STARSTEM

2020 Newsletter

Shedding new light on regenerative medicine

STARSTEM is developing a nanotechnology-enhanced optoacoustic imaging approach, using a novel nanostar contrast medium, which will deliver unprecedented imaging depths and levels of sensitivity in identifying and tracking mesenchymal stem cells and extracellular vesicles and their healing function in osteoarthritis after administration into an affected joint.

STARSTEM will, for the first time, enable objective measurement of functional markers of healing, including vascularisation, oxygen saturation, and inflammation, over time and at significant depth. Understanding the hallmarks of the healing process will ultimately help patients to benefit from new cell therapies.

STARSTEM is a European project, with partners hailing from five countries; Ireland, Germany, England, Spain, and Italy. The project brings together leaders in the nanomaterials, regenerative medicine, osteoarthritis, and bio-imaging fields from across Europe.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 761214. The material presented and views expressed here are the responsibility of the author(s) only. The EU Commission takes no responsibility for any use made of the information set out.

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MESSAGE FROM THE COORDINATOR PROF. MARTIN LEAHY

STARSTEM is addressing major technology gaps to enable imaging of stem cells at clinically relevant depths. Our nanostar-enhanced multi-modal imaging approach will enable us to detect stem cell engraftment and tissue repair, and thus their activity and efficacy as a therapy.

We do this by using nanoparticles with novel optical properties. Gold nanostars will be attached to bone-marrow-derived mesenchymal stem cells (MSCs) and their extracellular vesicles (EVs) prior to their administration in a joint affected with osteoarthritis. We then harness the best properties of light and sound with the optoacoustic imaging system to exploit the unique advantages of our nanostars for deep-tissue imaging. These labelled nanostars greatly enhance molecular sensing and diagnostics by increasing the signal contrast during the visualisation process.

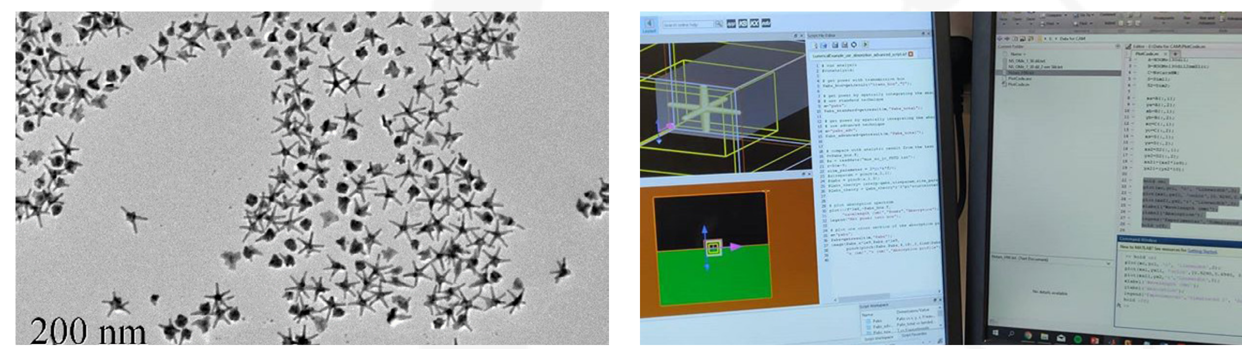
STARSTEM began in January 2018 and will run for four years. Since project kick-off, we have made significant progress on cell production; isolation of EVs from MSCs; nanostar design and production; labelling of cell products; and imaging technology development. We have also addressed necessary regulatory issues and secured ethical approvals and have held several consortium-wide meetings.

STARSTEM is off to a great start with the nanostar optimised for energy deposition at exactly the desired wavelength and much groundwork done for the cell preparation, animal studies and image acquisition, storage, co-registration and analysis. The next big challenge is to optimise uptake in the cells and hence push the sensitivity to cells in deep tissue in vivo to the limit.

Scientific Highlights | Nanostars

The STARSTEM nanostar is a novel gold nanoparticle, shaped like a star. Its unique composition and structure amplify the optoacoustic effect by concentrating the thermal response at the tips of the stars' arms. This helps to generate a strong optoacoustic imaging (OAI) signal. This effect is further enhanced by nanostars' responsiveness to long-wavelength OAI lasers, maximising thermal conversion and deep signal penetration. In effect, these nanostars greatly improve imaging capability while having no impact on the mesenchymal stem cell infusion and processes that are being monitored.

Initially, our work focused on the development of a reproducible synthesis protocol for the high-yield production of gold nanostars. To this end, we applied 'experimental design methods' to perform a systematic study of the influence of the different chemical conditions and parameters. Following several rounds of optimisation, we reached optimal synthesis conditions. The result was a very pure yield, meaning that most of the material in the batch is now nanostar-shaped, with only a small amount being non-nanostar gold nanoparticles. Substantial improvements were made in terms of the reproducibility of the geometrical dispersity (number and size of tips) to ensure that our nanostars will absorb light at ideal wavelengths for OAI. This optimisation phase included a systematic study of the surface modification of the nanostars and their stability in different media. This work is highly relevant to several of the tasks (culture, cell and animal studies) to be carried out by partners in the STARSTEM consortium.



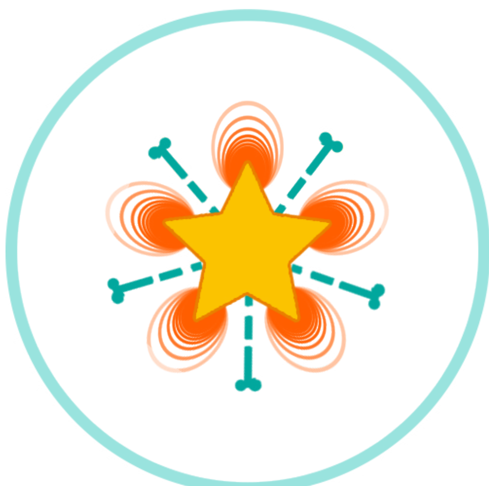
TEM image of nanostars and numerical simulations from ICFO and NUI Galway

In addition to optimising the way that we produce nanostars, we have also conjugated SPIONs (superparamagnetic iron oxide nanoparticles) to the nanostars. SPIONS are tiny magnetic nanoparticles that will enable us to visualise the nanostars in larger animal joints with MRI. This body of work will ensure that we attain images at unprecedented depths, with excellent sensitivity, and can identify and track our targets.

Scientific Highlights | Cell proudction and labelling

STARSTEM is interested in tracking mesenchymal stem cells, or MSCs and MSC-derived extracellular vesicles (EVs), labelled with novel nanoparticles.

MSCs secrete a variety of autocrine and paracrine factors that support regenerative processes in damaged tissue, induce blood vessel formation angiogenesis, protect cells from apoptotic cell death, and modulate the immune system to promote healing. The mechanisms through which this regenerative result is achieved appears to rely more on the secretion of specific bioactive factors, rather than the direct differentiation and engraftment of the transplanted stem cells in the host tissue.



The STARSTEM nanostar

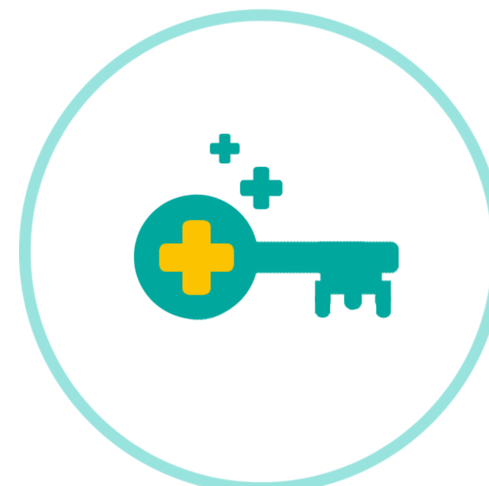
We have optimised our synthesis protocol to produce, with high yield and reproducibility, optimal gold nanostars resonant at around 1064 nm.

Cell production and osteoarthritis

Nanostars will be administered for *in-vitro* and *in-vivo* models of arthritis.

Imaging

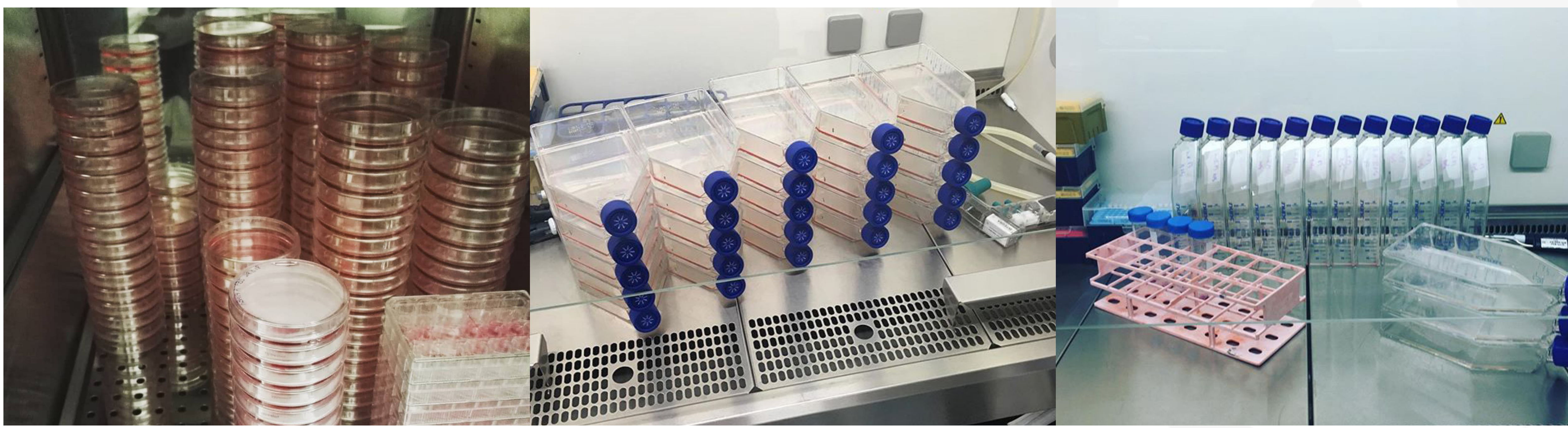
OAI and MRI are used to visualise and monitor the activity of the MSCs and EVs.



Hallmarks of healing

Better understanding of therapeutic efficacy can lead to new therapies and a subsequent clinical trial.

Extracellular vesicle (EV) is the generic term for particles naturally released from the cell. These vesicles are delimited by a lipid bilayer, cannot replicate and do not contain a functional nucleus. EVs represent a major element of the MSC secretome and have been implicated in the modulation of osteoarthritis development and the treatment of the condition. In STARSTEM, we are tackling the question of therapeutic agency - do mesenchymal stem cells lead to healing directly, or do they communicate with the body via sub-cellular particles and trigger healing ‘at a distance’? Initial work in STARSTEM focused on the production of MSCs and EVs for use in our in vivo and in vitro studies. In parallel, we developed a new heparin-free platelet-derived cell culture medium which improves the survival and proliferation of human cells and is a safer product that addresses recent regulatory concerns. Many current cell culture medium supplements are often based on animal sera, and as such are not ideal for the in vitro production of cells intended for human cell therapies. Our media supplement will be used in a variety of studies, from the sub-cellular to whole-animal scale. Importantly, we began labelling our MSCs with gold nanostars, to better understand the effects that this labelling process would have on cells. Through a series of in vitro studies, we examined how nanostars affected the functional properties of MSCs and began to look at how we could improve the cellular uptake of the nanostars.



MSC and EV production at the University of Genoa and the University of Cambridge

Scientific Highlights | Imaging

Optoacoustic imaging, or OAI, is an exciting imaging modality, with unique capabilities that have yet to be applied in a clinical setting. OAI takes advantage of the photothermal effect and is used to produce ultrasound in soft tissue caused by vibrations. When tissue is illuminated with a bright light, such as a pulsed laser, some of that light is absorbed and converted to heat. The heat leads to an expansion of the illuminated tissue. This expansion creates vibrations, which can be detected using ultrasound detectors. Because different substances expand in different ways, and in response to different light wavelengths, a sophisticated image can be assembled. OAI is non-invasive and non-traumatic. This means that the same study participant can be treated and tracked over multiple images or in real-time, reducing the number of study subjects required when carrying out research.

OAI is limited by its operating depth beneath the skin, which is why we are using nanostars to amplify the optoacoustic signal and increase its useful depth.

Optoacoustic Imaging (OAI) is also known as Photoacoustic Imaging (PAI). OAI uses ultrasound waves that are generated by transient light absorption.

Functional imaging using OAI will enable us to carry out real-time monitoring of engraftment and the therapeutic healing processes at the site of interest, while highly sensitive anatomical imaging using MRI (via magnetic nanoparticles as a contrast agent) will enable us to track the movement and retention of transplanted mesenchymal stem cells and extracellular vesicles in larger animals.

Initial imaging work in STARSTEM has focused on developing techniques to improve how our imaging modalities work and uses these tools to monitor and track nanostar labelled MSCs. Nano-sensitive Optical Coherence Tomography (nsOCT) techniques were developed specifically for MSC and EV monitoring.

Multispectral optoacoustic tomography (MSOT) was rolled out for imaging cells labelled with nanostars. MRI sequences have been developed to better identify and track SPIONs in joints. In addition, we began to develop algorithms capable of combining and analysing results from both OAI and MRI.

iThera and TUM generate sample images of the human finger with iThera's Medical's MSOT Acuity imaging system

Recent STARSTEM publications include:

Sergey Alexandrov, Paul M. McNamara, Nandan Das, Yi Zhou, Gillian Lynch, Josh Hogan and Martin Leahy. "**Spatial frequency domain correlation mapping optical coherence tomography for nanoscale structural characterisation**" Applied Physics Letters 115(2) 2019 DOI: https://doi.org/10.1063/1.5110459

Sergey Alexandrov, Nandan Das, James McGrath, Peter Owens, Colin J. R. Sheppard, Francesca Boccafoschi, Cinzia Giannini, Teresa Sibillano, Hrebesh Subhash, and Martin Leahy. "**Label free ultra-sensitive imaging with sub-diffraction spatial resolution**" Paper presented at 21st International Conference on Transparent Optical Networks, ICTON'2019, Angers, France, 09-13 July. DOI: https://dx.doi.org/10.1109/ICTON.2019.8840220

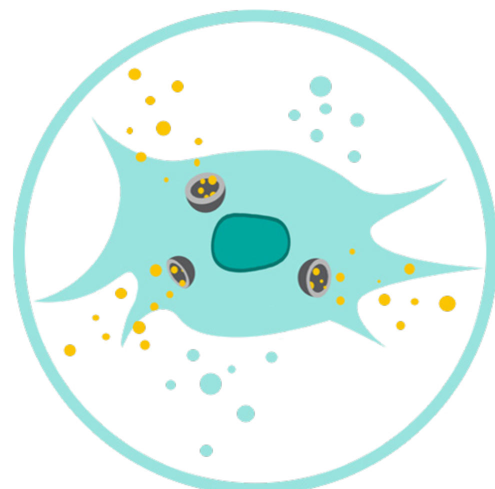
Resolution →

Vesicles

nsOCT - nano-sensitive Optical Coherence Tomography

nsOCT improves the optical microscopy resolution limit and dramatically improve sensitivity to nanoscale structural changes.

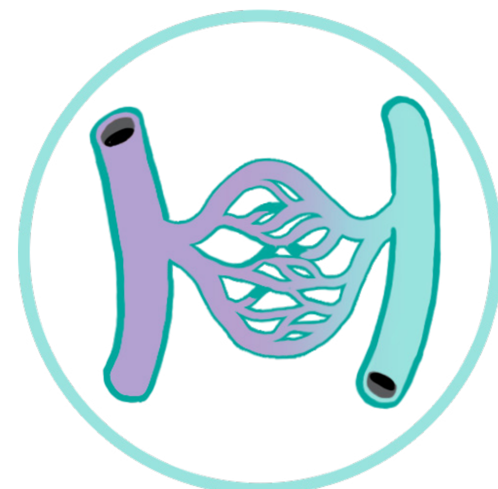
Cells



RSOM - Raster-Scanning Optoacoustic Mesoscopy

RSOM combines optical illumination with high-frequency acoustic detection and ultrasound detection.

Vessels



MSOT - Multispectral Optoacoustic Tomography

iThera Medical's MSOT uses multiple laser wavelengths to image multiple substances and chromophores of interest. It combines this with the depth capabilities of conventional ultrasound imaging.

Joints

MRI - Magnetic Resonance Imaging

MRI uses a magnetic field to create an image of the body.

MEET THE TEAM | PARTNER SPOTLIGHT: ICFO

Created in 2002, ICFO – The Institute of Photonic Sciences is located in the Mediterranean Technology Park in the metropolitan area of Barcelona, and hosts more than 350 researchers, organized in 26 research groups in 60 state-of-the-art research laboratories. Scientists work in diverse areas of both basic and applied research in which photonics play a decisive role. Themes range from studies relevant to medicine and biology, tunable and ultra-fast lasers, and advanced imaging techniques, to environmental sensing and quantum science and technologies, among others. ICFO is proactive in fostering entrepreneurial activities, spin-off creation, and creating collaborations and links between industry and researchers, with 7 start-up companies incubated and launched in the KTT LaunchPad to date. (www.icfo.eu)

STARSTEM PI, Romain Quidant, is an ICREA Professor at ICFO and is a European leader in plasmon nano-optics, nanoparticles and thermoplasmonics. He has received four ERC grants for advancing the fundamental science at the core of STARSTEM.

Prof. Romain Quidant, Dr Ignacio de Miguel and Arantxa Albornoz Grados have been responsible for the optimisation of production of our gold nanostars in collaboration with NUIG TOMI. ICFO plays an essential role in ensuring that STARSTEM nanostars reach clinical trial. Consistency, high-quality production, and regulatory compliance are particular areas of expertise for ICFO.

"STARSTEM is a wonderful example of an interdisciplinary research project that brings together experts from fundamental science, technology and medicine to solve a complex problem with direct impact on patients. Despite the numerous challenges of the project, STARTSTEM is becoming, thanks to a great team effort, a very fruitful collaboration that pushes the limits of knowledge towards unprecedented grounds." Prof. Romain Quidant

ICFO

Meet the Team

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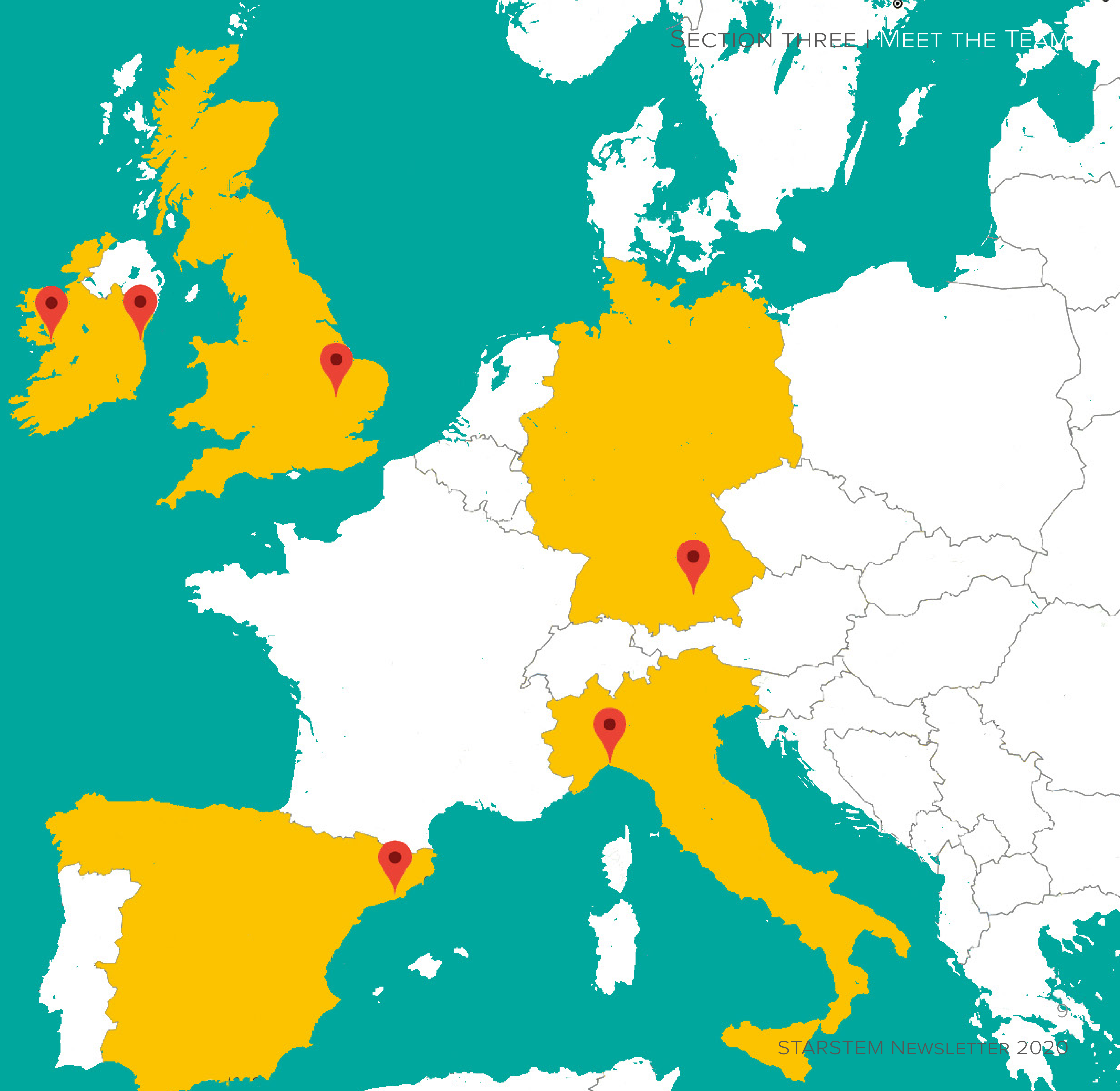

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