

35 mm

NEWSLETTER AUTUMN 2019

Dear Readers,

It is a pleasure for me to introduce you a new issue of the newsletter of our Laboratory of X-ray micro and nano computed tomography CEITEC BUT. You can read here about some of our recent analyzes, including a measurement of linear fibrous assemblies with submicron resolution, an investigation of an unusually large piece of meteorite, and a morphological analysis of bones. We will describe selected tomographic artefacts, and another topic to cover are new possibilities of our laboratory, especially in the field of digital radiography on GE phoenix microme | x.

Enjoy the reading!

Tomáš Zikmund
Head of the laboratory



CTLAB
X-ray Computed Tomography



CEITEC

Central European Institute of Technology
BRNO | CZECH REPUBLIC

NEW POSSIBILITIES OF THE CT ANALYSIS USE

SUBMICRON CT MEASUREMENT OF LONG THIN SAMPLES

In cooperation with the Department of Technologies and Structures at Faculty of Textile Engineering, Technical University of Liberec, a tomographic analysis of linear fibrous assemblies with a diameter of 0.5 mm and a length of 5 mm was carried out in order to visualize the winding of individual fibres. It was necessary to perform tomographic measurements with submicrometer resolution (i.e. voxel size $\approx 0.5 \mu\text{m}$) for visualization and detection of individual thread fibers which are approximately $10 \mu\text{m}$ in diameter. For this purpose, a procedure was developed involving a special fixation of the sample, increasing the fibre contrast and joining a series of datasets along the axis of linear fibrous assemblies.

In the first step, the sample was fixed in a plastic tube to prevent any movement and also to prevent the fibers from unfolding. For CT measurement, the Rigaku Nano3DX system was used, which is limited to $0.9 \times 0.7 \text{ mm}$ field of view to achieve the given resolution. In order to cover the desired length of the sample, it was necessary to perform 8 CT measurements. The sample was shifted in the direction of the thread axis between

subsequent measurements. To improve the contrast between the fibers and the background, the data adjustment for phase contrast visualization was used (Fig. 1c). The resulting CT data were equalized to unify the intensity values between the individual datasets (Fig.1a) and were subsequently stitched using the registration tools in VG Studio MAX. The 3D fiber model allows studying the fiber orientation (Fig. 1b).

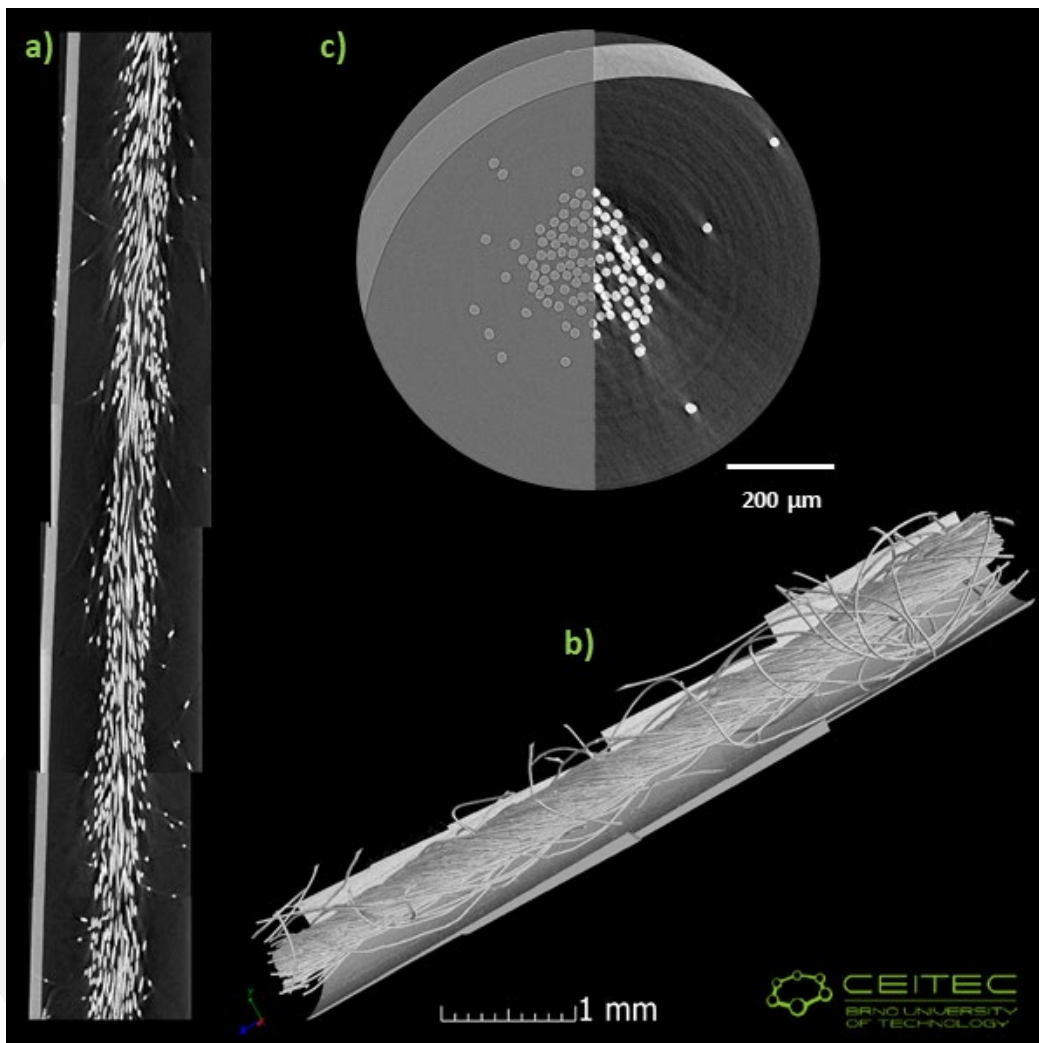


Figure 1: Results of developed scanning procedure applied on sample of linear fibrous assemblies: a) longitudinal slice over stitched CT volume; b) 3D surface render of stitched CT volume and c) comparison of visualization in absorption contrast (left part) and visualization in phase contrast after intensity values equalization (right part).

CT FOR HOLOGRAM OF METEORITE

Using a microCT with a voxel resolution of 80 μm , we analyzed an unusually large piece of meteorite weighing 1320 g with dimensions approximately $11 \times 15 \times 4$ cm. The meteorite was found in 2016 in Stubenberg, a village near Simbach, Lower Bavaria, Germany. The aim of the study was to display its virtual 3D model in the RiesKraterMuseum in Nördlingen, Germany. For this purpose, the meteorite surface was determined in CT data, and the metal and sulphide particles from which the meteorite is composed, were segmented. Based on the data, a holographic model of both meteorite and internal structure was created. The visitors of the museum will be able to switch between the views of the individual structures to perceive the complex composition of the meteorite.

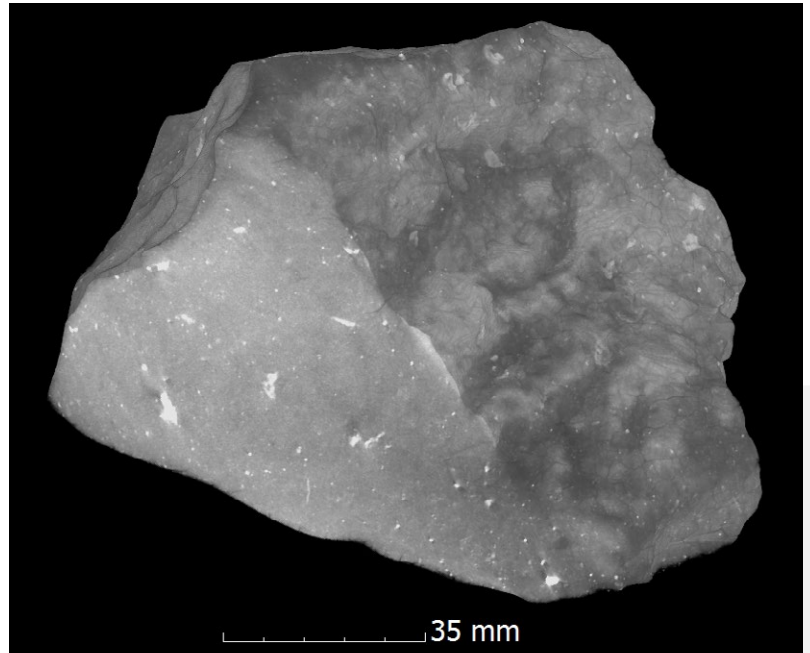


Figure 2: Virtual slice through 3D model of Stubenberg meteorite, the light parts represent metal particles.

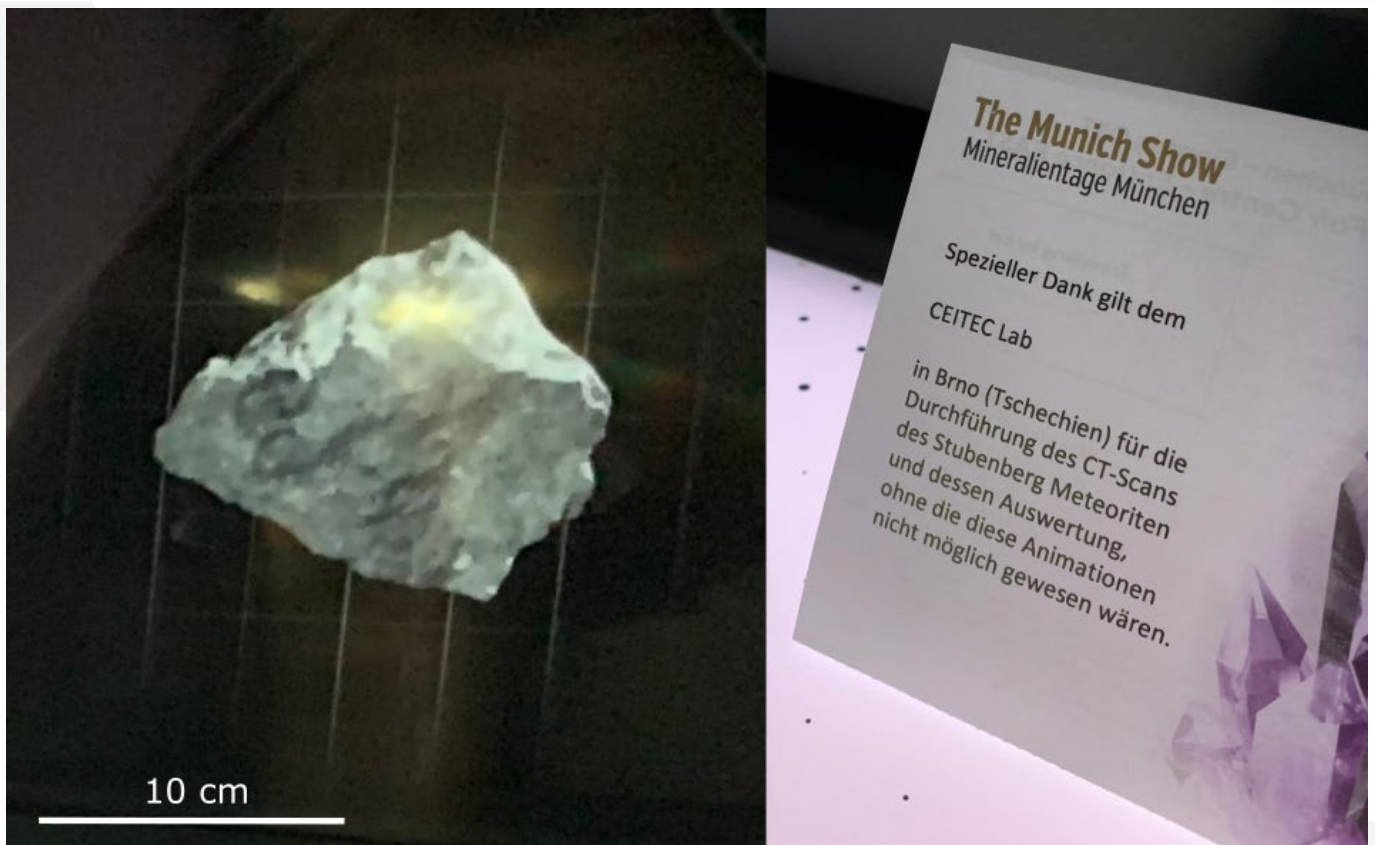


Figure 3: 3D holographic model of meteorite, exhibited at Munich Show 2019.

MORPHOLOGICAL ANALYSIS OF MOUSE BONES BY X-RAY MICRO-COMPUTED TOMOGRAPHY

X-ray micro-computed tomography has been proven as a very useful tool to better understand the effect of various biological processes on the development of mammalian bones. With its resolution in the range of micrometres, we are able visualize and analyze the bones in fine detail. In case of the morphological analysis of bones, biologists are often interested in the comparison of several samples. In Fig. 4 you may see an example of bone images that we analyzed: two femur bones, each from a different mouse.



Figure 4: Selected slices and 3D models of the bones scanned on micro CT.

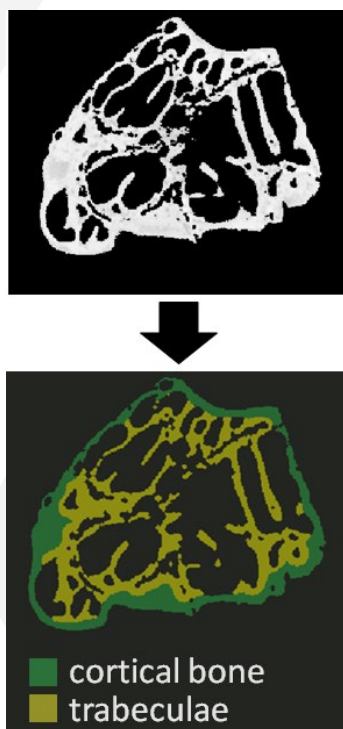


Figure 5: Automatic differentiation of trabecular and cortical bone in a selected micro CT slice.

We use VG Studio Max to visualize the bones in 3D and to perform their initial visual evaluation and dimensional measurement. The next step is the differentiation of the trabecular and cortical bone, where we employ our segmentation algorithm that we are currently developing (see Fig. 5). We then use an ImageJ plugin BoneJ for further analysis of the bones. BoneJ provides tools that enable an evaluation of trabecular bone geometry and a cortical bone shape analysis. In the case of trabecular bone, we are able to calculate metrics like the degree of anisotropy, connectivity of the trabeculae, analyze the skeleton of the trabeculae network and much more. For cortical bone, we can for example calculate the surface area and the volume of the bone or even measure its thickness.

The analysis of bones by X-ray micro computed tomography is a complex task. By combining the visualization capabilities of VG Studio Max, our trabecular bone segmentation algorithm, and the tools provided by BoneJ, we are able to tackle a wide variety of cases and gain as much information as possible about the bone differences.

TOMOGRAPHIC ARTEFACTS

An undesirable element of the image that damages the information of tomographic data is considered as an artefact. It is possible to group the origins of these artefacts into several categories: physics-based, scanner-based and sample-based artefacts. In this paper, we show a representative of each group.

1. The first type is circular artefacts, sometimes also called ring artefacts (Fig. 6). These artefacts are caused by a different detection efficiency or sensitivity of pixels on the detector of the instrument. Significant circles or semicircles are created in the images with the centre in the axis of rotation. Larger amounts of circular artefacts are eliminated by calibrating of the detector before each measurement, or shifting the detector position for each image during the acquisition.

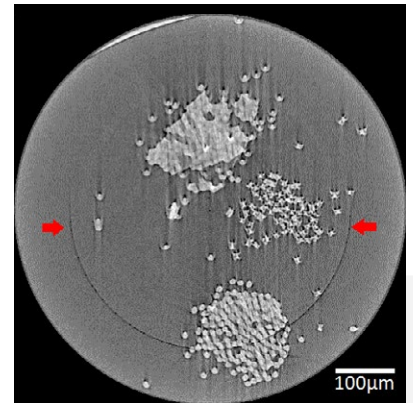


Figure 6: Circular artefacts: textile, voxel size 0.27 μm .

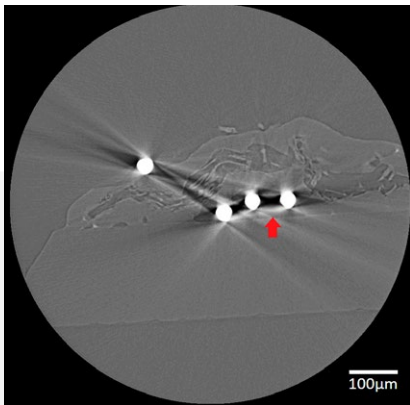


Figure 7: Metal artefacts: molybdenum fibers in the polymer, voxel size 0.27 μm .

2. The second type is called metal artefacts (Fig. 7). These are caused mainly by the phenomenon of beam-hardening, scattering and photon starvation. These artefacts appear in the resulting images mainly as dark and white streaks between high-density objects.

The main approaches reducing metal artefacts include filtration of X-ray spectra and, more recently, the use of dual-energy which our laboratory also deals with.

Filtration consists of placing a copper or tin plate in front of the X-ray source. The filter attenuates part of the spectra with the lowest energy, the centre of gravity of the spectrum is shifted toward higher energies.

The dual-energy approach is based on obtaining two datasets, each at different X-ray energy. So-called monoenergetic images are then extracted from these datasets and artefacts are significantly reduced.

For the correct analysis of the measured data, it is crucial to prevent artefacts as much as possible by applying optimal instrument settings and appropriate measurement strategy. Only in the second step, we try to correct artefacts using image analysis algorithms.

3. Motion artefacts appear at some places as light streaks, artificially adjusting the actual shape of the structure and blur the edges of the pattern. Therefore, it is necessary to prevent the sample from moving and to keep the temperature stable throughout the acquisition process. A correct fixation of the sample is very demanding, especially when measuring with a resolution in a range of a micrometre and less, where the possible movement in the data would be significantly reflected. (Fig. 8)

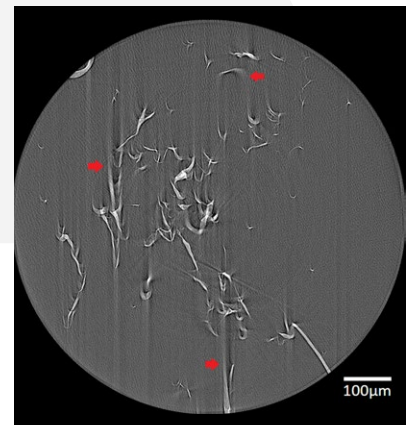


Figure 8: Movement artefacts: polymer structure, linear voxel size 0.27 μm .

NEW PUBLICATIONS

1. PUBLICATION OF PHASE CONTRAST IMAGING DEVELOPMENT

We published a study presenting possibilities of phase contrast imaging (PCI) with laboratory nanoCT device in IEEE Transactions on Instrumentation and Measurement journal. The conditions which each CT device has to fulfil in order to be able to utilize PCI are explained, evaluated and discussed in terms of different properties and settings of CT machine. Propagation-based PCI is demonstrated on a sample of polymer composite measured with a CT RIGAKU nano3DX at CEITEC laboratory.

The full paper is available here: <https://ieeexplore.ieee.org/stamp/stamp.jsp?tp=&arnumber=8686191>

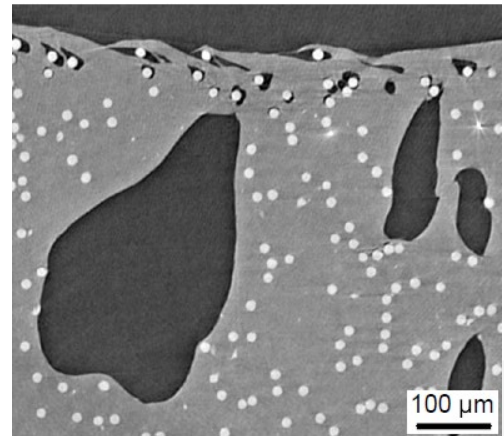


Figure 9: Tomographic slice of a polymer sample with enhanced phase contrast.

2. MOUSE MODEL OF CONGENITAL HEART DEFECTS, DYSMORPHIC FACIAL FEATURES AND INTELLECTUAL DEVELOPMENTAL DISORDERS AS A RESULT OF NON-FUNCTIONAL CDK13

In cooperation with research group lead by Marcela Buchtová from Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, the high resolution X-ray computed microtomography was used as one of the methods to describe the phenotype of two genetic mutations in CDK13 gene on a model of mouse embryo. CDK13 gene mutations have recently been associated with congenital heart defects, dysmorphic facial features and intellectual developmental disorders (CHDFIDD) syndrome in humans. On the mouse model with mutated CDK13 gene, an improper development of

multiple organs was documented, partly resembling defects observed in patients with mutated CDK13. In particular, the overall developmental delay and kidney failure accompanied by congenital heart defects were detected (Fig. 10). Based on the results published in the paper, the Cdk13 mutant mice might be a very useful model for further studies focused on revealing the molecular mechanisms underlying CHDFIDD caused by mutation in CDK13 gene.

The full paper is available here: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6694211/pdf/fcell-07-00155.pdf>

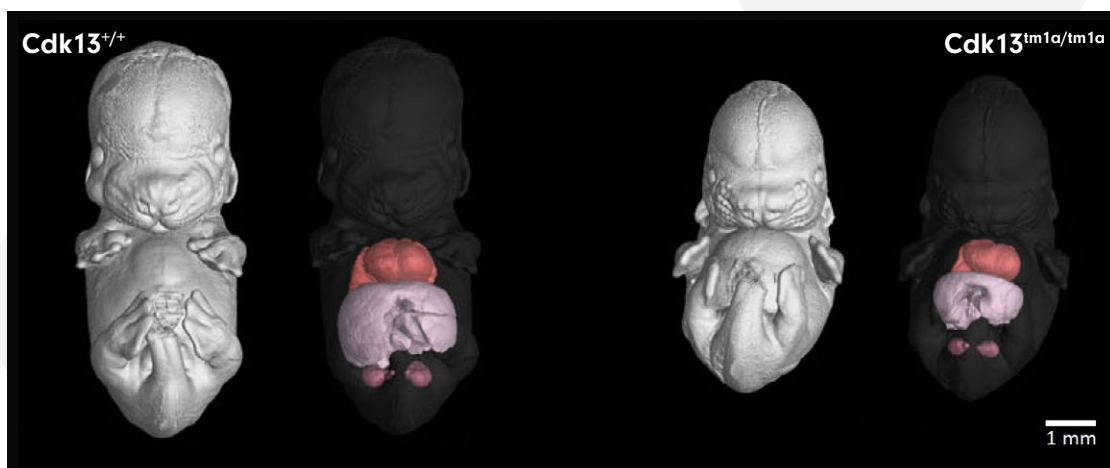


Figure 10: Comparison of Cdk13^{+/+} and Cdk13^{tm1a/tm1a} of 13.5 days old mouse embryo, segmented organs are heart (red colour), liver (light pink colour) and kidneys (dark pink colour).

LATEST NEWS

NEW EXPERTISE FOR ELECTRONIC DEVICES: GE MICROME|X

Our portfolio of non-destructive testing expertises has newly expanded by digital radiography (X-ray imaging). This extension is a result of a long-term cooperation with PBT Rožnov p.R., s.r.o., within which we can use a professional inspection system GE phoenix microme|x (<https://www.industrial.ai/phoenix-micromex>) in a shared laboratory regime.

Microme|x is designed especially for inspection of complicated printed circuit board and SMT (Surface Mount Technology) assemblies. A high resolution is achieved thanks to the open X-ray tube. The inner arrangement guarantees unbeatable magnification that enables to inspect soldered printed circuit boards or even microcracks in the balls of BGA (Ball Grid Array). Maximal available voltage 180 kV allows also for inspection of challenging assemblies with coolers or metal cases mounted.

X-ray images are post-processed by unique software tools for local contrast enhancement in order to reveal material defects. The software also allows quantifying porosity in the soldered joints. Inspection of bigger amount of identical samples can be partially automated for NOK/OK samples sorting. Last but not least, microme|x can use advanced methods such as planar CT (laminography), enabling to visualize multilayer structures in 2D cross-sections (more about this method will be published in next issue).

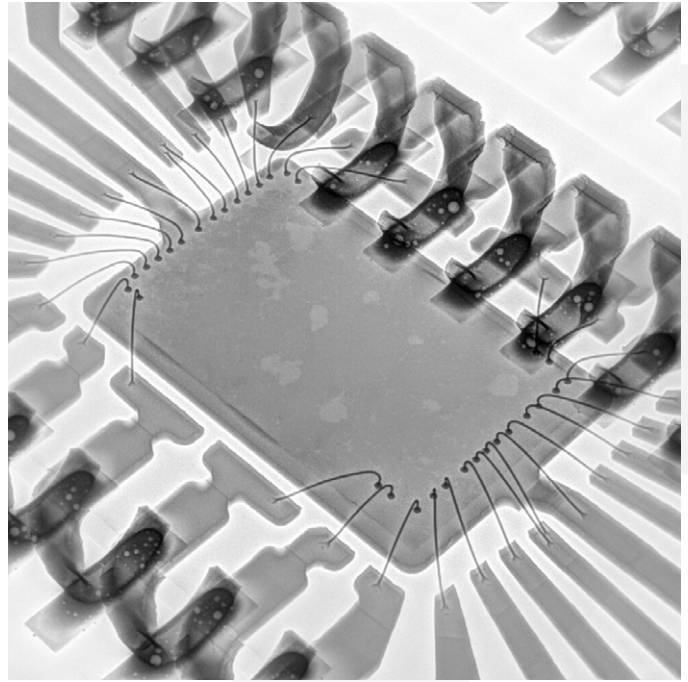


Figure 11: Filtered X-ray image of a microchip soldered to the flat board.

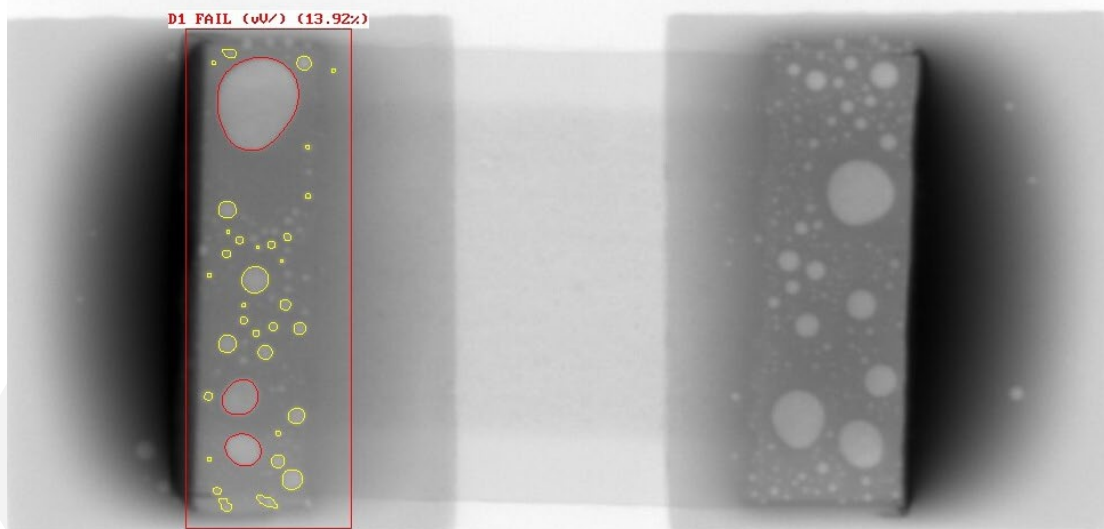


Figure 12: Determination of porosity of soldered joint from X-ray image.

CERTIFICATED ANALYSES IN CONFORMITY WITH AUTOMOTIVE STANDARDS

Our laboratory fulfils requirements of the newest standard IATF 16949:2016 (Automotive quality management standard) for external laboratories of automotive companies. The standard defines quality management system requirements for automotive production, design and development. Standard was developed as an extension of ISO 9001 according to automotive manufacturers requirements. It emphasizes the development of quality management system which is oriented on continual improvement, defect prevention and the reduction of variation and

waste in the supply chain. Most importantly, rules for external laboratory facilities used for inspection, test or calibration services are defined by automotive manufacturers. It is newly required to perform these tests in laboratories accredited to ISO/IEC 17025.

Our laboratory is authorized to perform tests using X-ray computed tomography to standard ISO/IEC 17025 from 2006. For our partners, we can offer metrological measurements on two top CT devices (<http://ctlab.ceitec.cz/accreditation/>).

PARTICIPATION

ICTMS 2019 CONFERENCE, CAIRNS, AUSTRALIA

On 22nd – 26th July 2019, PhD student Dominika Kalasová represented our laboratory at International Conference on Tomography of Materials and Structures (ICTMS) in Cairns, Australia. She gave a talk called „Propagation-based phase contrast imaging with a lab-based CT system with helical space-filling trajectory“, where she presented initial results of the project TACR NCK that we work on together with Thermo Fisher Scientific.

More info about the conference at <http://ictms2019.org/>.

INTERNATIONAL SOCIETY OF VERTEBRATE MORPHOLOGISTS (ICVM), PRAGUE, CZECH REPUBLIC

We attended ICVM conference with 2 contributions concerning the field of developmental biology with following topics: Bone Pedicle Development and its Association to the Tooth Germ in Acrodont Chameleons, and 3D-Quantitative Morphometrics in Craniofacial Development by X-ray Computed Microtomography. The ICVM conference is held every 3 years, this year the conference was hosted in hotel Grandior in Prague and the number of participants was the highest in the history with 700 scientists present. The conference programme topics covered paleontology, evolutionary developmental biology, functional morphology, morphometrics, evolutionary morphology, and descriptive morphology.

All of the abstracts from ICVM conference were published in Journal of Morphology in special issue contributed to the conference, the abstracts are available at <https://doi.org/10.1002/jmor.21003>.

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