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**UNIVERSITY  
OF BERN**

*u<sup>b</sup>* X-ray microtomography

485018-HS2024-0: Advanced Course II  
Ultraprecision Engineering

**David Haberthür**

Institute of Anatomy, September 25, 2024

# *u<sup>b</sup>* Grüessech mitenang!

- David Haberthür
  - Physicist by trade
  - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
  - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
  - Post-Doc II: µCT group, Institute of Anatomy, University of Bern, Switzerland.

*u*<sup>b</sup>

# Grüessech from the μCT group



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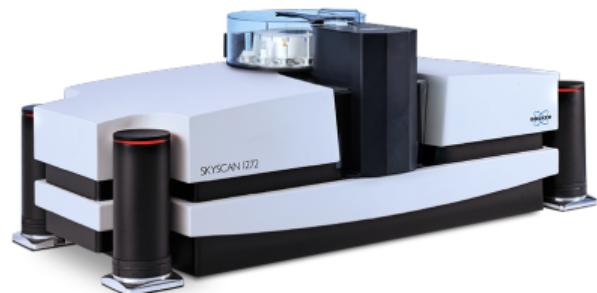


[Oleksiy.Khoma@unibe.ch](mailto:Oleksiy.Khoma@unibe.ch)

$\mu$ <sup>b</sup>

# $\mu$ CT-group

- microangioCT [1]
  - Angiogenesis: heart, musculature [2] and bones
  - Vasculature: (mouse) brain [3], (human) nerve scaffolds [4], (human) skin flaps [5] and tumors
- Zebrafish musculature and gills [6]
- (Lung) tumor detection and metastasis classification [7]
- Collaborations with museums [8] and scientist at UniBe [9] to scan a wide range of specimens
- Automate *all* the things! [10], [11]



[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

# Contents

## Tomography

- Interaction of x-rays with matter
- Tomography today

A scan, from *getting started* to *nice image*

## Examples

- A study about teeth
  - Overview
  - Materials & Methods
  - Results

Metal foam analysis

A study on fish

*u<sup>b</sup>*

# Biomedical imaging

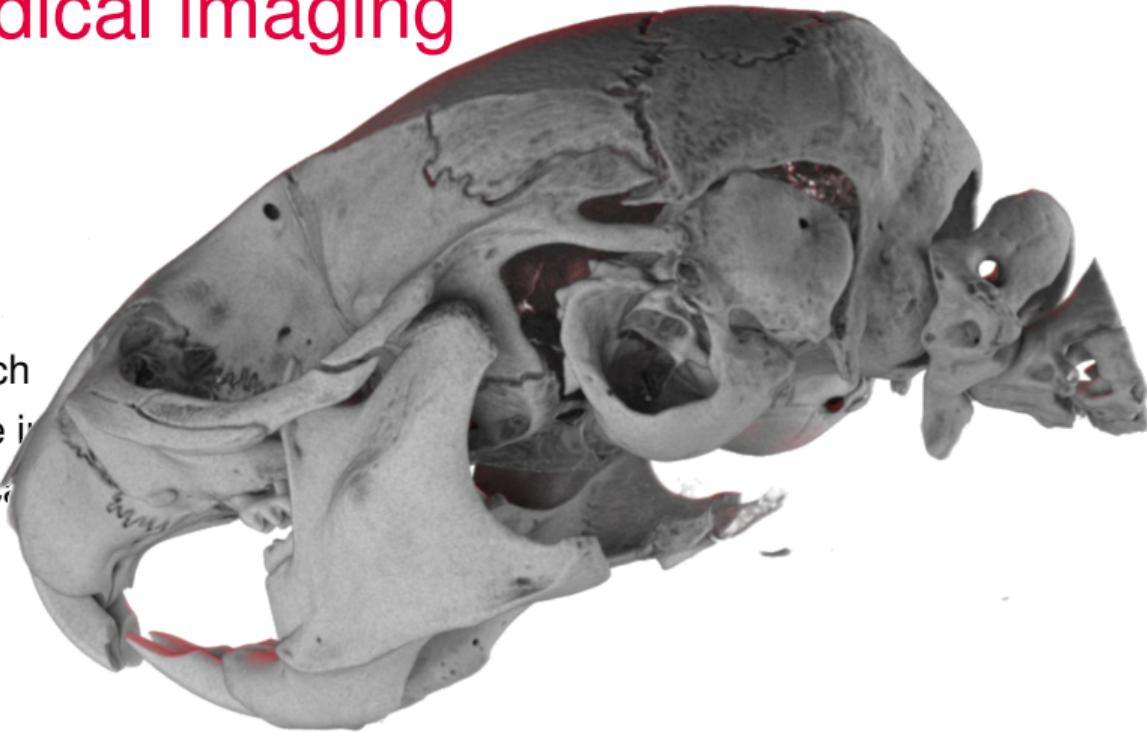
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



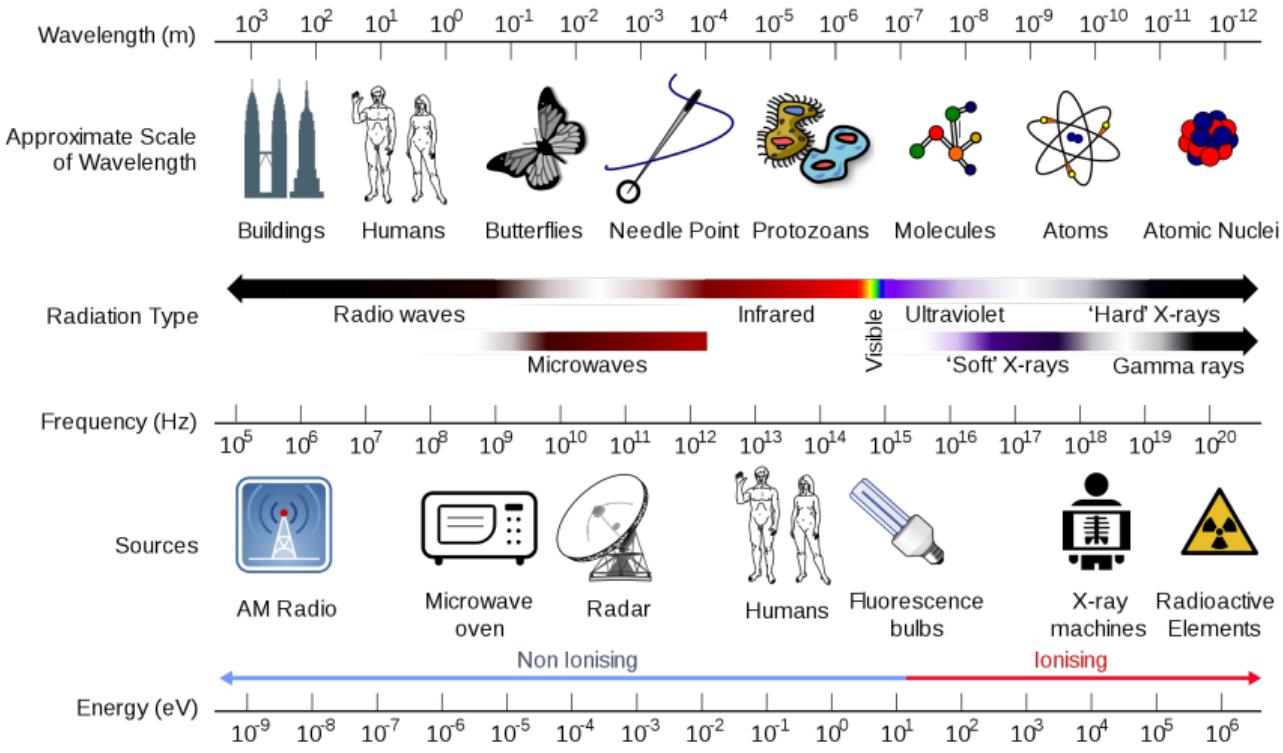
w.wiki/7g4 CC BY-NC-SA

# Biomedical imaging

- Medical research
- Non-destructive i
- (Small) Biological

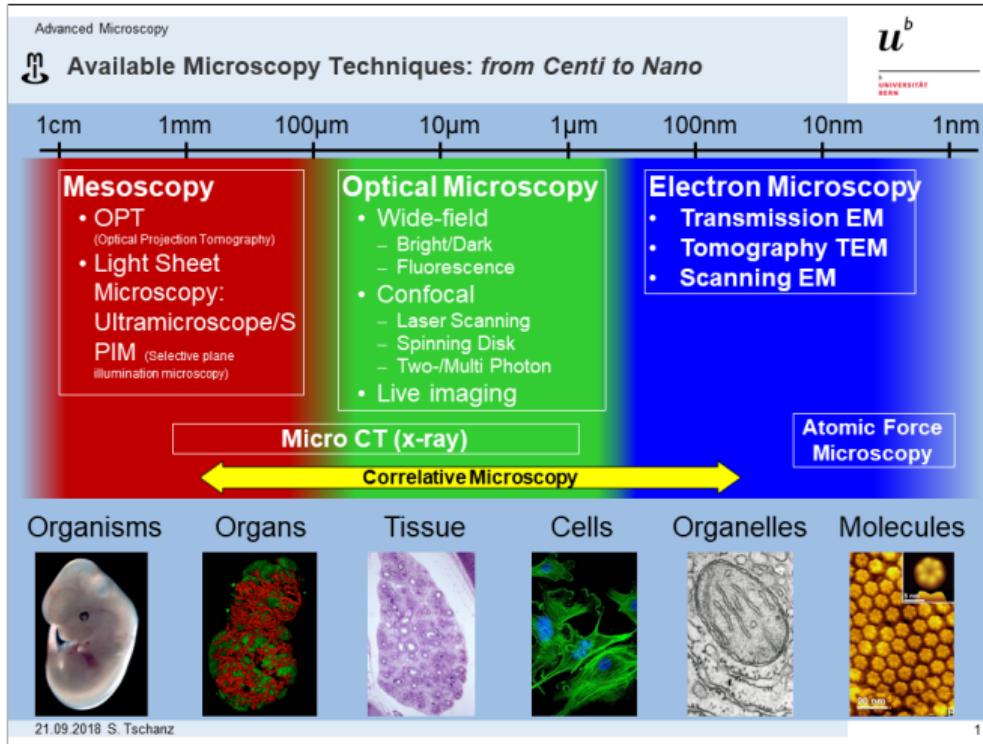


# $u^b$ Wavelengths & Scales



w.wiki/7fz @①②

# $u^b$ Wavelengths & Scales



Stefan Tschanz, with permission

# Imaging methods

- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy
  - *Analytical electron microscopy* by Dimitri
  - *SEM Grundlagen* by Sabine Kässmeyer and Ivana Jaric
  - *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan

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# CT-Scanner



[youtu.be/2CWpZKuy-NE](https://youtu.be/2CWpZKuy-NE)

*u<sup>b</sup>*

# CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays

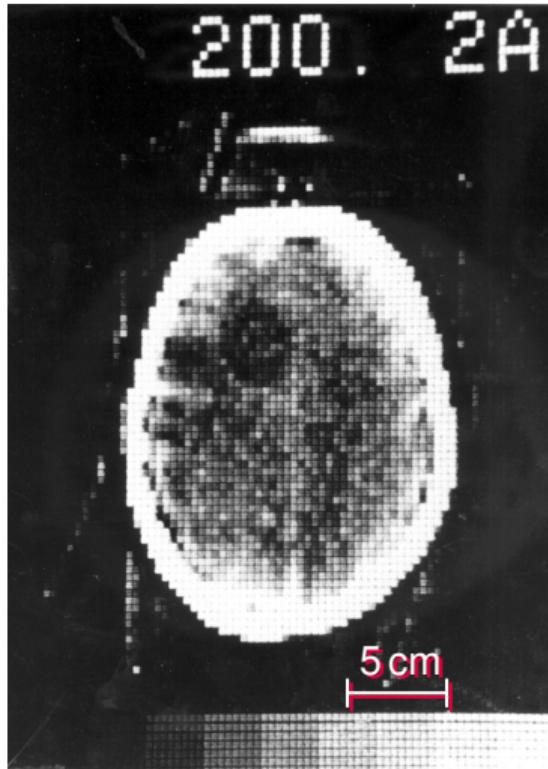


w.wiki/BHAN

*u<sup>b</sup>*

# CT History

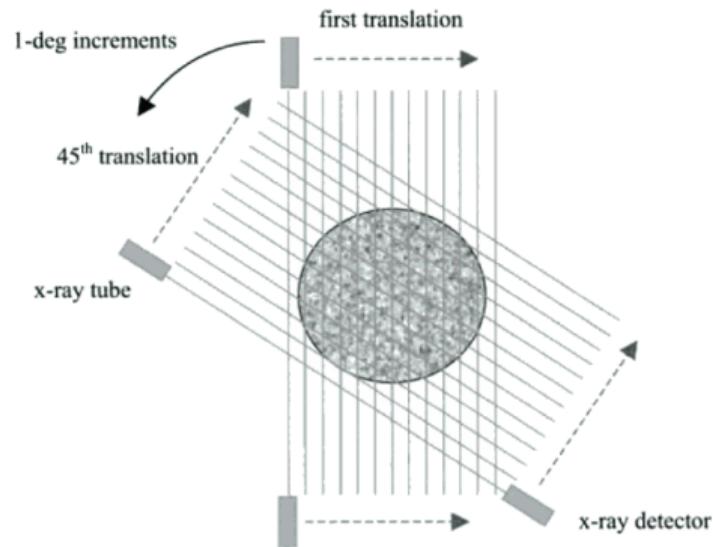
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]



From [14], Figure 5

# CT History

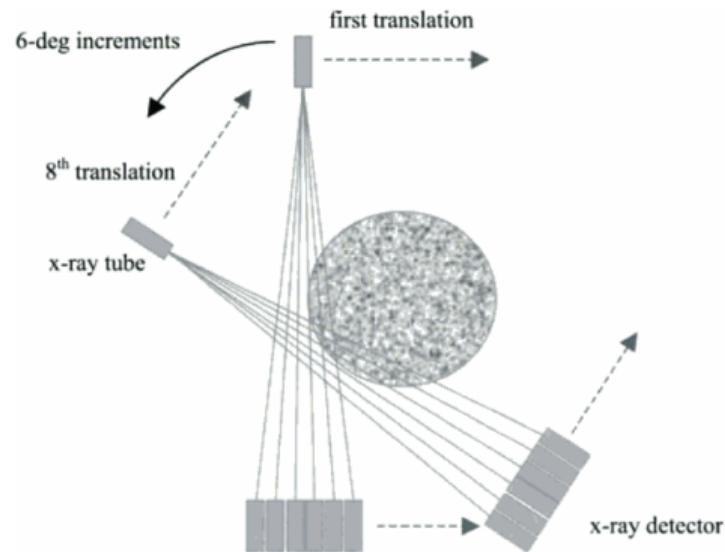
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- 1976: Hounsfield worked on first clinical scanner [13]
- CT scanner generations
  - First generation



From [15], Figure 1.12

# CT History

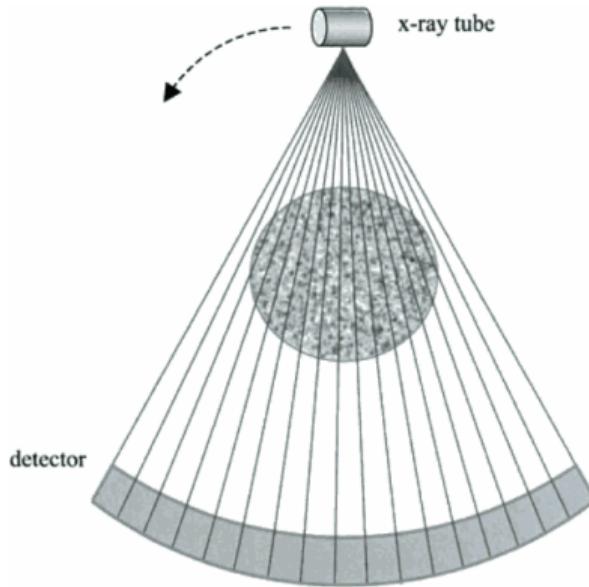
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- CT scanner generations
  - First generation
  - Second generation



From [15], Figure 1.13

# CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays
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- 1976: Hounsfield worked on first clinical scanner [13]
- CT scanner generations
  - First generation
  - Second generation
  - Third generation



From [15], Figure 1.14

# $\mu$ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [16]
- In the early 1980s, Lee Feldkamp [17] developed one of the early laboratory microCT systems by assembling a micro-focus cone beam x-ray source, specimen holder and stages, and an image intensifier at Ford Motor Company's Scientific Research Laboratory to nondestructively detect damage in ceramic manufactured automobile parts
- Met with scientists at Henry Ford Hospital and University of Michigan interested in understanding bone microstructure **Feldkamp1989** and osteoarthritis **Layton1988**
- 1987: The first commercially available  $\mu$ CT scanner was developed by SkyScan (now Bruker)

# $\mu$ CT History II

- Today:  $\mu$ CT is widely used in various fields for non-destructive imaging
  - quantifying the microstructure of organic materials, particularly mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
  - Material Science: internal structure of composites, foams, and other materials. Understand the relationship between structure and properties.
  - Biomedical Research: microarchitecture of bone tissue, dental research, and small animal imaging. Since the 1990s,  $\mu$ CT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
  - Paleontology and Archaeology: study of fossils and archaeological artifacts
- $\approx$ 2500  $\mu$ CT systems are in use worldwide with over 1000 publications annually

# X-ray interaction

- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([18])
  - Photoelectric absorption ( $\tau$ ) is strongly dependent on the atomic number  $Z$  of the absorbing material:  $\tau \propto \frac{Z^4}{E^{3.5}}$
  - Compton scattering is one of the principle forms of photon interaction and is directly proportional to the (electron & physical) density of the material. It does *not* depend on the atomic number:  $\lambda' - \lambda = \frac{h}{m_e c} (1 - \cos \theta)$
- Lowering x-ray energy increases contrast
- X-ray penetration decreases exponentially with sample thickness [19, i. e. Beer-Lamberts law]:  $I(t) = I_0 e^{-\alpha z}$

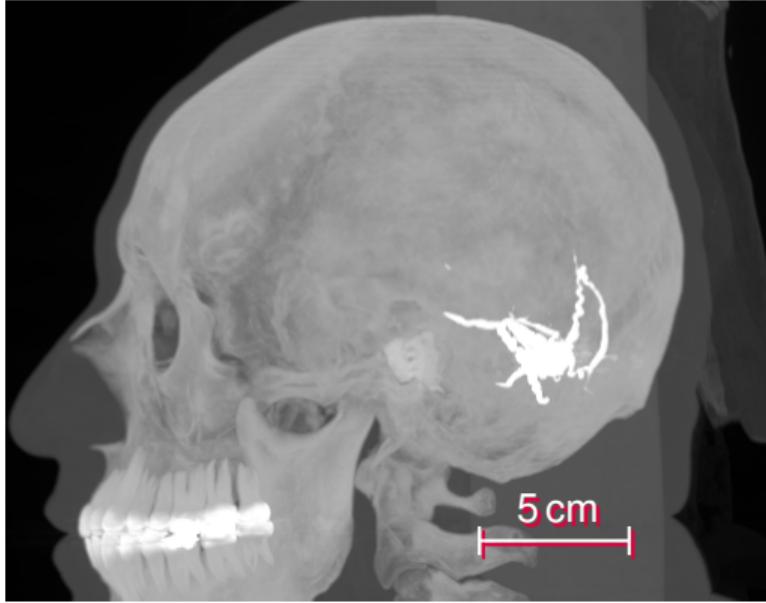
# Composition of biological tissues

Tissue: content by mass percentage

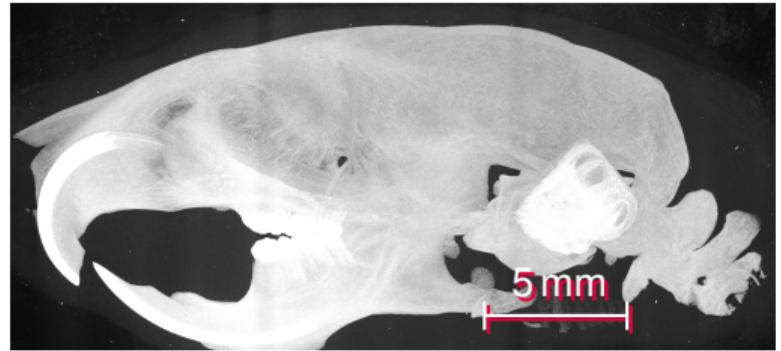
Element Atomic number	H 1	C 6	N 7	O 8	Na 11	P 15	S 16	Cl 17	K 19	Ca 20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3			22.5

*u*<sup>b</sup>

# Why $\mu$ CT?

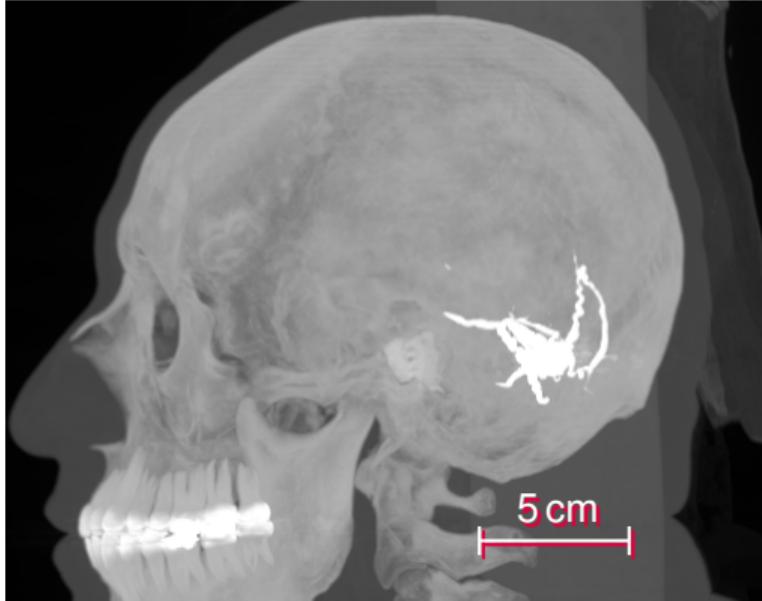


From [20], Subject C3L-02465



$u^b$

# Why $\mu$ CT?

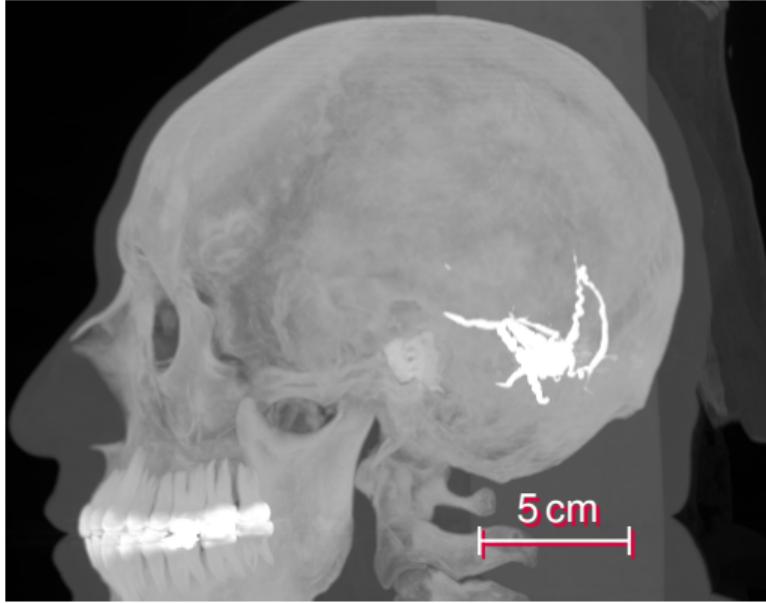


From [20], Subject C3L-02465

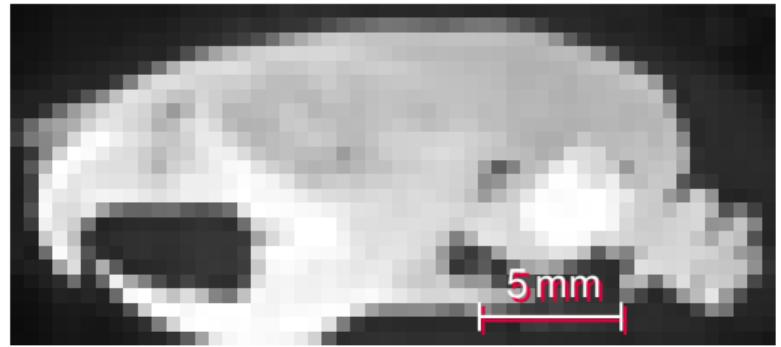


$u^b$

# Why $\mu$ CT?

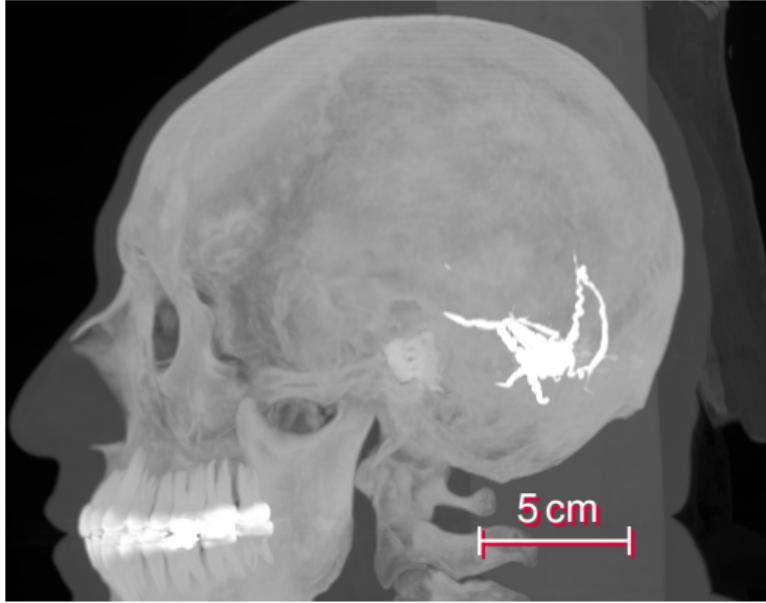


From [20], Subject C3L-02465

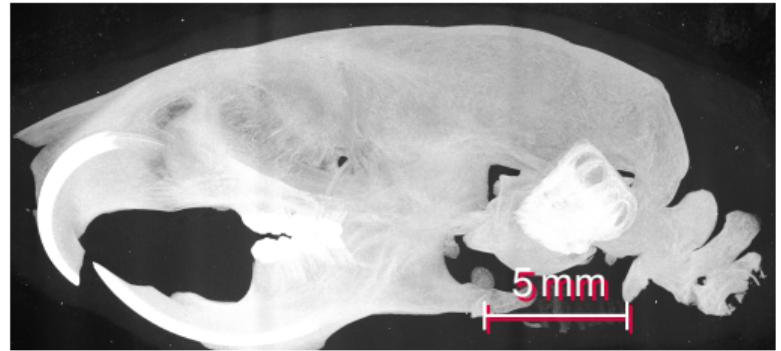


*u*<sup>b</sup>

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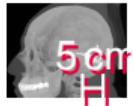


From [20], Subject C3L-02465

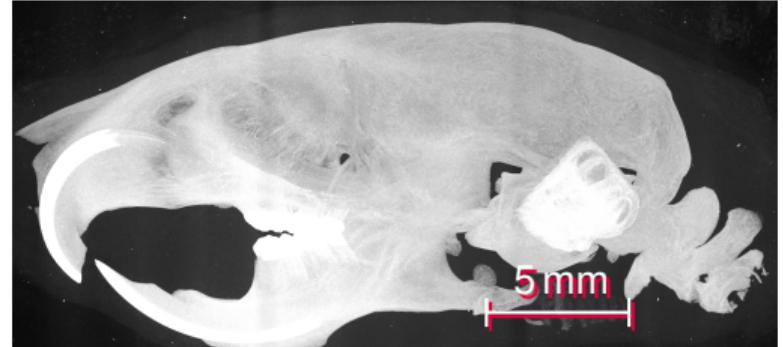


$u^b$

# Why $\mu$ CT?



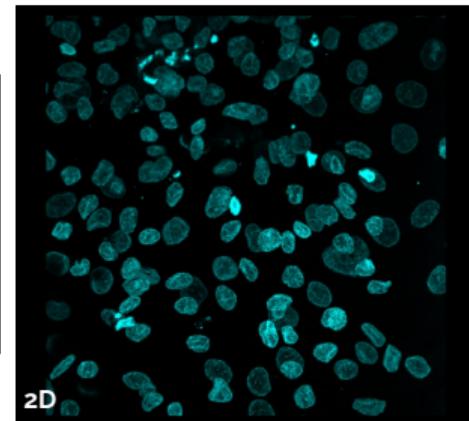
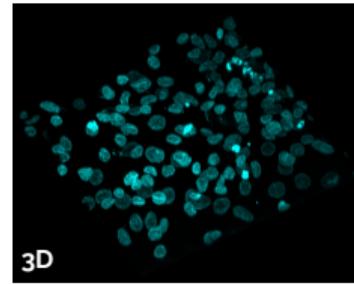
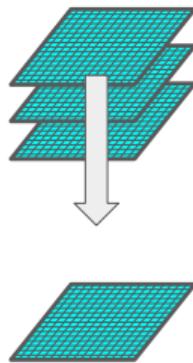
From [20], Subject C3L-02465



# Maximum intensity projection

## Projections

Reducing the dimensions of a dataset. For example projecting a volume (3D) to a surface by taking the maximum value across planes for each pixel.



*u*<sup>b</sup>

# Machinery

- Hospital CT
  - Voxel size around 0.5 mm
- Lab/Desktop CT
  - Voxel size around 7  $\mu\text{m}$  (*in vivo*) or 0.5  $\mu\text{m}$  (*ex vivo*)
- Synchrotron CT
  - Voxel size down to 160 nm



trim=<left> <lower> <right> <upper>

flic.kr/p/D4rbom ☺☺☺☺

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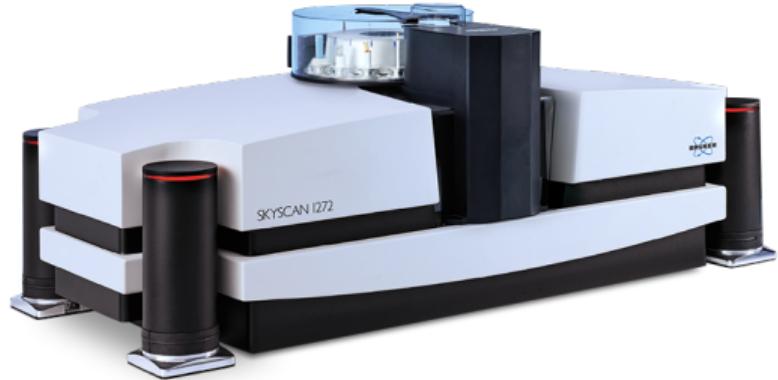


flic.kr/p/fpTrGu

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v. (None)

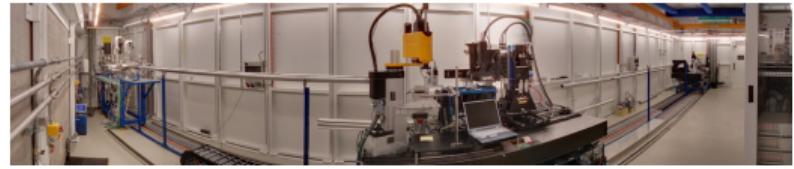
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trim=<left> <lower> <right> <upper>

[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

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trim=<left> <lower> <right> <upper>

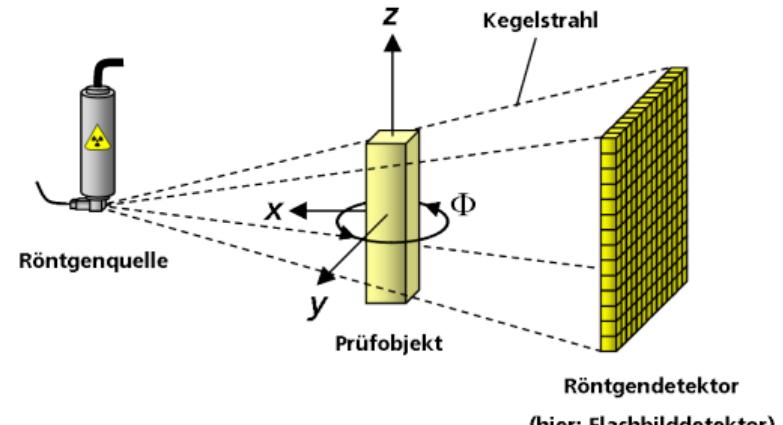
flic.kr/p/7Xhk2Y

*u<sup>b</sup>*

# What is happening?

No matter what kind of machine, the basic principle is always the same

- an x-ray source
- a sample
- a detector



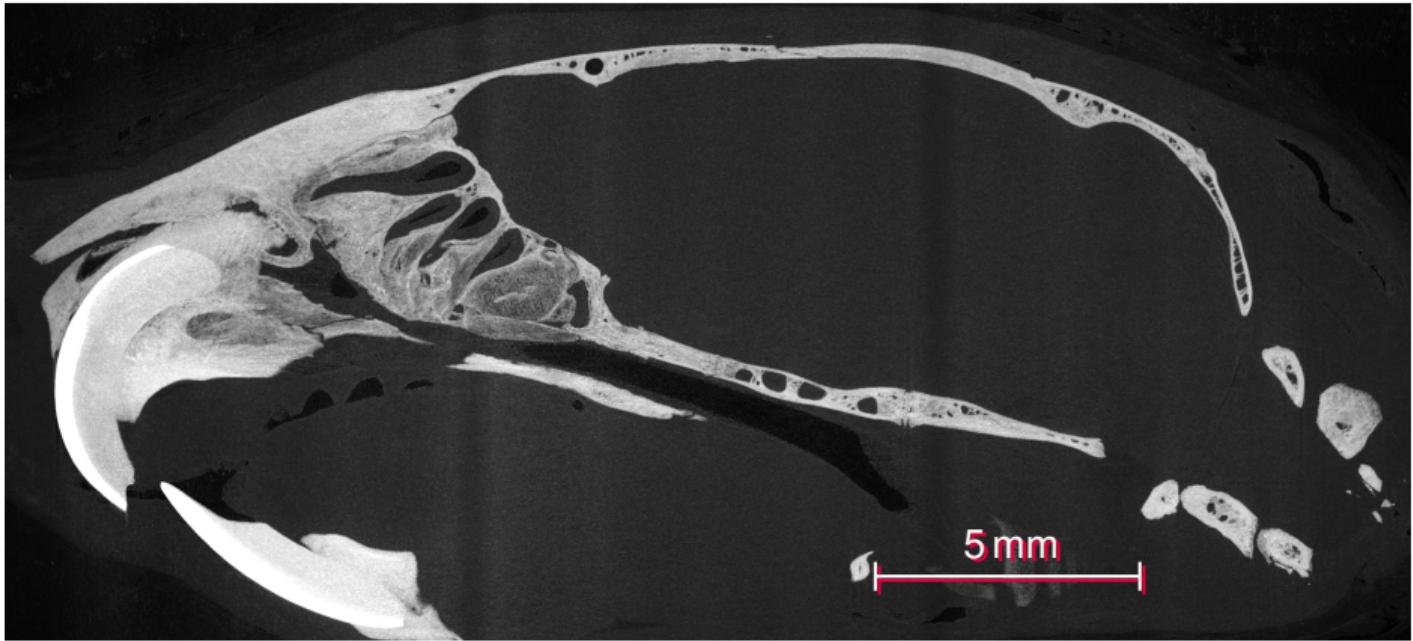
w.wiki/7g3

*u<sup>b</sup>*

# Machinery

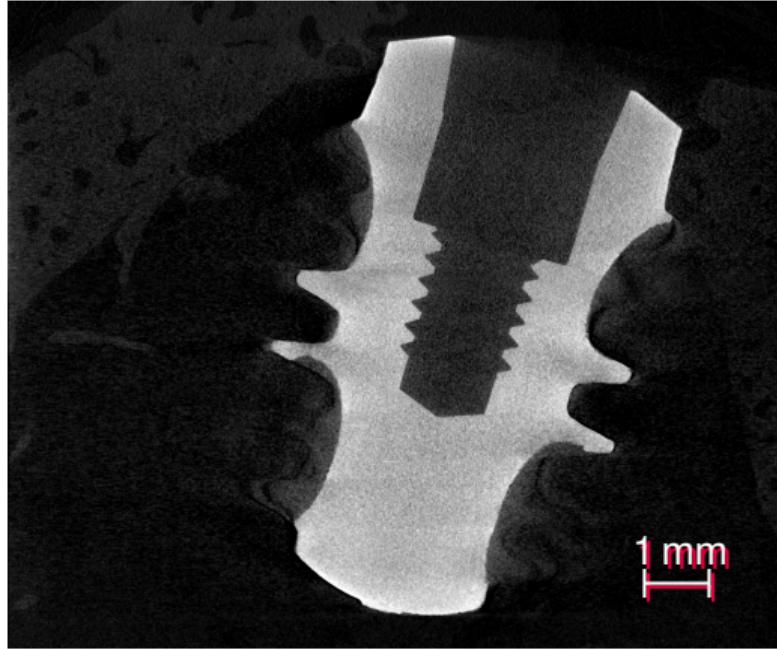
*u*<sup>b</sup>

# Examples



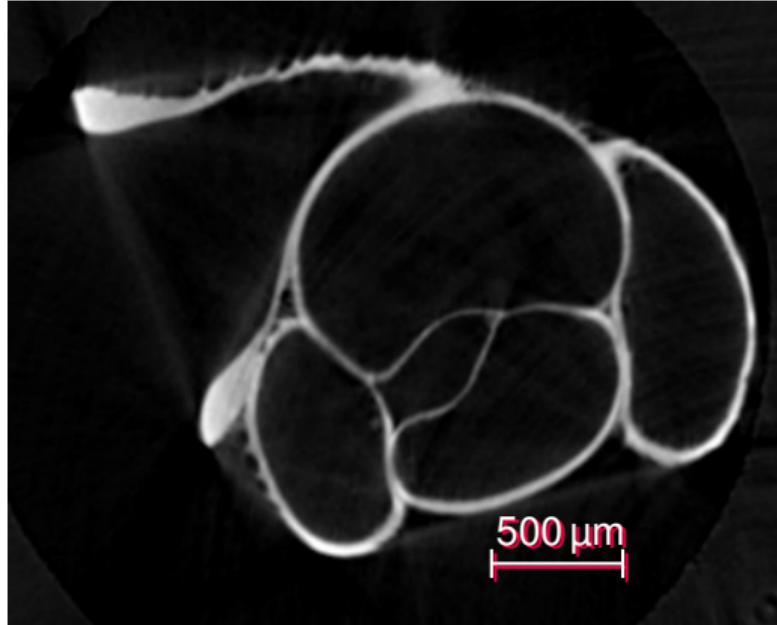
*u*<sup>b</sup>

# Examples



*u*<sup>b</sup>

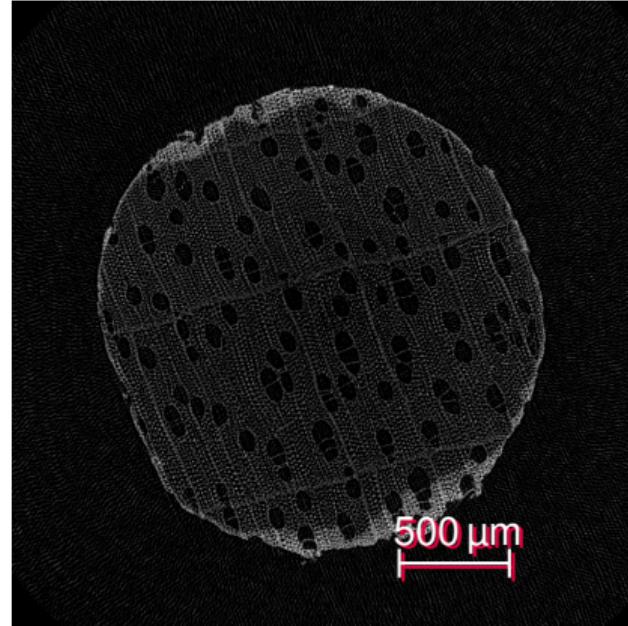
# Examples



From [8], *Diancta phoenix*

$u^b$

# Examples



*u*<sup>b</sup>

# Examples



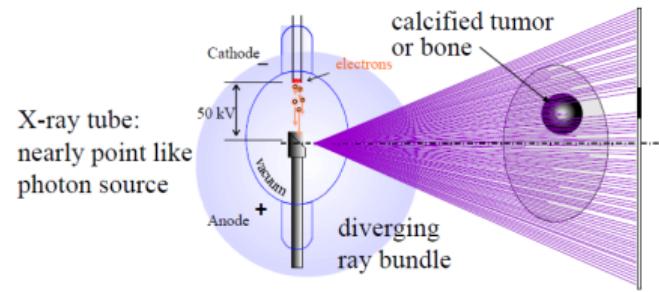
*u<sup>b</sup>*

# Preparation

- Study design
- Sample preparation

# Projections

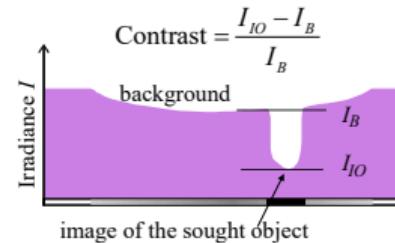
## X-ray generation and contrast



X-ray tube:  
nearly point like  
photon source

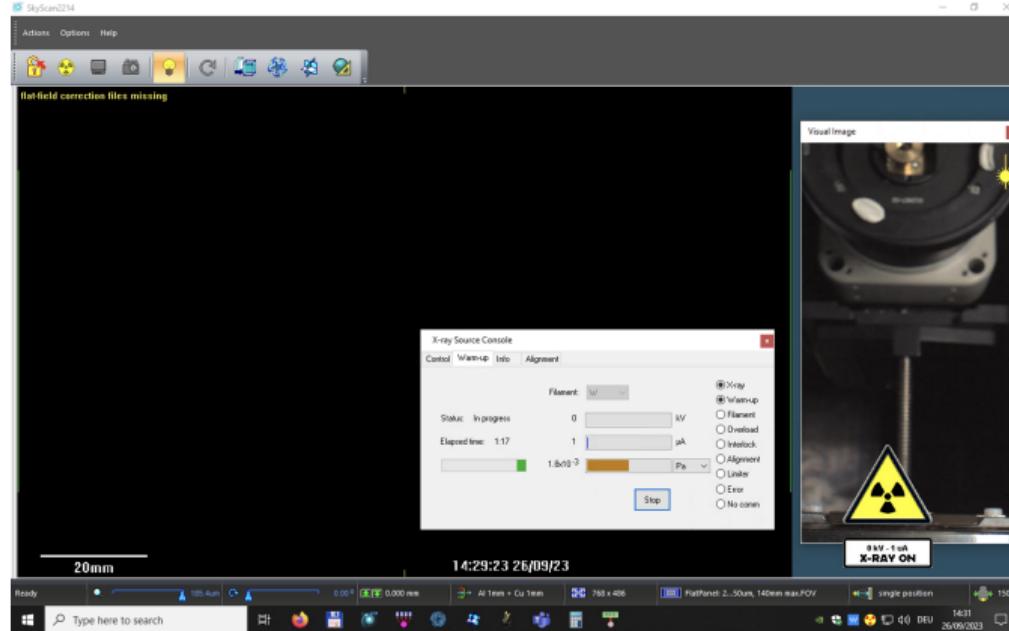
Contrast is given by  
absorption of intensity I

Note that contrast is negative  
X-ray shadowgraphy  
is a bright field technique



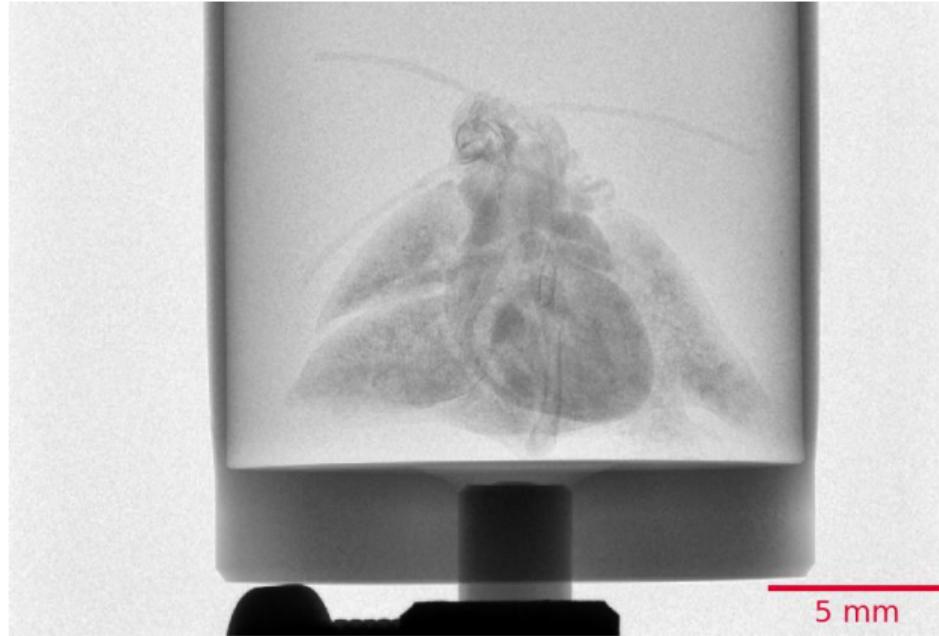
*Contrast, Magnification and Resolution—Laws of Physics for Microscopists (1, 2022) by Martin Frenz, Slide 21*

# $u^b$ Projection acquisition



*u*<sup>b</sup>

# Projections

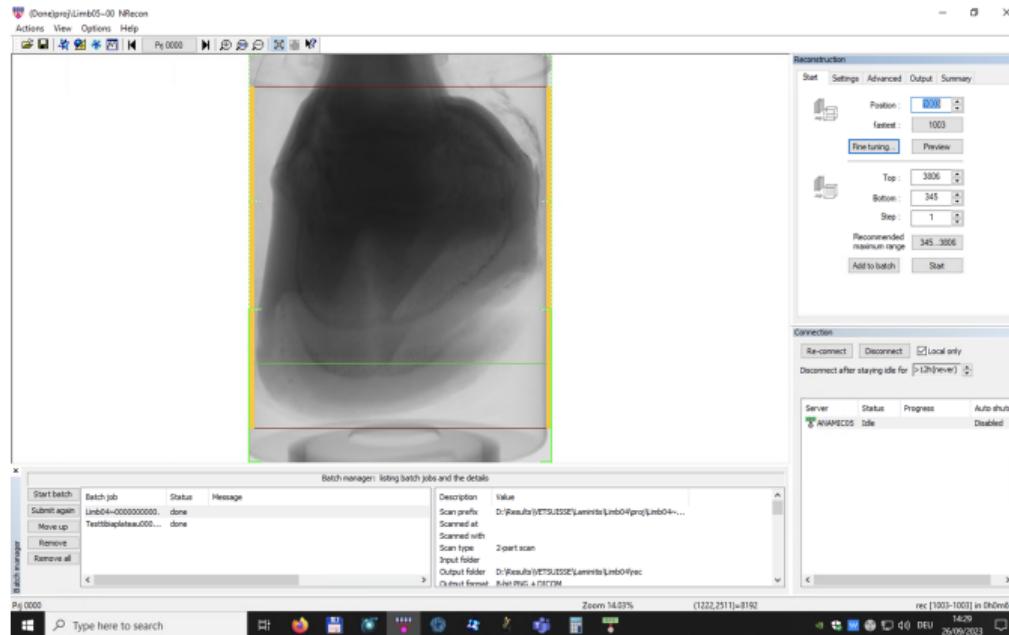


# Projections

- A (micro-focus) x-ray source illuminates the object
- The x-rays penetrate the sample and are attenuated
- A scintillator converts the x-rays to visible light
- A (planar) x-ray detector collects (magnified) projection images.
- The projections are recorded on disk

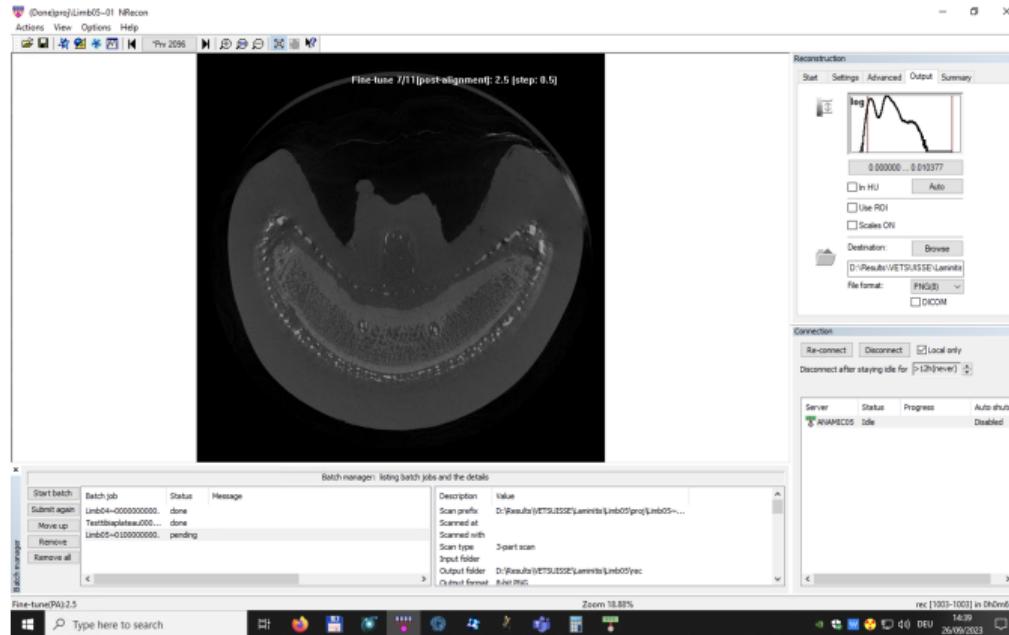
*u<sup>b</sup>*

# Reconstructions



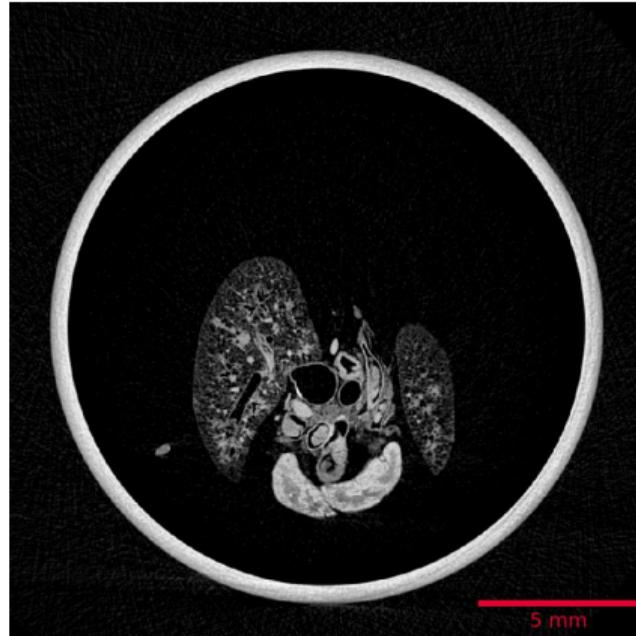
$u^b$

# Reconstructions



*u*<sup>b</sup>

# Reconstructions



# Reconstructions

- Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.
- Radon Transformation
- Filtered back projection
- Fan beam reconstruction
- Corrections (beam hardening, etc.)
- Writing to stack

$u^b$

# Visualization



# Visualization

- Based the on reconstructions, a computer synthesizes a three-dimensional view of the scanned sample

*u*<sup>b</sup>

# What to use?

- ImageJ/Fiji [21]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  -  in Jupyter [22]
  - **git**
  - Script all your things!
  - Data repositories; i. e. sharing is caring!

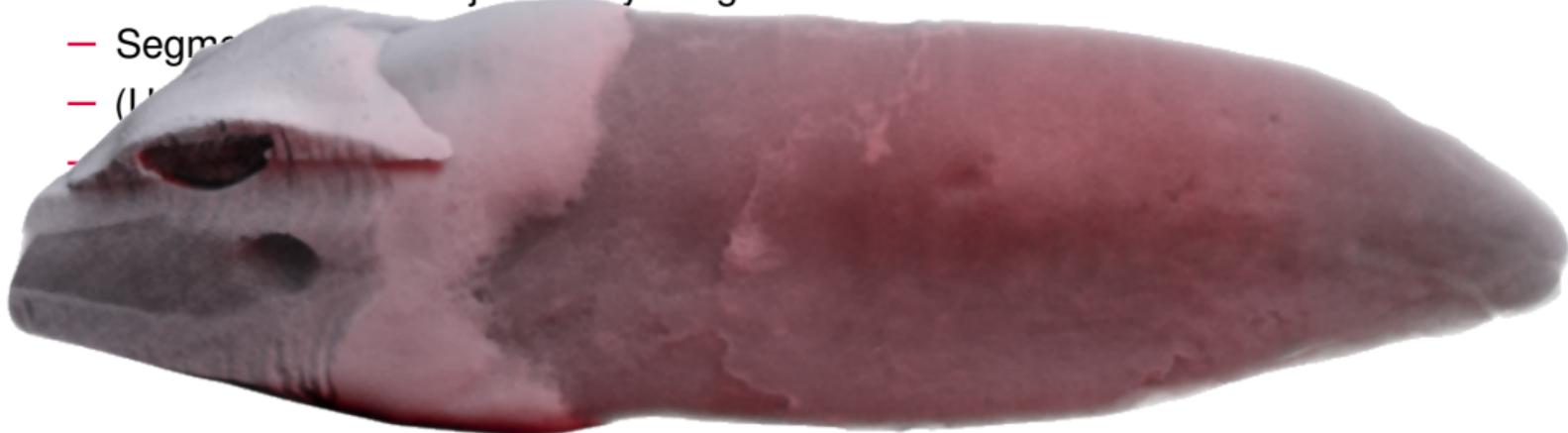
# Quantitative data

- Pretty images are nice, but we need quantitative numbers
- Segmentation
- Characterization

# Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers instead of just pretty images
- Segments
- (L)
- 



# Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers instead of just pretty images
- Segmentation of teeth and root canal
- (Unbiased) Characterization
- Reproducible and automated image analysis ( in Jupyter [22])
- Two publications:
  - [10], BMC Oral Health, doi.org/gjpw2d
  - [23], Scientific Reports, doi.org/g7r8

*u*<sup>b</sup>

# How?

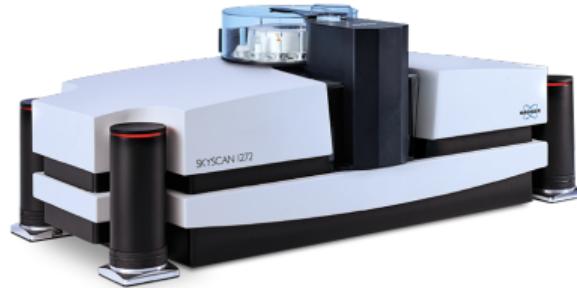
- 104 extracted human permanent mandibular canines
- $\mu$ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [24]
- *Reproducible* analysis [25], e. g. you can click a button to double-check or recalculate the results yourself!



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# How?

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[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

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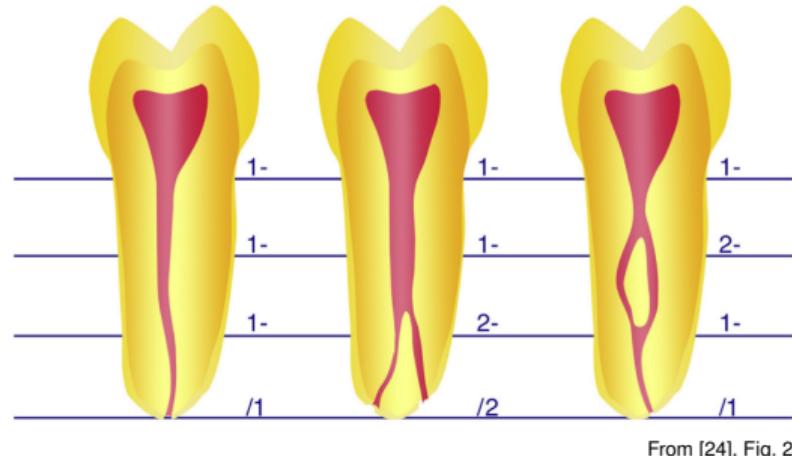
```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files= 482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)= 80
Source Current (uA)= 125
Image Pixel Size (um)=9.999986
Exposure (ms)=950
Rotation Step (deg)=0.400
Frame Averaging=ON (3)
Filter=Al 1mm
Study Date and Time=02 Jul 2020 08h:23m:34s
Scan duration=0h:39m:51s
```

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*Sample changer on the SkyScan 1272*  
In total:

- 13 days of *continuous* µCT scanning
- 819 GB of raw data  
230 648 TIFF projections
- 326 GB data as input for analysis  
282 062 PNG reconstructions

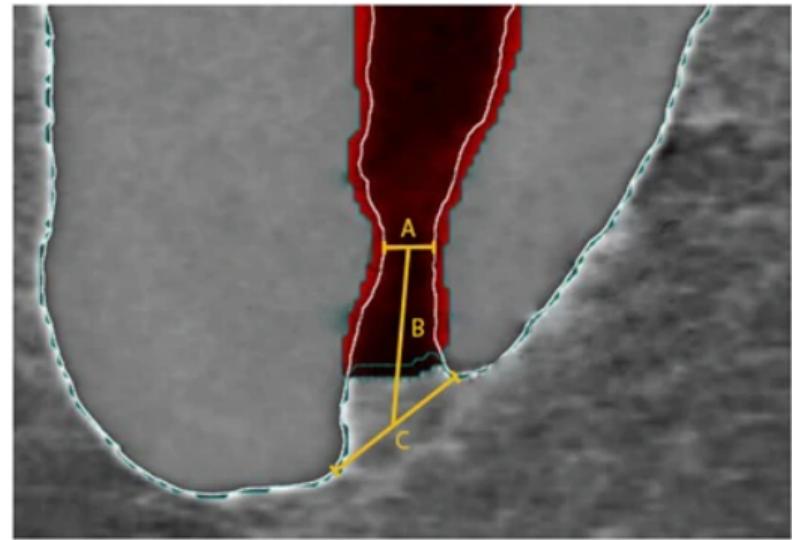
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From [26], Fig. 1

*u*<sup>b</sup>

# How?

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[gph.is/2nqkple](http://gph.is/2nqkple)

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The screenshot shows a GitHub repository interface. At the top, there are buttons for 'master', '1 branch', '1 tag', 'Go to file', 'Add file', and 'Code'. Below this is a list of files under the 'habil' user:

File	Description	Last Commit
.github/workflows	Update actions file	20 days ago
.gitignore	Only 'mode' changes	2 months ago
DownloadFromOSF.ipynb	Clean run of download script	22 days ago
README.md	Typo in Binder badge & link to full repo on Binder	22 days ago
Tooth.Border.jpg	Only 'mode' changes	2 months ago
Tooth.Characterization.jpg	Only 'mode' changes	2 months ago
ToothAnalysis.ipynb	Only select a subset if we actually have data.wq	22 days ago
ToothAxisSize.ipynb	Clean run of notebook	22 days ago
ToothDisplay.ipynb	Display Tooth045 for manuscript	22 days ago
requirements.txt	We also need this	2 months ago
treebeard.yaml	Add treebeard configuration	20 days ago

Below the file list is the 'README.md' page, which contains the following content:

```
README.md
```

DOI: 10.5281/zenodo.3999402 treebeard.yaml failing

[launch binder](#)

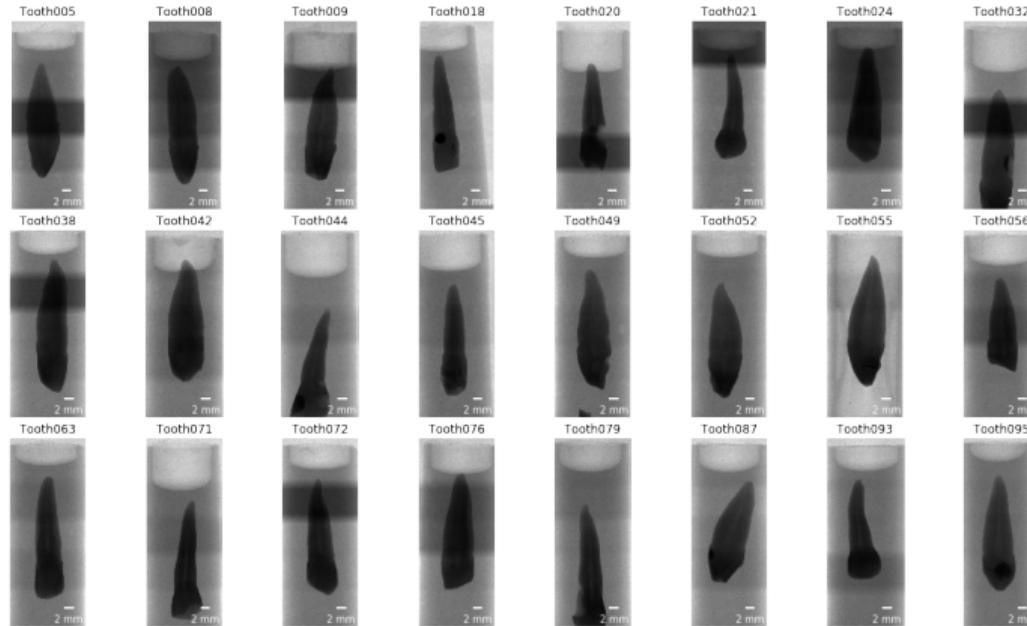
**A big tooth cohort**

We scanned a *big* bunch of teeth for a team of the dental clinic of the University of Bern.

To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the ~100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of any software. If you'd like to start a Binder instance with the full repository, you can click [here](#).

$\mu$

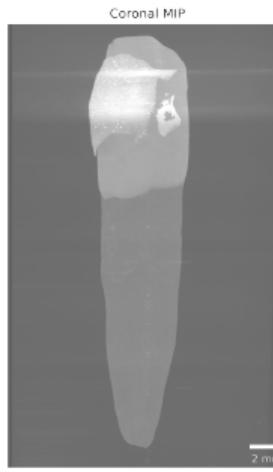
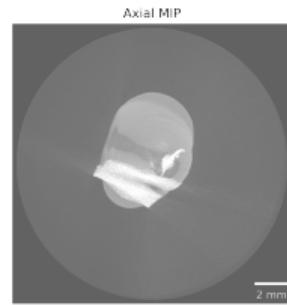
# $\mu$ CT imaging



*u<sup>b</sup>*

# Dataset cropping

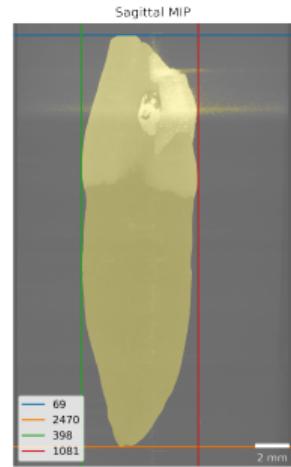
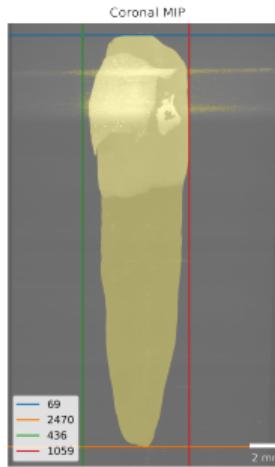
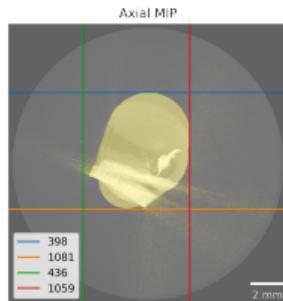
- Full datasets: 326 GB
- Cropped datasets: 115 GB



*u<sup>b</sup>*

# Dataset cropping

- Full datasets: 326 GB
- Cropped datasets: 115 GB



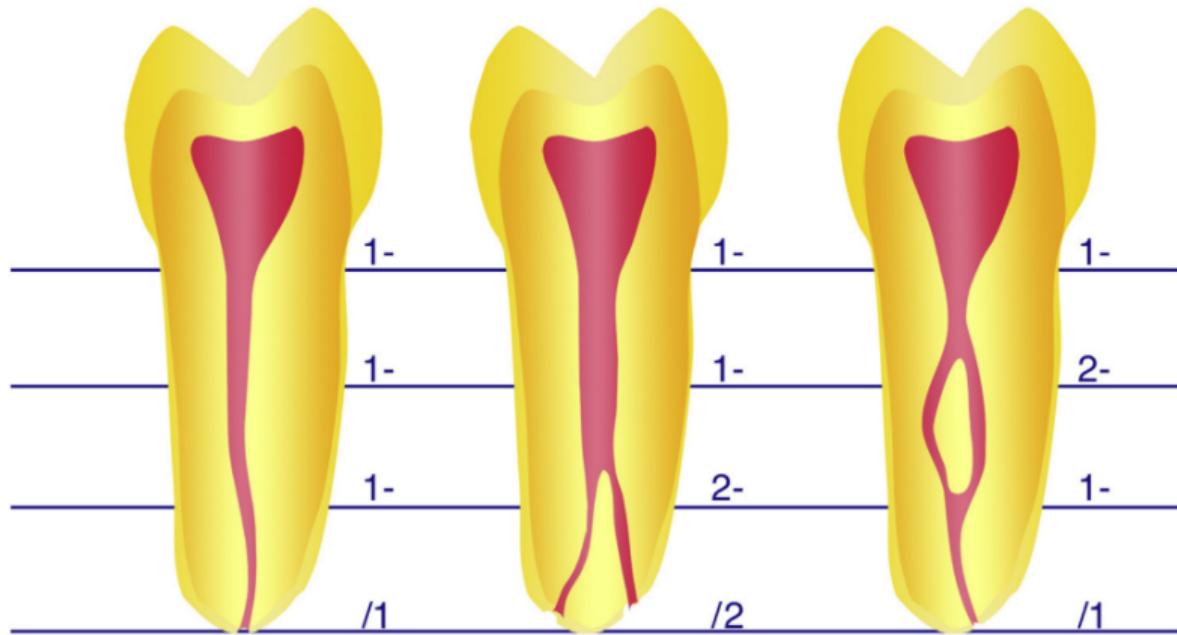
*u<sup>b</sup>*

# Tooth morphology



*u<sup>b</sup>*

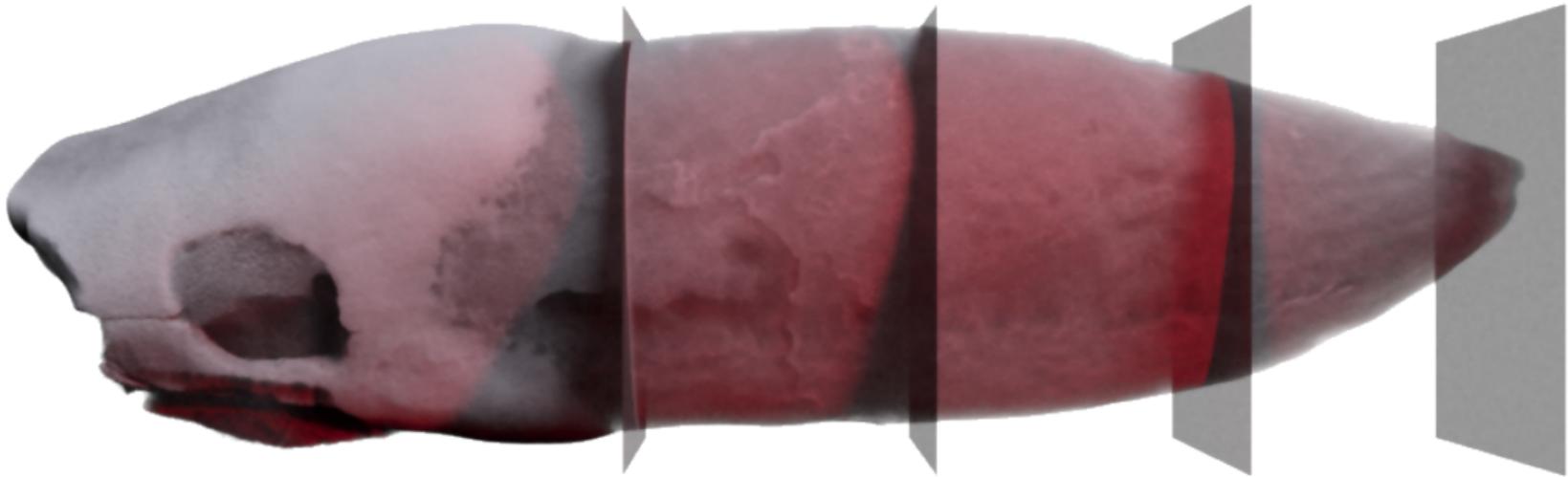
# Tooth morphology



From [24], Fig. 2

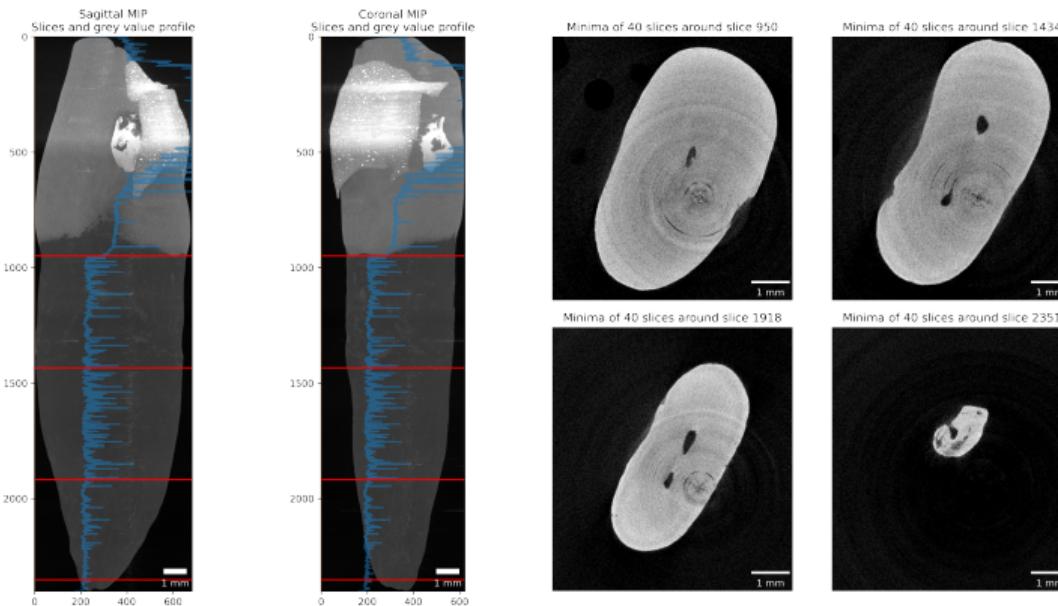
*u<sup>b</sup>*

# Tooth morphology



*u<sup>b</sup>*

# Detection of enamel-dentin border

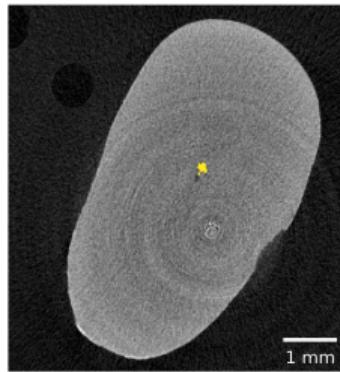


*u<sup>b</sup>*

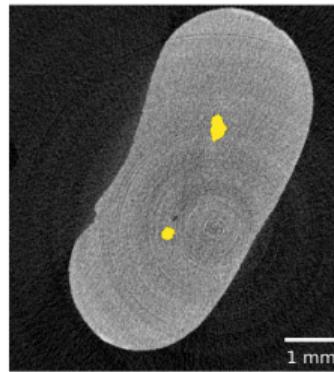
# Detection of enamel-dentin border

Tooth045

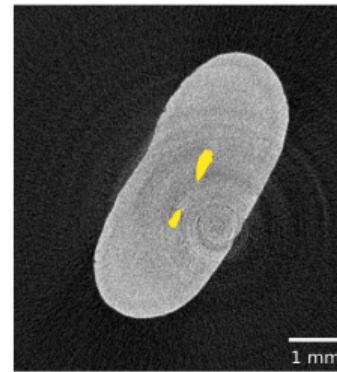
Tooth045, Slice 950: 1



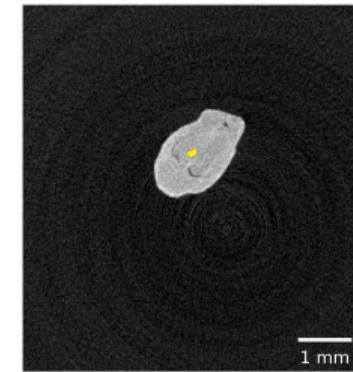
Slice 1434: 2



Slice 1918: 2



Slice 2351: 1



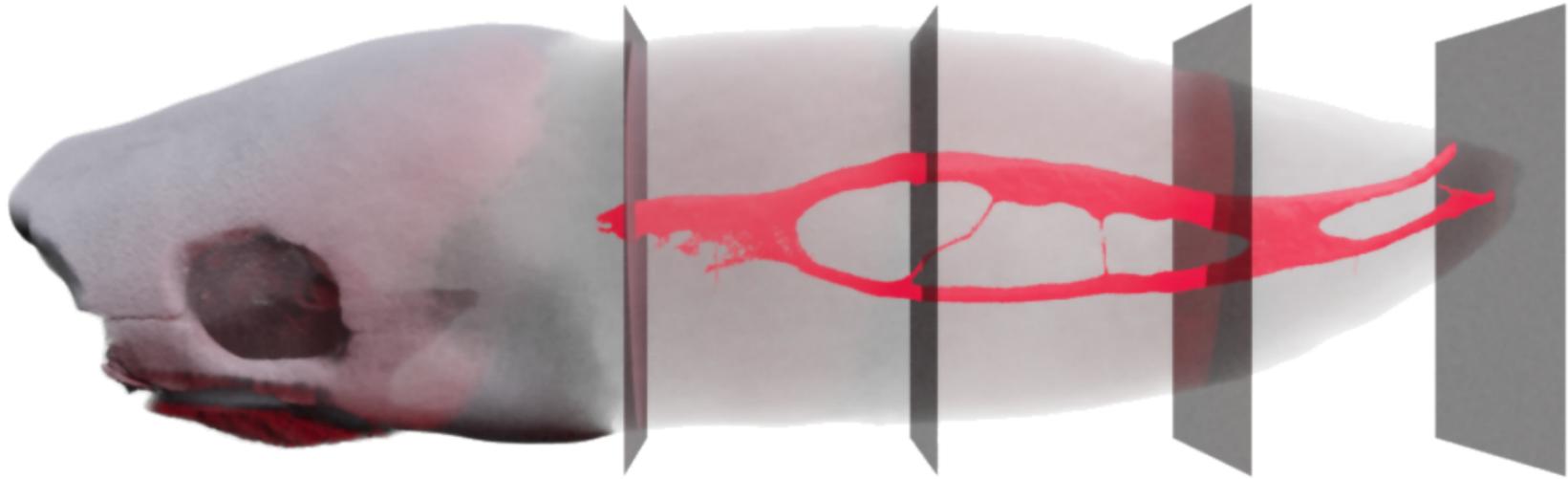
*u<sup>b</sup>*

# Outcome root canal configuration classification

Roots	RCC	#	%	
Single (N=98)	1-1-1/1	73	74.5	
	1-1-1/2	14	14.3	
	1-1-1/3	1	1.0	
	1-1-1/4	2	2.1	
	1-1-2/1	1	1.0	
	1-2-1/1	4	4.1	
	1-2-1/2	1	1.0	
	1-2-2/2	1	1.0	
	2-3-1/1	1	1.0	
Double (N=3)	Buccal	1-1-1/1	2	66.6
		1-2-1/1	1	33.3
	Lingual	1-1-1/1	2	66.6
		1-1-1/2	1	33.3

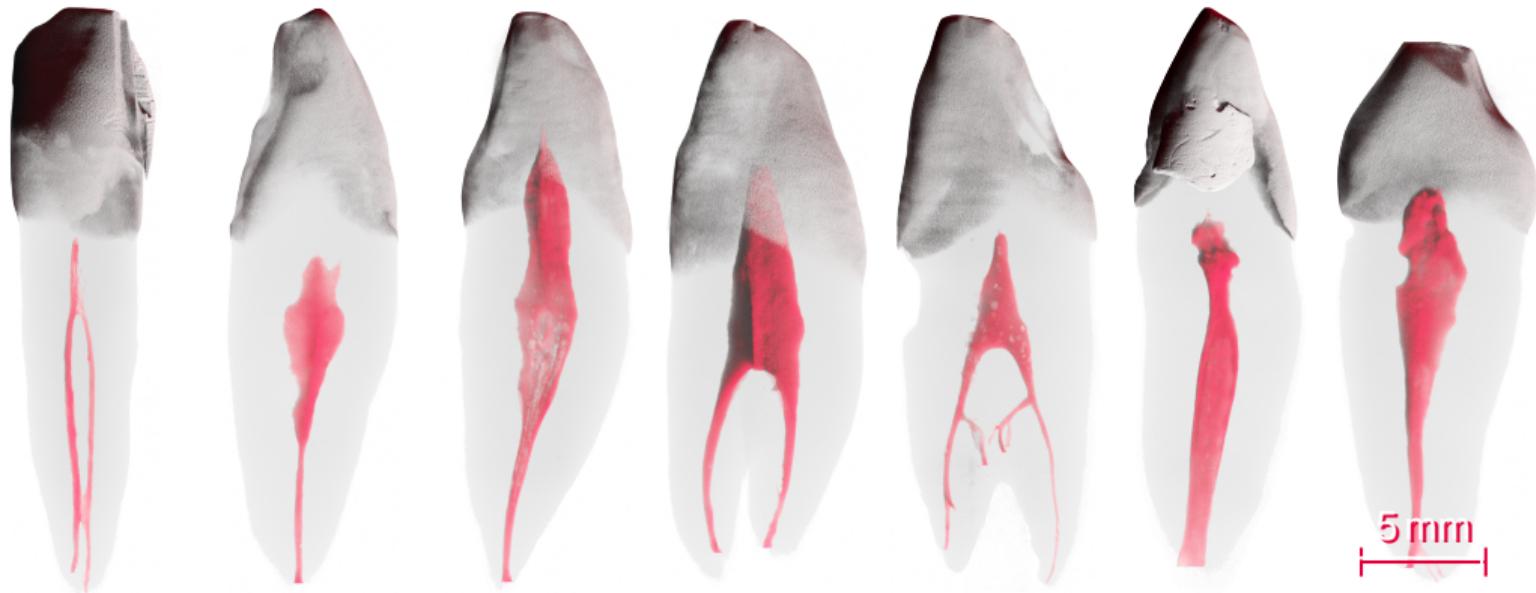
*u<sup>b</sup>*

# Extraction of root canal space



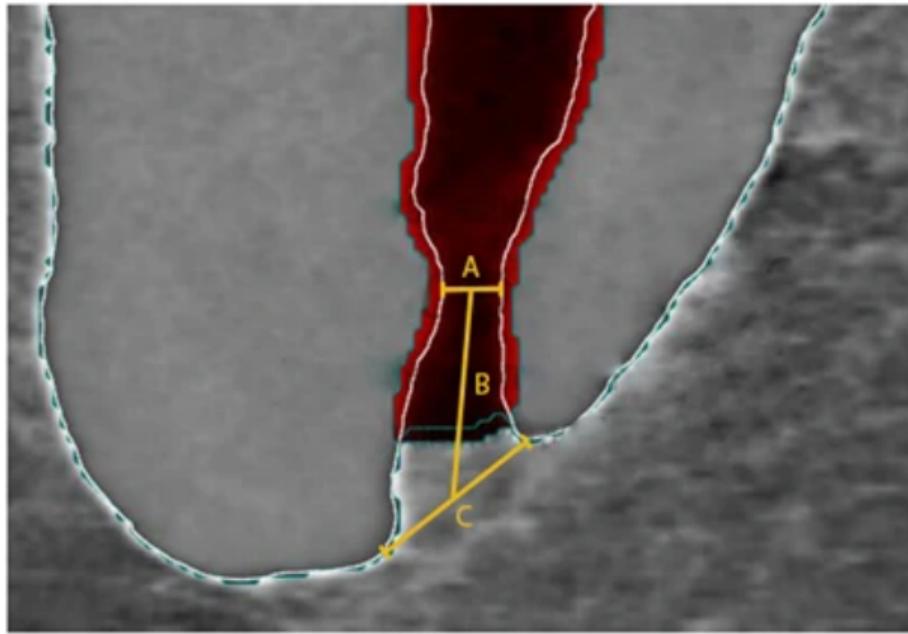
*u<sup>b</sup>*

# Results of root canal space extraction



*u<sup>b</sup>*

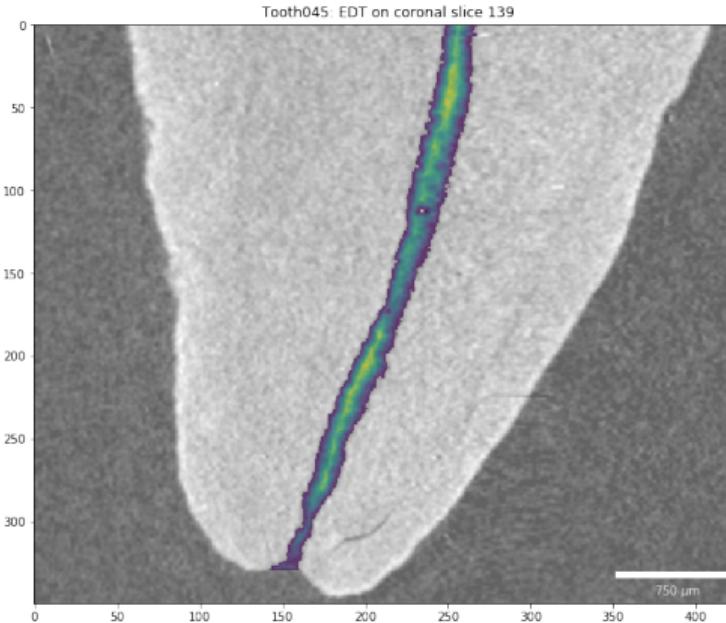
# Analysis of the physiological foramen geometry



From [26], Fig. 1

*u<sup>b</sup>*

# Analysis of the physiological foramen geometry

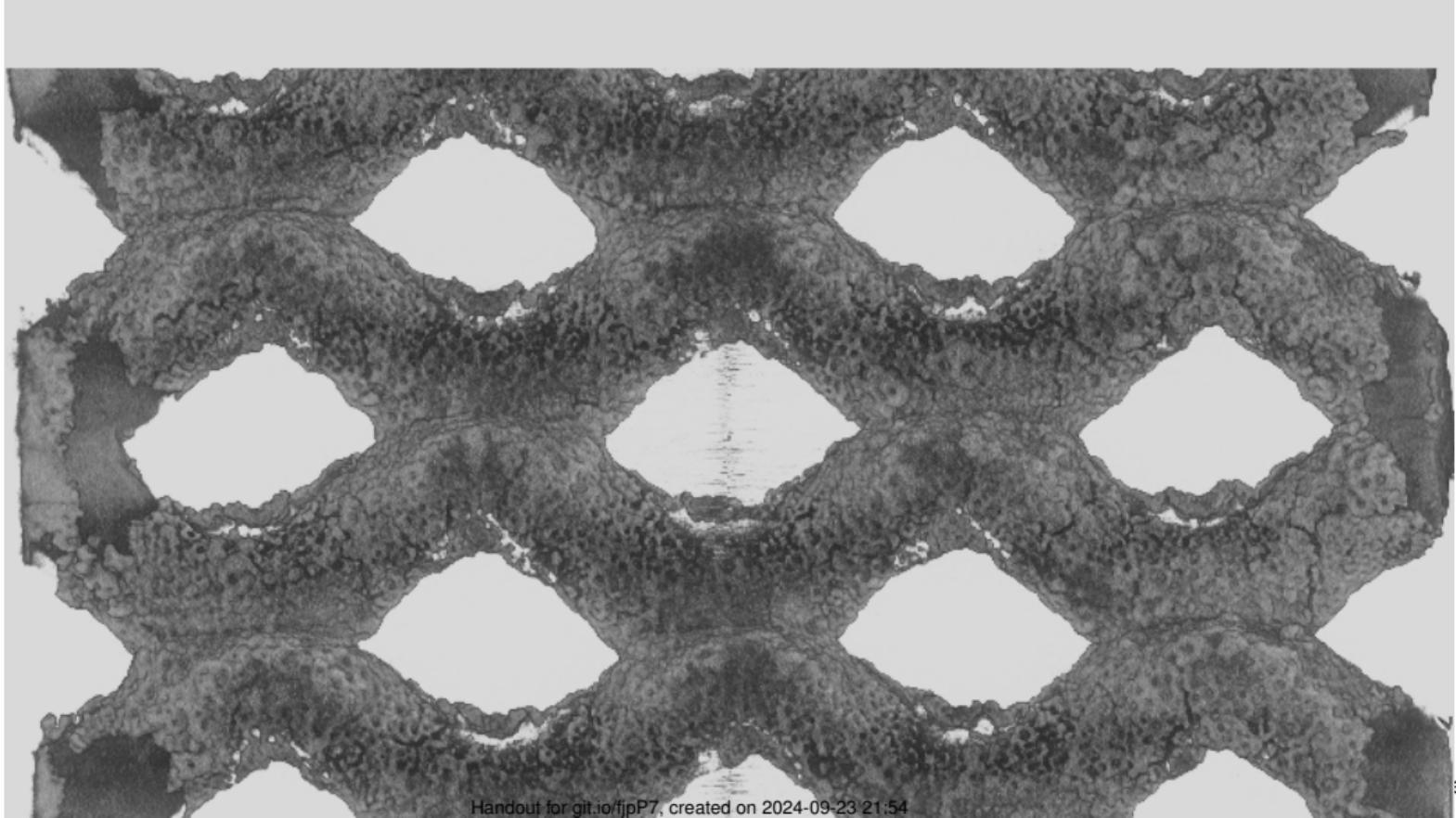


# Conclusion ZMK

- Efficient use of time, e. g. more teeth does not mean more (human) work
- Reproducible analysis with *free and open-source* software, usable by *anyone*
- Objective analysis, e. g. no operator bias

$u^b$

# Metal foam

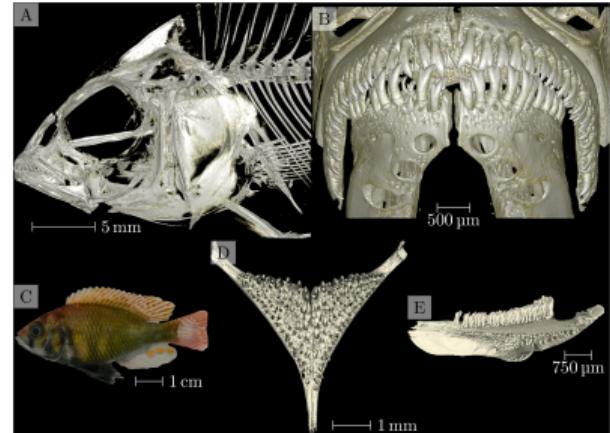


*u<sup>b</sup>*

# Data wrangling by example: Cichlids

Collaboration with team of *Aquatic Ecology & Evolution*,  
from the Institute of Ecology and Evolution [11]

- 133 Cichlids from Lake Victoria, East Africa
  - Functional anatomy of the skulls and jaws
  - 6–18 cm in size
- 375 scans in total
  - Voxelsizes from 3.5–50 µm
  - 46 days of scanning time
  - 9.8 TB of raw data
  - 1.5 TB/+1 000 000 reconstructions

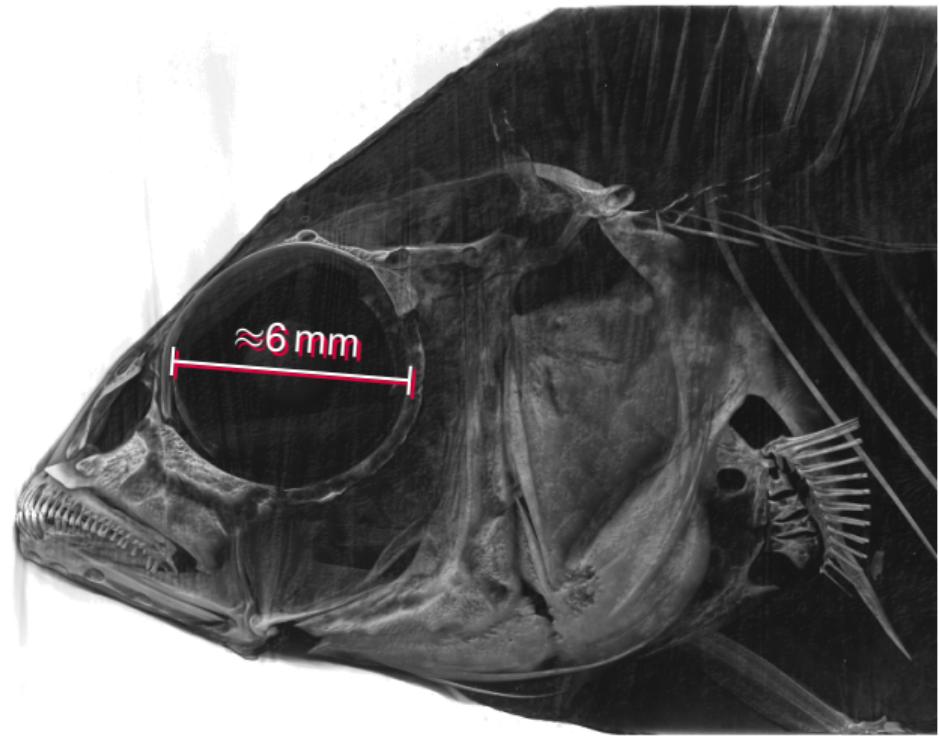


DOI:gsst8t, Fig. 1

[11] D. Haberthür *et al.*, Sep. 2023. DOI: gsst8t.

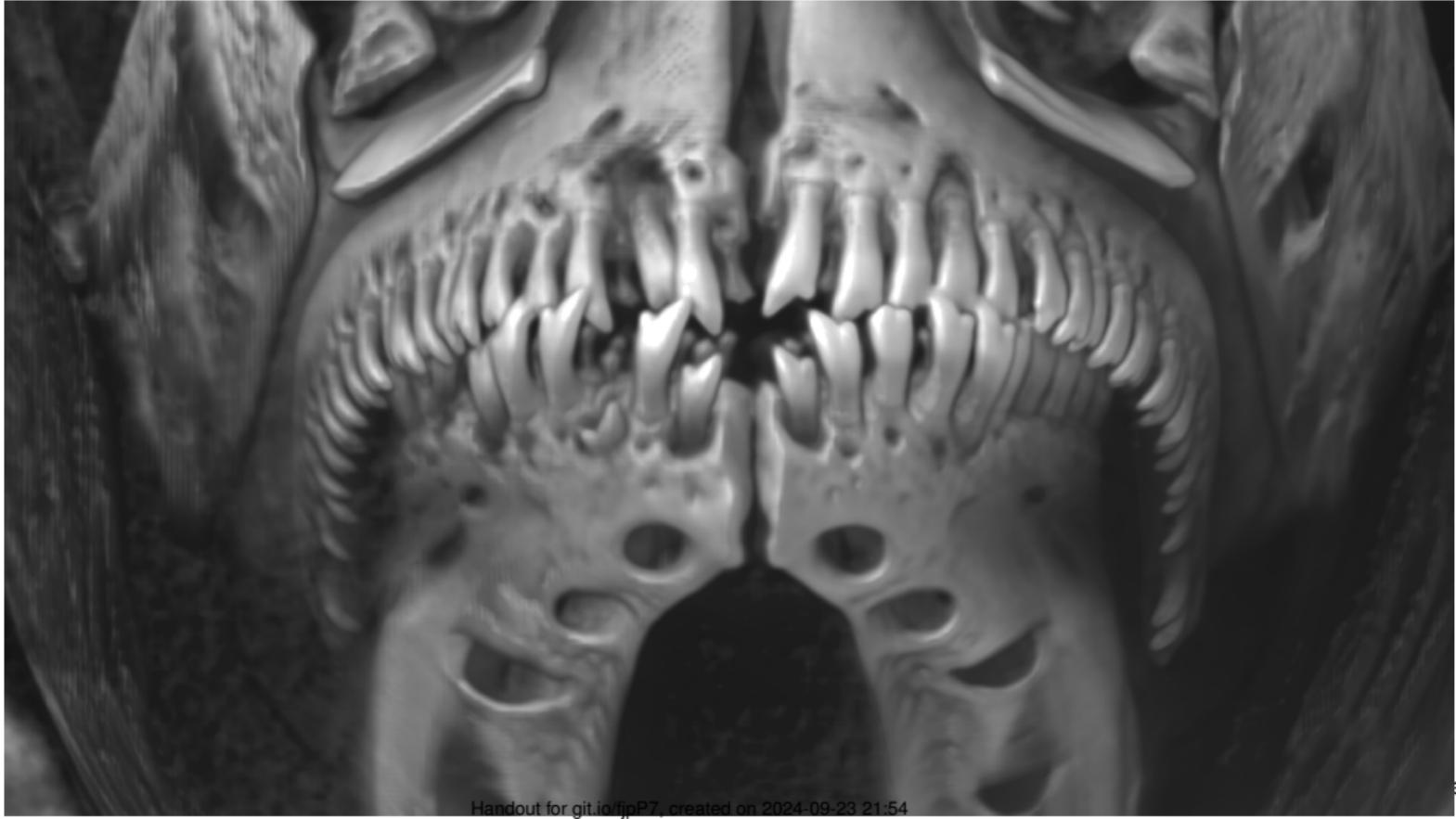
*u*<sup>b</sup>

# Visualization of cichlid head



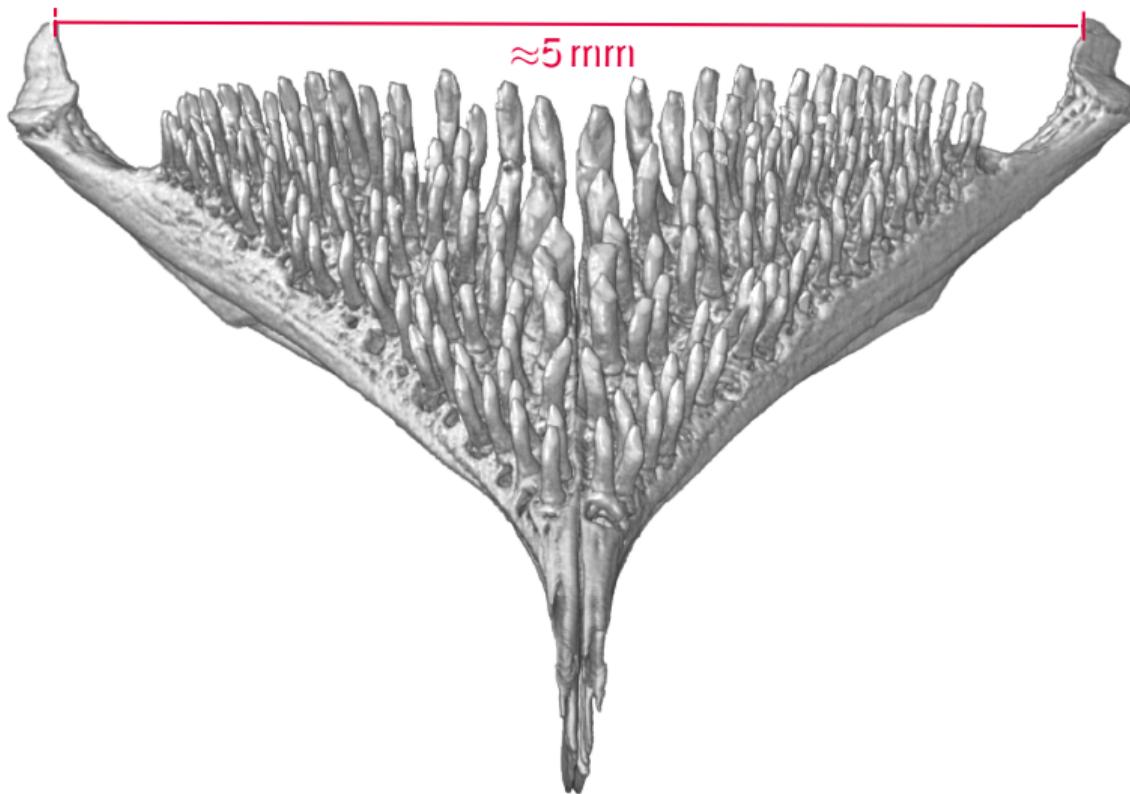
*u*<sup>b</sup>

# Visualization of cichlid head



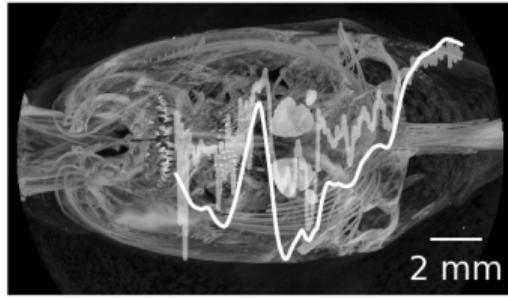
*u*<sup>b</sup>

# Visualization of segmented pharyngeal jaw



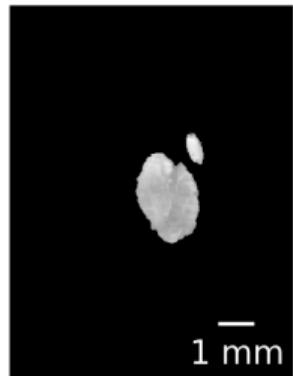
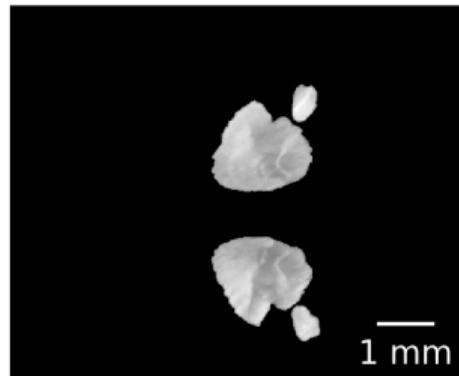
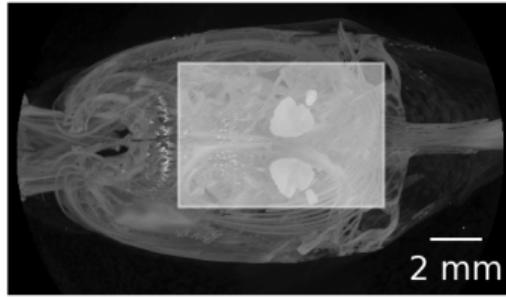
*u*<sup>b</sup>

# Data wrangling by example: Cichlids



*u*<sup>b</sup>

# Data wrangling by example: Cichlids



*u*<sup>b</sup>

# Thanks!

- Thanks for listening to me!
- What questions do you have for me?

# Colophon

- This BEAMER presentation was crafted in  $\text{\LaTeX}$  with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
  - Complete source code: [git.io/fjpP7](https://git.io/fjpP7)
  - The  $\text{\LaTeX}$  code is automatically compiled with a GitHub action to a (handout) PDF which you can access here: [git.io/JeQxO](https://git.io/JeQxO)
- Did you spot an error?
  - File an issue: [git.io/fjpPb](https://git.io/fjpPb)
  - Submit a pull request: [git.io/fjpPN](https://git.io/fjpPN)
  - Send me an email: [david.haberthuer@unibe.ch](mailto:david.haberthuer@unibe.ch)

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