PhD proposal 1: Imaging and characterization of tissue fibrosis

1. Consortium

- University awarding the degree: University of Trieste
  Proposed academic supervisor: Dr. Giuliana Tromba/ Dr. Loredana Casalis

- Proposing CERIC partner facility: SYRMEP beamline, Elettra-Sincrotrone Trieste, Italy
  Lead proponent: Dr. Giuliana Tromba

- Contributing CERIC Partner facilities: Graz University of Technology, Graz, Austria
  Lead collaborator: Prof. Dr. Heinz Amenitsch

- Contributing CERIC Partner facility: - NanoInnovation Laboratory, Elettra-Sincrotrone Trieste, Italy
  Lead collaborator: Dr. Loredana Casalis

- Contributing CERIC Partner facility: SISSI-Bio beamline, Elettra-Sincrotrone Trieste, Italy
  Lead collaborator: Dr. Lisa Vaccari

- Contributing CERIC Partner facilities: National Institute of Chemistry, Ljubljana, Slovenia
  Lead collaborator: Prof. Dr. Janez Plavec

2. Scientific background

Fibrosis is the formation of excess fibrous connective tissue in an organ or a tissue, produced by an inflammation process or a damage and can affect many tissues within the body. This increased deposition changes mechanical properties like stiffness which may affect the function of the organ like in lung fibrosis. In later stages the increased fibre content will supplant the cells of that organ which yields to a loss of functionality like in liver fibrosis. The diagnosis of fibrosis in histology is the gold standard. Since the pathomechanisms behind fibrosis are not completely understood and treatment is far from being optimal, preclinical research (especially in life animal models) is crucial and often performed utilizing mouse models.

The smallness of the mouse in combination with the diffuse and patchy appearance of fibrosis render the application of imaging techniques extremely challenging, which on the other would be extremely helpful especially if they could be applied *in-vivo* in longitudinal studies.

Thus this thesis we be focused on developing better *ex-vivo* analysis tools to quantify fibrosis in organs (lung, liver, heart and kidney) of mouse fibrosis models, by utilizing new cutting edge technologies provide

by CERIC/ERIC such as SAXS and phase contrast microCT. In order to provide a proof of concept if these techniques could be also potentially applied in patients it is also foreseen to work with larger animal models such as pigs.

References

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3. Outline of the experimental protocol

Mouse lung and heart fibrosis models will be setup at the University Medical Center Goettingen Germany (UMG). Mice will be studied using *in-vivo* microCT and *in-vivo* x-ray based lung function. The organs will be explanted: one part will be embedded in paraffin and scanned at the SYRMEP beamline in order to perform 3D virtual histology at micron scale resolution. The other part of the tissue will be analyzed using SAXS. Bio-mechanics studies will be performed using Atomic Force Microscopy (AFM) of tissue stiffness at NanoInnovation Lab. The search of specific metabolites in tissue specimens will be performed at the CERIC NMR facility. Fibrosis tissue alterations will be also characterized biochemically and spectroscopically using IR analysis at the SISSI beamline. Subsequently classical histology will be performed at UMG using fibre specific staining protocols such as Masson-Goldner-Tricrome.

In an additional set of experiments lungs of a porcine model of idiopatic pulmonary fibrosis (IPF) will be mounted in a human chest phantom and scanned with phase contrast CT at SYRMEP to mimic a potential application in patients as close as possible. Biopsies will be taken afterwards and scanned again with the high resolution white/pink beam microCT setup at SYRMEP as well as analyzed using SAXS and AFM. Like in the mouse models, the analyzed specimens will be further processed by classical histology.

The aim of the study beside the optimization of the image acquisition pathway is the correlation of the multi-parametric data regarding:

a) a potential earlier diagnosis of the above mentioned fibrotic diseases

b) a better understanding of the ongoing mechanism

The proposed results are: a) (with respect to the mouse experiments) a pipeline for effective testing of anti-fibrotic treatments and b) (in case of the porcine experiments) an alternative imaging strategy for improved diagnosis.

4. The expected impact of the proposed research on the overall quality and capability of CERIC

The aim of the project is the design and optimization of a novel protocol for the characterization of tissue fibrosis based on an integrated multi-technique approach. The research will fully exploit the potentials of CERIC/ERIC, involving 5 experimental facilities of 3 partner Institutions and will benefit from the collaboration with the group of University Medical Center Goettingen, holding a high experience in pre-clinical research with different animal models. In the framework of CERIC funded Integra project, the proposed research will contribute to establish new connections and facilitate further collaborations within the consortium research groups. Furthermore, the proposed activity will have a natural link with the project for low dose phase contrast lung CT under development for the new bio-medical imaging beamline foreseen by the Elettra 2.0 upgrade project.

5. **Estimated cost, if relevant** (tuition fee, salary/stipend, travel)

Year	Tuition fees	Salary	10% *	Stay abroad	Mobility	Tot per year
1st	€ 496.00	€ 18,850.00				€ 19,346.00
2nd	€ 496.00	€ 18,850.00	€ 1,534.00	€ 4,712.00¥	€ 600.00	€ 26,192.00
3rd	€ 496.00	€ 18,850.00	€ 1,534.00		€ 600.00	€ 21,480.00
Adm Costs						€ 1,800.00
Total						€ 68,818.00
* Funds available for the research activity of the PhD, per regulation						
¥ Funds available for 6-months abroad						

Annex 1: CV of supervisor

Giuliana Tromba is the principal beamline scientist of the SYRMEP hard X-ray imaging beamline and Qualified expert for Radiation Protection of Elettra and FERMI laboratories. She has several years of experience in the development and application of novel phase contrast imaging techniques to different areas of biology, bio-medicine and clinical mammography.
Her recent main research topics concern the development of Synchrotron-based imaging procedures for the study of small animals models, the use of high resolution CT for virtual histology, the imaging of bones, biomaterials and scaffolds, the protocols optimization for tissue staining, cell tracking and low dose phase contrast CT imaging.
She is author of more than 200 publications on International Journals, h-index=30 (Scopus).