Analysing soft tissue calcification

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Aims

Normally the only calcified tissue in the body is bone. The actual mineral in bone is hydroxyapatite, a calcium phosphate. Mineralisation of bone is a carefully regulated process, and one of the limiting factors in this process is the availability of phosphate. The phosphate is generated by enzymes called phosphatases. They release the phosphate from phosphorylated proteins, or by cleaving a substance called pyro-phosphate into 2 phosphates. This last process has an additional stimulatory effect on phosphorylation, as pyrophosphate is a mineralisation inhibitor.

One of the phosphatase enzymes involved in the mineralisation of bone is phospho-1. Mice lacking this enzyme have poorly mineralised, soft, bendy bones¹. In addition, they also have inappropriate calcium phosphate deposits in soft tissues, most notably the aorta. Normally these calcifications are demonstrated by histological techniques. However, as they are sparse and randomly distributed, it would require serial sectioning and analysis of the entire aorta to reliably quantify the amount of mineralisation. Our aim was to visualise the soft tissue of the aorta and the mineralised deposits by µCT, and quantify the amount of mineralisation.

Method

The mouse aortas were carefully dissected out and cleaned of any adhering tissue and the blood rinsed out before fixing overnight in 4% buffered formalin, rinsed in PBS and stored in 70% ethanol. Although soft tissue has sufficient X-ray contrast in air, scanning hydrated soft tissue like these aortas in air leads to several problems due to drying out of the sample. First the drying process leads to shrinking of the sample, which creates movement artefacts in the scan. Second the drying results in poor morphology in the subsequent histological analysis. Scanning the samples in an aqueous medium leads to almost complete loss of contrast. However, oil has a different X-ray attenuation from water, and suspending the sample in oil prevents drying out. We therefore scanned the samples suspended in corn oil on a Skyscan 1172 µCT scanner. The fixation resulted in reasonable stiffness of the sample, and it was sufficient to keep the sample in place in a plastic tube with two plugs of parafilm to avoid sample movement. The inside of the aorta was carefully filled with corn oil using a 1 ml syringe fitted with a 28 gauge needle.

Scans were reconstructed using NRecon and analysed using CTAn and CTVol.

Results

For the first run, the aortas were scanned at µm resolution, 40 kV µA, no filter and a 0.3° rotation angle, scan time min. As shown in figure 1a this lead to sufficient contrast to distinguish the soft tissue. However, as shown in figure 1b, at these settings there are the distortions in the areas with calcifications, probably due to X-ray depletion. Scanning the same sample using a 0.5mm Al filter distinctly improved the appearance of the calcifications, however, the contrast of the soft tissue was rather poor, and the increased noise made thresholding problematic.
Figure 1: Mouse Aorta scanned in oil.
Fixed mouse aorta was scanned at a 10 µm resolution on a Skyscan 1172 system. 
A- C: no Al filter, 40 kV, 250 µA, 0.3° rotation angle, frame averaging=3. Scan time 33 min.
A: Projection image. B and C: Reconstructed images. Very good imaging of the vessel wall of the aorta, and clearly visible lumen of the blood vessel. Note imaging artefacts around the calcification in C.
D, E: 0.5mm Al filter, 60 kV, 167 µA, 0.3° rotation angle, frame averaging=3. Scan time 50 min. 
D: Projection image. E: Reconstructed image. Note improved imaging of the calcification, loss of contrast and increased noise.

The improve the contrast of the soft tissue, we incubated the sample for 5 min in a iodinated medical CT contrast agent, Iopamidol (final concentration 15% in water). The sample was rescanned using the 0.5 Al filter. This resulted in a clear contrast between the soft tissue and the oil, and good imaging of the calcium deposits (Fig. 2). An added benefit of the oil in this setting is that it prevents the contrast agent from dissolving out of the tissue, as it is not oil soluble.

Figure 2: Aorta scanned after incubation in contrast agent.
0.5mm Al filter, 60 kV, 167 µA, 0.3° rotation angle, frame averaging=3. Scan time 50 min.

The resulting images were analysed using CTAn. As shown in Fig. 3, the images were easily segmented using straightforward thresholding of the main two tissues: the calcifications and the soft tissue of the aorta. Using the 3D analysis of the custom processing tab in CTAn, we
were able to measure tissue volume (10.2 mm$^3$), calcification volume (0.09 mm$^3$) and the percentage calcified aorta tissue (0.88%). The individual object analysis allowed us to calculate the distribution of the calcification sizes (Fig. 4).

Figure 3: Thresholding of aorta and calcification. The reconstructed images allow for easy thresholding of the aorta (a) and the calcifications (b). The area in (a) shows part of the tissue without calcifications, allowing the setting of appropriate thresholds for soft tissue.

Figure 4: Analysis of the distribution of particle sizes.
Conclusion
Using a combination of corn oil as a scanning medium and an iodinated contrast agent we were able to visualise both the soft tissue of the aorta, and the calcifications. This method visualises the entire sample, and allows relatively rapid and easy quantification of the tissue mineralisation. As the method is non-destructive, the samples can still be used for additional histological analysis. The method of scanning soft tissue samples in oil may be useful for other specimens as well. If there are no additional x-ray dense particles (such as calcifications), the application of contrast agents may not be necessary.

References:
Three Dimensional Analysis of Cardiovascular Development in Mouse Embryos Using Micro-CT

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Aims
Micro Computed Tomography (µCT) has become an important tool in medical imaging due to its high resolution, non destructive nature and its ability to generate tomographic data. This makes it an ideal technique for examining mouse embryos in 3D, the motivation for imaging embryos comes from studies of their vascular system. Cardiovascular sciences play a major role in modern research as cardiovascular related diseases are the leading causes of death worldwide according to the world health organization. Embryos form a good model of study as we can witness the development of their vessels and alter their genetic to produce defects. Due to the morphology of blood vessels, 2D imaging modalities such as histology fall short but µCT provides the volumetric data required.

The aim of this study is to devise a suitable and simple method to introduce contrast in blood vessels with use of µCT. This can allow for useful comparison and quantitative analysis of embryos with normal and defective vascular systems. Iodine and barium are common agents used in radiography; these will form the basis of this study. Iodine can be dissolved and hence will be able to diffuse through tissue, whereas barium is insoluble but this is what gives its inherent specificity to blood vessels.

Method
Mouse Embryos. Embryos were derived from mice with alleles that produce offspring with a genetic defect that causes blood vessels to seep into the lymphatic system which normally does not occur. In this study only embryos without the defect were used as proof of principle. All animal experimentation was performed in accordance with UK Home Office Regulations. Females mated overnight with males were checked for the presence of a postcoital plug. Those found with a plug were considered to be at half embryonic day of development (E0.5). At the required days postcoitum, the mice were culled according to home office regulations and embryos extracted. After which, they were placed in cold phosphate buffer solution (PBS) for approximately 20 minutes and removed from their membranes.

Iodine Staining. E17.5 embryos were fixed in 4% paraformaldehyde (PFA) and washed to remove any residual PFA. The samples were dehydrated to increase the uptake of iodine. This is done by playing the embryos in a graded series of ethanol for one hour in each; 30%, 50%, 70% and another stage of 70% left overnight. After dehydration the embryo was placed in a 1%w/v iodine solution and left over two days at 4ºC then washed several times with 70% ethanol and stored in 70% until scanning.

Barium Staining. Two embryos at E14.5 and E16.5 were used for this method. Barium sulphate was ground to a fine powder by using a pestle and mortar and a 30% w/v PSB mix. Due to its insolubility, it requires direct injection into vessels. To ensure the particles of
barium were sufficiently small, the mixture was passed through a series of syringes each with decreasing diameter until it flows readily through an insulin gauge. Glass needles were made by placing capillary tubes over a Bunsen flame and pulled apart; this creates needles with much smaller diameters. The glass needle is then connected to the insulin syringe via a thin plastic tube. The vitelline vessels used for injection were those found on the umbilical cord as these are found outside of the main body of the embryo and was performed under a bright field microscope due to the minute sizes of vessels. After a sufficient amount of barium is injected, the embryos were placed in 4% PFA.

**μCT Scanning.** All scans were performed on a SkyScan 1172 high resolution micro-CT and images reconstructed with SkyScan NRECON 1.6.3.3. Image processing was performed using ImageJ such as mean filtering, thresholding and generating 3D renderings. Iodine and barium stained embryos were scanned with a pixel size of 4.08µm and 13.59µm respectively.

**Results**
The E17.5 iodine stained embryo showed very poor tissue contrast and most of the iodine solution has congregated on the surface of the specimen. Due to the age of the embryos the iodine proves difficult to penetrate into all other regions of the sample.

![Image](image1.png)

Figure 1: (a) Transverse slice of the abdominal region with the greatest intensity coming from the bone. (b) 3D rendering from the slices such as from (a), this detail of the surface can be seen due to the aggregation of iodine onto the skin. (c) The same 3D rendering angled to show the internal structure. Scale bar: 1mm

The barium injected samples have shown good specificity to major and minor blood vessels but have shown some leakages and ruptures due to the injections. Due to the greater attenuation of barium, the images can be rendered without any soft tissue through simple thresholding. The ruptures of contrast agent were removed by applying basic filters in ImageJ. This would cause a problem in areas where there should be large amounts of barium such as the heart but the main feature of this study if for the vessels. Quantitative measurements can also be made such as blood volume and length or width of specific vessels by simply counting the voxels or pixels.
Figure 2: (a) E14.5 embryo injected with barium, major vessels can be seen from the surface and accumulation in the primordial kidney region (white arrow). (b) Reconstructed image of E16.5 embryo, complete removal of soft tissue can be seen along. Note the large leakage in the abdomen. (c) Reconstructed image of (b) using a mean filter showing removal of large deposits of barium. Scale bar 1mm

**Conclusion**

The iodine stains are much simpler but lacks specificity with its ability to diffuse through tissue, whilst the bariums is technically more challenging this yields interesting results. To improve the problem of barium ruptures this should be done preemptively through the injection process such as using micro manipulators or anti coagulants to prevent blood clots etc. Never the less these results demonstrate that the vasculature of embryos can be isolated and imaged successfully with relative ease. This may allow for further studies with specimens that have abnormalities for comparative review.
Monitoring of *Cryptococcus* lung infection with micro-CT and micro-MRI

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**Background**

*Cryptococcus* is an encapsulated yeast that causes life-threatening disease in both immuno-competent and immuno-suppressed individuals. The two predominant pathogenic strains of this yeast, *Cryptococcus neoformans* and *Cryptococcus gattii*, are generally found in soil, bird excrement and in the bark of certain trees and enter the host via inhalation. Cryptococcosis mostly affects the lung of a host and may spread to the brain, manifesting itself by cryptococcal meningitis and/or pseudocystic lesions (cryptococcoma) in the brain often with fatal outcome. It remains still unknown why, how and when the cryptococci are able to cross an apparently intact blood-brain barrier (BBB). Histological techniques will remain essential to confirm and unravel cellular and molecular interactions, but imaging techniques are indispensible to define the relevant time frames for histological analysis and this for each animal individually, to investigate crucial events in pathogenesis. As currently imaging tools to evaluate pneumonial cryptococcosis with good temporal and spatial resolution *in vivo* are lacking, advances made in lung µCT and µMRI techniques to follow-up disease progression non-invasively will greatly enhance the cryptococcosis research.

**Aims**

It is our objective to dynamically monitor cryptococcosis non-invasively in individual animals, in a mouse model for pneumonial and cerebral cryptococcosis. Thereby, we will establish the kinetics of cryptococcal lung infection with µCT and µMRI and the time profile of *Cryptococcus* spreading to the CNS, thereby defining the most critical time points for histological and immunological analysis of key events in the pathogenesis of cryptococcosis.

**Methods**

Balb/C mice were infected by inhalation of a *C. gattii* R265 cell suspension (500 cfu’s) in PBS (n = 7) or with PBS alone (control, n = 3). Mice were scanned with MRI and CT at baseline and weekly until 45 days post infection. **MRI images** were acquired on a horizontal Bruker Biospec (9.4T, 20 cm) in combination with a 7cm quadrature coil using a retrospectively gated FLASH sequence IntraGate (Bruker Biospin, Ettlingen, Germany) with the following parameters: TR/TE = 30/1.26 ms, 17 deg flip angle, 5 slices covering the lung, slice thickness 1 mm and gap of 0.5 mm, FOV = 4 cm x 4 cm, matrix 256 x 256, in plane resolution of 156 µm, 80 repetitions resulting in a 10 min acquisition; the navigator slab was 1cm wide excited with a 0.8 ms sinc10H pulse with a 1.5 deg flip angle. For reconstruction, 70% of the respiration and ECG period was used (Paravision 5.1, Bruker). **CT images** were acquired on a dedicated small animal µCT scanner (SkyScan 1076, Kontich, Belgium) with the following parameters: 50 kV, 0.5 mm Al filter, 200 µA source current, 35 µm isotropic resolution, 120 ms exposure time, 9 projection images per 0.7° rotation step and
retrospectively gated. Image analysis, segmentation and quantification of CT data were performed with custom written algorithms using SkyScan software. After the last time point, mice were sacrificed followed by sterile CNS removal and lung isolation for histological analysis (HE and PAS-stainings) and quantification of fungal load.

Results
Pneumonial cryptococcosis was successfully and reproducibly induced in immunocompetent mice. While the mice showed no phenotypical signs of cryptococcosis, the progression of the lung pathology could be non-invasively visualized using the here evaluated protocols for IntraGate MRI and µCT at different time points post infection (see figure 1). After optimization of quantification protocols, MRI and CT images were quantified (total lung volume, aerated lung volume ...) and the data correlated for cross-validation. Cryptococcal invasion of the lung and brain (meningitis) was confirmed by histochemical analysis (see figure 1, last panel), lung fungal load was quantified and compared with CT and MRI data.

Figure 1: Imaging cryptococcal lung infection with MRI and CT. MRI (first column), µCT (second column) and histology (PAS-staining, third column) images of a mouse 6 weeks post instillation of a Cryptococcus suspension (first row) or PBS (second row). White arrows point at cryptococci in the lung tissue.

Conclusions &Perspectives
To the best of our knowledge, this is the first study showing that non-invasive monitoring of pneumonial cryptococcosis is feasible with retrospectively gated µMRI (Intragate, Bruker) and µCT resulting in high resolution and contrast images. This imaging approach will allow longitudinal screening of animals, without radiotoxicity concerns when using the MRI, and with even better resolution when using the CT, thereby visualizing infection onset and progression on an individual basis and far before the appearance of any phenotypical signs of disease. We will further finetune the timing of disease onset and correlate this with the time of traversal of Cryptococcus cells to the CNS. MR imaging of cryptococcosis will greatly help unraveling the still enigmatic pathogenesis of this life-threatening disease.
References:
Multi-modality approach to ameliorate stereotactic brain surgery planning in rodents

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Background
Stereotactic neurosurgery has applications in various domains, like therapeutic stem cell transplantation and Deep Brain Stimulation (DBS). The latter is stimulation by implanted electrodes and is applied worldwide in therapeutic-resistant patients with movement and affective disorders. In humans, preoperative planning, to avoid hitting blood vessels in the brain, is an important part of the intervention as risks of side effects, such as bleeding, are significant. Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) are used to image the brain vasculature for this purpose. Despite the fact that this information is equally important in pre-clinical neurosurgical research, extensive imaging isn’t always attainable in animals. Instead, a 2D atlas of the brain is widely used to plan stereotactic surgery in rodents. Although providing good anatomical information, this method lacks sufficient information regarding neurovasculature. Thus stereotactic surgery could lead unintentionally to deleterious effects when hitting a blood vessel, followed by morbidity or even mortality, and decrease in validity of the study.

Aims
The focus of the current study is to develop a probabilistic atlas of the rat brain vasculature, which accounts for inter-individual heterogeneity in blood vessel architecture, to lower the chance of bleeding during brain surgery. To obtain this, a multi-modality approach, using MR and CT, is being used to combine anatomical and vasculature information. Unraveling the vascular anatomy is difficult and the choice between MR Angiography (MRA) or CT Angiography (CTA) remains a matter in dispute. This study aims to clarify when the two approaches differ, conform or add each other. Assessment of the intra-strain variability in vasculature and the relative position to the Bregma skull reference point contribute to the quality of the module. The atlas will be validated and used for preoperative neurosurgical planning in rodents and defines the risk of damaging a blood vessel in any user-defined electrode trajectory.

Methods
The animals used in this study, were all adult male Wistar rats with weights ranging from 270-310g. In vivo images were acquired under general isoflurane anaesthesia. In vivo 3D anatomical MR, reconstruction resolution 117x153x148µm, was used to normalize angiographic images to common space. 2D multislice MR angiography (MRA, FLASH-TOF, flow compensated) was acquired in both axial and coronal orientation, with an in-plane resolution of 59µm. All MR images were acquired pre- and postoperative. In vivo CT skull images were acquired on a SkyScan 1076 small animal CT scanner. Image parameters were 35 µm isotropic resolution, 49 kV source voltage, 200 µA source current, 0.5 mm Al filter, 180 ms exposure time, 0.8° rotation step, 2 averages and 2 connected scans to cover the complete rat skull length. Requirements for these images were clear visualization of coronary, sagittal and lambdoid suture, which make determination of Bregma and Lambda possible. Ex vivo contrast enriched CT angiography images were acquired as ground truth for in vivo MRA. An injection of 400 units heparin in the tail vein was given before the animals were sacrificed by administration of an overdose Nembutal. Transcardial perfusion with heparinized sucrose solution in dH2O, in order to flush the blood away, followed by
Paraformaldehyde (4% in PBS) to fix the tissue and a final flush with sucrose solution in dH2O, was performed before injecting the contrast agent. The contrast agents investigated were BaSO₄ in 2g gelatin solution or liquid latex in different ratios which were all supplemented with 5% black indian ink. Also a commercial available contrast-agent, Microfil, was used. A blunt needle, fixed in the aortic arch, was used for perfusions with contrast enriched mixtures after which both aortic arch and vena cava superior were clamped to prevent any leakage. Ex vivo dual-energy CT (DECT) images were acquired with parameters 18µm isotropic resolution, 48 kV/100 kV source voltage, 200/100 µA source current, 0.5/1.0 mm Al filter, 1000ms exposure time, 0.6° rotation step, 3 averages and 2 connected scans to cover the complete rat skull length. Before coregistration of the images, a RF intensity inhomogeneity correction was applied to MR images using our in-house developed software. CT images were coregistered to the RF intensity inhomogeneity corrected MRI images, before warping to a common atlas template. Intra-subject registration of images, MR-MRA, MRA-CTA, CTA-CT, was performed via a rigid registration with a histogram based mutual information (MI) similarity measure. All images were warped to common Paxinos space using an affine, maximizing MI similarity based transformation which preserved the normal anatomical variability in the population. The risk of perforating a vessel was then calculated as being reversed proportional to the Euclidean distance between vessels and electrode. This information is presented to the user through an interface in which the user can define stereotactic coordinates and gets the optimal surgical path and the associated risk as output. (figure 1) Using the coordinates for an assumed risky and safe trajectory, stereotactic surgery under general anaesthesia using Ketamine and Domitor.

Results
By combining MR and CT data, we provide for the first time information about the relative position of Bregma to neurovasculature. Vasculature was most consistently visualized with 30% BaSO₄ in 2g gelatin solution (figure 2). Connectivity, number of branches visualized and easy practical use, made this mixture superior to others. Histology proves no extravasation of the contrast agent, which prevents overestimation of vessel size. Intra-strain vascular variability of large vessels appears minimal, which indicates that the use of a probabilistic vasculature atlas for neurosurgery planning could be useful. By co-registering both in-vivo and ex-vivo images in atlas space, we present a 2D automatic ray casting approach in which the risk of a potential user-defined trajectory is displayed. By choosing a calculated safe trajectory, of which entry point, angle and depth can be deducted, the risk of morbidity or even mortality upon neurosurgery can be minimized. Performing MRA acquisition for both axial and coronal orientation proves useful as more details are visualized in the combined image. But MRA nor CTA protocol are yet sufficient as a stand-alone technique. The two techniques complement each other. The module is currently being validated by evaluating risky and safe trajectories by stereotactic surgery.

Conclusion & Perspectives
The presented study is still a work in progress but has the potential to improve validation of pre-clinical neurosurgical studies on rodents. A general conclusion about imaging is that the brain vasculature can be visualized but protocols for both modalities, CTA and MRA, are not sufficient as a stand-alone technique yet. Ex-vivo contrast-enhanced CT angiography is difficult and results can differ enormously. Though, the described mixture offers consistent results combined with the proposed DECT protocol. Stereotactic surgeries already performed were not able yet to make a distinction between a safe and risky trajectory. Problems to address are the noticed variability in coordinates of planned and performed electrode injection. This variability increases proportional with the angle of insertion and researcher dependent error cannot be excluded. Intra-strain variability appears minimal though this needs to be objectively quantified to justify usage of a reference atlas. Current research involves the implementation of more subjects, as well as postoperative validation of the method through imaging, behavioral and motor-function studies. Important conclusions about usage of Bregma reference point can be drawn from currently performed experiments which
investigate intra- and interobserver variability in determination of this reference point. Ongoing experiments will further expand and increase the quality of this new method. The research performed also offers important insight in the validity of atlas-guided, stereotactic neurosurgery in preclinical research.

References

Figure 1 with courtesy of Ir. J.R. Rangarajan. In-house developed module to visualize calculated safe and risky trajectories. These trajectories are presented as colored lines with thickness equal to electrodes used. The module can combine MR(A) and CT(A). The future atlas incorporated in this user-friendly module can be used in pre-operative planning. Figure 2 Dual-energy CT with BaSO₄ in gelatin solution, allows to visualize neurovasculature in proportion to Bregma.
Multiscale lung imaging

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Aims
High-resolution computer tomography (HRCT) allows acquiring 3D image stacks of lungs in patients with a sub-mm resolution leading to a better understanding and categorization of diffuse interstitial lung diseases (ILD), a group of lung disorders in which the deep lung tissues become inflamed. HRCT is currently an important aid in the diagnosis and follow-up of patients. The radiologist’s interpretation of HRCT images relies on the recognition of subtle patterns in the lung tissue. Many of these patterns are currently inventoried and for several of them the correlation with pathology is already known. There are, however, still many pathological conditions that are not visible on CT images despite the increasing image resolution. Moreover, several conditions are indirectly visible but cannot be related to the pathology because the deviating features are above the physical resolution limit of a clinical CT. Ground-glass opacity (GGO) is an example of this where the CT images show a brighter smeared-out region which may either be the result of air space disease (filling of the alveoli) or interstitial lung disease (i.e. fibrosis). Knowing that 60-80% of patients with ground-glass opacity on HRCT have an active and potentially treatable lung disease, while in the other 20-40% of the cases the lung disease is not treatable and GGO is a result of fibrosis, it proves the importance of a better understanding of the patterns visible on HRCT. Already closer to the microscopy resolution, X-ray micro-CT allows to make image stacks of the lung parenchyma within the micrometer resolution range¹ when looking at small samples. In this study, the main goal is to register the different image stacks, from patient to micro-CT, in order to bridge the resolution gap between HRCT and microscopy.

Method
Explant lungs were obtained from patients who got a donor lung because of interstitial lung disease. These explant lungs were frozen after inflation and cut in 2 cm thick sections. HRCT images were taken of the patient before lung transplantation, of the full explant lung after operation and of the sliced explant lung using a Siemens Sensation system of the University Hospital of Gasthuisberg in Leuven. The HRCT images (512 x 512) have a pixel size ranging from 0.742 mm to 0.575 mm with a slice thickness of 1 mm and are shown in Figure 27.

![Figure 27: (a) Pre-operative HRCT scan of the patient, (b) Registered explant lung, (c) HRCT of the sliced explant lung, the holes are from the cylindrical samples taken for X-ray micro-CT imaging.](image-url)
Out of the frozen lung sections, small cylinders of ~2 cm length and with a diameter of ~ 1.5 cm were extracted and fixed with glutaraldehyde-aceton, dehydrated with ethanol and hexamethyldisalazane followed by air-drying. These lung samples were then imaged using the SkyScan 1172 high resolution X-ray micro-CT system. To obtain a good contrast in this soft tissue material, a voltage of 40kV was chosen without filtering the X-ray beam. The camera binning was set at 2000 x 1000 pixels and, with a pixel size of 8.4 micron, two connected scans were needed to cover the length of the cylinder. The exposure time per frame was set at 295ms and 4 frames were taken at every rotation step of 0.2°, in short scan mode. The reconstruction of the images was done using NRecon. The micro-CT result of one cylindrical lung sample is shown in Figure 28.

![Figure 28: Lung sample taken from an explant lung of a patient with BOS disease scanned with SkyScan 1172 (8.4μm pixel size)](image)

In order to combine the CT data from the medical HRCT system and the X-ray micro-CT on a single platform, which will be used in the hospital, it was necessary to convert the reconstructed micro-CT images to the standard medical format, DICOM.

**Results**

For one patient with BOS disease (Bronchiolitis obliterans syndrome) all the different datasets were obtained. BOS is a rare and life-threatening form of non-reversible obstructive lung disease in which the bronchioles or small airway branches are compressed and narrowed by scar tissue, also called fibrosis, and/or inflammation. Fibrosis results in stiff and X-ray dense regions in the lungs as can be seen in Figure 28.

In the first step of combining the different datasets, the patient images need to be registered with the full explant lung. Even after inflation of the explant lung, the shape and size does not match the patient data. In Figure 29 the volume rendered lungs are shown of the patient (green) and the inflated explant lung (red). Since these differences are from a different nature than the normal inspiration-expiration cycle, a rigid registration was chosen in order to avoid incorrect deformations on the explant lung.
In order to register the two datasets the patient lung was segmented using region growing. A principal component analysis (PCA) was performed on the isosurfaces of the segmented lungs leading to an automated registration procedure. In Figure 27 (a,b) the registered datasets are shown.

In a second step the sliced lung was registered with the full explant lung, in order to find the position of the extracted cylindrical samples, which were imaged using X-ray microtomography. The holes in the sliced lung were segmented and indicated on the explant lung.

In a last step the micro-CT results were visualized next to the corresponding region in the explant lung and patient data. This allows us to get an accurate view on the anatomy and pathology of multiple sections of the lungs on both HRCT and micro-CT scale, which will help us understand the correlation with the deviating features only indirectly visible on the HRCT images. In Figure 30 a screenshot is shown of the platform integrating the different image data. Using this platform we can zoom in and out at the locations where micro-CT images are available and better understand the pathology behind the abnormalities visible on HRCT.
Conclusion
This first step in the joint visualization of clinical HRCT and X-ray micro-CT images already allows the medical expert to better study interstitial lung diseases and proves micro-CT to be an interesting tool to bridge the gap between medical CT and microscopy.

Acknowledgements
This research was done within the ICON-IBBT AIR project. AIR stands for “analysis of images from the respiratory system” and is focused on the development of a number of innovative imaging, image reconstruction and analysis techniques for lung diseases. Research groups involved are MiC – KULeuven, Visionlab – UA, ETRO – VUB and SMIT – VUB. The industrial partners are SkyScan, Philips Medical Systems, UZ Brussel and UZ Gasthuisberg

References:
Detection of Lung Metastasis of Cancer Cells in Mice Using High Resolution Micro Computed Tomography

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Aims
For cancer research and chemotherapeutic drug development, non-invasive detection of the progress of cancer lung metastasis in live animal is needed. In this study, we compared wild-type (WT) mice and a certain gene knockout (KO) mice, which we supposed to be easier for the metastasis of cancer cells into lung tissue by using micro-CT.

Method
Animal and Cell culture
Female C57BL/6 mice (WT) or a certain gene KO mice were aged 5 to 6 weeks, weighing 20-25g. They were provided free access to food and water at room temperature. In cell culture, LL/2 murine lung carcinoma cells were maintained in the humidified incubator (5% CO₂, 37°C) in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT), 100 U/Ml penicillin and 0.1 mg/mL streptomycin (Invitrogen, Carlsbad, CA). To compare the metastasis of WT mice with that of knockout mice, LL/2 cells (6 × 10⁴ cells / mL) were injected into the femoral vein of mice. Two–Three weeks later, the mice were subjected to micro-CT scanning and then sacrificed for the count of lung nodules.

Micro-CT imaging of mice
The mice were anesthetized with isoflurane and scanned by Skyscan 1176 (Kontich, Belgium) at 35 μm resolution (50 KeV, 497 μA, 0.5 mm aluminum filter, 50 ms exposure, 0.8° per image, image averaging = 2) with respiratory synergism (5 ms after respiratory signal). At the end point of study, mice were sacrificed by overdose of trichloroacetaldehyde (TCA) and rapidly tracheotomised. 1 ml of air was injected into lung and the sacrificed mice were then scanned.

Results
To monitor the tumor metastasis into lung tissue in wild-type (WT) mice and gene knockout (KO) mice, living mice were scanned by Skyscan 1176 at week-3 after intravenous cell injection. Micro-CT imaging showed that the metastasis of LL/2 lung carcinoma is more prominent in gene KO mice than in wild-type mice (Fig. 1A). It was also found that there were more tumor nodules on lung surface in KO mice than in wild-type mice (Fig 1B). To gain better resolution of images, mice were sacrificed and scanned again. As shown in Fig.2, a gene KO mouse with moderate cancer lung metastasis was scanned representatively. Two tumor colonies were found at the bottom of lung space (Fig. 2A). Fig. 2B (left colony) and Fig. 2C (right colony) showed coronal and sagittal images of these tumor. The whole lung pictures were shown in Fig. 2D.
Figure 1: Severe cancer lung metastasis in gene KO mice
(A) Micro-CT imaging (Coronal and sagittal) in living animals showed that the lung space of gene KO mice was critically occupied by tumor tissues and less severe metastasis was found in wild-type mice. (B) More tumor nodules were found on the surface of lung in gene KO mice. Scale bar: 1 mm.

Figure 2: Micro-CT images of mice after sacrifice
(A) The transaxial image revealed two tumor colonies at the bottom of lung space in gene KO mice. (B and C) Micro-CT images showed the phenotype of metastatic tumor. (D) Representative images photographed using dissecting microscope. V: ventral view, D: dorsal view

Conclusion
For the detection and monitoring of the progress of lung metastasis in living animals, micro-CT can provide imaging for studies such as cancer research and anti-cancer drug development. Micro-CT is also suitable for monitoring the spontaneous lung cancer or lung metastasis of cancer cells which are not labeled with luciferase or fluorescent dyes.

References:
Calcium determination of CTO in samples from patient and animal model using Micro-CT

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Aims
Chronic total occlusion (CTO) can occur in the coronary arteries increasing the risk of major cardiac events or in the peripheral arteries (e.g. femoral or popliteal arteries) leading to intermittent claudication or amputation. Percutaneous revascularization with angioplasty and stents could improve patient outcomes, including quality of life. However, calcification in CTO is a significant obstacle to revascularization. The aim of this study was to determine the extent and distribution of calcification within CTO using high resolution of micro-CT and also to compare the calcification characteristics in CTO between patients and an animal model. Greater understanding of calcification could be beneficial to design of new clinical treatments to increase the successful revascularization of CTO.

Method
A total of 6 human peripheral artery CTO samples from amputation and 5 rabbit femoral artery CTO samples were included.
1) Amputation samples: Peripheral blood vessels were flushed with saline then injected with Microfil (Flowtech, Carver, Massachusetts).
2) CTO model: The femoral arteries of New Zealand White rabbits were injected thrombin to create the CTO [1]. Rabbits received calcium carbonate (75mg/day), vitamin D (50,000 units/day) and a high cholesterol diet (0.5%/day). In addition, bone morphogenetic protein (BMP-2), dipotassium phosphate, and calcium chloride were injected into the site of femoral occlusion. After 12 weeks the rabbits were euthanized; the rabbit femoral arteries were treated in a manner similar to the amputation samples washed with saline followed by a microfil perfusion. When the microfil solidified (in 90 min), the vessels were isolated and excised carefully.
All samples were embedded in 2% Agar and scanned using a GE Healthcare Explore SP (London, Ontario) at a spatial resolution of 14 um. The three dimensional (3D) volume images were reconstructed and the analysis was conducted in the Amira 3D visualization software for windows (Visage Imaging Inc., Andover, MA) [2]. The samples from rabbit model were cut for histological study.

Results
We have successfully demonstrated calcification within CTO three-dimensionally both in patients and in the animal model. The micro-CT data sets provided detailed information of the calcium distribution within CTO of peripheral. The calcifications were deposited irregularly around the wall of the blood vessels (Fig. 1). A cross-sectional image of the CTOs indicated that the calcium was deposited around the lumen with different thicknesses (Fig. 2). It is clearly shown that the characteristics of the calcification of CTO in the animal model were similar to that in the patient samples (Fig. 3); tube-like structures of calcium were distributed irregularly around the walls of the blood vessels in both groups. Furthermore, the results from micro-CT matched well histological results (Fig. 4). One limitation is that the calcium and microfilm in the lumen of the vessels may have some overlapping characteristics in micro-CT because of similar x-ray attenuation characteristics.
Figure 1: Micro-CTs of peripheral CTO arteries from a patient. Left: Peripheral artery wall shown in purple and the calcification indicated in red. Right: the calcification is shown in red and the lumen is shown in yellow.

Figure 2: Cross-section from a micro-CT of a peripheral artery; blue line in left image indicates the location of the cross section; red in right panel shows the irregular calcification on the wall of the blood vessel. 3D Vessel wall is shown in light blue.

Figure 3: Sample from the femoral artery of a rabbit. Left: A femoral artery with branches in yellow and with the calcification in the CTO shown in dark red. Right: Zoomed segment of CTO, calcium shown in red (irregular calcification in the wall of the vessel) and blood vessel outline shown in grey.
Conclusion
High resolution micro-CT is a valuable and reliable technique to detect the calcium within a CTO. The 3D images demonstrated in fine detail the calcium distribution both in patients and in an animal model. Our animal model of calcification within CTO demonstrated characteristics of calcification similar to that in the patient samples. These results could provide important information for designing novel treatments for patients with CTO; this animal model provides a foundation for associated basic and clinical research.

References:
Structural analysis of calcified aortic valve deposits by microtomography

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Aims

Mineralization is a physiological process, which consists in the deposition of minerals in an organic matrix. Minerals are composed mainly of calcium phosphate (hydroxyapatite crystals). Mineralization is a complex and multistage process requiring an interaction of many physicochemical factors. The mechanism responsible for deposition of minerals has not been fully elucidated, yet. Mineralization can be a pathological process, when it develops outside osseous tissue. Mineralization in aortic valves (AVs) is an example of such process. Degree of AV mineralization depends on many features, including the characteristics of the valve itself. It is commonly accepted [1] that the first stage of the aortic valve calcification requires the precipitation of small mineral seeds within tissue structures. Next, the seeds grow and ripen that leads to the massive mineralization and to the valve destruction. A number of factors, alone or in synergy, may contribute in the initiation and further development of the process. A large number of anti-calcification treatments have been tested [2]. Until now, however, no method has proven its efficacy in clinical applications [3]. Recent data suggest that soft tissue mineralization is a process similar to osteogenesis [4]. Therefore, some authors proposed the hypothesis that soft tissue mineralization and bone calcification may be influenced by similar factors. Many studies have been conducted to investigate chemical composition of mineralized AV cusps [5,6]. Microtomography offers unique capabilities for studies of localization, shape and distribution of calcified deposits in aortic valve cusps. Results obtained with this method can provide a key link between the type of calcified deposits and specific pathologies of aortic valves.

Method

The studies were conducted on 21 aortic valves excised during aortic valve replacement. Aortic valves were collected 1 hour after operation and stored in Parker's Medium 199 (Gibco). Before measurements, the samples were taken out and washed in physiological saline. AV were collected from 11 patients with aortic stenosis, additional 4 patients suffered also from inflammatory states and 6 patients from vulvular incompetence and aortic aneurysm.

Computer microtomography (µCT) was applied to establish geometrical dimensions localization and structure of the AV calcifications. Additionally, X-ray fluorescence (XRF) was used to find out the elemental composition of the samples. Moreover, chemical content of the cusps' mineral was characterized by infra-red spectroscopy (IR). The samples were scanned with a Skyscan 1172 microtomograph (Skyscan, N.V, Belgium). The scanner was equipped with an X-ray detector: 11 Megapixel (4024 x 2680 in total; 4000 x 2400 effective), 12-bit digital X-ray camera with 24 x 36 mm field of view. The X-ray source voltage was set to 40 kV and current to 100 µA. No filter was used during data collection. The image pixel size was 13 µm. The projection images were acquired over an angular range of 180° with an angular step of 0.5°. Projections were reconstructed using a conebeam reconstruction software (Nrecon SkyScan, Belgium) based on the Feldkamp algorithm.
Subsequently, binarization was performed (CT-Alanyzer, SkyScan, Belgium) based on density distribution histograms in the whole sample volume. This procedure allowed for determination of geometrical parameters of deposits and valve tissue, and for creation of 3D models. The results were presented using CT-Volume program (SkyScan, Belgium). After tomographic measurements, the calcified deposits were extracted from the cusps, dried under vacuum and ground in a mortar. The powder was used to XRF and IR examinations. The elemental compositions of the cusps were determined using the energy dispersive X-ray fluorescence method (ED-XRF). The Oxford 2000 spectrometer (Oxford Instruments, UK) was applied. The spectrometer was equipped with silver anode X-ray tube and Si(Li) detector (area 30 mm², thickness 3 mm, and 8 μm thick Be window, FWHM at 5.9 keV = 145 eV). The working conditions were: the tube voltage 5 (for light elements) and 35 kV (for heavy elements). The tube current was 1 mA and 250 μA. Procedure for calculation of elemental concentrations was described earlier [7].

Fourier transform infrared (FTIR) spectra were acquired on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, USA). Spectra were recorded for KBr tablets. Each tablet was made from the dry powder prepared using approximately 2 mg of calcified tissue per 200 mg KBr. A total of 32 scans were acquired with a spectral resolution of 4 cm⁻¹. The complex bands have been deconvoluted, and curve fitting was performed using an Origin software (OriginLab, USA) to calculate the following parameters [8]:

1. mineral/matrix ratio - integrated area for phosphate (900–1200 cm⁻¹)/Amide I (1585–1720 cm⁻¹) bands.
2. carbonate/phosphate ratio - area for carbonate band (865–880 cm⁻¹)/phosphate
3. collagen maturity - intensity ratio of subbands at 1660/1686 cm⁻¹

Results

A representative AV calcium deposit X-ray spectrum is presented in Fig. 1. The following elements were present in the cusps: P, S, Cl, K, Ca, Fe, Cu, Zn and Sr. It should be noted that the above-mentioned elements were detected in all AVs, while Mn, Ni, Rb and Br were observed only in some samples. Average concentrations of the elements in the samples are shown in Table 1. Standard deviations did not exceed 26%, while for elements actually building the deposits (Ca, P), SD did not exceed 13%. SD for the Ca/P ratio, which is commonly considered to be an indicator of mineral maturation in tissues was lower than 4%. These results indicate that calcium deposits in AV cusps had the same chemical composition. Similar results were obtained using IR spectroscopy. A representative IR spectrum for a calcified deposit in a cusp is presented in Fig. 2. Based on the estimated parameters (Tab. 2), it was concluded that the contents of the main chemical groups: PO₄, CO₃ and collagen (Amid I) did not differ by more than 20%. Moreover, the data from spectral database indicated that the deposits were composed mostly of hydroxyapatite and carbonatehydroxyapatite and of collagen. IR spectra showed also the presence of CaCO₃. Therefore, calcium deposits in AV cusps at advanced stage of calcification did not differ in terms of chemical composition, however, there were conspicuous differences between the structure of the deposits and their distribution in soft tissue, as can be seen Fig. 3. Based on microtomographic data, statistically significant differences in geometrical parameters were observed between aortic valves excised from patients.
Figure 1: A representative characteristic X-ray spectrum of a calcified deposit extracted from an aortic valve cusp.

Table 1. Average elemental concentrations in deposits from AV cusps. Min and Max indicates the lowest and the highest concentration, AVG = average value, SD = standard deviation.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>9.37</td>
<td>4333</td>
<td>1232</td>
<td>134</td>
<td>18.14</td>
<td>31.67</td>
<td>0.37</td>
<td>130</td>
<td>44</td>
<td>1.77</td>
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<tr>
<td>Max</td>
<td>14.60</td>
<td>6001</td>
<td>4374</td>
<td>427</td>
<td>28.18</td>
<td>53.40</td>
<td>3.88</td>
<td>295</td>
<td>95</td>
<td>2.06</td>
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<tr>
<td>AVG</td>
<td>11.97</td>
<td>4248</td>
<td>2955</td>
<td>349</td>
<td>22.36</td>
<td>37.37</td>
<td>1.86</td>
<td>196</td>
<td>69</td>
<td>1.87</td>
</tr>
<tr>
<td>SD</td>
<td>1.61</td>
<td>684</td>
<td>770</td>
<td>62</td>
<td>2.84</td>
<td>9.57</td>
<td>0.49</td>
<td>49</td>
<td>16</td>
<td>0.07</td>
</tr>
<tr>
<td>100*SD/AVG</td>
<td>13</td>
<td>16</td>
<td>26</td>
<td>18</td>
<td>13</td>
<td>25</td>
<td>26</td>
<td>25</td>
<td>23</td>
<td>4</td>
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</table>

Figure 2: A representative infrared spectrum of a mineral deposit from an AV.

Table 2. Parameters determined from IR spectrum.

<table>
<thead>
<tr>
<th></th>
<th>Phosphate/Amid I</th>
<th>Carbonate/Phosphate</th>
<th>Collagen Maturity</th>
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<tr>
<td>Min</td>
<td>1.28</td>
<td>0.025</td>
<td>1.05</td>
</tr>
<tr>
<td>Max</td>
<td>2.50</td>
<td>0.048</td>
<td>2.88</td>
</tr>
<tr>
<td>AVG</td>
<td>1.96</td>
<td>0.035</td>
<td>1.87</td>
</tr>
<tr>
<td>SD</td>
<td>0.26</td>
<td>0.007</td>
<td>0.35</td>
</tr>
<tr>
<td>100*SD/AVG</td>
<td>13</td>
<td>19</td>
<td>19</td>
</tr>
</tbody>
</table>
suffering only with aortic stenosis and those diagnosed with aortic stenosis accompanied by inflammation. No differences were found between AV of patients with stenosis and those with stenosis and vulvular incompetence and aortic aneurysm. Statistically significant differences were identified using an analysis of variance (ANOVA) with 99 % confidence level. Average values of geometrical parameters are presented in Tab. 3. Aortic valve cusps that originated from patients suffering only from aortic stenosis contained massive calcified deposits characterized by high-density calcification foci visible in tomographic images and lower-density mineral infiltrations with concentric cusp hypertrophy. Mineralization developed mostly in the cusp base (Fig. 3 A). In AV from patients with accompanying inflammatory states, mineralized deposits were tiny and were scattered along cusp edges (Fig. 3 C, D).

![Figure 2: Calcium deposits in aortic valve cusps. A – AVs from patients with aortic stenosis, B - AVs from patients with aortic stenosis with accompanying inflammatory states.](image)

Table. 3 Geometric parameters of calcified deposits calculated based on microtomographic measurements.

<table>
<thead>
<tr>
<th></th>
<th>Volume of calcified deposits [mm³]</th>
<th>Surface of calcified deposits [mm³]</th>
<th>Surface / Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVs from patients with aortic stenosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVG</td>
<td>236</td>
<td>1310</td>
<td>6.19</td>
</tr>
<tr>
<td>SD</td>
<td>183</td>
<td>690</td>
<td>1.54</td>
</tr>
<tr>
<td>100*SD/AVG</td>
<td>76</td>
<td>53</td>
<td>25</td>
</tr>
<tr>
<td>AVs from patients with stenosis accompanied by inflammatory states</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVG</td>
<td>175</td>
<td>777</td>
<td>4.44</td>
</tr>
<tr>
<td>SD</td>
<td>88</td>
<td>123</td>
<td>2.12</td>
</tr>
<tr>
<td>100*SD/AVG</td>
<td>50</td>
<td>16</td>
<td>48</td>
</tr>
</tbody>
</table>

**Conclusion**

The study should be considered as a pilot investigation, which aimed to optimise analytical and image processing procedures. Our results have indicated that hydroxyapatite and carbonatehydroxyapatite form the main inorganic phase at advanced calcification stage. Differences between the cusps consist in diverse distribution of the deposits in the cusp tissue which corresponds to clinical diagnosis. Ongoing studies attempt to quantify distribution of deposits in the cusp tissue.

**Acknowledgment**

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**References:**
Monitoring anticancer therapies against lung tumor in experimental models using X-ray MicroComputed Tomography
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Background
Three-dimensional micro Computed Tomography (µCT) offers the opportunity to capture non-invasively images of lung structures and lesions in mice with a high spatial resolution allowing for accurate calculation of lung lesion volume. Longitudinal imaging overcomes the limitation of single time-point imaging because it enables tracking of the natural history of disease and provides quantitative assessments of the effects of an intervention in every single mouse.

ALK (Anaplastic Lymphoma Kinase) is a transmembrane protein and a member of the family of insulin receptor tyrosine kinases. Oncogenic fusion proteins harbouring the ALK kinase domain have been identified in different cancer types. In lung cancer, the most frequent chimeric protein involves fusion of the ALK kinase domain at the C-terminus to part of the EML4 (echinoderm microtubule-associated protein-like 4) protein at the N-terminus in approximately 3-13% of human non-small cell lung cancers (NSCLC). The pivotal role of EML4-ALK in the carcinogenesis of NSCLC was demonstrated in experimental models such as the transgenic mouse model described by Soda, expressing EML4-ALK specifically in lung alveolar epithelial cells under the control of the Surfactant Protein C (SPC) promoter (1). A similar transgenic model was developed internally using the lung specific Clara Cell Secretory Protein (CCSP) promoter.

A dual ALK/c-Met inhibitor (Crizotinib Xalkori®) was approved by FDA in September 2011 for non-small cell lung cancers (NSCLC) expressing ALK. Escape from Crizotinib treatment linked to mutations in the ALK kinase domain has been reported (2) and novel second generation Alk inhibitors active on Crizotinib resistant cancer are under development. Here we report the evaluation of an ALK inhibitor (hereafter compound-A) in comparison with Crizotinib, in the CCSP-EML4-ALK-TG16 transgenic mouse model using µCT.

Method
73 CCSP-EML4-ALK-TG16 transgenic mice were received at 6 weeks of age. For baseline imaging, mice were imaged twice at 1 week interval using µCT to identify growing lung tumor nodules. Three homogenous groups were randomized on the basis of the tumor size of one selected tumor nodule per mouse, and treated according to the following protocol:

Mice were monitored longitudinally for 5 imaging sessions of 7 min each using the Skyscan 1076 ® scanner (35um, Ti 0.025mm, 72kV; 145uA, exposure 316ms, rot step 1°). During imaging, the mice were anesthetized with Aerane® (0.2%, O2:2l/min) and kept warm. Images
were reconstructed using NRecon ® and Regions Of Interest (ROI) were manually drawn over the selected tumor nodule using the image analysis software CTAn®. Tumor volume was calculated by stacking 2-dimensional ROI. Statistical analysis was done on tumor volume from day 47 to day 77 using 2 way analysis of variance with repeated measures on one factor, with significance level set at p<0.05. Partial tumor Regression (PR) corresponds to reduction of 50% of the tumor size compared to baseline and Complete tumor Regression (CR) is declared when the tumor is no more visible on the scan.

Results
The figure below shows longitudinal µCT monitoring of the selected lung nodule in one representative mouse of control (top row) and compound-A treatment (bottom row) groups.

![Tumor Monitoring Images](image)

Compound-A had a significant effect on tumor nodule volume vs control from day 54 (p=0.0098) to day 77 (p<.0001) with 96% tumor growth inhibition on day 77, 3/10 CR and 10/10 PR. Crizotinib had significant effect on tumor nodule volume vs control from day 61 (p=0.0191) to day 77 (p=0.0295) with 44% tumor growth inhibition on day 77, 0/10 CR and 3/10 PR. Compound-A was found significantly more active than Crizotinib on day 61 (p=0.013), day 70 (p=0.0025) and day 77 (p<.0001).

Conclusion
Longitudinal monitoring of lung tumor nodules using X-ray µCT in CCSP-EML4-ALK-TG16 transgenic mice developing ALK+ lung tumor nodules, revealed superior activity of the Alk Inhibitor compound-A compared to Crizotinib at 50 mg/kg given daily.

References:
µCT visualization of BMP7 mediated kidney regeneration

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Aims
The disruption of kidney structure and functional units, nephrons, leads to potentially lethal disease. Chronic kidney disease (CDK) is a condition characterized by fibrosis of the kidney tissue which leads to impaired kidney function. The role and the production of bone morphogenetic protein 7 (BMP7) in kidneys is well documented¹,². In this experiment we wanted to visualize the vascularization of kidney tissue and regeneration mediated by systemically administered BMP7 with the use of µCT.

Method
Male Sprague-Dawley rats, 4 months old and weighing around 300g, were used in this experiment. To create an animal model of chronic kidney disease animals underwent surgery in which five sixth of the total kidney mass was removed³. To monitor the progression of the renal failure, serum creatinine was measured weekly until the end of the experiment. After surgery animals were assigned in two groups: 1) control group, receiving vehicle (N=4) and 2) BMP7 group, receiving 50µg of BMP7 once a week i.v. (N=4). After 5 weeks of therapy animals were scanned with the µCT device.

To scan the kidneys with the µCT we administered contrasting agent in the blood stream (vena jugularis) of the animals. As a contrasting agent we used Omnipaque, which is based on the compound iohexolulm. The animals were scanned in vivo after which we administered another injection of contrasting agent and the remnant kidney was clamped, excised and scanned ex vivo.

The scans were performed on SkyScan 1076 µCT device. For in vivo scanning conditions include: scanning width of 68mm, 18 µm pixel size, aluminum 0.5 mm filter, frame averaging of 1 and a rotation step of 0.9 degrees. Ex vivo scanning conditions include: 9 µm pixel size, aluminum 0.5 mm filter, frame averaging of 2 and a rotation step of 0.5 degrees. Data was reconstructed with the newest NRecon software. Functional vasculature of the kidney was analyzed by CTAn program while the 3D model was created with the CTVox software.

To compare the µCT results we prepared histological sections of the remnant kidney that were stained with Sirius Red for fibrous tissue analysis and hemalaun-eozin staining of the blood vessels.

Results
Administered BMP7 improved kidney function in CDK through the measurement of serum creatinine levels which were significantly lower in the BMP7 treated group (Figure 1.). On histological slices the amount of fibrous tissue was decreased and the extent of blood vessels was increased in BMP7 treated group.
Healthy kidney filters the Omnipaque contrasting agent far too quickly for a quality image of the kidney obtained with µCT. Ex vivo scan of a clamped healthy kidney gives us detailed vascularization and the division on the medulla and the cortex of the kidney (Figure 2.).

Conversely, impaired kidney function in CDK results in slower filtration of the contrasting agent and a possibility to scan the kidneys in vivo (Figure 3.).
Through *in vivo* and *ex vivo* scans the vascularization of the remnant kidney was greatly improved in the BMP7 treated group compared with the control group.

**Conclusion**
Systemic administration of BMP7 mediated regeneration and angiogenesis in the chronic kidney disease which can be distinguished with µCT scan using the vascular contrasting agent. Level of creatinine in the serum indicates improved renal function in animals receiving BMP7.

**References:**
Functional and morphological changes of the urinary bladder in ApoE−/−/LDLR−/− double knockout mice

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Aims
Atherosclerosis and overactive bladder dysfunctions (OAB) have increasing prevalence in elder patients. This study aimed to demonstrate the manifestation of OAB in a mouse model of atherosclerosis, based on functional data acquired from cystometry and morphological data acquired from micro- and nano-CT measurements.

Method
Mice lacking apolipoprotein E (ApoE) and low-density lipoprotein receptors (LDLR) show severe atherosclerosis. Cystometric measurements in 6 female 60 weeks old ApoE−/−/LDLR−/− mice were performed. Another 10 female C57B/6J mice served as control. Maximum bladder pressure (P_max), peak to peak interval of detrusor activities (PP), micturation intervals (MI), micturation volume (MV) and residual urine volume (RUV) were determined. Also, urinary bladder was analysed by micro- and nano-CT in 13 ApoE−/−/LDLR−/− mice and 14 controls. Cannulation of the left ventricle was followed by injection of heparinized saline (10 ml, 0.9% sodium chloride with 1000 IU heparin) until the venous effluent from the incised right atrium was aqueous. A lead containing polymer (Microfil MV-122, Flow Tech, Carver, MA, USA) was injected into the left ventricle. Vaseline filled bladders were fixed in formalin and scanned in a micro-computed tomograph (micro-CT, SkyScan1072_80kV) manufactured and developed by SkyScan (Kontich, Belgium). For more detailed analysis, sections of the bladder wall were scanned in a nano-computed tomograph (nano-CT SkyScan2011), manufactured and developed by SkyScan (Kontich, Belgium). Quantitative measurements were performed using ANALYZE Software packages (ANALYZE 9.0/10.0, Mayo Clinic, Rochester, MN, USA) and CTAn (SkyScan, Kontich, Belgium).

Results
ApoE−/−/LDLR−/− mice showed a significant increase of maximum bladder pressure (p=0.045) and a significant reduction of peak-peak interval (p=0.001), micturation interval (p=0.001) and micturation volume (p=0.002). Micro-CT measurements showed a significant reduction of in bladder wall volume (p=0.047). Analysis of bladder segments using nano-CT showed a significant reduction of vascular volume fraction (p=0.011), segment volume (p=0.001) and bladder wall thickness (p=0.001).
Figure 1: Micro-CT of bladders (vessels filled with Microfil, bladder filled with vaseline). Imaging depicts a reduction of wall thickness in bladders of ApoE<sup>−/−</sup>LDLR<sup>−/−</sup> mice (C&D) compared to controls (A&B).

Figure 2: 3D figures of Micro-CT Data demonstrate a reduction of vascular volume in ApoE<sup>−/−</sup>LDLR<sup>−/−</sup> mice (E/F) compared to controls (A/B). High resolution images from Nano-CT (controls: C/D,
ApoE<sup>−/−</sup>LDLR<sup>−/−</sup> mice allow a precise measurement of vascular volume even in small vessels and depict a significant reduction of vascular volume fraction in ApoE<sup>−/−</sup>LDLR<sup>−/−</sup> mice compared to controls (see results).

**Conclusion**

Our study demonstrates a relationship between atherosclerosis and overactive bladder. ApoE<sup>−/−</sup>LDLR<sup>−/−</sup> mice have considerable changes in bladder function, which seem to be linked to morphological changes as seen in micro- and nano-CT. Our results suggest deterioration of bladder wall vascularisation as a factor in pathogenesis of OAB.
Experimental micro-CT based spleen and liver imaging with nanoparticulate and iodine-based contrast agents

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2 Department of Radiology - UZ Brussel
3 Electronics and Informatics - ETRO - IRIS - Vrije Universiteit Brussel

Aims
The purpose of this study was to characterize the time course of contrast enhancement of three iodine-based contrast agents (ExiTron P, U, V) and two alkaline earth metal-based nanoparticulate contrast agents (ExiTron nano 6000 and 12000) developed for experimental micro-CT imaging.

Method
We injected all contrast agents intravenously in C57bl/6 mice (n=37). As indicated on the manual, we injected initially at a dose of 0.1 ml/25g (n=22). In case of decease, 0.05/25g was injected (n=15). Animals were anesthetized with Isoflurane. The anesthesia was induced with 5% isoflurane and maintained at 2% during the scan with spontaneous breathing via a mask. Imaging was performed using micro-CT (SkyScan 1178 micro-CT system; SkyScan, Kontich, Belgium) at a resolution of 83 µm before contrast injection, immediately after contrast injection and at 15 min, 30 min, 45 min, 1h, 2h, 3h, 4h, 24h and 48h after contrast (scan duration: 121 sec). Images were analysed using Amide. Regions of interest were drawn in spleen, liver, and left ventricle. The contrast enhancement was measured and expressed in function of time.

Results
Our results reveal that the recommended dose of 0.1 ml/25g was lethal after intravenous injection of ExiTron P, ExiTron nano 6000, and ExiTron nano 12000: the animals died immediately after contrast injection. After injection of only the half of the recommended dose (0.05 ml/25g), most animals survived, but injection of ExiTron P was lethal. The ExiTron U and ExiTron V contrast enhancement of the spleen reaches a maximum just after injection, while the maximum liver enhancement occurs at 1h after ExiTron V and at 3h after ExiTron U. Maximum contrast enhancement of the spleen after ExiTron nano 6000 and ExiTron nano 12000 occurs after 48h, while maximum liver enhancement is after 4h for ExiTron nano 6000 and after 24h for ExiTron nano 12000. Enhancement in the cardiac cavity is the highest after ExiTron nano 12000.
Conclusion
The nanoparticulate contrast agents ExiTron nano 6000 and 12000 provide strong contrast enhancement of the spleen and liver. Contrast-enhanced angiography is feasible after injection of ExiTron nano 12000. However, there is a limitation in the injectable volume of contrast.

References:

Figure: Micro-CT images after injection of ExiTron nano 12000 with visualization of spleen, liver, and heart.