PhD in Information Technology and Electrical Engineering

Università degli Studi di Napoli Federico II

PhD Student: Maria Agnese Pirozzi

XXXIII Cycle

Training and Research Activities Report – Second Year

Tutor: Prof. Mario Cesarelli

co-Tutors: Dr. Mario Quarantelli (MD), Ing. Mario Magliulo (PhD)

Information

I am **Maria Agnese Pirozzi** and I received a **M.Sc. degree cum laude in Biomedical Engineering** from the University of Naples "Federico II" on January 31, 2017. From April 2017 I am the grantee of a **research fellowship at the Italian National Research Council – Institute of Biostructure and Bioimaging (CNR-IBB)** of Naples. From January 2018 to today I am also a **PhD Student of XXXIII cycle in Information Technology and Electrical Engineering (ITEE)** at Department of Electrical Engineering and Information Technology (DIETI) of University of Naples "Federico II" **without fellowship**. My tutor is Prof. **Mario Cesarelli** and I have two **co-tutors** at CNR-IBB, Dr. Mario Quarantelli (MD) and Ing. Mario Magliulo (PhD).

Study and Training Activities

During the second year of PhD, I attended courses and seminars within the ITEE program as reported below.

• Courses

Below are the courses included in the training plan of my second year of PhD. The ad-hoc modules organized and provided by DIETI Department are listed. Next to each item is reported the Department that provided the course, the lecturer(s), the start and end dates, the total amount of hours and ECTS.

Ad-Hoc Modules	Department	Lecturer/s	Start-End	H	ECTS
Data Science and Optimization	DIETI, Università degli Studi di Napoli "Federico II"	M. Gaudioso, L. Palagi, E. Messina	February 5, 2019 - February 7, 2019	6	1.2
Machine Learning	DIETI, Università degli Studi di Napoli "Federico II"	A. Corazza, F. Isgrò, S. Olivieri, R. Prevete, C. Sansone	May 6, 2019 - May 20, 2019	21	5
New directions in biomedical engineering research: neuroscience, machine learning and personalised medicine	DIETI, Università degli Studi di Napoli "Federico II"	P. Gargiulo, T. Helgason	May 16, 2019 - May 17, 2019	8	2
True Unipolar Electrocardiography and Application to Medicine	DIETI, Università degli Studi di Napoli "Federico II"	G.D. Gargiulo	July 1, 2019 - July 2, 2019	12	2.4

• Seminars

Below are the seminars part of my second-year training plan.

Seminars	Lecturer/s	Host/s	Date	H	ECTS
Medical Thermal Therapy and Monitoring Using Microwave Inverse Scattering	M. Moghaddam	A. Iodice	May 5, 2019	1	0.2

In September 2019, I attended the *XXXVIII Annual School of Bioengineering* held in Bressanone (Italy) organized by the Italian National Bioengineering Group (GNB). The PhD School overall included 30 hours of lectures, equivalent to 5 ECTS. During school I took part in a "pitch contest" on a creative topic. I was part of a group of 6 people from various Italian universities and we worked on the project entitled: "Ingestible sensorised pill for digestive function evaluation".

Seminars from PhD Schools	Start-End	H	ECTS
XXXVIII Annual School of Bioengineering organized by the Italian National Bioengineering Group (GNB)	September 9, 2019 - September 12, 2019	30	5

- ## Credits summary

Finally, I provide a table reporting a summary of the ECTS obtained in my second year of PhD.

	Credits year 2							
		1	2	3	4	5	6	
	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary
Modules	10	1,2	0	7	2,4	0	0	**10,6**
Seminars	5	0	0	0,2	0	5	0	**5,2**
Research	45	7	11	2,2	4	8	12	**44,2**
	60	8,2	11	9,4	6,4	13	12	**60**

Research Activity

Title

Development of innovative techniques to create anthropomorphic brain phantoms for morpho-functional imaging.

Introduction

Brain imaging is particularly challenging in many aspects and in functional imaging it is currently hampered by low-resolution issues, which lead to image "contamination" from surrounding structures. This problem becomes

more prominent in cases where atrophy is present, such as Alzheimer's disease. The need to validate the functional images and their analysis has led to the use of anthropomorphic brain phantoms, to overcome an intuitive and real disparity between the images usually obtained with simple geometric test brain phantoms and activity distributions seen in *in vivo* images [1]. The human brain is a functionally and topologically complex organ with deep fissures and sulci over its medial and lateral surfaces. Therefore, the creation of a physical model capable of depicting the form of the cerebrum is not trivial due in part to these deep structures. However, having a brain phantom containing the physical, geometrical and physiological information of the human cerebrum can be very useful for the development and testing of the medical imaging methods (such as CT, MRI, PET or SPECT) [2] [3]. There are several types of available brain phantoms which reflect the numerous imaging tasks, such as geometrical accuracy, dose algorithm accuracy, image quality, machine quality assurance, irradiation techniques, and calibration of measurements to required physical quantities. Traditional mould phantoms are used by researchers and radiologists, but these have several limitations. They are relatively expensive, and the complex geometry and structure of the human brain cannot be completely replicated. Thus, traditional phantoms do not have realistic structures, and therefore the evaluation on imaging methods and systems is often limited with their use. The solution to overcome these problems may be offered by most advanced 3D-printed phantoms which reproduce the exact anatomy of some brain compartments. In fact, computer-aided design (CAD) can be used to extract anthropomorphic brain phantom's shapes from MRI images providing the opportunity to clinicians, physicists, engineers and radiographers to develop much more complex brain phantom, that can be used even for multimodality systems [4].

Goals to achieve

The goal of this research activity is to design and produce a complex anthropomorphic brain phantom using innovative design and manufacturing techniques. Traditional production techniques of *Subtractive Manufacturing* do not allow creating such complex objects. While the *Additive Manufacturing* (AM), known as 3D printing, creating objects in an additive way, allows developing products no longer bound by design complexity. The designed phantom is a unique part, anatomically accurate in shape and proportions that, at the same time, can simulate three compartments of the human brain: Gray Matter (GM), White Matter (WM) and *Striatum*. The *Striatum* is a high-uptake structure in Nuclear Medicine (NM) studies, and, for this reason, it is interesting to emulate it.

Methodologies and developments

The chosen AM technology is the *Fused Deposition Modelling* (FDM), among the most advanced and effective available today, for the strength, durability and stability of the final parts. The material (in the form of the filament) is extruded through heated nozzles. A nozzle releases overlaid layers of a melted polymer; the other predisposes, if necessary, removable support structures.

To realize the 3D model of the phantom, automatic processing and manual editing were carried out to design a printable physical counterpart of the digital brain phantom *Phantomag* [5] (obtained from brain MRI images of a normal volunteer). Voxelised surfaces at the interface of compartments were extracted from images of digital phantom by selecting voxels (from the outside towards the inside) sharing vertices, edges and faces. Then, the 3D model of the brain phantom was obtained in the vector format STL (STereoLithography) for 3D printing. The interface surfaces between the GM, WM and Striatum were derived to create empty and fillable compartments. The specific filling solution for each compartment depends on the imaging modality, e.g. iodine solutions for Computed Tomography (CT), paramagnetic solutions for Magnetic Resonance Imaging (MRI), radioactive solutions for Nuclear Medicine (NM) studies.

Therefore, the phantom's design specifications and materialization were:

- Both internal and external supports are required to print the phantom: it is internally hollow and has complex and convoluted shapes [6].
- For each compartment, there are two tubes: one for the entry of the liquid, the other for the escape of air.
- The phantom must be sturdy and waterproof, having vertical wall thickness of less than 1 mm, preferably between 0.4 mm and 0.6 mm (not too visible to imaging).

In addition to representing a very complex geometry (the human brain), the designed phantom has structural characteristics that make the materialization process non-trivial. The experimentation conducted in my first year, with a technologically less advanced FDM printer, allowed me knowing better the limits and strengths of the FDM 3D printing. My research on more advanced 3D printing technologies (such as PolyJet) led me to conclude that the FDM is the only one that allows the dissolution of internal supports, so that the compartments of phantom are completely free for filling. Thus, during this year I chose to work with a technologically more advanced printer, the professional FDM printing *Statasys F370,* which could print the phantom without problems and predispose printing soluble supports. The first prints were obtained using the basic version of the slicing software *GradCAD Print*, but then I chose to optimize the printing parameters, first to obtain a greater impermeability of the printed phantom. For this reason, I attended an advanced course on the slicing software *Insight for GrabCAD*. Insight is a more complicated software, but since it's been developed for industrial printers it's incredibly powerful. Every finite detail of a print job can be customised down to each individual layer. Being able to optimise printing properties such as raster width and angle, number of contours and their widths, and amount of air gaps between them is indispensable to materialise such a complex object. Between toolpath parameters, the setting of the use of variable width remnant fill has been probably the most important ones.

I used to remove the supports the *Support Cleaning Apparatus* (SCA-1200HT), specifically designed for the removal of soluble support materials on 3D printed parts. The units are compatible with all Stratasys soluble support materials and corresponding build materials in conjunction with all recommended support removal

concentrates. Then, CT scans of the phantom allowed verifying that the supports removal system also dissolved the internal supports, completely emptying the internal volume.

Intermediate results and future perspectives of research activity

The experimental tests carried out to date highlight two critical issues for our application (due to the current limits of the FDM technology):

- The phantom requires submillimetre wall thickness and impermeability, while the generally printable minimum wall thickness is at least 1 mm thick.
- The required amount of internal support greatly extends the printing time. Trying to reduce it using slicing software, it does not always lead having efficient supports at critical points [6].

The large amount of support necessary for printing empty and complex forms such as those of anatomical phantoms, however, remains a problem to be studied in the future, to try to create ad-hoc supports for applications of this type. In this regard, I presented a pilot study at the MEDICON 2019 international conference [6].

A filling system consisting of three peristaltic pumps (one for compartment) connected to the tubes has been testing, highlighting that the greatest criticality of the filling phase is the need to avoid that air bubbles would be trapped inside the phantom. These would result in unwanted hypointense areas in imaging.
Waterproofing tests have shown that the water-tightness is not real because even according to the internal pressure the water could creep into the weft deposited during the printing process. To overcome the water-tightness problem, we have been developing an appropriate waterproofing technique. However, some waterproofing tests of partial realizations of the phantom with pure acetone, pure acetone vapours or dilutions of water and pure acetone have shown that these methods would make it possible to waterproof the parts printed in ABS.

In the next year, I will define the best technique for waterproofing the phantom, also exploring the possibility of using latex-like material. Then, I would like to improve the 3D model, trying new procedures of extracting the same from MRI images. The current phantom has walls of vertical and horizontal thickness ranging from a minimum of 0.5 mm to a maximum of 1 mm. This thickness is related to the resolution of the voxel in the phantom images, which was raised to 0.5 mm just to create a voxelised surface from which to directly derive the one in STL format, using 3D Slicer. Therefore, it must be considered that for prints with a layer height of 0.254 mm or more, for horizontal (or almost) surfaces there may be no more than one printed layer. This forces us to use, in the first instance, a layer height of 0.173 mm to make sure that more than one layer is printed for a horizontal wall of 0.5 mm. However, this choice would be impractical due to the lengthening of the printing times and consequently also due to the greater predisposition to hitches or other problems during printing. The solution could be to thicken the walls or only the horizontal walls to little more than 0.5 mm.

Once the phantom will be ready for use, the last step will be to define procedures for multi-analytical and related devices studies.

Title

Development of automatic brain segmentation software for multi-contrast magnetic resonance imaging.

Introduction

Magnetic resonance imaging (MRI) of the brain is widely used in clinical practice for diagnosis, patient follow-up, therapy evaluation and human brain mapping. MRI is non-invasive, has good spatial resolution and fast acquisition, and excellent performance when visualising differences in brain tissues. The segmentation of brain MRI images is very useful for the quantitative assessment of brain volumes, both for the characterization of normal brain ageing and for the study of chronic degenerative disorders of the brain (such as Parkinson's disease, Alzheimer's disease, Multiple Sclerosis, etc.). The automated segmentation of MR brain images is a challenging task due to image artefacts (such as intensity inhomogeneities and partial volume effects) and since different anatomical structures may share the same tissue contrast. Hence, *a priori* anatomical information is essential for simplifying the segmentation task. Prior information may be provided in different ways, for instance, as a set of predefined rules based on known tissue properties and topology, or as a set of manual expert annotations [7].

Automatic techniques are by far preferable for operator bias-free segmentation and to analyse large cohorts of images, but segmentation based only on MRI signal intensity data is associated with several problems and requires operator intervention. Strategies assessing multiple parameters (multi-feature procedures) are being developed in which the automated characterization of Multiple Sclerosis (MS) lesions seems to be more reliable [8], [9], [10]. Besides lesions, volumes of GM, WM, and cerebrospinal fluid (CSF) are also shown to be affected by the disease state in MS [10]. Also, the basal ganglia (caudate, putamen, pallidum, substantia nigra, red nucleus, thalamus and dentate nucleus), deep GM structures involved in cognitive tasks, are frequently affected in MS patients. Abnormal basal ganglia (BG) activation on functional MRI has been observed during cognitive testing and lower volume has been associated with cognitive dysfunction (such as episodic memory impairment or executive dysfunctions) [11]. For these reasons, a robust estimation of all brain tissues volumes is required to investigate these aspects.

Goals to achieve

The brain segmentation software that I am developing aims to recognize most of the brain tissues, as well as possible MS lesions, exploiting *a priori* knowledge contained in the MS digital brain phantom *Phantomag* [5] and

in look-up tables describing the tissues that define the physical and geometrical properties of the tissue to be segmented. The digital model replicates the real anatomy and tissue inhomogeneities reproduced from MRI images of a normal volunteer, representing both the anatomy and the relaxation rate (R1, R2) and PD distribution of 17 different healthy compartments, plus an eighteenth compartment that goes to simulate lesions (abnormal white matter, AWM).

The next evolution of the software aims to segment not only the QMCI maps, but also the MRI studies obtained from the sequences most used for MS (MPRAGE and FLAIR).

Methodologies and activities

During the first year, I developed the new procedure to segment *Quantitative Color Imaging* (QMCI) maps, based on physical MRI parameters (R1, R2, PD). These maps provide a reproducible position of voxel clusters of tissue in the multi-parametric space (R1, R2, PD) [11]. The distribution of pixels in the feature space (R1, R2, PD) allows separating a greater number of tissue components than the distribution of pixels in signal intensity space (T1, T2, PD). The segmentation procedure is descripted in the training and research activities report (TRAR) of my first year of PhD.

During the second year, I optimized the whole procedure and I developed a new algorithm to improve the recognition and segmentation of AWM. The developed method allows to identify and calculate the volume of demyelinated WM due to the pathology of MS. The Potential Lesions (PL) of the WM are identified within the segmentation algorithm based on maps of the relaxation rates (R1, R2) and Proton Density (PD). A preliminary assessment of the distribution of lesions from MS in the R1, R2, PD space shows that both the relaxometry and the geometric features must be used for the classification of the lesions. In fact, in the multi-feature space (R1, R2, PD) the voxels of AWM cover a wide range of values of R1 and R2, and appear to originate from the normal WM clusters that migrate to lower values of R1 and R2. Therefore, for the classification of AWM, other criteria have been added to consider also the 3D shape factor of the lesion and the composition of the surrounding tissues.

Intermediate results and future perspectives of research activity

The software was tested on a database of QMCI studies, obtained from conventional spin-echo sequences on MS patients. The lesion identification algorithm achieves a sensitivity of approximately 96% and an accuracy of approximately 94%.

In the last months of the year, I started to work on the extension of the software for the segmentation of studies obtained with the most modern MRI sequences. MPRAGE and FLAIR are those most used to date for the study of MS. These sequences have a much shorter duration and are currently much more widespread than the conventional spin-echo from which the QMCI are obtained.

In the next year, I will complete the extension of segmentation to other MRI sequences and I will test the software for multi-feature studies on MS patients.

- ### Collaborations

A collaboration is underway with Prof. Paolo Gargiulo from the Reykjavik University to create a prototype of the brain phantom using PolyJet technology.

Products

I am preparing journal publications on this year's research activities and other conference publications. A lot of work has been done on both topics, so the last few months have been dedicated to writing articles that will be submitted to international journals.

- ### International Conference Publications

1. **Pirozzi M.A.**, Andreozzi E., Magliulo M., Gargiulo P., Cesarelli M., Alfano B. (2020) Automated Design of Efficient Supports in FDM 3D Printing of Anatomical Phantoms. In: Henriques J., Neves N., de Carvalho P. (eds) XV Mediterranean Conference on Medical and Biological Engineering and Computing – MEDICON 2019. MEDICON 2019. IFMBE Proceedings, vol 76. Springer, Cham.
2. Andreozzi E., **Pirozzi M.A.**, Sarno A., Esposito D., Cesarelli M., Bifulco P. (2020) A Comparison of Denoising Algorithms for Effective Edge Detection in X-Ray Fluoroscopy. In: Henriques J., Neves N., de Carvalho P. (eds) XV Mediterranean Conference on Medical and Biological Engineering and Computing – MEDICON 2019. MEDICON 2019. IFMBE Proceedings, vol 76. Springer, Cham.

- ### Journal Publications

1. Canna A., Prinster A., Fratello M., Puglia L., Magliulo M., Cantone E., **Pirozzi M.A.**, Di Salle F., Esposito F., A low-cost open-architecture taste delivery system for gustatory fMRI and BCI experiments, *Journal of Neuroscience Methods* (2018), https://doi.org/10.1016/j.jneumeth.2018.10.003.

Conferences and Seminars

- ### Conferences

1. Participation at XV Mediterranean Conference on Medical and Biological Engineering and Computing – MEDICON 2019 (September 26-28, 2019, Coimbra, Portugal) with 2 papers.

2. Oral presentation at XV Mediterranean Conference on Medical and Biological Engineering and Computing – MEDICON 2019. The presented paper is: ***Pirozzi M.A.**, Andreozzi E., Magliulo M., Gargiulo P., Cesarelli M., Alfano B. (2020) Automated Design of Efficient Supports in FDM 3D Printing of Anatomical Phantoms. In: Henriques J., Neves N., de Carvalho P. (eds) XV Mediterranean Conference on Medical and Biological Engineering and Computing – MEDICON 2019. MEDICON 2019. IFMBE Proceedings, vol 76. Springer, Cham.*

Activity abroad

I did not carry out any activity abroad in my second year of PhD.

Tutorship

- Assistant for the BSc course of "Elaborazione dei Dati e Segnali Biomedici", held by Prof. Francesco Amato, **20 hours.**
- Assistant for the BSc course of "Ulteriori conoscenze: laboratorio di bioingegneria", held by Prof. Mario Cesarelli, **6 hours**.
- Assistant for the MSc course of "Elaborazione di Segnali e Immagini Biomediche", held by Prof. Mario Cesarelli, **4 hours.**

Reference:

[1] E. J. Hoffman, P. D. Cutler, W. M. Digby e J. C. Mazziotta, «3-D Phantom to Simulate Cerebral Blood Flow and Metabolic Images for PET», IEEE TRANSACTIONS ON NUCLEAR SCIENCE, vol. 37, n. 2, 1990.

[2] X. G. Xu e K. F. Eckerman, «Handbook of Anatomical Models for Radiation Dosimetry», in Series in Medical Physics and Biomedical Engineering, CRC Press, 2009, pp. 4-41.

[3] M. Caon, «Voxel-based computational models of real human anatomy: a review», Radiat Environ Biophys, vol. 42, pp. 229-235, 2004.

[4] Filippou, V. and Tsoumpas, C. (2018), «Recent advances on the development of phantoms using 3D printing for imaging with CT, MRI, PET, SPECT, and ultrasound». Med. Phys., 2018, 45: e740-e760. doi:10.1002/mp.13058.

[5] B. Alfano, M. Comerci, M. Larobina, A. Prinster, J. P. Hornak, S. E. Selvan, U. Amato, M. Quarantelli, G. Tedeschi, A. Brunetti, M. Salvatore, «An MRI digital brain phantom for validation of segmentation methods», Medical Image Analysis 15, 2011, 329–339.

[6] M.A. Pirozzi, E. Andreozzi, M. Magliulo, P. Gargiulo, M. Cesarelli, B. Alfano. «Automated Design of Efficient Supports in FDM 3D Printing of Anatomical Phantoms». In: Henriques J., Neves N., de Carvalho P. (eds) XV

Mediterranean Conference on Medical and Biological Engineering and Computing – MEDICON 2019. MEDICON 2019. IFMBE Proceedings, vol 76. Springer. (2020).

[7] Cabezas, M., Oliver, A., Lladó, X., Freixenet, J., & Bach Cuadra, M. «A review of atlas-based segmentation for magnetic resonance brain images». Computer Methods and Programs in Biomedicine, 104(3), 2011. e158–e177. doi:10.1016/j.cmpb.2011.07.015

[8] Danelakis A, Theoharis T, Verganelakis DA. Survey of automated multiple sclerosis lesion segmentation techniques on magnetic resonance imaging. Comput Med Imaging Graph 2018; 70:83–100.

[9] Alfano B, Brunetti A, Larobina M, Quarantelli M, Tedeschi E, Ciarmiello A, Covelli EM, Salvatore M. Automated Segmetation and Measurment of Global White Matter Lesion Volume in Patients with Multiple Sclerosis. Journal of Magnetic Resonance Imaging. 12:799-807 (2000).

[10] Datta S, Narayana PA. A comprehensive approach to the segmentation of multichannel three-dimensional MR brain images in multiple sclerosis. NeuroImage Clin 2013; 2:184-96.

[11] Brochet B, & Ruet A. (2019). Cognitive Impairment in Multiple Sclerosis with Regards to Disease Duration and Clinical Phenotypes. Frontiers in Neurology, 10. doi:10.3389/fneur.2019.00261.