ESRF	**Experiment title:** High-resolution 3D phase imaging of intestinal structural and ultra-structural characteristics for innovative regenerative medicine applications	**Experiment number**: **MD-693**
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Report:

Tissue engineering (TE) is an emerging sub-discipline of regenerative medicine with the potential to overcome major issues connected with organ replacement in humans: the shortage of suitable donor organs, and the problem of organ rejection and related need for immunosuppression. Several approaches towards tissue engineered replacement organs exist, all of which are based on using acellular scaffolds with the objective of populating them with stem cells of the organ recipient, either post-operatively through natural growth or through pre-operative seeding. Acellular scaffolds can be generated from biocompatible materials ("smart polymers") collagen constructs, or the matrices of the replacement organs themselves. The latter (natural acellular matrices, ACMs), have been suggested as the optimal choice for tissue engineered organs due to the fact that native biomechanical properties are naturally present. ACMs are typically obtained via a decellularization procedure. The exact method is organ specific, and the main challenge is the preservation of the organ structure while eliminating the entire cell population and thus DNA of the donor. The main focus of current TE research is on the decellularization of complex organs such as the liver, lungs, oesophagus and further organs of the gastrointestinal tract. The visualization of ACMs is crucial for the assessment of whether or not a decellularization method successfully preserves the structure of a specific organ; however, there is currently a lack of appropriate imaging modalities. The challenges are the 3D nature of the scaffold, the small scale of the structural features, poor soft tissue contrast, and the requirements of high throughput associated with clinical practice. Several imaging modalities have been tested (including electron microscopy and MRI), however, they all have shortcomings regarding the 3D nature of the ACM, the demand for a high throughput, or both. MicroCT imaging is not suited due to insufficient soft tissue contrast.

X-ray phase contrast imaging (XPCI) offers a potential solution to this problem, thanks to the substantially increased soft tissue contrast it provides. Combined with CT, XPCI can allow volumetric renderings showing an ACM in its 3D nature.

To the best of our knowledge, 3D phase contrast imaging of acellular organ scaffolds had not been performed yet, and the proposed experiment has been dedicated to bridge this gap. Initially, we had planned to image acellular scaffolds obtained from a few selected organs. Shortly before the experiment, the opportunity has emerged to broaden the organ selection and include organs that had not been considered previously, but are

of high interest to the TE community. This was due to developments in the acellularization process which took place between the proposal submission and the experiment itself. The final list of specimens included ACM s obtained from colon, small intestine, oesophagus, lung, liver and different types of muscular tissue. While initially the use of an Analyzer Based phase contrast imaging setup had been suggested for the experiment, the opportunity to be able to image specimens originating from a larger variety of organs has triggered the requirement for a "faster" method with a higher sample throughput. As this is directly linled to the available flux at the detector, Propagation Based imaging, which does not involve additional x-ray optics, was chosen for the experiment.

The experimental setup was as follows. The sample was placed approximately 150 m from the source. The beam was monochromatized by a double Laue/Laue silicon (1,1,1) crystal to 26 keV ($\Delta E/E \sim 0.02\%$) and filtered using 0.8 mm of copper and 3 mm of aluminium. The object-to-detector distance was 3.45 m. The sample had been placed on a PI miCos rotation stage (PI mi- Cos GmbH, Eschbach, Germany). Images were recorded by a FReLoN CCD camera coupled to a 47 μm thick GGG scintillator. The effective pixel size was 3.5 x 3.5 micrometres^2.

Samples were prepared as follows. The organs were harvested from rabbits and rats in accordance with UK Home Office guidelines under the Animals (Scientific Procedures) Act 1986 and the local ethics committee. Organs were washed with phosphate buffererd saline (PSP) containing 5% antibiotic antimycotic solution, then subjected to a detergent enzymatic treatment in order to eliminate their entire cell population.

In preparation for imaging, the specimens were fixed in 2% glutaraldehyde, followed by a procedure known as "ultra-drying". Tissue segments of approximately 1 cm length were mounted on a support while presenting their full thickness (in the transverse direction) to the beam.

For each sample, 2000 projections were acquired, with a 0.18 degree angular step and over a total range of 360 degrees. Exposure time for each projection was 2 seconds. The projections have been corrected for detector gain and offset, and reconstructed via the standard Filtered Back Projection Formula in combination with the Paganin filter to account for the phase nature of the projections and the standard ramp filter. The reconstruction has been performed using the free pyHST (High Speed Tomography in PYthon) software (https://forge.epn-campus.eu/html/pyhst2/). No further image processing has been used post reconstruction. Subsets of the reconstructed datasets have been extracted showing transverse slices of the samples.

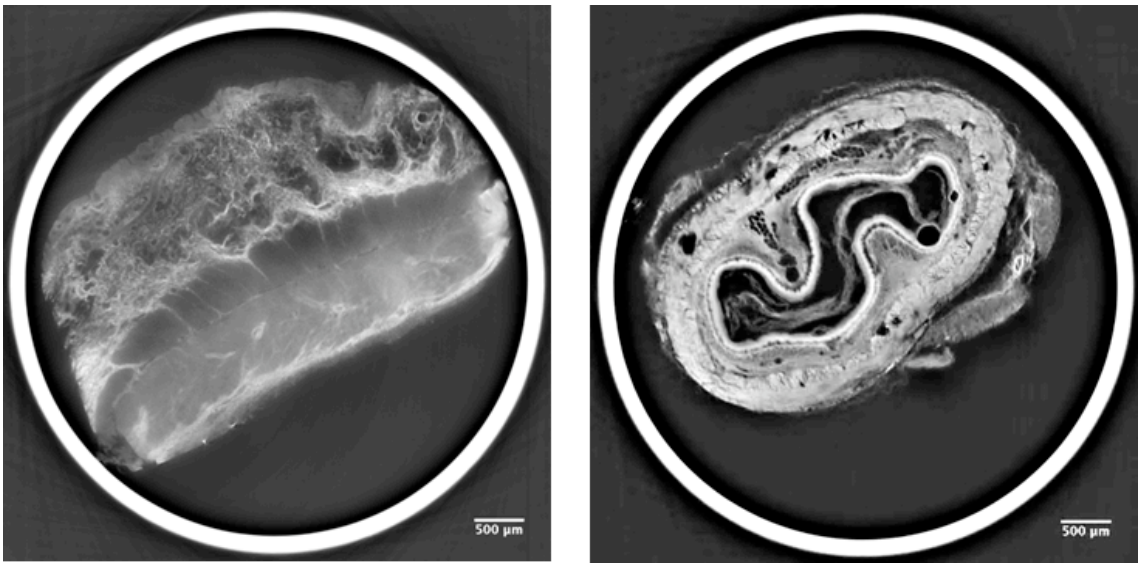

Figure 1. Reconstructed transverse slices showing acellular matrices obtained from a rat colon (left) and a rabbit oesophagus (right).

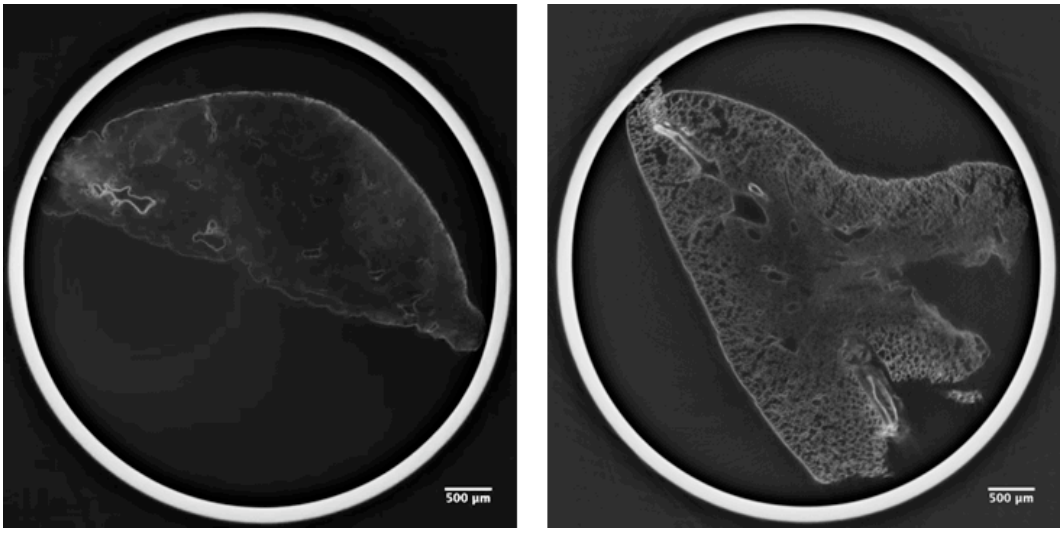

Figure 2. Reconstructed transverse slices showing acellular matrices obtained from a rat liver (left) and a rat lung (right.)

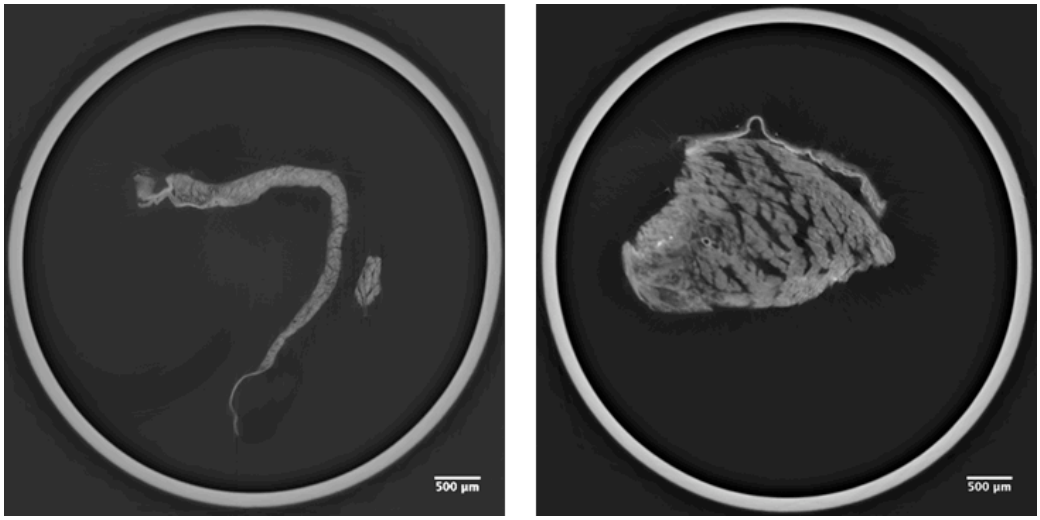

Figure 3. Reconstructed transverse slices showing acellular matrices obtained from rat muscular tissue (left: diaphragm, right: quadricepts).

Above we show some significant examples of the reconstructed CT images (figures 1-3). All images have been scored by clinicians. The feedback was extremely positive, in particular with respect to the high level of visible soft tissue detail. This enabled confirmation that all components are present and intact in the ACM; an important conclusion that has later been confirmed by histological analysis (not shown in this report). As an example of visualization of structural details, the image of the colon (fig. 1 (left)) has revealed the presence of villi on the inner surface of the acellular colon scaffold, a feature that is always present in a healthy colon and highly important for the digestive functionality of the organ. As an example of soft tissue contrast particularly appreciated by the clinicians, one should note the enhanced image contrast between similar tissue types present in the image of the oesophagus (fig. 1 (right)). Although almost identical in composition, the mucosa and sub-mucosa vary significantly in grey value, and can therefore be differentiated.
In conclusion, PC-CT imaging was applied to the ACMs of various organs obtained from a rabbits and mice, with the purpose to address the need for a fast, cheap, easily accessible, high resolution and high contrast 3D imaging modality. Propagation based imaging setup in tomographic mode was used to image the specimens, and volumetric datasets was reconstructed using basic CT reconstruction concepts together with a method-specific phase retrieval procedure (Paganin filtering). The resulting images have been assessed by clinicians and rated valuable with respect to the assessment of the structural preservation within the ACMs. The success of this proof-of-concept experiment opens the way to future developments which will include *in vivo* extension of the synchrotron-based method and ultimately translation to lab-based systems.