

# Instituto de investigação e Inovação em Saúde - i3S

## Unit Report 2022

# Index

<b>Summary .....</b>	<b>3</b>
<b>Description of the Unit .....</b>	<b>3</b>
<b>Major highlights of 2022 .....</b>	<b>4</b>
<b>Highlights of the Research Programs .....</b>	<b>5</b>
<b>Training Activities .....</b>	<b>9</b>
<b>Scientific Platforms .....</b>	<b>9</b>
<b>Porto comprehensive cancer centre .....</b>	<b>20</b>
<b>National and international infrastructures .....</b>	<b>20</b>
<b>Transversal services .....</b>	<b>20</b>
<b>Clinical initiatives .....</b>	<b>28</b>

## Summary

i3S is a very large research unit which aims to promote interdisciplinary research in three major areas of critical importance for the health sciences, namely, neurobiology and neurodegenerative diseases, molecular and cellular biology of cancer, and infection, immunity and regenerative medicine. It is our strong belief that only through the integration of different fields of research that major advances in the health sciences with impact to patients, can be developed. In this context, i3S promotes a virtuous cycle in which fundamental curiosity-driven research provides the basis for the translation into new and innovative solutions that can then be applied to the clinic and result in benefit for patients. During the past year i3S continued to make major advances in research with a very significant number of peer-reviewed publications and obtained a very large number of projects with competitive funding from national and international sources. We continue to collaborate with the University of Porto in the training of PhD students in the context of 5 PhD programs and also host a large number of postdoctoral fellows. We actively promote the economic valorization of research results which lead last year to the creation of new start-ups and more patents. Our researchers have developed more clinical studies or trials in collaboration with big Pharma and our partner hospitals, and together with the Portuguese Institute of Oncology in Porto, the Porto Comprehensive Cancer center Raquel Seruca was granted five more years after an international evaluation. I3S continues to develop its clinical initiatives that involve molecular diagnostics of cancer, neurodegenerative diseases and infection. Finally, in the area of outreach we have re-started and continue to develop new activities with the aim of promoting the public understanding of science and health literacy. Thus, after two and half highly demanding years imposed by the COVID pandemic i3S has emerged stronger and more determined to fulfill its objective of becoming a major hub for the health sciences nationally and internationally.

## Description of the Unit

i3S was created in 2016 and it is run by a private-non for profit Association. It has over 450 integrated researchers with PhD that are organized into 74 research groups organized into three research programs: Cancer, neurobiology and neurodegenerative diseases; Infection, Immunity and regeneration, all major areas of critical importance for the future of the health sciences. Our integrated researchers are supported by institutional funding, competitive contracts and fellowships provided by national and international agencies but we also host academics and clinicians from the University of Porto and the major hospitals in the region. Our research effort is supported by a large number of scientific platforms and transversal units. Since its creation i3S has followed a policy of sharing large equipment not only within our researchers but has also made the scientific platforms available to the wider community of the University of Porto, other researchers in the country, hospitals and industry. i3S runs 13 Scientific platforms that include: the only animal facility certified by ALACC and able to train young scientists under the FEALSA organization; Advance Light Microscopy with state-of-the-art equipment, Biochemical and Biophysical Technologies mostly for proteins production and characterization, Bioimaging, a new Bioinformatic platform for genomic and transcriptomic analysis, Biointerfaces and Nanotechnology for the study of biomaterials, a high throughput Biosciences screening system for identification of new biologically active

compounds, Cell Culture and Genotyping, Genomics, Histology and Electron Microscopy, in vivo CAM assays, Proteomics, Translational cytometry and x-ray Crystallography. All these platforms are run by highly skilled personnel that not only maintain the equipment but also provide training and help with experimental design. The transversal units are responsible for supporting researchers and students in all other aspects of their activity. The overall infrastructure is supported by the maintenance unit together with the Health and Safety unit and the lab support unit. The research innovation unit ensures that our researchers are fully aware of all the funding opportunities and support the preparation and submission of proposals. Through the knowledge transfer office it provides support for management of intellectual property, creation of start-ups and business development plans. The translational research and industry partnership office it guides researchers in the implementation of clinical and studies or trials. Students and postdoctoral fellows are supported by the career development unit. i3S also organizes a very large number of training activities, workshops, conferences and seminars through the Advance Training unit and the Events Management Unit. The administration provides support for the management of projects, human resources and financial aspects of the research unit. The data protection officer and the unit for responsible conduct in research ensure that we maintain the highest ethical standards at all levels of activity. The information systems and technology unit maintains a highly professional digital infrastructure that supports all of our activities. Finally, i3S has three fully dedicated clinical services that provides extensive support for the national health service in the areas of molecular diagnostics of cancer and neurodegenerative diseases, pathology, genetic counselling and molecular identification of pathogens. Thus, i3S is a complex and rich ecosystem that covers the full cycle of research and innovation and during the past year has made significant advances in all areas of its activity.

## **MAJOR HIGHLIGHTS OF 2022**

During 2022, researchers at i3S continue to produce a significant number of publications which totaled 511 original peer-reviewed articles of which 80 were in journals with Impact Factor higher than 9 and 228 other publications including reviews, book chapters and proceedings. Collaborative work between different research groups of the institute resulted in 134 publications and our international collaborations resulted in 343 articles between researchers from i3S and international collaborators. The institutional H-index of i3S is now 115 with a total number of citations of 134656 for the same period. Currently i3S has more than 200 projects running. In 2022 there were 41 projects approved by FCT with a total funding of 3.75M€. We also obtained 7 projects funded by the EU with a total budget of 8.6M€ including our first ERC grant. There was also significant success with La Caixa Foundation with the approval of 4 projects totaling 0.9M€. Applications to the national calls by FCT for scientific employment was very productive and we obtained a total of 22 new contracts, 7 junior, 11 Assistant and 4 Principal investigators. With respect to technology transfer the activity of i3S researchers continues to increase. In 2022 the portfolio of innovative solutions developed out of research at i3S, including diagnostics (12), medical devices (19) and therapeutics (11) is now very significant. We hold 25 patents of which i3S obtained 10 last year and there were 23 that have now been licensed. Of major significance was the licensed agreement for leishmaniosis treatment that resulted in a 1.3M€ deal with health Pharma. During 2022 i3S researchers obtained 400K€ in royalties and raised 1M€ in funding from

innovative programs. Our involvement with the healthcare industry has also continued to increase and last year 3.2M€ were obtained for the development of over 30 clinical studies or trials involving some of the major players in the Pharma Industry like Merck, Pfizer, Gilead, Roche and Astrazeneca. Overall, researchers at i3S continue to intervene at all stages in the cycle of innovation from fundamental research to clinical applications with great success. Finally, during 2022 i3S organized more than 80 national or international events including workshops, courses and conferences that brought more than 8000 participants to the institute.

## HIGHLIGHTS OF RESEARCH PROGRAMS

### Cancer

The Cancer Program aims to unravel fundamental genetic, molecular, and cellular mechanisms relevant to cancer biology, and to translate this knowledge into practical and tangible benefit for cancer patients. The Program includes research groups centred on specific oncobiology questions using diverse cancer models and on fundamental cellular and molecular biology mechanisms key to our understanding of cancer. During 2022, in the oncobiology area, we have demonstrated that tumours establish an organized communication network between subpopulations of cancer cells using extracellular vesicles. This network is plastic and reshapes in response to its environment, and communication within the network occurs preferentially from stem to non-stem cancer cells. In gastric cancer, we have shown that activation of Laminin  $\gamma 2$  by *Helicobacter pylori* promotes invasion and survival, and that integrin  $\beta 1$  orchestrates abnormal cell-matrix attachment and invasive behaviour of cells with E-cadherin defects. Still in the context of cancer cell invasiveness, we have shown that mutant KRAS modulates colorectal cancer cells invasive response to fibroblast-secreted factors through the HGF/C-MET axis. In the field of cell division, we have demonstrated that misaligned chromosomes that satisfy the spindle assembly checkpoint, may represent a mechanism of formation of micronuclei and drive chromosomal/genomic instability during cancer cell division. Finally, using an *Atrx* conditional knockout mouse model, we have demonstrated that ATRX loss, a chromatin remodeller that maintains telomere homeostasis, surprisingly causes endocrine dysfunction rather than cancer.

In the study of fundamental molecular and cellular mechanisms, we demonstrated that aPKC, essential in the regulation of apical-basal polarity, prevents rupture of epithelium tissues by regulating apical constriction. We have also shown that the scaffold protein JIP3 interacts with dynein and kinesin-1 to regulate bidirectional organelle transport. In the field of cellular aging, we demonstrated that cyclic induction of the FOXM1 transcription factor significantly extends health span and ameliorates the senescence-associated histopathology driven by repression of the endogenous protein. In the functional genomics field, we demonstrated that multidimensional chromatin profiling of zebrafish constitutes an excellent approach to uncover and investigate disease-relevant cis-regulatory genetic elements. In a different study, we have shown that alternative polyadenylation in the 3' untranslated region of the MCL1 mRNA has an essential function in cell viability and mitochondria dynamics. Finally, using whole exome sequencing enriched for untranslated regions, we demonstrate that surveying

consanguineous populations is essential for the identification of new pathogenic variants that are not efficiently eliminated by genetic drift.

On the translational side of our research, we have developed patent applications on methods of fabrication of organ-on-a-chip elastomeric-based devices and microactuators, and on the use of polymeric nanoparticles targeting sialyl-Tn for the treatment of cancer. On a more clinical side, we highlight a study explaining disparities in survival outcomes and highlighting predictive biomarkers of early gastric cancer aggressiveness. Finally, we have participated in publication of recommendations of a European Alliance for Personalised Medicine (EAPM) expert panel discussion on how to improve knowledge on thyroid cancer, to accurately identify cases at an early stage that can benefit from effective intervention, optimize therapy, and reduce the risk of overdiagnosis and unnecessary treatment.

### **Infection, Immunity and Regeneration**

The IIR Thematic Line aims to conduct research to comprehend and tackle infectious diseases, autoimmune and degenerative disorders and tissue injuries. These are top leading causes of death and disability worldwide and result in part from the interaction and deregulated response of our organism to different aggressions. We relay on internationally recognized researchers with multidisciplinary expertise in complementary areas, from pathogenesis, immunity, clinical and population genetics, bioengineering, and regenerative medicine. Our broad aims are to identify and comprehend virulence mechanisms of pathogens, to identify the molecular and cellular processes underlying immune responses, in the context of infection and tissue repair/regeneration, to develop innovative/reliable disease models, new tools for disease prevention and diagnosis and advanced anti-microbial, immunomodulatory and pro-regenerative therapies.

Identify and comprehend virulence mechanisms of pathogens: Some pathogens produce pore-forming toxins (PFTs) that disrupt plasma membrane integrity by forming transmembrane pores. We showed that, during intoxication by cholesterol-dependent PFTs produced by different pathogenic bacteria, PFTs are eliminated through the release of extracellular vesicles (EVs), and we identified that EVs shed during intoxication are composed by proteins important for plasma membrane repair, including Copine-1 and Copine-3. *Photobacterium damsela* subsp. *piscicida* (*Phdp*) is a bacterium that infects a large number of marine fish species, both in aquacultures and in the natural environment, including sea bream and sea bass. AIP56, a secreted AB-type toxin that induces apoptosis of sea bass phagocytes during infection, is the major *Phdp* virulence factor. We showed that the effect of AIP56 is different in sea bream, being its phagocytes resistant to AIP56 cytotoxicity due to an inefficient internalization of the toxin by those cells.

Identify molecular and cellular processes of host immune responses: Malaria is a major public health concern, as a highly effective human vaccine remains elusive. The efficacy of a subunit vaccine targeting the most abundant protein of the parasite surface (CSP) has been hindered by difficulties in generating an effective humoral response. We showed that immunization with CSP adjuvanted with 5'ppp-dsRNA, a RIG-I agonist, confers early and long-lasting sterile protection in mice against stringent parasite and mosquito bite challenges, demonstrating

5'ppp-dsRNA as an adjuvant to CSP vaccines inducing effective anti-*Plasmodium* humoral immunity. Within the thymus, thymic epithelial cells (TECs) provide microenvironments for T cell development. Because TEC functionality is sensitive to aging and cytoablative therapies, unraveling their thymopoietic role has fundamental and clinical implications. We showed that *lamp2*-deficiency in thymic stroma conditions the development and TCR repertoire formation of CD4 T cells, diminishing the T-cell mediated immune response to *Listeria monocytogenes*. These results suggest that LAMP2 interconnects autophagy and lysosomal generation of selecting self-peptides in cTECs, with implications for CD4 T cell selection. Group B *Streptococcus* (GBS) remains a major neonatal life-threatening pathogen. We initially identified GAPDH as a promising vaccine against GBS, and then investigated whether GBS GAPDH maternal vaccination interferes with the offspring. An altered gut microbiome is observed in pups born from vaccinated dams. These pups present deregulated expression of immune regulators, and marked reduction of immune cells. In addition, male mice born from vaccinated mothers present serious behavioral abnormalities during adulthood, highlighting the danger of using ubiquitous antigens in maternal human vaccines against neonatal pathogens.

Develop innovative disease models: Bone diseases, such as osteoarthritis, are extremely complex, comprising the action of inflammatory mediators leading to unbalanced bone homeostasis and de-regulation of sensory innervation and angiogenesis. *In vitro* platforms mimicking this complexity are still missing. We developed a microfluidic-based neuro-vascularized bone chip (NVB chip) to model mechanistic interactions between innervation and angiogenesis in the inflammatory bone niche, and screen for novel strategies targeting inflammatory diseases. This reliable *in vitro* tool will enlighten novel mechanisms of inflammatory bone diseases, bone destruction, and pain, opening new avenues for more effective therapies. Drug development requests reliable *in vitro* tools for early screening phases, reducing the need for animal experiments. Understanding the absorption pattern of new drugs in the small intestine is paramount. We developed an advanced 3D *in vitro* intestinal model to test absorption that better mimics the native environment. Our model is composed of a collagen-based stromal layer with embedded fibroblasts mimicking the intestinal lamina propria, supporting an epithelium composed of enterocytes and mucus-secreting cells, and an endothelial layer, surrogating the absorptive capillary network. This new 3D *in vitro* intestinal model presents permeability outcomes comparable to those observed *in vivo*. The epicardium, the outer epithelial layer that covers the myocardium, derives from a transient organ known as pro-epicardium, crucial during heart organogenesis. We developed a self-organized human multilineage organoid that recreates the co-emergence of pro-epicardium, septum transversum mesenchyme and liver bud. By co-culturing these organoids with cardiomyocyte aggregates, we generated a self-organized heart organoid, opening the path to advance knowledge on how myocardium-epicardium interaction progresses during heart organogenesis in healthy or diseased settings.

Develop new prevention, diagnosis, anti-microbial, immunomodulatory and pro-regenerative therapies: *Leishmania* are parasites responsible for leishmaniasis, a neglected disease that affects individuals in developing countries and animal populations all over the world. Amphotericin B (AmB) is a highly hydrophobic drug with significant leishmanicidal activity whose use is limited by its poor water solubility and adverse effects. By covalently linking AmB



to periodate-oxidized hyaluronic acid (HA), we increased water solubility and reduced cytotoxicity, and demonstrated the safe and effective use of HA-AmB conjugates for leishmaniasis treatment. Half of the world's population is infected with *Helicobacter pylori*, a gastric bacterium that is responsible for 90% of non-cardia gastric cancers. Antibiotic therapies fail in up to 40% patients and antimicrobial peptides (AMPs) are appealing alternatives, but their high susceptibility to degradation *in vivo* limits their clinical translation. We demonstrated that immobilization of MSI-78A (one of the few AMPs with activity against *H. pylori*) onto chitosan microspheres strongly enhances its antibacterial activity even at acidic pH (gastric settings), highlighting its strong potential as an antibiotic alternative for *H. pylori* eradication. Hemochromatosis (HC) is a genetically heterogeneous disorder responsible for disabling and life-threatening complications such as arthritis, diabetes, heart failure, hepatic cirrhosis, and hepatocellular carcinoma. Current HC classification is based on different molecular subtypes, mainly reflecting successive gene discovery and very difficult to adopt in clinical practice. With the BIOIRON Society, we presented recommendations for unambiguous HC classification, deemphasizing the use of molecular subtype criteria in favor of a classification addressing both clinical issues and molecular complexity. The HIR Line hosted two prestigious ERA Chairs (MOBILISE and ImmunoHub). In 2022, the two ERA chair holders were recruited to form new outstanding research groups working in Immunology and Molecular Bioengineering, while at the same time bringing high-quality human resources and implementing structural changes necessary to achieve excellence on a sustainable basis.

## Neurobiology and Neurological Disorders

In 2022 members of the Neurobiology and Neurological disease Program reported that rewired glycosylation activity promotes scarless regeneration and functional recovery in spiny mice after complete spinal cord transection, an observation that opens new therapeutic avenues for spinal cord regeneration. Our work also showed that sensory neurons have an axon initial segment that initiates spontaneous activity in neuropathic pain, a finding providing mechanistic insight into the cellular mechanisms that cause pathological pain and identifying a new potential target for chronic pain management. Work on chronic pain showed that altered prefrontal-striatal theta-band oscillatory dynamics underlie working memory deficits in neuropathic pain rats. Work on neuro inflammation showed that astrocyte-targeted gene delivery of interleukin 2 specifically increases brain-resident regulatory T cell numbers and protects against pathological neuroinflammation. This work not only shows that IL-2 gene delivery protects against neuroinflammation, but also describes a versatile platform for delivery of diverse biologics to neuroinflammatory patients and that neuron-microglia contact-dependent mechanisms can attenuate microglia reactivity induced by methamphetamine and that this enhances neuronal plasticity. A finding that provides evidence that neuron-microglia contact-dependent mechanisms have the capacity to attenuate pro-inflammatory events. We also described the evolution of vitamin C biosynthesis and transport in animals, focusing on the evolution of the GULO, Regucalcin and SVCT transporters and developed an AAV-based approach to deliver single domain antibodies against BACE-1 that could alleviate Alzheimer's disease pathology in a rodent model of AD. A robust assay to monitor the first steps of ataxin-3 amyloid fibril assembly that will help further studies with cell and animal models of spinocerebellar ataxia type 3 was developed and a



novel self-assembled multi-siRNA nanocaged architecture with controlled enzyme siRNA release that might be useful for therapeutic delivery. In addition, we found that the LIM-domain protein Pinch2 is a key regulator of central nervous system myelination – its function is essential to regulate myelin production and growth, but also to maintain myelin stability. Interestingly this was the first time that a biological function is described for Pinch2. Finally, we revealed an altered cytosolic PAS/CNBh assembly during hERG channel gating, using a conformation-sensitive antibody. Members of the Program also won prestigious prizes, including de Melo e Castro Award with the project TARGET: Translating *Acomys* regenerative capacity and the Mantero Belard award with the project NeuroSpark - Neuromorphic devices for adaptive real-time neuromodulation in brain electrical stimulation therapies, both from the Santa Casa da Misericórdia de Lisboa; the Grunenthal Foundation Award, Prize on Basic Research, and the Prize Alfredo da Silva from Fundação Amélia de Mello. They also secure competitive funding from Fundação LaCaixa with projects aiming at improving Deep Brain Stimulation in Parkinson's Disease with Personalized Electrophysiological Biomarkers and Data Driven Stimulation. In addition, researchers of the Program also secured significant competitive funding from different H2020 EU funded projects. An important highlight was also the inauguration in 2022 of the NCBI ERA-Chair project headed by Mathew Holt which major objective is to contribute to build a hub of excellence for (non-neural) glial biology at i3S. Finally, researchers from the Program played an essential role in setting up the new bioinformatics platform at i3S.

## TRAINING ACTIVITIES

### PhD students

i3S is engaged in the training and development of the next generation of scientist offering a multidisciplinary and vibrant environment to all our students by a very active participation in 5 different PhD programs run by the University of Porto ranging from fundamental Biology to Applied Clinical Research, namely: MCbiology, BiotechHealth, PRODEB, PGBioMed and NeuroSciences. During 2022 i3S hosted more than 350 PhD students most of which are funded by fellowships from the FCT all of which are integrated with the different research groups of i3S. Through the Advance Training Unit (see below for details) students of the different PhD programs are now allowed to attend different modules providing an excellent opportunity so that each student can design, together with the supervising team, a more personalized curricula. PhD students are also challenged every year to organized a conference entitled “PhDday” that involves all students from different programs, with international speakers and poster presentations. Also, during 2022 there 26 different courses offered to students covering all technical and scientific areas of our activity (see below Advance Training Unit).

## SCIENTIFIC PLATFORMS

Research at i3S is heavily supported by our scientific platforms. With cutting-edge equipment and highly qualified professionals, the i3S Scientific Platforms are at the forefront of technology to promote and sustain the highest standards of scientific research and

development. Experienced and accomplished specialists coordinate and manage these infrastructures, who are available to provide personalized guidance and help in the processes of experimental design and implementation. All our scientific platforms are run on an open access policy and we regularly host both national and international researchers. During this year i3S have continued to expand its technological infrastructure, specially through the acquisition and implementation of new equipment and hiring human resources to support the new technologies at the scientific platforms. Altogether, the scientific platforms participate in 8 national research infrastructures, coordinating three of these (PPBI – Portuguese Platform of Bioimaging, PT-OPENSREEN – National Infrastructure for Chemical Biology and Genetics and PCCC – Porto Comprehensive Cancer Center Raquel Seruca). Further, they fully integrate 15 international networks, of which the national delegates of the *Euro-Bioimaging* and *EU-OPENSREEN* European Research Infrastructures (ERIC) are from i3S scientific platforms. In 2022, staff from the scientific platforms have participated as team members of 13 scientific funded projects and co-authored 39 scientific publications and have been acknowledged in many more. The scientific platforms have organized 5 advanced courses and lectured in several others by invitation.

### **Advanced Light Microscopy**

The Advanced Light Microscopy (ALM) scientific platform specialises in imaging technologies for the study of biological systems from the macro to the nanoscale, enabling applications such as the analysis of the expression and interaction of biomolecules, cell proliferation, cell and tissue morphology, dynamic behaviour of cells and subcellular structures, cell signalling, molecular activity, embryonic development, cell response to perturbations, etc. The technologies available are high-end, advanced light microscopy systems designed to visualise and measure living and fixed biological specimens with high resolution and sensitivity. The equipment includes widefield fluorescence microscopes, laser point scanning and spinning disk confocal microscopes, a light sheet microscope and a TIRF microscope. The ALM also provides resources (hardware, software and expertise) for digital image data analysis. The ALM has a strong focus on the characterisation of dynamic biological processes in living cells and common samples include prokaryotic and eukaryotic cells, tissues, embryos of small model organisms, 3D cell cultures, biofilms and biomaterials. A team of two highly qualified scientists with expertise in the field provide training in bioimaging, hands-on training in instrument operation, technical support in image data acquisition and analysis, and scientific advice on project design and execution. The ALM is also involved in the development and implementation of new imaging applications and technologies. The scientific platform is available to academia, research institutes and industry under an open access policy. In 2022, the ALM provided access to 264 users from sixty-two i3S groups and 8 external users. In addition, 150 hands-on training sessions were given to users on the operation of the systems. The work carried out in the ALM resulted in at least 27 peer-reviewed publications in international journals, of which 7 were co-authored by the ALM team, and 6 preprint publications. The ALM team was also actively involved in i3S training activities: organising the "13th Course on the Optical Microscopy Imaging for Biosciences" and the "5th Introduction to Digital BioImage Analysis", as well as collaborating on the "2nd Advanced Course on Organoid Models" and the "6th High Throughput Screening and Image Analysis for

BioSciences". Also contributed to the course "Fundamentals of Confocal Microscopy" at the University of Bern. Furthermore, bioimaging was taught in the PhD programs MCBiology (ICBAS/FCUP) and Neurosciences (FMUP); and in the master courses on Cell and Molecular Biology (FCUP) and on Neurobiology (FMUP). The team also co-organised the NEUBIAS Academy and EOSC-Life training school "Defragmentation: bringing Image Analysts to the cloud. The ALM is a core facility of the national research infrastructure PPBI – Portuguese Platform of Bioimaging (ppbi.pt), which is a node of the Euro-Bioimaging ERIC (eurobioimaging.eu). The ALM is member of the European Light Microscopy Initiative (elmi.embl.org). The ALM team participates also in the Network of European Bioimage Analysts – NEUBIAS (neubias.org), in the COMULIS - Correlated Multimodal Imaging in Life Sciences (comulis.eu) and in the QUAREP-LiMi - Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy (quarep.org).

### **Animal Facility**

The i3S animal facility reached in 2022 a number of animals similar to the admitted before the pandemic, with 3000 rodent cages. The facility team followed this increase by reinforcing the number of caretakers. Some services, such as the production of genetically engineered mice also saw a significant increase in the number of requests. Among internal and external projects, a total of 73 project licenses were actively being performed at the facility during 2022. The facility had also the opportunity to acquire several new devices, through the Raquel Seruca Porto Comprehensive Cancer Centre, some being installed yet in 2022: IVIS Spectrum CT and a hematological analyzer. A total of 4 external services involving universities and companies were performed in distinct areas such as infection, cancer and wound healing. The i3S animal facility has also joined EARA this last year, in an effort to support openness and improve communication of laboratory animal science.

### **Biochemical and Biophysical Technologies**

The Biochemical and Biophysical Technologies platform develops and implements methodologies within the scope of recombinant protein expression and purification, biochemistry and molecular biophysics. Recombinant protein expression projects are planned and mentored throughout the stages of construct design, molecular cloning, expression screening, scale up, protein purification, stability assessment, size homogeneity and structural integrity. An array of biochemical technologies are available including ultracentrifugation and preparative and analytical chromatography allowing for the separation and characterization in terms of size, charge and hydrophobicity of proteins and small molecules like metabolites and drugs. Molecular biophysics characterization of macromolecules and ligands include the study of molecular interactions in terms of binding affinities, kinetics and thermodynamic profile as well as the characterization of structure, with technologies like surface plasmon resonance, isothermal titration calorimetry and circular dichroism. During 2022 the vast portfolio of resources and methodologies offered by the platform were used by 59 Research groups. Frederico Silva was part of the team of 3 research projects and co-authored 2 papers. Organization of a one week advanced course on "Protein

expression, purification and characterization” of the Doctoral Program in Molecular and cell Biology. Member of the Protein Production and Purification Partnership in Europe (<https://p4eu.org/>) and management committee member in charge of advanced training of the Association of Resources for Biophysical Research in Europe (previous <https://arbre-mobieu.eu/> now <https://www.arbre-biophysics.eu>).

## Bioimaging

The mission of the Bioimaging Scientific Platform is to provide a set of solutions for *in vitro*, *ex vivo*, and *in vivo* imaging to advance in the integration and use of bioimaging solutions from molecules to small animals on the fields of Biomaterials, Nano- and Regenerative Medicine, in Biology and Medical sciences. Techniques available include: chemical analysis (Raman spectroscopy); fluorescent microscopy (Widefield microscopy, CLSM and Imaging Flow Cytometry); topography and biomechanics (AFM); *in vivo* imaging (Ultrasounds and MicroCT); and sample preparation (Critical Point Dryer). Offline workstations for image processing and analysis with dedicated software are available. The main functions of our staff include: maintenance and training of the different equipment; support on experimental design and project development, digital image analysis, and data analysis; and provision of services on the different technologies. In 2022, a total of 179 i3S researchers (from 31 i3S groups) and 9 external researchers (from the University of Porto: Faculty of Medical Dentistry, Faculty of Sports, Faculty of Engineering, ICBAS, and INESC) took advantage of the Bioimaging Scientific Platform’s technologies and services. In this year, the equipment were running 4,594 hours (services for external users represent a 7% of it). The work developed at the Platform, by the users and the staff, contributed to 28 published papers, with an average impact factor of 7.6. From these works, a maximum impact factor was obtained by a publication on Gut (23.09) and the publication with the minimum impact factor was published at Arthritis Research & Therapy (1.788). The staff from the Platform co-organized one course: “High Throughput Screening and Image Analysis for BioSciences”, 23-27 May 2022; and the training school: “Astrotech Training School”, 26<sup>th</sup>-28<sup>th</sup> September 2022. We were also invited to participate as speaker in the “13<sup>th</sup> Course in Optical Microscopy Imaging for Biosciences”, 28<sup>th</sup> March-1<sup>st</sup> April 2022. The Bioimaging Scientific Platform also contributed to training activities within the scope of different PhD and MSc programs with focus on biomedical engineering (FEUP), a visit of students from the Metallurgical and Materials Engineering course at FEUP, and different visits from students from secondary schools. In 2022, the staff from the Bioimaging Scientific Platform collaborated as co-authors in 4 papers published in this year. We also contributed as team members in one successful application for FCT projects granted in 2022, and are part of the team from an FCT project ending in 2024. The Bioimaging Scientific Platform participated as a node of the Portuguese Platform of Bioimaging (PPBI) and the EuroBioimaging. In 2022 we also contributed to different writing proposals to EU grants with EuroBioimaging (as part of the PPBI node). In addition, the Platform participated in the COMULIS COST action (Correlated Multimodal Imaging in Life Sciences).

## Bioinformatics

The i3S Bioinformatics platform was created in the last trimester of 2022 to support i3S researchers in the field of bioinformatics, particularly on next-generation sequencing technologies. The platform runs a continuously growing list of protocols used frequently in the fields of (but not limited to) genomics, transcriptomics, arrays, and phylogenetics. Protocols are made available on a website (<https://i3s-bioinformatics-service.github.io/>), specifically designed for this purpose, where we provide technical details on analysis, what will be obtained and suggest follow-up approaches. All protocols were designed using Docker images and the Common Workflow language (CWL) to ensure reproducibility and long-term support of analysis. Docker images, as well as the implementation of the protocols, are performed in close collaboration with the pegi3S Bioinformatics Docker Images Project, one of the services of the Portuguese ELIXIR node. At present, the i3S Bioinformatics platform is free for i3S researchers. The platform has a dedicated cluster composed by 2 AMD EPYC 7252 8-Core processors, 514 Gb of RAM and 24Tb of storage. Although recent (November 2022), the Bioinformatics platform has had a total of 8 users in 2022, all from different research groups.

## Biointerfaces and Nanotechnologies

The mission of Biointerfaces and Nanotechnology (BN) is the study of materials, materials' surfaces, and interfaces of materials with cells and tissues, allowing to reach the micrometric, and nanometric levels through the development and improvement of advanced physical, chemical, mechanical, and structural characterization techniques. BN is mainly focused on the fields of Biomaterials and Nano- and Regenerative Medicine. BN technical staff also provide advanced training in specialized areas. Its target audience is researchers in the area of health sciences. BN also offers services to external users (industry, technology centers, etc.) who can benefit from existing technologies; however, the internal ones have privileged conditions and access. The activity developed at BN differs from other core facilities of other research centers since the tests are personalized. Both types of customers (internal and external) are encouraged to follow the tests and tailor their experiences with BN's technical staff. Therefore, the objective of BN is to offer excellent personalized service to its customers. Some of the main features of BN include the following: chemical, physical, and structural characterization tests; knowledge through training (training independent qualified internal users) and advanced training (through specific courses and participation in higher education programs for masters and doctoral students within the scope of their curriculum area).

A Quality System is implemented at BN according to ISO standards (NP ISO 17025) and Good Laboratory Practices (GLP). This contributes to ensuring the provision of strict procedures followed at the BN core facility, thus enabling each technique to be performed according to high-quality standards. Its technical staff supports the platform's day-to-day activities, and a Scientific Coordinator assures its governance. Fifty-three groups from i3S used the services of BN in 2022, including 96 PI. The characterization techniques existing at BN were used in 24 papers in nanomedicine, biomaterials, regenerative therapies, and cancer, with an impact factor (IF) average of 10.536 (the IF varied between 6.953 and 14.119). Several master's and doctorate theses and oral presentations, and posters used the characterization techniques

available. 5203 were the total number of samples analyzed in 2022. Compared with the 4752 analyzed in 2021, there was an increase of 8%. The number of hours used was 4228. In comparison with the 3511 used in 2021, there was an increase of 17%. Synergy and the Rheometer were the most used techniques. The number of qualified users in 2022 (176) increased compared with the number of qualified users in 2021 (122), showing an increase of 31%. Additionally, several external users (other universities besides UPorto and private companies) also used the BN services: UÉvora, UCoimbra, Stemmatters, Fluidinova, Rodrigo Marquez, and Mesosystem). Students from the UPorto, and IPP, were received, and classes were provided to create a curricula plan for Mechanics Engineering (ISEP) and Biomedical Engineering (ISEP) courses. Two public tenders were performed to acquire a biodynamic system with a bioreactor (BioSys) and an atomic force microscopy (AFM). Both were prepared in the framework of the PCCC.Raquel Seruca project. The BioSys (Electroforce 5175, Waters/TA Instruments/ USA) was installed in November 2022. The equipment allows the dynamic mechanical stimulation of samples in 3D in a physiological environment (temperature, CO<sub>2</sub>, and humidity) and the respective quantification of mechanical properties. Waters/ TA Instruments has already trained nine users. The AFM selected was the NanoWizard V (JPK/ Bruker/ USA) and will be assembled to an inverted fluorescence microscope (Observer Z7/ Zeiss/ Germany) during April of 2023. The work to be carried out with this system will be focused on biological samples (molecules, cells, and tissues), using the potentialities of high-speed imaging with high-resolution modes; nanomechanics; force spectroscopy, and molecular recognition using fluorescence simultaneously.

### **BioSciences Screening**

The i3S BioSciences Screening scientific platform provides to the internal and external scientific community and industry, state of the art instruments and competence to solve challenging (biological) questions with high throughput and high content technologies. Highly qualified scientists with expertise on project evaluation, assay development, liquid handling, automated microscopy, multimode microplate readers, image and data analysis, work with project teams to successfully run medium-to-high throughput screens. Examples are genetic and chemical screens for the purpose of target and/or drug discovery. The platform facilitates access to genetic and compounds screening libraries and is actively establishing collaborations in order to receive and expand its own libraries. Training is given to all the users and regular workshops are organized for the global scientific community. Through national funds attributed to the national research infrastructures PT-OPENSREEN and PCCC-Raquel Seruca, we added to our equipment portfolio an automated screening workstation (Cell::Explorer), equipped with a high-end high content screening confocal microscope (Opera Phenix Plus) allowing to address biological questions using 3D cell models such as organoids. Furthermore, we acquired a new widefield high content screening microscope (Operetta CLS) expanding our offer in high throughput image acquisition and analysis. Also, a new multimode microplate reader has been acquired (Spectramax iD3) increasing the available offer for this technology. We were also able to hire an assay development specialist allowing us to increase the training in equipment, accommodate more users and reduce response time. We coordinated the joining of Portugal to the European Research Infrastructure Consortium (ERIC) EU-OPENSREEN and i3S has been recognized as a Partner Site, enabling the



institution to compete for European projects and receive services from this ERIC. We started to build the national screening library with approximately 300 cyanobacteria extracts and 300 pure compounds. We also obtained a copy of the European pilot library with about 5000 compounds, of which about half have proven biological activity and can be used for repurposing, the other half being compounds chosen for their structural diversity. Of relevance, since the installation of the new high throughput screening equipment, in the last trimester, we have already carried out two screening projects and are developing the test for a third one. From one of these projects we have signed a non-disclosure agreement with the UPorto Faculdade de Ciências to study the activity of a very promising compound, coming from the newly built national library of compounds.

We coordinate the national infrastructure for chemical biology and genetics (PT-OPENSREEN), participate in the national infrastructure Portuguese Platform of BioImaging (PPBI) and in 3 running COST Actions as workgroup member, workgroup leader and management committee. Internally, we worked with 47 research groups and services, summing more than 148 frequent users in all our technologies and applications. Furthermore, we are team members of 3 FCT projects, coordinators of 1 NORTE2020/FCT project (>2M EUR) and coordinators of 1 H2020/INFRADEV/EU project (10k EUR). In 2022, we co-authored 4 original articles and been acknowledged in several publications. We also participated as lecturers in 3 i3S Advanced courses and organized the 6<sup>th</sup> edition of the “Course in High Throughput Screening and Image Analysis for BioSciences”, credited with 2 ECTS by the Faculdade de Engenharia da Universidade do Porto.

## Cell & Tissue Culture

The Cell & Tissue Culture (CTC) is an integrative i3S Scientific Platform of different, but complementary, competencies supporting i3S scientific research related with cell and tissue cultures. This platform offers technical and scientific expertise, as well as training and support in experimental planning and design. Thus, the platform includes the following facilities: Cell Culture Quarantine (CCQuar), the Cell Line Bank (CLBank), the In Vivo CAM assay (CAM) and the Cell Culture and Genotyping (CCGen). The CCQuar is exclusively intended for carrying out specific work involving the handling of cell lines purchased commercially or provided by external collaborators until the guarantee of their microbiological status relative to infection by *Mycoplasma* spp. or other agents. In this room, the decontamination of cell cultures for *Mycoplasma* can also be carried out, if necessary. These procedures guarantee the good laboratory practices of the remaining cell culture rooms at i3S. In 2022, the CCQuar was used by 16 different groups from i3S and by 34 i3S users. External are not applicable, since this facility is intended to be used only by i3S research groups. The CLBank includes a collection of parental cell lines, obtained from tumoral and non-tumoral tissues of various histological topographies, provided by the i3S research groups and represent an unquestionable heritage of the Institute. All of them are characterized for their genetic profile and microbiological status, in particular infection by bacteria of the genus *Mycoplasma*. The vast majority of CLBank cell lines were commercially obtained. However, there are some exceptions, such as cell lines established at the institute and other non-commercial provided by external institutions under scientific collaboration. Each cell line has its own technical sheet, supported by photographs, and accompanied by information regarding its origin, existence of mutations



and culture conditions. In 2022, the CLBank was used by 11 different groups from i3S and by 13 i3S users. External are not applicable, since this facility is intended to be used only by i3S research groups. Cell lines obtained by the CLBank are to be used only in internal or in i3S collaborative research projects. CLBank provides reports that genetically authenticate the used cell lines for the submission of research manuscripts to peer-reviewed journals. In the context of the PCCC project, the following equipment was acquired: a freezer (-80°C), a liquid nitrogen storage container and an automated cell counter. This year cell lines were also started to be provided in culture, in addition to frozen aliquots. The CLBank has received 26 new cancer cell lines during 2022 (some from the NCI60 Cell Line Cancer Panel). The CAM facility provides scientific expertise and services using the chick embryo model, more specifically, assays based on the chorioallantoic membrane – the CAM. This facility offers researchers additional or alternative *in vivo* tools (complying with the 3Rs policy), that are reliable and cost and time efficient. Due to the CAM structure and its easy access, CAM assays constitute attractive preclinical *in vivo* tools for drug screening and/or vascular studies (angiogenesis and permeability). Associated with the chick natural immunoincompetence, CAM assays can also be used to study complex human cancer features, such as tumorigenesis, invasion, metastasis and cancer stem cell activity, and the effect of potential therapeutic molecules. In 2022, the CAM was used by 8 different groups from i3S and by 10 i3S users. The service was also used by an external national company, as well as by an external international research institute. The work of each user was integrated into their respective projects (number of projects=10). Additionally, Marta Teixeira Pinto participates as part of the PTDC/BIA-MIC/5343/2020 project team (PI: Ana Tomás). In addition to the previously established portfolio already described, new and customized assays were developed, in 2022, to assess Leishmania infection in the CAM. During 2022, 2 publications arose from work performed at CAM. Moreover, although it is not yet a formal network, Marta Teixeira Pinto is part of a team of international experts in CAM assays, which work already resulted in a publication. In 2022, Marta Teixeira Pinto still integrated the working group, led by Anna Olson, to set up the i3S 3Rs Knowledge Center, as CAM is an excellent replacement model. The Cell Culture and Genotyping Platform (CCGen) aims to provide i3S researchers with state-of-the-art advances in cell culture, genotyping, and gene expression technology. The facility offers expert consultation, cell culture facilities and training, genotyping, and gene expression. Highly qualified experts are available to the scientific community to help solve questions that may arise from technical and/or data interpretation. A high-quality genotyping service is also available. i3S joined recently the program Laboratory Efficiency Assessment Framework, UCL (LEAF) and CCGen entered this pilot program having received the bronze prize certificate in October 2022. Tailor-made conditions are available for the culture of organoids, cell lines, primary cells, clinical samples, and viruses, including lentivirus-based transfections. Training is mandatory to access this facility. The organoids cell culture room, implemented in June 2021, was used by 19 users from 10 different research groups in 2022, confirming the need for this structure at i3S. Its implementation involved the simultaneous establishment of a clinical samples room, as the main organoids protocols require hospital samples. The cell culture room dedicated to lentivirus, provisionally allocated on the first floor in 2020, has been used by several researchers making it mandatory to revise its infrastructure concerning practical working conditions including space, safety, and quality. Regarding mouse genotyping, new research needs with new animal models and the arrival of new researchers led to the implementation of 20 new protocols in 2022. The total number of

users raised to 31 from 29 groups. The service genotyped near 60000 amplified samples. The existence of the 384-well real-time equipment (CFX 384), allowed researchers (20% of the total users) to work with more challenging genetic analysis.

## Gene Core

The Genomics platform is a full service facility dedicated to provide investigators state of the art technological solutions in the field of genomics and high throughput analysis. We offer technical expertise and support with experimental design, protocol development, and data analysis guidance. The laboratory is proficient in providing: Sanger/NGS sequencing, cell line genotyping, exome sequencing, targeted DNA and RNA sequencing, small genome sequencing, metagenome sequencing, whole transcriptome sequencing, real-time PCR, digital PCR and microarray analysis. Since late 2022, the scientific platform provides single-cell sequencing. The platform processed around 55 000 samples, provided by 49 internal groups and 9 external institutions (academic and non-academic) with recurring users.

The facility is accredited by IPAC norms ISO 15189 and ISO/IEC 17025. It is also an Ion Torrent certified service provider and a member of GenomePT project (POCI-01-0145-FEDER-022184).

## Histology and Electron Microscopy

HEMS – “Histology and Electron Microscopy” is focused on optical microscopy and electron microscopy providing ancillary equipment. The service provides the equipment, technical support and guidelines to researchers needing high level optical and electron microscopy to tackle studies of either cells, tissues or material sciences. The HistoPathology Service will offer a full range of macroscopic and histopathology services, able to support research groups using animal models or human tissues in histopathology studies. The main application of the histology is to provide Grossing & Processing & Embedding & Sectioning, [Special stains \(monochromatic / polychromatic\)](#), histology image analysis, cryosectioning, cytobloks / histogel, optimization of immunohistochemistry and/or immunocytochemistry, tissue sections for DNA extraction. Transmission electron microscopy (TEM) has been widely applied to characterize morphology, crystalline structure, and elemental information of several elements. The main application of a transmission electron microscope is to provide conventional ultrastructure, immunoelectromicroscopy, negative and cytochemistry stains, Energy Dispersive X-ray Spectrometry (EDS), Grossing & Processing & Embedding & Sectioning, Consultation in the analysis. In 2022, the platform participated in the scientific work of 63 internal groups (421 internal users) and 22 external groups (62 external users) with 5 publications in international peer-reviewed journals and 1 book chapter.

Besides the training courses for researchers, masters (BioImaging-FCUP) and PhD programmes (PDMCbiology), the unit also offered internships for higher education students (Instituto Politécnico Porto e Bragança). HEMS was in the final phase of implementing the CLEM application – “Correlative light and electron microscopy” at the institute, in collaboration with Paula Sampaio (ALM), and we aim to improve other applications such as “Imuno Electron Microscopy” and “Scanning Electron Microscopy – SEM”. The platform is a member of the

national infrastructure PPBI - Plataforma Portuguesa de Bioimagem (PPBI-POCI-01-0145-FEDER-022122), and is represented on the board of SPMicros - Portuguese Society of Microscopy. In addition, it is involved in international networks COST Actions COMULIS (CA17121).

## **Proteomics**

The i3S Proteomics platform provides access to mass spectrometry analysis of protein samples from extracts, solutions and gel bands. It is equipped with a LC-MS system comprehending a nanoUHPLC on-line coupled to a high-resolution accurate-mass Orbitrap mass spectrometer (Q Exactive, Thermo Scientific) with a nano electrospray ionization source (nanoESI). The Proteomics platform is prepared to work in variety of experimental workflows in order to provide answers to scientific questions and addressing the researchers needs. It offers scientific and technical expertise with protocol design, experimental execution, results/data interpretation and consultancy. Its members are available to offer assistance in grant proposals and project execution. It also provides training in workshops and pre-/post-graduate courses. The Proteomics platform analyzed a total of 1196 samples in 2022. 33 research groups from i3S performed proteomics analysis. External Institutions also requested proteomics studies namely, Minho, Aveiro, Coimbra, Beira Interior, Lisboa, Algarve and Católica Universities, Hospitals, IPO - Portuguese Institute of Oncology, IMM and ITQB research Institutes and two external companies. In 2022, the Proteomics platform integrated 5 FCT funded research projects from i3S groups. It also started its participation in a novel Project – Neurosense which has been selected for funding by the European Union. This Project, led by i3S, aims to create the first sensor to anticipate life-threatening epileptic seizures and trigger automatic drug delivery to prevent sudden death. The funding received allowed to purchase a novel LC-MS equipment fully dedicated to the analysis of samples related to this project. A liquid chromatography instrument (Vanquish, Thermo Scientific) together with a Triple Quadrupole Mass Spectrometer (Altis Plus, Thermo Scientific) were installed. This equipment performs highly sensitive and accurate quantitative molecule measurement. It is fully dedicated to the Neurosense project. We organized proteomics curricular units/modules, including theoretical and practical classes, for the MSc degree from Medicine Faculty and for the MCbiology Ph.D. program of the University of Porto. We participated in the Ph.D. advanced course “Advanced Analytical Tools: Multiple Applications for Mass Spectrometry” at the University of Lisbon. The Proteomics platform is a node of the Portuguese Mass Spectrometry Network- RNEM integrated into the FCT Research Infrastructures RoadMap (ROTEIRO/0028/2013; LISBOA-01-0145-FEDER-022125). The Head of the Facility is a member of the board of the Portuguese Proteomics Association – Procura and RNEM node director.

## **Translational Cytometry**

The mission of the Translational Cytometry Scientific Platform (TraCy) is to provide efficient and reliable flow cytometry services with the highest standards of quality control and throughput. TraCy provides equipment and support for cytometric data acquisition and

analysis, as well as cell sorters that are capable of physically separating individual cells or populations based on specific molecules marked by fluorescence. During 2022, TraCy continued to have a high usage rate being acknowledged in 20 research papers including co-authoring one of them. Overall, the equipment was in use for 4525 hours serving 197 different users from 45 research groups. Furthermore, TraCy organized 1-2 training sessions per month/ equipment, capacitating around 50 new users in 2022. Most of the year, the platform operated with 3 cytometers: 2 analysers (FACS CANTOII and Accuri C6) and 1 sorter (FACS ARIAll). At the end of the year, the TraCy platform significantly improved its capacity, first by incorporating the analyzer LSR Fortessa in November. Albeit already being used by different i3S users in the past years, this equipment was officially allocated within Thymus Development and Function group, as part of a legal obligation of an ERC grant completed in October 2022. The platform was also equipped with two new instruments, a cell sorter (BD FACSAria™ Fusion) and a full spectrum cell analyser (Cytek® Aurora), acquired by the Porto Comprehensive Cancer Center (P.CCC)/Raquel Seruca project. We also improved our analytic workstations by purchasing more licenses for specific cytometry analysis software such as FlowJo, FCsexpress and SpectroFlo, with a dedicated powerful computer. All these actions improved the skills of the platform staff, promoting several training sessions for the new instruments and the analysis software. The new equipment is fully operational since the end of 2022 and TraCy staff was immediately able to operate them and to offer the corresponding training. Additionally, TraCy gives experimental support, including in the design of panel of markers, fluorescence combination and post-acquisition analysis, organizes workshops and webinars. The platform participates in flow cytometry networks and societies, such as Sociedad Ibérica de Citometría (SIC) and International Society for Advancement of Cytometry (ISAC). TraCy is the coordinator of the recently created national flow cytometry network, named PT-FlowCyt, which involves 19 national institutions and will submit a proposal to the National Roadmap for Research Infrastructures (RNIE) as soon as the FCT call is opened.

## **X-ray Crystallography**

The X-ray Crystallography platform offers to the wide scientific and industrial communities, through an open-access policy, access to state-of-the-art facilities and instrumentation for crystallization and crystallographic characterization of small molecules, macromolecules and macromolecular complexes, including membrane proteins. Its technical resources (temperature-controlled crystallization chambers; a sub-microliter scale, lipid cubic phase-compatible crystallization robot; and a dual-wavelength, high angular resolution single-crystal X-ray diffractometer with sample cryostat) allow the crystallization of small molecules, biomolecules and biomolecular complexes, as well as crystallographic data collection and processing from these samples. In 2022 the platform hosted 28 users from 6 internal groups, to whom specialized training is provided on demand. 2 publications arose from work performed at the platform. The platform integrates the Portuguese Centre for Integrated Structural Biology (PCISBIO), which is an Affiliate Centre of Instruct-ERIC. The Scientific Coordinator of the platform integrates the Scientific and Executive Committees of PCISBIO.

## **PORTO COMPREHENSIVE CANCER CENTRE RAQUEL SERUCA**

P.CCC Raquel Seruca was formed between IPO Porto and i3S in 2017 and has the general aim to improve cancer prevention, diagnosis, and treatment innovation by combining excellent research and patient care. The project is built around the "From Bed to Bench and Back" (B3) concept. "Bed" represents the daily contact of the IPO Porto with the real cancer patients' needs. These needs then drive research development by the main research institution that makes up the P.CCC ("Bench") - i3S, with the aim of finding solutions in the form of clinical trials. Thus, the patient returns ("Back") to the clinical trials developed in the Clinical Research Unit of P.CCC. During 2022 the P.CCC Raquel Seruca was evaluated by an international panel approved to continue its excellent initiatives for the next 4 years. During the past year P.CCC has seen a significant increase in collaborative projects, currently 25, funded through competitive calls, and the number of PhD students, currently 25, co-supervised by researchers from i3S and clinicians from IPO has continued to increase. The collaborative publications between researchers of both institutions in 2022 has steadily increased to 17 last year. More over in the context of the national infrastructure road map P.CCC Raquel Seruca was awarded a total of 16M€ for new equipment of which 8M€ are being executed by i3S allowing us to maintain our scientific platforms equipped with state-of-the-art technology that will allow us to maintain the highest standard of performance.

## **NATIONAL AND INTERNATIONAL INFRASTRUCTURES**

The i3S is integrated in the National Roadmap for Research Infrastructures of Strategic Interest created by the Foundation for Science and Technology, capitalizing on existing state-of-the-art technological facilities. i3S coordinates the PPBI– Portuguese Platform of Bioluminescence and the PT-Openscreen-Portuguese Network for screening; and is an active node of: RNEM-Portuguese Mass Spectrometry Network; RNCCC – National Network of Comprehensive Cancer Centres; CRYOEM-PT - National Advanced Electron Microscopy Network for Health and Life Sciences; BioData.pt - Portuguese Biological Data Network; BIOBANCO.PT - National Biobanks Infrastructure; HCP- Health Cluster Portugal; and GenomePortugal - National Facility for Genome Sequencing and Analysis. In the future we will continue to play a leading role within the landscape of the national infrastructure road map, with i3S hosting new infrastructures, as detailed further in this document.

## **TRANSVERSAL SERVICES**

The transversal services aim to provide support for all the researchers that carry out their work at i3S, including technicians, Master and PhD students and researchers. These services are absolutely fundamental to allow researchers to devote all their energy in research and release them from administrative duties but also to maintain and run the large infrastructure that supports all of our activities.

## Research and innovation unit (RIU)

The RIU is divided into a Research Funding Office, Knowledge Transfer office and the Translational Research And Industry Partnerships Office, each one with its own agenda.

The Research Funding Office (RFO) at i3S is committed to attracting high-level competitive funding for research activities, providing comprehensive support to the i3S research programmes and to the individual researchers in their project applications. The RFO aims to facilitate every step of the pre-award process, including grant advising, partner search, grant preparation, submission, and contract negotiation, in compliance with institutional, government and sponsor policies and regulations. Early in the grant life cycle, the RFO is responsible for providing information on funding programmes, identifying and mapping, open and forthcoming calls for proposals by national and international, public or private funding agencies, which are later matched to the interest of i3S researchers. Also, in this regard, the RFO organizes or collaborates in the organization of sessions on strategic funding opportunities or related topics, such as capacity building events. In addition, the RFO operates in coordination with the Knowledge Transfer Office and the Translational Research and Industry Partnerships Office to ensure matching the most appropriate type of funding to the type and maturity of i3S knowledge/technology. To gain further insights on the funding opportunities, the RFO acts as liaison with relevant funding bodies, in particular with the European Commission, Regional and National funding bodies, academia, research organizations, enterprises, business stakeholders, among others. During the year 2022, the RFO communicated hundreds of national and international funding opportunities, ranging from projects and fellowships to awards and mobility support actions.

As part of the pre-award operations, the RFO organized and participated in different events directed at the i3S community: (i) organized the MSCA Postdoctoral Fellowships info session and (ii) ERC Starting and Consolidator Grants info session; (iii) hosted the 9th meeting of the Research and Fundraising Working Group of the University of Porto (“GT IAF, Grupo de Trabalho para a Investigação e Angariação de Financiamento”); and (iv) participated in the Round Table “Grantsmanship, the art of successful proposals” at the i3S Annual Meeting 2022. Attesting the i3S competitive profile, during 2022 the RFO supported the submission of 623 proposals. About 30% were submitted to international funding agencies, whereas of the latter 32% were submitted to Horizon Europe calls and 21% regard La Caixa Banking Foundation submissions. Regarding the application for national funds, 77% of the applications were submitted to FCT calls, including the call for Project Grants in all Scientific Domains, the Individual Call to Scientific Employment Stimulus, the Health Marathon Awards 2022, the 2022 Call for Exploratory Research Projects under the UT Austin Portugal Program or the L'Oréal Portugal Honor Medals for Women in Science. A total of 66 projects submitted during 2022 were funded, securing more than 4.2 M€ from competitive national funding and 7.5 M€ from international funding opportunities (including Horizon Europe and la Caixa Health Research projects). Moreover, 22 applications to the FCT Individual Call to Scientific Employment Stimulus were approved. Regarding applications submitted in 2022, the i3S success rate was set at 18% for national applications and 7% for international applications.

The mission of the Knowledge Transfer Office (KTO) is to transfer the knowledge developed at i3S to the Healthcare & Biotech Ecosystems by promoting its valorisation and commercialization in the form of knowledge-intensive products or services. Valorisation



actions encompass Intellectual Property (IP) support and licensing, scouting and coaching of early-stage innovation projects, regulatory issues and certification assistance, support to applications for innovation awards and accelerator programs; launch and follow-up of spin-offs (including business plan writing and presentation to investors), as well as development of exploitation plans for European grants. KTO also provides templates and revises IP terms in several agreements. Through the KTO, in 2022 we filed 12 new patents, extended 4 previous applications to an international PCT filing, and 2 previous patent applications have been granted. We achieved 3 technology licencing/acquisition agreements which, along with royalties from previous contracts summed up to 408k€. We obtained an overall of 1090k€ in funding from 9 acceleration/innovation programs including Caixa Impulse and EIT initiatives and, noteworthy, we achieved financial support to launch an i3S-based technology transfer accelerator program RESOLVE-HEALTH, 2.0. One new start-up was incorporated in 2022. The KTO also carried out Patent Surveys, Stakeholder Engagement Plans and Exploitation Plans for European projects.

The Translational Research and Industry Partnerships Office (TRIP) develops strategical interfaces with health-related industry and clinical centres to conceive and implement matched and innovative academic-industrial-clinical translational R&D Projects that fulfil clinical and healthcare needs. Within the RIU, the i3S Translational Research and Industry Partnerships (TRIP) Office is at the interface between the Health Care Industry, academic research teams and clinicians, potentiating the Institutes' scientific knowledge as a means of delivering innovative clinical and translational research in diseases of the institute's scientific expertise. By generating added value partnerships with pharmaceutical and biotechnology companies, in convergence with the emergent drug R&D pharma model and framed in the new pharmaceutical industry academic collaboration policy, this unit has been setting up i3S as a partner in research and co-development of breakthrough projects. This Office has been contributing not only to strategically accelerate proof-of-concept in health-related companies and, most importantly, has been opening new funding sources to i3S by contracted research and providing human resources for academic groups in the context of these contracts. The TRIP is also stimulating the organisation of events that include pharma experts, clinicians and academic community in order to promote the execution of translational partnerships.

During the year of 2022, the TRIP managed several health-related projects with bio/pharma companies establishing service agreements, contracted research and consultancy agreements. As a result of a formal protocol established by TRIP between i3S and Pfizer, the first Think Tank meeting between Pfizer's Medical Department, clinicians and i3S researchers took place in January 2022. In this meeting four clinical studies were presented and discussed: GALLERIA, IPlus, Drive and IBD Clinical Study. Two of these (iPlus and IBD) are in the phase of scientific and strategic alignment within global Pfizer and are expected to kick-off during the first half of 2023. TRIP has celebrated two new contracted research projects during 2022: - the CarboX-WP1, a preclinical development project celebrated with the German company CarboCalyx, and PRESSING, a project funded by Gilead and involving the UMC – Utrecht – NL. In 2022, TRIP managed an active translational research project portfolio including the following Projects/Clinical Studies: Immunomimetic (Astrazeneca/CHSJ), ITCA90 (Pharmis/CHSJ), iCOVPlus (Gilead), NanoThERM (Gilead), TTRinAxon (Pfizer/CHSJ), BioHeat90 (Pharmis/CHSJ), SAFE90 (Pharmis, pre-clinical), Duo-Diu (Gilead), ResistNet (Merck, IPOP).



Concerning the TRIP sourcing strategy for establishing bridges between renowned international academic institutions, health industry and clinicians, the unit organized and hosted the XXVIII Porto Cancer Meeting, a renowned translational research-driven two-day event that in this year was held in online format, gathering about 250 participants, including clinicians, academic and healthcare company's researchers. In 2022 event explored the topic of Extracellular Vesicles, Cell Communication and Cancer and brought to i3S international renowned scientists such as Robert Weinberg (MIT, USA), Raghuram Kalluri (MD Anderson Cancer Center, USA), Jacco van Rheenen (NKI, Netherlands), Clotilde Théry (INSERM, France), Hector Peinado (CNIO, Spain), Wei Guo (University of Pennsylvania, USA) and Michele de Palma (EPFL, Switzerland). The XXVIII PCM attracted the sponsorship and the participation of medical staff from pharma companies such as Takeda, Janssen, BMS, Gilead, Astrazeneca, Merck, Bayer and Lilly.

Taking advantage of the integration of i3S in EATRIS by TRIP, the unit was invited to co-lead the organization of the EATRIS-PLUS Personalised Medicine Summer School, which was held at Infarmed in Lisbon between May 23-26, 2022. EATRIS Plus is a flagship project that aims to consolidate the field of Personalised Medicine to better serve academia and industry and increase the number of EATRIS Innovation Hubs with major pharmaceuticals, expand strategic partnerships with research infrastructures and other relevant stakeholders. The EATRIS-PLUS Personalised Medicine Summer School focus on how translational medicine can be used for Personalised Medicine applications, bringing to the same discussion senior academics, healthcare providers and industry/companies that focus on more advanced aspects of translational medicine, and how translational research can be used for Personalised Medicine.

### **Events Management Unit**

2022 has been marked by the return of face-to-face events, even though hybrid and online events have still been held, which will certainly be maintained. Events and online courses' outcome is positive, allowing the participation of foreign participants, especially in short-term initiatives. In 2022, EMU organized 44 events with the participation of about 3000 participants. Additionally, EMU organized 24 courses in collaboration with Advanced Training Unit. These courses involved more than 500 participants. Seminars have been held initially in hybrid format, but returned to the face-to-face format later in the year. In 2022, 85 seminars were organized in i3S, according to the following typologies: 16 Young Scientists Seminars, 14 Postdoc Seminars, 28 Group Leaders Seminars, 12 Friday noon Seminars and 15 Satellite Seminars. Regarding the external events, we could verify an increase of initiatives when in comparison to 2021, mainly due to the relief of the constraints imposed by the pandemic. This year we had 12 external initiatives.

### **Advance Training Unit**

The i3S Advanced Training Unit (ATU) offers continuous, professional and post-graduate training in several research and related support areas. In collaboration with i3S researchers

and scientific and transversal units, ATU offers advanced, high-quality and skilled training directed to academic and industry researchers, PhD students, technicians and members of scientific community. The unit major goals are to support scientific community at the different careers stages by stimulating and promoting the exchange of scientific knowledge, practical competencies, techniques and training to maintain high standards of excellence in research practice, endorsing research and scientists throughout their careers.

Through the courses and workshops with main focus on biomedical and life sciences, a wide range of disciplines are covered. During the 2022-year the training unit offered more than 26 courses (around 550 participants registered), covered the different areas of expertise at i3S with special focus in areas of Laboratory Animal Science, Advanced Light Microscopy, High Throughput Screening, Image Analysis, Neuroengineering, Machine Learning and Cancer, among other relevant topics. i3S coordinates the only Advanced Laboratory Animal Science course in Portugal with international FELASA accreditation. Nonetheless, ATU continued in 2022 the investment in the development of courses covering Transferable Skills Training for Researchers such as Ethics & Integrity and Career development.

To provide basic and fundamental training to new members and continuous professional training to researchers in house-training modules have been also developed by ATU.

### **Career development Unit**

i3S Career Development Unit (CDU) works closely with all i3S community and institution constituents to create a culture that recognizes career development as a part of the overall experience at i3S. CDU focus on ensure a i3S offers a supportive working environment and resources to prepare researchers to make informed career decisions, gain lifelong career management skills, and achieve their professional goals in academic research or beyond.

The CDU has offered one-to-one sessions with the officer to all level i3S researchers. As in previous years, independently of the career stage, women request the service more than men (around 90% of the users were women). The service more requested by both, students and researchers are mainly related with career exploration for jobs beyond academic research, application preparation and training with mock interviews with the CDU officer for participants in selection process. Issues related to working in the lab / team and planning for academic careers. The CDU organized and delivered group training sessions on career planning, exploration and professional development, namely: PhD Career Leader Program 1<sup>st</sup> edition (January-April 2022). Peer group career exploration program PhD students. Research Project Management (June 2022). Three-day course for PhD students and postdocs on early-career stage aiming to equip participants with basic skills in managing research projects. Funding information sessions. The CDU in collaboration with i3S funding office has organize information session for international individual funding calls, namely MSCA IF (June 2022) and ERC Starting and Consolidator Grants (July 2022) to encourage i3S community to apply for funding that supports their career development. Ethics and integrity course talk. (July 2022) CDU officer delivered a seminar on mentoring and supervision for the Postdoctoral Appreciation Week (PAW). (September 2022) CDU participated in the postdoctoral

appreciation week with a talk on Career and Professional Development: What is it and why is important? I have a PhD. What next? (Oct. 2022). 3 day- workshop on career planning and exploration for 3<sup>rd</sup> and 4<sup>th</sup> year PhD students with additional 1-day training on grant writing. Life Science Intellectual Property: Management and Exploitation, 1st edition (Nov. 2022). In collaboration with the i3S Research Innovation Unit the unit organized a one-day workshop to promote understanding of the meaning and value of Intellectual Property in academia. Welcome sessions Together with the i3S Unit for Responsible Conduct in Research, CDU deliver training sessions for newcomers on professional development three times a year (January, April and Oct. 2022). Visits to groups. Following the work done in previous year the CDU continuous working with specific research groups in the implementation of the individual development plans. (IDP). Additional seminars were delivered ad-hoc for specific research group, namely on time management and skill analysis workshops. i3S maintain the compromise to work through the new ERA Policy Agenda common values and principles guiding research and innovation in Europe. CDU actively participate in the i3S working group for the Gender Equality Plan preparation and implementation. The Unit was actively involved in the i3S signature of the Coalition for Advancing Research Assessment CoARA and the Gago Manifesto Fostering Early Career Researchers' careers in Europe. CDU officer is a member of the ReMO COST Action CA19117: "Researcher Mental Health" and participated in the ReMO Ambassador Programme and associated 1<sup>st</sup> Training School (Grenoble 27<sup>th</sup>-30<sup>th</sup> June, 2022). i3S CDU integrated the working group responsible for the design of a career Development program framework for PhD students to be established in the University of Porto.

## **Ethics and Responsible Conduct in Research**

The Unit for Responsible Conduct in Research (URCR) has been working close to i3S researchers, answering their queries and promoting their training in Ethics and Responsible Conduct in Research. It has also provided support to various researchers and to the Research and Innovation Unit concerning the submission of projects to Ethics Committees, Grant Applications and the making of Data Management and Sharing plans; it is member of the Ethics Advisory Board of the PhasAge Project (WP 6). Working close to research groups: URCR held meetings with different research groups on different themes, namely on Misconduct (Falsification; Fabrication and Plagiarism) and other unacceptable practices: Authorship and Publication Ethics, Data management and Manipulation, Open Science, Mentoring, Privacy and Confidentiality, Ethical issues in human experiments and Ethical Self-Assessment in European projects. It is a collaborative approach, within a bottom-up approach together with a top-down normative enforcement addressing the pillars of the EU policy on responsible conduct. Guidelines for Responsible Conduct for Researchers: URCR has issued the *Checklist for Research Integrity*, which aims to be an easy tool for new incomers, raising awareness of the main areas and documents of Responsible Conduct at i3S. Other documents that were issued by this Unit regarding Responsible Conduct at i3S were: i3S Research Integrity Checklist; i3S Data Management Checklist; Tips for good Data Management Plans; i3S Authorship Guidelines; i3S Guidance on Authorship Planning; Procedures for Reporting Research Misconduct | Unacceptable Practices – Currently articulated with the Reporting Channels; Guidelines to handle conflicts of interest in Research and in Relation to Recruitment

& Promotion general ethics&integrity policy; i3S general ethics & integrity policy; i3S Guidelines on Incidental Findings Policy. Collaboration with national and international institutions of excellence: I3S Unit for Responsible Conduct in Research is part of the following national and international Research Integrity Networking: Steering Committee of the Portuguese Reproducibility Network; The Austrian Agency for Research Integrity; “Institut International de Recherche e d’Action sur la Fraude e le Plagiat Académiques” (IRAFPA) ; The Embassy for Good Science: Virt2UE - Virtue based ethics and Integrity of Research: Train-theTrainer program for Upholding the principles and practices of the European Code of Conduct for Research Integrity” [https://embassy.science/wiki/AboutCertifiedTrainers, ConfOA](https://embassy.science/wiki/AboutCertifiedTrainers,ConfOA) , [BioData.pt](https://www.biodata.pt) Training in Research Ethics and research integrity on a regular basis: The Training Course in Research ethics&Integrity started in 2020-2021, with a target audience of researchers from all career levels and has been followed up by a second edition from October 2021 to July 2022 and a currently ongoing 3<sup>rd</sup> edition that started in December 2022 and will finish in June 2023. The course has integrated not only i3S members but also external members from the University of Porto, providing the opportunity for discussion of ethical and integrity issues among researchers from different institutions. Moreover, there have been Welcome Sessions on ethics & integrity in research for all new members, led by this Unit together with the Unit for Career Development. The unit also organized numerous events for internal and external researchers to provide training in all aspects of ethics and responsible conduct in research. Round-table: Sharing Views on Research Integrity in Portugal 28th April 2021 | 12:00-13:15 | ONLINE <https://www.i3s.up.pt/training-detail.php?v=171> Training Session on Informed Consent, Course Data Stewardship for life and health sciences, organized by BioData.pt – Infraestrutura Portuguesa de Dados Biológicos, together with the University of Minho and FCCN. Training the Trainers Virt2UE Course (2, 9, 16, 23 and 30 June 2021 | ONLINE COURSE) <https://www.i3s.up.pt/training-detail.php?v=169>. Round-table: Fair Data: Challenges and Opportunities| 8 July 2021, 12:00-13:15. Workshop “Research Integrity - Insights and Initiatives”, 17th November 2021, Rectorship of the University of Porto. Communication: “Integrity Officers in Research: Challenges and Opportunities” 18-22 April 2022 PhasAge Training School, i3S: The Ethics of Authorship and Publication. 8th International Conference | European Conference on Academic Integrity and Plagiarism, Faculty of Medicine of the University of Porto (FMUP), Porto, Portugal, May 4th-6th, 2022. Communication: Responsible Scientific Research: Challenges and Opportunities for Ethics/Integrity Officers 6th Dash Symposium Humanism in Surgery (19, 20, 21 maio, 2022), i3S, UP (Organizing and Scientific Committee). “Cups of Ethics” for PhD Students (June and July 2022). 7th World Conference On Research Integrity (29 May-June 2022), South Africa (online participation). Communication: Building and reflecting on a framework for research integrity at a Portuguese biomedical research institution: why dialogue and narrative matter”. In collaboration within the EU project INTEGRITY, a “Diner Pensant” was organized in June 2022 and a “Picnic Pensant: talking about research integrity” in July 2022, i3S. Consensus Conference on Scientific Integrity (FMUP), 21 October 2022, Communication: “Conflicts Management”. 15th Meeting of Internal Medicine Residents, 16 e 17 december 2022, Vila Real; Communication: “Ethics and Integrity of Publications”. Responsible for organizing, together with the Faculty of Medicine of the University of Lisbon, the [Data Management Plan Webinar | FAIR data management – from plan to practice](#) 24 January 2023 | 15:00 - 16:30, by Femmy Admiral, Leiden University, i3S.. 4 hour lecture on Research Integrity at the Doctoral Program of

BiotechHealth and McBiology 2 February 2023 | 14.30 – 18.30, i3S.. Bioengineering Hub: “In vitro, In vivo and in Silico research” (moderator) 24th January | 9.00-10.15, i3S.

## Communication and outreach

2022 was a year of recovery for i3S, as it marked the return to regular activities after two pandemic-impacted cycles. Though the early months of the year saw some caution in the organization of public events due to restrictions, by March regular activities resumed with a few precautions in place. By September, everything was back to normal, and i3S's accountability and scientific integrity helped strengthen its recognition and presence in the media. Despite some hiccups, i3S's Educational Program had a successful year. The indicators for i3S's activities and projects over the past year demonstrate a strong commitment to education and outreach, as well as institutional support for research and communication. In terms of activities for students and pupils, the numbers are impressive: 58 visits and 1380 students to i3S, 187 sessions and 5799 students in the Science Ambassadors Program, 99 workshop sessions with 1821 participants, 6 summer internships and trainee programs with 21 students, 26 sessions with 250 students in grade school partnerships, 179 sessions with 3268 students in the Open Laboratory, and 182 sessions with 3071 students in LABS Maia. The institute also hosted 10 international and/or higher education visits, totaling 231 students. In addition to student programs, i3S has also offered training and workshops for adults, under- and postgraduate students. In January 2022, the workshop "Triggers and Trends in Communicating Science for Society" was held, followed by "Introduction to information design for life sciences" in February 2022. These workshops provide valuable skills and knowledge to those who seek to communicate science effectively.

i3S has been successful in reaching the general public through media mentions, social media followers, and other channels. With 1106 media mentions, including 39 TV features, i3S has been making headlines and promoting its work to a wider audience. In addition, the social media platforms of Facebook, Twitter, Instagram, and LinkedIn have a combined following of over 60,000, with impressive growth rates and engagement. This level of visibility and engagement helps to raise awareness of i3S's mission and impact, and to build support for its future work. In 2022, i3S experienced notable growth on social media platforms, reflecting the shift in usage patterns away from Facebook, which has been seeing a sharp decline in users, and towards other social networks like LinkedIn. Over the past few years, i3S has significantly increased its presence on LinkedIn, placing among the top three Portuguese competitors and leading in terms of engagement and growth rate. This growth has also been observed on Twitter and Instagram, despite i3S accounts having been created much later than its competitors. The Communication Unit carries on its partnership with the Animal Facility to improve the visibility of i3S's good practices regarding animal welfare and experimentation, and also teamed up with the i3S Green Lab Initiative to spread their actions to a wider audience.

The aforementioned 1,106 media mentions throughout the year covered a host of notable achievements, including the selection of four projects coordinated by i3S researchers by the CaixaResearch program, the attainment of an ERC Synergy Grant worth €10 million, and several other major awards and distinctions attributed to i3S researchers, such as EMBO

grants, a Rui Osório de Castro Award, Gilead Génese grants, a Wellcome Trust grant, a L'Oréal Portugal Medal of Honor for Women in Science, a Grünenthal DOR Award, Maria de Sousa Awards, two Santa Casa Neurosciences Awards, and many more. Finally, i3S researchers were featured twice on the IPO-promoted show "Segundas de Conversa" on Facebook, where they discussed their research and work.

Throughout the year, i3S held several Art&Science activities targeting both the i3S community and the wider public: a live music session where i3S researchers participated in an action painting event, leading to the CoPraxis exhibit, which was displayed from April 1st to 22nd; a public session reflecting on previous artistic residencies; an exhibit entitled "Solitude Fictions", from September 19th to October 22<sup>nd</sup>; the return to the Open House Porto circuit, welcoming about 100 visitors on July 2nd. The pandemic situation strongly affected the STEAM education ERASMUS+ project (HYBRID LAB NETWORK), but in 2022 in-person workshops became possible. The project served as the impetus for a partnership with ICBAS and FCUP to introduce the Biolaboratory - Multidisciplinary Experimentation Project (ongoing training). As a direct result, new international projects/collaborations are starting to develop.

Another important aspect of i3S's work is institutional support to and involvement in EU- and FCT-funded projects, such as MIRACLE, RESTORE, FIBRALUNG, PhasaGE, INTEGRITY, and ERA Chairs. These projects provide funding and resources for innovative research, while i3S manages communication work packages to ensure that research findings are effectively disseminated and impact society.

## **CLINICAL INITIATIVES**

### **Centro de Genética Preditiva e Preventiva (CGPP)**

CGPP, IBMC, is a clinical initiative of i3S, in the area of Medical Genetics, offering services to the community in the healthcare sector: molecular genetic testing (one of the main providers to the NHS); medical consultations of various specialties; and genetic (clinical and laboratory) training for residents and interns of several specialties and other health professionals.

Its services are highly differentiated in the diagnosis, follow-up and genetic counselling of patients and families. CGPP provided an extension of clinical services in CHUSJ (one of the largest NHS hospitals) and has protocols with multiple patient associations. As a result of the combination of its scientific, clinical and technical skills, its main objectives are to Carrying out clinical molecular genetic tests, to apply a multidisciplinary pre-symptomatic genetic testing protocol for healthy family members at risk for late-onset hereditary diseases (includes Genetic Counselling, Clinical Psychology and Psychosocial assessment, as well as genetic testing), to provide differentiated consultation services for the diagnosis of hereditary diseases, in the areas of Medical Genetics, Neurology, Neuropediatrics, Psychiatry, Paediatrics and Haematology; to carry out Undergraduate and postgraduate education (in collaboration with UP and other universities) and continuous training for Family Medicine doctors and other health professionals; to maintain of a Biobank (DNA, cell lines and other biological products); to provide support for basic and clinical research on genetic diseases at the IBMC, as well as other institutions that may request it.



Its clinical staff is composed of Medical Geneticists, Neurologists, Psychiatrists, Clinical Psychologists, Genetic Counsellors, Nurses and Social Service professionals. Its technical staff comprises health technicians specialized in Human Genetics, Molecular Genetics, and/or Bioinformatics. CGPP is fully licensed by ERS. CGPP has a quality management system and participates in annual external evaluations of quality (EQAs) for a wide range of genetic tests (n=14 in 2022), through the EMQN. It is accredited by IPAC (through NP EN ISO 15189) to perform blood sampling and molecular genetic testing, since 2014. As for the laboratory, it has been supplementing the growing need for genetic testing, using next-generation sequencing (NGS), more specifically whole-exome sequencing (WES) based on Illumina technology. For this, CGPP developed and validated dedicated bioinformatics pipelines, following international guidelines and best practices. Since 2016, over 11,000 WES have been processed and analysed. Given this vast experience, in 2022, CGPP was able to expand the accreditation of genetic tests to include multigene panels based on WES, being the first at the national level to achieve it. Further to genetic testing, with one additional year of outstanding growth (16% increase in genetic test reports), a significant amount of work was made in the three key areas of action: 7,643 genetic tests requests (3,588 for WES-based studies), including 5,879 new biological samples; and 6,027 reports issued. There were also 23 clinical sessions to discuss clinical cases (n=72) and laboratory methodologies, in 10 different hospital services. The unit performed 683 medical consultations carried out of which 216 at the CGPP and 467 at the CHUSJ. As a direct outcome of genetic services and extended collaborations, 21 articles published in peer-reviewed scientific journals; and 34 communications made at medical congresses (18 invited oral communications, 5 other oral presentations and 11 posters). The unit organized 2 scientific meetings including the 10<sup>th</sup> Meeting on Neurogenetics at the HDE, and the 7<sup>th</sup> Course of Genetics for Primary Health Care physicians and we participate in the i3S Science Ambassadors' Program. There were also 4 curricular internships for medical residents of Genetics (n=2), Neurology (n=1) and Haematology (n=1); 4 other scientific and/or professional internships: Genetic counsellor (n=1), Psychologists (n=2), Clinical laboratory Geneticist (n=1);

### **Ipatimup Diagnostics**

In 2022, Ipatimup Diagnostics maintained the high quality standards successfully achieved in the previous years through CAP accreditation being its recognition reinforced by the IPAC accreditation renewal in two different standards (ISO 15189 and ISO/IEC 17025). The unit participates in international networks, professional training centre through its nomination by the Portuguese Physician's Association (*Ordem dos Médicos*) as a reference centre in molecular pathology of the Anatomical Pathology internship, it is also involved in research projects with health institutions and pharmaceutical companies and maintains a strong participation in Molecular Tumour Boards. During 2022 it carried out 32620 diagnostics in the area of pathology, genetics analysis of 6643 cases and 5522 molecular pathology diagnostics. During 2022 Ipatimup Diagnostics has participated in proficiency tests and quality assurance programs for 47 distinct parameters covering all laboratory areas and methods with 96.5% of satisfactory results for the anatomic pathology laboratory and 98,8% satisfactory results for the genetics laboratory. These external quality assessment schemes were performed in collaboration with several accredited organizations such as CAP, ESP,



GenQA - NEQAS, Instand, EMQN and RfB. In terms of training 48 MD or technicians underwent training at Ipatimup Diagnostics, from Portugal (36), Mozambique (1), Brazil (7), Turkey (1), Ukraine (1) and Spain (2). The unit participated in 42 scientific meetings and presented scientific works in 18 of these meetings and were involved in the organisation of 6 scientific events.

### **i3S Diagnostics**

i3S Diagnostics is a clinical service created in March 2020 to mitigate the COVID-19 pandemic through the screening of the SARS-CoV-2 virus, demonstrating the i3S capacity to explore the established scientific and technical excellence to respond to public health emergencies. i3S Diagnostics is Registered to the Health Regulatory Entity (ERS) and approved as COVID-19 diagnostic Laboratory by the National Institute of Health (INSA). The i3S Diagnostics technical staff is composed by highly specialized technicians in Molecular Biology and Bioinformatics. i3S Diagnostics has a quality management system and carries out annual external evaluation of quality, through the National Program for External Quality Assessment (PNAEQ) and the WHO Global Round of Laboratory Proficiency Testing. Since its creation, the i3S Diagnostics has carried out more than 100 000 SARS-CoV-2 detection tests. In 2002, the i3S Diagnostics opened a collection center for PCR and antigen tests for all patients with medical prescriptions. In January 2022, challenged by ARS Norte, i3S Diagnostics opened, in less than a month, a unity to collect nasopharyngeal swabs for COVID-19 diagnosis, developed with the i3S Communication and IT Units all the publicity material and dedicated web site, created a platform to register appointments and send results to patients and SINAVE, registered to the platform Exames Sem Papel (SPMS) and performed both SARS-CoV-2 rapid antigen and RT-qPCR tests for the community during the high demand on COVID-19 diagnosis. It was also involved in the training of technicians on new equipments and technics. It externalized during several months (March-Mai 2022) some of its technicians to Basel (Switzerland) in recognition of their expertise in SARS-CoV-2 molecular testing and performed more than 200 DNA extractions for i3S research groups. The unit also coordinated a research and development project aiming not only at supporting i3S Diagnostics to carry out faster and more reliable diagnostic tests, but also at accelerating the research on SARS-CoV-2 co-infections and infectiveness of SARS-CoV-2 strains. To evaluate seasonal co-infections, we analyzed the microbiome of 600 nasopharyngeal swab samples positive for the presence of SARS-CoV-2 virus and collected from 04/2020 to 02/2023. To ascertain infectiveness/aggressiveness of circulating SARS-CoV-2 strains along the pandemic, we sequenced 200 virus collected during 2020-2021 and assessed their infectiveness on Calu-3 cells. During this year it was also decided that part of the i3S Diagnostics mission should include the development of new diagnostic methodologies for infectious diseases and the preparedness and response to future infectious disease outbreaks. i3S Diagnostics is thus pursuing the diversification of its activities to ensure its sustainability. In particular, i3S Diagnostics is developing the use a metagenomic next-generation sequencing (mNGS) approach for the identification of infection causative agents, by sequencing circulating cell-free DNA (small DNA fragments released by pathogens into the bloodstream). i3S Diagnostics is also developing whole genome analysis to identify pathogens and multi-drug resistance organisms from culture

isolates, and body fluids or tissues (blood, fecal swabs, heart valves, local of infection, etc.). Sequencing is performed using two platforms, Illumina and Oxford Nanopore Technologies. This development phase is performed in interaction with health professionals from several hospital units to identify and solve gaps in the diagnosis of infectious diseases.