From the finest meshes, voxelized grids were generated with the purpose of obtaining realistic anatomical models as similar as possible to each other. This represented a fundamental prerequisite for the purpose of the paper, which was to compare computational results and characterize numerical errors in rectilinear voxel grids and tetrahedral meshes.

In summary, for each individual, four tetrahedral meshes were created with a mean edge length of 2.14 mm, 1.60 mm, 1.31 mm, and 1.19 mm. Additionally, four voxelized head models were generated with spatial resolutions of 2 mm, 1 mm, 0.5 mm and 0.25 mm. To compare the results obtained in the voxelized and tetrahedral models, the induced electric fields were interpolated on a surface at the midpoint between the surfaces of the grey and white matter. This made it possible to avoid calculating the field on the boundary of tissues having different conductivities. In this context, a nearest-neighbor interpolation scheme was used, i.e., we assigned the electric field to each point of the surface based on the value of the enclosing voxel/tetrahedron. Modest discrepancies between the computed electric fields induced in these meshes/grids were observed, and the discretization error decreased as the resolutions of the models became finer. In this context, the use of voxelized models reduced significantly the computational costs in terms of CPU time needed to solve the FEM systems. However, both voxelized and tetrahedral head models were affected by numerical errors in the evaluation of the peak electric field strengths. These numerical artefacts derived from the staircase approximation errors in the voxelized models, and from low-quality elements in the tetrahedral meshes. Results showed that the computational artifacts could be suppressed by either spatially averaging the electric fields over $2 \times 2 \times 2$ mm$^3$ cubes [1], and/or by calculating a suitable percentile (99th or higher) of the induced electric field. Please note that excluding data above a certain percentile, by definition, causes an intrinsic loss of information. Here we showed that the use of higher percentile values could reduce the underestimation of the highest electric field strengths, especially for localized exposure. Thus, the selection of a proper percentile to be used is rather a compromise between computational accuracy and underestimation of the maximum electric field strength. Please note that when the ICNIRP averaging was performed, the use of a regular grid of 2 mm provided higher electric field strengths compared to the other resolu-
Among the finer resolutions, the ICNIRP averaging over the 0.5 mm voxel grids produced an uncertainty in the 99th percentile up to 1% depending on the exposure direction [118]. The obtained results represent a meaningful contribution to the improvement of the reliability in the human exposure assessment at LFs.

(III) Publication III investigated the variability in the induced electric field strengths between different anatomical induction models when exposed to spatially uniform magnetic fields at 50 Hz. This analysis is relevant for LF human exposure as the limits for both the ICNIRP and IEEE were established without considering the actual variability in the induced electric fields among individuals. In this context, the present study recruited a total number of 118 participants, for each of which high-resolution head models were reconstructed from T1- and T2-weighted images. After exposing the anatomical models to magnetic fields directed in the top-bottom (TOP), left-right (LAT), and antero-posterior (AP) directions, the induced electric field strengths were computed numerically. A statistical analysis was then performed to evaluate the effect of individual characteristics and anatomical differences on the maximum electric field strengths. Results showed that larger skull volumes as well as exposure in the lateral direction produced higher electric field strengths in the brain (Figure 4.4). This derives from larger cross-sectional areas in the sagittal plane that produce higher strengths according to Faraday’s induction law.

Aging also had an effect, older individuals producing higher field strengths (Figure 4.4). The latter effect might be explained by the growth in CSF volume with aging, as observed in the present study.

Figure 4.4. Relationship between the maximum electric field strength with age (a) and skull volume (b) for each exposure direction (AP, LAT and TOP). Lines represent the expected values from the statistical analysis, with the shaded areas showing the corresponding 95% confidence intervals, and the dots are the partial residuals.
Summary of publications

(see appendix A) and in previous investigations [142, 143]. This produces an increase in the total induced current (Figure 4.5–(a)) and thereby the corresponding induced electric field strength (Figure 4.5–(b)). Most notably, the obtained average maximum electric field strength for the LAT direction (22.1 mV m\(^{-1}\) per mT) was significantly lower than the CNS induction factor (33.0 mV m\(^{-1}\) per mT) used by the ICNIRP for deriving the reference level from the basic restriction. Using the same approach, the CNS induction factor derived here would give a higher reference level. On the other hand, the elliptical model used by the IEEE would produce an induced electric field of 16.3 mV m\(^{-1}\) for LAT exposure, indicating a large difference between the strengths computed in the ellipsoidal and anatomical models. The results derived in this investigation will be useful for the next revision of the international exposure guidelines.

![Figure 4.5](image)

**Figure 4.5.** (a) Relationship between the total induced current and the CSF volume, when the head models of publication III were exposed to spatially uniform magnetic fields (1 mT) at 50 Hz along the LAT direction. The fitted linear regression model showed a significant increase in the total induced current with CSF volume \([R^2 = 0.6, F(1,116) = 172.2, p = 2 \times 10^{-16}]\). (b) Maximum electric field strength as a function of the total induced current. The fitted linear regression model showed a significant increase in the maximum electric field strength with the total induced current \([R^2 = 0.4, F(1,116) = 70, p = 2 \times 10^{-13}]\).

(IV) In Publication IV, the authors studied the effect of variations in the electrical conductivities of the tissues on the electric fields induced in the brain of twenty-five individuals who were exposed to uniform magnetic fields at 50 Hz. According to the values reported in Table 3.1, three conductivity data sets were employed in this investigation. In particular, the McCann data set included new estimations based on an extensive meta-analysis review of the latest studies that focus on measuring the electrical conductivities of the head tissues. On the other hand, the Gabriel and Dimbylow data sets contained commonly used values in LF dosimetry. As clear from Table 3.1, the new estimations of the brain tissue conductivity values were considerably higher than those usually employed. Such a difference had
a major effect on the induced electric field strengths, as the higher brain conductivities produced significantly lower strengths. For LAT exposure, an average electric field strength of $15.5 \pm 0.8 \text{ mV m}^{-1}$ was found using the McCann data set, which was considerably lower compared to those obtained with the Gabriel ($20.8 \pm 1.7 \text{ mV m}^{-1}$) and Dimbylow ($22.0 \pm 1.7 \text{ mV m}^{-1}$) data sets. This difference becomes even more significant when comparing our results with the CNS induction factor ($33.0 \text{ mV m}^{-1} \text{ per mT}$) used by the ICNIRP to derive the reference level from the basic restriction. On the other hand, the use of higher brain conductivity values reduced the difference between the doses in the ellipsoidal and anatomical models. A sensitivity analysis also showed that the peak induced electric fields in the brain were mainly affected by variations in the conductivity of WM and GM, and only marginally from those of the other head tissues. Our results characterize the uncertainty in computational dosimetry due to tissue electrical conductivity variations and are useful for the harmonization of international exposure standard/guidelines.
5. Discussion

The present thesis consists of four scientific papers focused on characterizing several sources of error and uncertainty that affect computational dosimetry at LFs. Previous works investigated different and/or related issues which are relevant for LF human exposure. In [21], the authors studied the effect of spatial averaging/percentile filtering on the induced electric fields for canonical and anatomical voxelized anatomical models, showing that (i) the maximum value of the spatially averaged electric field was a stable peak approximation for grid sizes lower than 0.5 mm, and (ii) the use of the 99th percentile could lead to an underestimation of the exposure. This underestimation was shown to be even more significant in localized exposure scenarios [23]. However, those investigations only examined voxel-based anatomical models, which are prone to numerical artifacts associated with the rectilinear voxel discretization. On the other hand, meshes consisting of tetrahedral elements are exempt from staircasing approximation errors. Nonetheless, low-quality tetrahedra and discretization errors might have an adverse effect on the accuracy of numerical solutions [25, 26].

In this context, no comparison has ever been made between the computational results obtained with tetrahedral and voxelized-based methods. For this purpose, publication II investigated numerical errors affecting both tetrahedral meshes and staircase grids. Several post-processing approaches were studied to mitigate these numerical errors, with the aim of improving the reliability of human exposure assessment at LFs. Based on the results, computational artifacts could be removed by spatially averaging the electric field strengths (as long as finer resolutions than 2 mm were used) and/or by further applying a suitable percentile value. Our findings confirmed that the additional percentile filtering produced an intrinsic underestimation of the peak strengths, especially in the case of localized exposure, indicating that this underestimation is exposure-specific. As a consequence, it is not feasible to adapt the BRs depending on the different exposure conditions. Although we adequately characterized numerical errors, additional sources of uncertainty related to individual characteristics
and anatomical differences still needed to be further investigated.

In an early work, Bakker et al. [20] studied the variability of induced electric fields between eight high-resolution human models consisting of two adults and six children. Results showed discrepancies among the individuals up to 40% when comparing the lowest and highest electric fields induced in the CNS tissues. Similar results were obtained in [19], where a total number of six individuals were recruited (five adults and one child). However, the number of anatomical models in those investigations was still inadequate to derive results for a larger population. In addition, although it has been widely shown that a larger cross-sectional area of the body induced higher electric field strengths [21, 22, 38, 47], the effect of individual characteristics and other anatomical factors still requires further investigation.

For this reason, 118 individuals were enrolled in publication III to assess the effect of different factors, such as incident magnetic field direction, age, skull volume and gender, on the electric field strengths induced in the brain. No study in this field has ever counted such a high number of individuals. Despite the statistical significance of skull volume and age, these factors did not considerably affect the peak electric field strengths in the brain. On the other hand, the incident magnetic field directed in the lateral direction produced significantly higher induced field strengths. Additionally, the factor used in the ICNIRP guidelines to convert the basic restriction for CNS tissues to an external magnetic field was found to be approximately 1.5 times higher than that derived in our investigation. Note that one limitation of this study was represented by the fact that the authors did not estimate the variability of the calculated electric field strengths due to the uncertainty in the electrical conductivity of the tissues.

The latter represents another important uncertainty factor in LF computational dosimetry. On this basis, publication IV intended to lessen dosimetric uncertainty due to the variability of the electrical conductivity of the tissues. To investigate this effect, three conductivity data sets derived from the McCann [61], Gabriel [54] and Dimbylow [38] investigations were considered. Although the conductivity values of brain tissues estimated by McCann were significantly different from those derived by Gabriel and Dimbylow, they are in good accordance with experimental data based on in vivo measurements. For instance, the WM conductivity value (0.22 S/m) estimated by McCann [61] is in a good agreement with that measured by Latikka et al. [59] during brain surgery (0.25 S/m). Similarly, the GM conductivity value (0.47 S/m) is in the same range as observed in [60], where the authors obtained a value of 0.404 S/m after performing invasive measurements on the visual cortex of monkeys.

In addition, the skin conductivity value (4.5 × 10^{-4} S/m) derived from the dispersion model developed by Gabriel [54] is significantly lower even compared to that employed by Dimbylow [38]. This is due to the fact that the
skin conductivity values were measured on the skin surface, i.e., the stratum corneum [124], and therefore they do not represent inner layers, such as epidermis and dermis, which have a higher conductivity value [8, 123]. In [38], Dimbylow modeled the skin as a layer consisting of skin and subcutaneous fat, without providing any explanation for using a conductivity of 0.1 S/m. Presumably, this value was calculated approximately as the average between (i) the skin conductivity value estimated by Yamamoto and Yamamoto [68] for the epidermis (0.22 S/m) and (ii) the conductivity value of fat (0.045 S/m) determined by Gabriel [54]. On the other hand, the skin conductivity value (0.41 S/m) estimated by McCann [61] is in the upper bound of the range of values reported in LF literature, which spans from 0.0002 S/m to 0.465 S/m [125]. Please note that the skin is considered a potential target tissue for PNS stimulation at LFs [1] as it contains peripheral nerve endings. For low-frequency exposure of peripheral nerves, previous investigations showed the importance of an accurate modeling of the skin [8, 124], especially in those cases where the body posture makes large induction loops with skin-to-skin and skin-to-metal contact regions [24], in which the current density increases, leading to higher maximum electric field strengths.

Additionally, in publication IV we exposed the brains to spatially uniform magnetic fields as this corresponds to the reference exposure scenario considered by both ICNIRP [1] and IEEE [2, 3]. Particularly, ICNIRP derived the conversion factors for obtaining the reference levels from the basic restrictions based on the study performed by Dimbylow [38], who exposed anatomical models to spatially uniform magnetic fields at 50 Hz along three different directions. The set of conductivity values employed in this work was also used in publication IV, and the results compared with those obtained with the other two conductivity data sets. To investigate how the reference levels are affected by the use of higher brain conductivity values, the same exposure conditions as in [38] had to be considered. In this regard, different exposure scenarios would not be suitable for the purpose of comparing our results with those used as a basis for developing the safety guidelines. Our findings show that the conversion factor for the CNS would be significantly lower using new estimates of the brain conductivity values. More generally, a decrease in the electric field strengths is expected even in the case of localised exposure due to non-invasive stimulation when higher brain conductivity values are employed.

The sensitivity analysis performed in publication IV revealed that variation in the conductivity of grey matter and white matter significantly affect the induced electric field strengths in the CNS. Therefore, additional investigations based on in vivo measurements are needed to further reduce the large variability in brain conductivity values currently present in the literature. Future studies should also include infants and children, as the conductivity decreases with age. This is related to the water content in the
tissues which is higher for children than adults, as found in [139, 140]. For example, a significant variation in water content was observed for rather young individuals, approximately under 12 years old in [140], whereas it remained invariant for an older population. Please note that none of our investigations recruited such young individuals. Therefore, the age-related changes in the water content of the tissues does not have any effect on our results. The changes in the electrical conductivity with age were also investigated in [141] at intermediate and high frequencies based on measurements performed on rat tissues. However, there is still a lack of data for the extremely low frequency range. As the conductivity of the CNS tissues is expected to be higher in the young population, this will possibly produce lower field strengths.

**Additional limitations**

In publication I, stimulation thresholds of the CNS tissues were estimated at the IFs through a computational approach that combined TMS experiments, electromagnetic dosimetry and biological axon models. The IEEE International Committee on Electromagnetic Safety [28] addressed the importance of employing this kind of methodology for assessing the stimulation thresholds in a scientific manner. On this basis, the results produced in publication I are particularly important considering the lack of scientific data at IFs.

It is worth mentioning that the BRs defined by the IEEE standard and the ICNIRP guidelines were set in terms of magnitude of the induced electric field under the assumption that the neuronal excitation takes place within a locally uniform electric field at the axonal terminations or bends [28], where a strong variation of the component of the electric field along the axon occurs. In this context, it is still questionable whether including the spatial derivative of the electric field along the neuron as a metric for human protection in the standard/guidelines [28]. The hypothesis of uniform electric field at the axonal level is also generally accepted for non-invasive stimulation [149], and therefore it was used in publication I, where TMS was employed to investigate the electro-stimulation thresholds at IFs, i.e., the minimum electric field needed to produce a cortical response. The reasoning behind the choice of this technique lies in the fact that is capable of investigating the excitability of the axons non-invasively. In this regard, it has been shown that TMS is likely to activate myelinated axons in the motor cortex [88]. Based on the derived electro-stimulation thresholds, we employed two biological axon models, namely the SENN and NIT models, to estimate the diameters of axons with straight and bent shapes. As a final step, we used the modeled axons to extrapolate the stimulation thresholds of the CNS tissues for sinusoidally varying electric field at various frequencies. Therefore, the extrapolation of these
stimulation thresholds cannot be considered TMS-specific.

Please note that tissue heterogeneity was not considered, as it was found to have negligible effects on activation sites for TMS [105]. In particular, it was shown that at WM–GM interface, where local changes in the induced electric field might occur due to charge accumulating at the boundaries of tissues with different electrical conductivities, stimulation never happened due to heterogeneity [105]. Therefore, this work confirmed the importance of geometrical factors, i.e. axonal bends and terminations, in stimulation of neurons, as previously observed in other investigations [103, 104].

In publication I, the authors opted to employ the SENN model as it was used by the IEEE standard to derive the basic restrictions [2, 3]. In order to verify the reliability of the results, we used an additional biological axon model (NIT) [136]. Results showed that both models produced comparable excitation thresholds of the CNS tissues. However, other kinds of biological axon model are currently in used. In this context, a survey [94] between different electro-stimulation models was conducted to compare the excitation thresholds obtained in different conditions, i.e., characteristics of the neuron and the stimulation waveforms. This survey included the SENN, NIT and other electro-stimulation models. A large variability on the results was observed, suggesting that an experimental validation under certain conditions of the electro-simulations model is recommended [94].

The electro-stimulation thresholds estimated in publication I largely exceeded the current basic restriction/dosimetric reference limits, denoting a certain margin of conservatism. Please note that for extremely low frequencies, the current limits for the induced electric field are intended to avoid the stimulation of retinal phosphenes, as well as alterations of synaptic activity in the CNS. Below 100 Hz, synapses in the cerebral cortex are in fact sensitive sites that could be effected by externally applied electric fields, possibly leading to excitatory or inhibitory effects [28]. Those effects, which are expected to take place at lower thresholds than those estimated for the axonal stimulation, require further investigations. In this context, the inclusion of synaptic terminations in biological axon models will be essential for assessing the effects of an applied electric field at the neuronal conjunction level [6].

Another important source of uncertainty that requires additional investigation is represented by the anisotropic properties of WM. Our electromagnetic simulations are based on the hypothesis that all electrical conductivities are isotropic. If this assumption holds for certain tissue such as GM and CSF [144], the same is not actually valid for WM. In the latter case, the conductivity varies depending on the direction of the neurons, being higher along the fibers than in the perpendicular direction [145, 146]. However, the inclusion of the anisotropic properties of the tissues strongly complicates the computational modeling that would require information about the orientation of nerve fibers. For this purpose, DTI represents a
valid technique for modeling WM conductivity anisotropy [61], that could be used in combination with numerical dosimetry to investigate the effect of anisotropy properties on the electric field strengths.

The segmentation of medical images into a limited number of tissues plays an important role in the assessment of the induced electric field. In this context, our semi-automatic segmentation pipeline enables the reconstruction of anatomical models consisting of 24 tissues, which denotes a high level of detailedness. The more realistic the anatomical models, the more accurate the computed electric fields. To study the effect of model detailedness on the computed electric fields induced by TMS, each of the 19 head models employed in publication I were used to generate less detailed anatomical models. In particular, different tissues of the original head models were combined together to constitute a larger compartment. For simplicity, we will refer to these simplified models as (i) M1, obtained by assigning blood and dura to the CSF, and (ii) M2, where cortical bone, cancellous bones, vestibule and mucous membrane were combined in a single compartment of cortical bone. The head models were stimulated at the HS, and the distribution of the induced electric field was calculated, as in publication I, in the left hemisphere on a surface lying 2 mm below the surface of the GM. Results showed that the average relative difference between the electric fields computed in the more detailed and the less accurate head models were in the order of 4% (M1) and 1% (M2). This seem to confirm the moderate sensitivity of CSF to segmentation errors when evaluating the electric field induced by TMS [153].

To study the effect of model detailedness even in the case of uniform exposure, the same approach was used to generate the less detailed anatomical models M1 and M2 for each of the 118 head models considered in publication III, which were then exposed to uniform magnetic field (1 mT) at 50 Hz along AP, LAT and TOP. For each head model/exposure scenario, the maximum electric field strength was calculated in accordance to publication III. Results are provided in table 5.1, which shows that changes in the CSF produced slightly higher maximum electric field strengths. On the other hand, changes in the skull did not have an effect on the results.

Table 5.1. Statistical data of the highest 99th percentile (mean±standard deviation) of the ICNIRP-averaged electric fields over all the brain tissues (mV m⁻¹) derived for the head models employed in publication III (baseline), along with those obtained using the less detailed models M1 and M2.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Baseline</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>18.0±1.4</td>
<td>18.7±1.3</td>
<td>18.0±1.4</td>
</tr>
<tr>
<td>LAT</td>
<td>22.1±2.1</td>
<td>22.7±2.1</td>
<td>22.0±2.0</td>
</tr>
<tr>
<td>TOP</td>
<td>16.8±1.3</td>
<td>17.5±1.2</td>
<td>17.0±1.3</td>
</tr>
</tbody>
</table>

Lastly, our results were obtained under the quasi-static assumption,
which derives from the fact that in the LF range the wavelength is much larger than the dimension of the body. It has been shown that this approximation holds even for frequencies close to the upper bound of the LF interval [147, 148], which is set to be equal to 5 MHz in the IEEE standard [2, 3] and 10 MHz in the ICNIRP guidelines [1]. Around those frequencies, the stimulation of nerves might still occur [34]. In addition, between 100 kHz and 10 MHz the dominant effect of electromagnetic field exposure changes from nerve stimulation to heating [150]. Therefore, for frequencies higher than 100 kHz, the ICNIRP and IEEE exposure levels protect against both stimulation of nerve and heating effects. Please note that the limits associated to thermal effects are expressed in terms of the specific absorption rate (SAR, W/kg), which is the energy absorbed by human tissues per unit mass when exposed to electromagnetic fields. For frequencies higher than 10 MHz [1], heating becomes the major effect of absorption of electromagnetic energy. In this context, the applicability of the quasi-static assumption at 10 MHz was investigated in [147], where the authors compared the SARs obtained using the quasi-static and full-wave approaches. Results derived from the exposure of a homogeneous cylindrical human model to a wireless power transfer system showed differences of up to 15% in the SAR computed with the two approaches [147]. More recently, an analogous investigation was conducted using realistic anatomical human models for whole-body uniform exposure at frequencies between 100 kHz and 100 MHz [148]. Both free space and grounded conditions were considered in the study. Up to 1 MHz, the results revealed a good agreement between the whole-body averaged SARs calculated using the quasi-static approximation and the full-wave modeling. For higher frequencies, the results started to diverge reaching a difference of 10% at 10 MHz and at 30 MHz for grounded and free space exposure, respectively. Violation of the quasi-static assumption was visible for higher frequencies, which resulted in a strong overestimation of the SAR. Please note that we also assumed the displacement currents to be negligible. This approximation resulted in a rather small error when evaluating the maximum electric field strengths, which was in the order of 2% at 50 Hz [151]. For frequencies between 1 kHz and 1 MHz, these discrepancies reduced and remained well below 1% [151].
6. Conclusion

The present thesis characterized open issues in computational dosimetry at LFs with the purpose of producing quantitative data useful for the future revision of human exposure standard/guidelines. This includes the estimation of stimulation thresholds of brain tissues at LFs using a combined approach between electric field modeling and biological axon models [6], characterization of numerical artifacts in voxelized and tetrahedral models [118], assessment of the effect of anatomical differences/individual characteristic on the induced electric field strengths [127], and evaluation of the uncertainty produced by the variability in tissue electrical conductivity values [29].

However, additional topics need to be further investigated. For instance, consistency among different biological axon models has to be carefully characterized with the purpose of reducing discrepancies in the predicted excitation thresholds. Also, inclusion of neuronal conjunctions will be important for studying thresholds for the alteration of the synaptic activity. The accuracy of LF dosimetric studies will be further improved by reducing the uncertainty in the dielectric properties of the tissues. This can be achieved by performing new in vivo measurements based on advanced non-invasive methods. In this context, imaging techniques seem to be promising [61]. Additionally, the inclusion of the anisotropic properties of WM in computational methods will be particularly of interest to evaluate its effect on the induced electric field strengths.
References


References


[17] F. S. Salinas, J. L. Lancaster, and P. T. Fox, “3D modeling of the total electric field induced by transcranial magnetic stimulation using the


References


References


References


References


Errata

Publication I

In section 2.2: The AMT was defined as the minimum intensity required to elicit a MEP of 200 $\mu$V in at least 5 out 10 consecutive trials during a slight isometric contraction of the FDI muscle.
Characterization of open issues in low-frequency computational dosimetry