# MicroCT for high throughput phenotyping: perspectives and obstacles

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## Aims

MicroCT is becoming more and more spread as a tool in the mouse molecular genetics due to its three-dimensional (3D) data recording and importantly non-invasive nature, which makes microCT scaning compatible with other phenotyping procedures. These two characteristics are providing much better data quality aquirement in phenotyping parameters from skeletal morphology and body composition analysis.

There are also some drawbacks though: a large amount of data and time limitations for analysis. These obstacles can force researchers to use more conventional, two-dimensional (2D) methods like X-ray radiography for morphology and dual-energy X-ray absorptiometry (DXA) for body composition and bone mineral density analysis, although the amount and quality of information is far more compromised.

The Czech Centre for Phenogenomics (CCP) is member of International Mouse Phenotyping Consorcium[1] with the aim to systematically annotate mouse genome. As such, we are following its high-throughput approach according to which we should be supposed to analyse several cohorts of novel mouse mutants a week. As we are advocating in-vivo microCT approach as the standard morphological phenotyping procedure, we need to accelarete and automate the data postprocessing steps and data analysis workflow accordingly. We believe the automation and machine learning are the way.

To do so, we have determined several problems to be solved more or less separately:

- data flow
- reconstruction set-up
- region of interest (ROI) selection
- transfer of analysed results
- bone morphology analysis and morphometrics

## Method

***Standard working procedure***

For our morphology / body composition analysis each mouse is anesthetized, weighted and scanned with help of SkyScan 1176 in-vivo microCT (Bruker, Belgium) with voxel size of 35 µm, voltage of 50 kV, current of 160 µA, and 0.5mm aluminium filter with 180° rotation. After scanning, some basic body measurements are recorded with the help of digital dial caliper. This basic procedudre takes some 25 minutes for every mouse in cohort.

Primary microCT data are processed with NRecon software (Bruker microCT, Belgium) to create two reconstruction subfolders with parameters set-up according Bruker's recomentadation[2, 3, 4] and our own experiments: First one for whole-body bone morphology and BMD analysis (smoothing = 3, ring artifacts correction = 4, beam hardening = 36%, intensities = 0.0047 – 0.1230), and the second one for core-body composition analysis (i.e. without head, tail, and

paws: smoothing = 7, ring artifacts correction = 5, beam hardening = 10%, defect pixel masking = 5%, intensities = 0.0040 – 0.0200). See Fig.1A, B, D. Although there is a possibility to store more reconstructions in a batch in NRecon and to load reconstruction parameters, the set-up of ROI has to be done manually, as connection with structures is very tight sometimes to be done automatically.

Morphology is evaluated with the help of CTvox (Bruker microCT, Belgium). For body composition analysis we use CT analyzer software (CTan: Bruker microCT, Belgium), where first the ROI is cleared manually as there are some problematic parts which would be hard to remove automatically (e.g. paws to close to body, tape etc. – see Fig.1 B, C, D). Especially this part is a real bottle-neck of our analysis, as experienced operator is able to process the data in 25 minutes per mouse (i.e. some 12 hours per cohort), but usual time is about 45 minutes per mouse (i.e. some 21 hours per cohort). For analysis only, we have developed a macro according to Bruker's suggestions[3] with respect to core-body analysis, which is able to estimate volumes of fat, lean, and bone in the body in one run. With connection with Batch Manager software (BatMan: Bruker microCT, Belgium) the analysis is not time-demanding for operator. This isn't true for aquaring analysis results though, where analysis of each of the mice is saved in a separate CSV-file in its own subfolder together with all procedures and in different formate. To go through one cohort (28 animals) means another 2 hours of operator's time. ROI file from this analysis is applied for data from bone morphology to compute BMD.

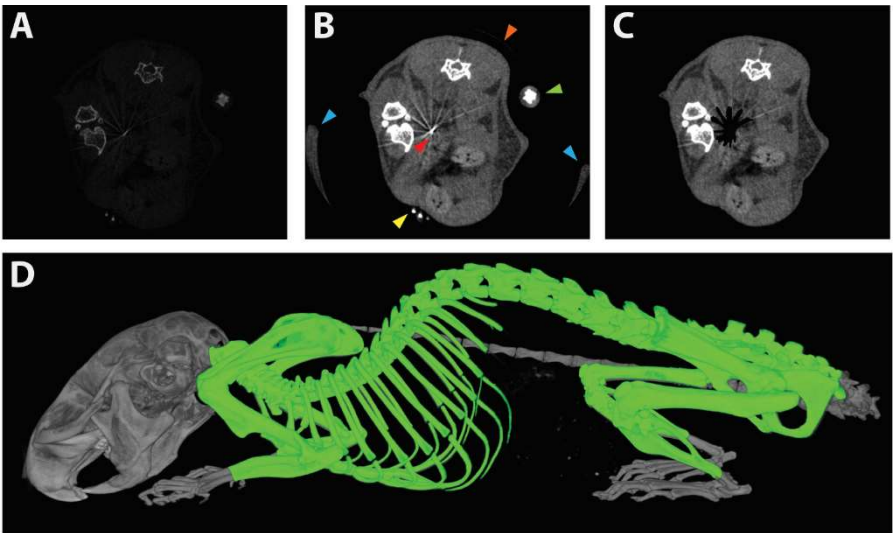


Figure 1: Mouse reconstruction for phenotyping. A-C one virtual section through mouse body – reconstruction for bone morphology (A), body composition analysis (B) with arrowheads marking parts to be removed: tape (orange), tail (green), holder (blue), paw (yellow), food particle (red), and result for body composition analysis (C). D: reconstruction of mouse skeleton with highlighted core-body for body composition analysis in green

More than 500 whole body microCT scans of wild type mice and about the same number of various mutants are stored in our database nowadays, which makes perfect match for developing and testing automatic tools for analysis.

***Data flow automatization***
To overall time we also need to add time for copying and removing data to the central diskspace for storage and back to operators' computers for processing and back, which demands full concentration too as mistakes can couse several demage to the whole analysis. To do it as automatically and safely, we need to 1) be sure everything we want to is copied to the right position, and 2) be sure nothing is deleted, which hasn't its backup yet.

***Automatic ROI selection as the first step to automatic picture analysis of microCT data***
As ROI selection for body composition analysis is now the most time-consuming procedure, our capacities are focused primarily to this point.

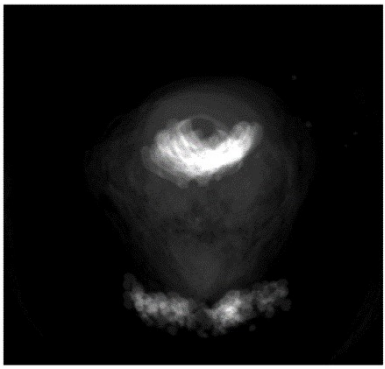

Figure 2: Average picture for the first section of body composition analysis

The automatization can be divided into two parts, firstly finding the start and end of the region of interest, secondly removing unwanted parts of animal (Fig.1 B, C, D). For the first part we used averaging model. We take the average image of start/end region and find the best hit on analysis picutres. For the second (see Fig.2) part we use the deep leaerning process to recognize region of interest and cut the others.

Similar approach could be used in the future for automatization of bone morphology analysis and data reconstruction (Fig.3).

***CTan results extraction***
A Microsoft Excel macro is used to extract and summarize the data from the standardized output CSV-files.

## Results
Although the whole automatization is still in progress, as it isn't always easy to translate the researcher's decision process in a way a software works, we have already reached some first successes. In the case of ROI selection we have success rate about 10 sections from ideal (researcher-based) possition, which means some 1-2 mm difference in reality (i. e. one or two cervical vertebrae of mouse), which is quite promising.

The Excel macro for data extraction is written in a way to be able go through all subfolders (specimens) in a project, pick up the values we are interested in, write warning messeages, if it is not able to find them, and built up a table where first column contains extracted numbers of specimens in increasing order and the rest are values of extracted variables. This form of table is directly importable for various statistical software. But the main point is, it shortens laborious work of a research or technician lasting up to 2 hours to a few clicks on your computer mouse. The only precondition is the same subfolder structure in a project.

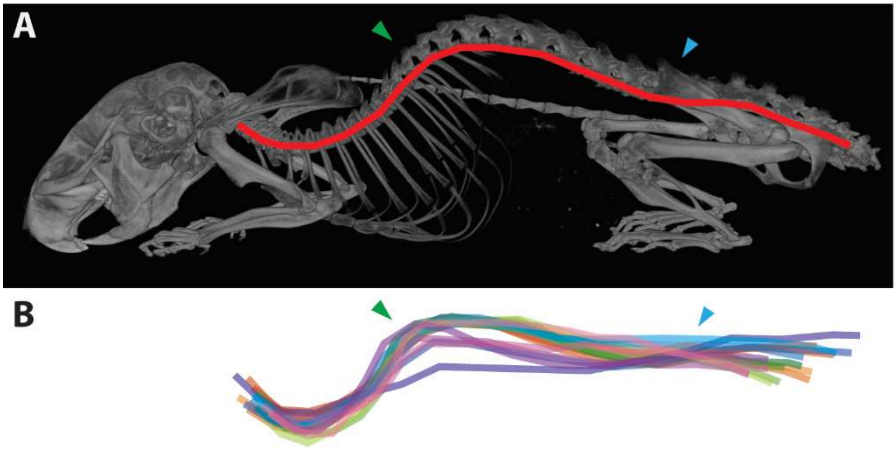


Figure 3: Spine shape analysis. Mouse skeleton with highlighted shape of spine (A). Overlay of lateral spine shape in multiple mice in various positions (B). Green arrowhead = kyphosis; blue arrowhead = lordosis.

## Conclusion

The automation in microCT image analysis is a strong tool sparing a lot of time of researches as it is able to process routine work with microCT data with quite a high precission and efficiency, and minimalize the biggest bottle-neck in finalizing any microCT analysis, i. e. time demands for data analysis.

## References:

11. http://www.mousephenotype.org/
12. Bruker microCT, “An overview of NRecon: reconstructing the best images from your microCT scan”, 48 pp., 2014
13. Bruker microCT, “Quantifying adipose tissue (fat) in a mouse or rat by *in-vivo* microCT”, 26 pp., 2013
14. Bruker microCT, “Bone mineral density (BMD) and tissue mineral density (TMD) calibration and measurement by micro-CT using Bruker-MicroCT CT-Analyser”, 30 pp., 2013