

# X-ray microtomography

**David Haberthür**

September 27, 2023 | 485018-HS2023-0: Advanced Course II Ultraprecision Engineering

# Grüessech mitenang!

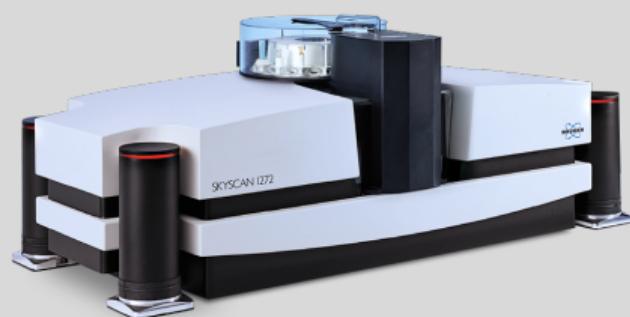
- David Haberthür
  - Physicist by trade
  - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
  - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
  - Post-Doc II:  $\mu$ CT group, Institute of Anatomy, University of Bern, Switzerland.

# Grüessech from the $\mu$ CT-group



## $\mu$ 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

**Overview**

**Imaging**

**Tomography**

History

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

# µCT

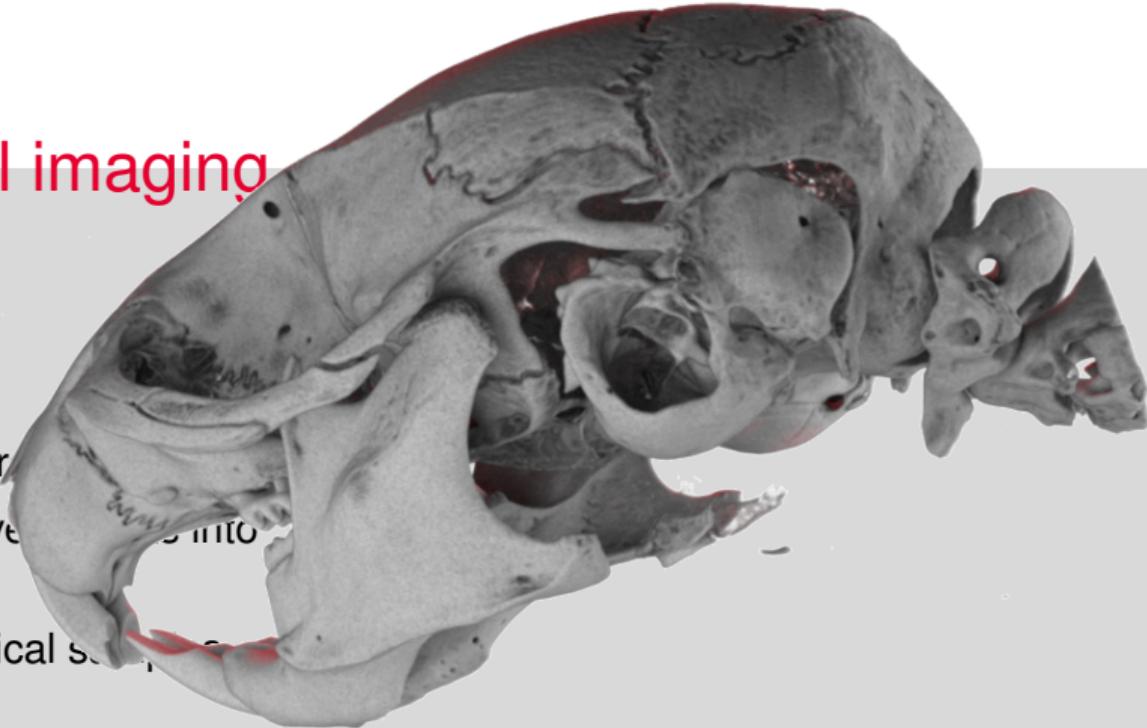
- Dense and/or non-transparent samples
- Calibrated & isotropic 3D images at micron resolutions
- Covers a very large range of sample sizes
- Gives information at different length scales
- Nondestructive imaging, thus compatible with routine sample preparation.  
Enables correlative imaging pipelines, scanning of museum & collection material

# Biomedical imaging

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

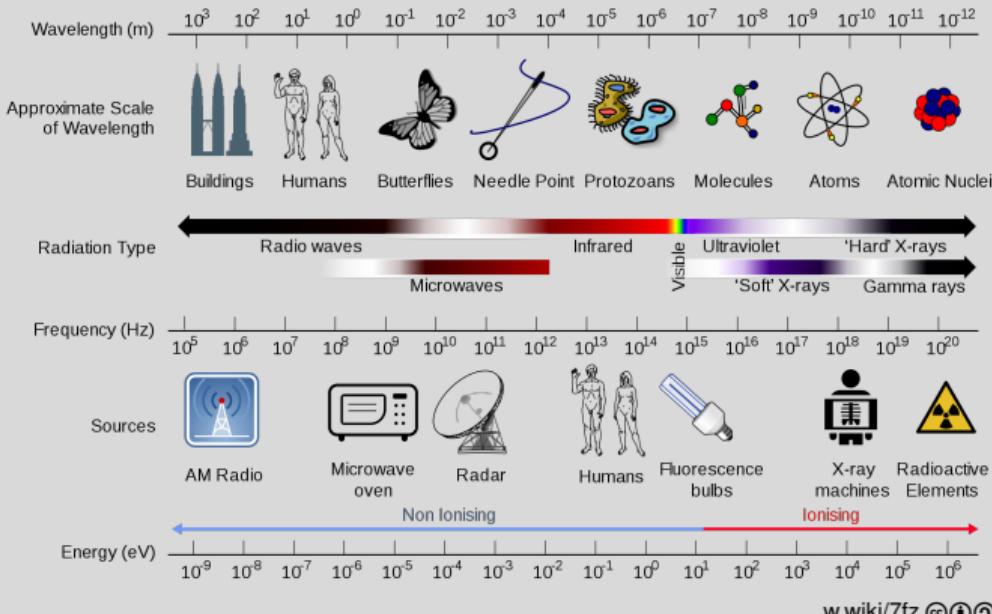


## Biomedical imaging

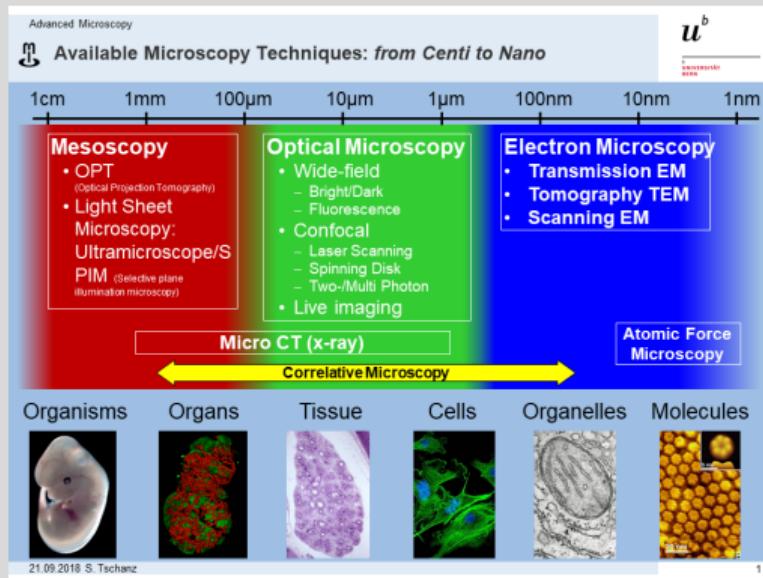


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# Wavelength & Scale



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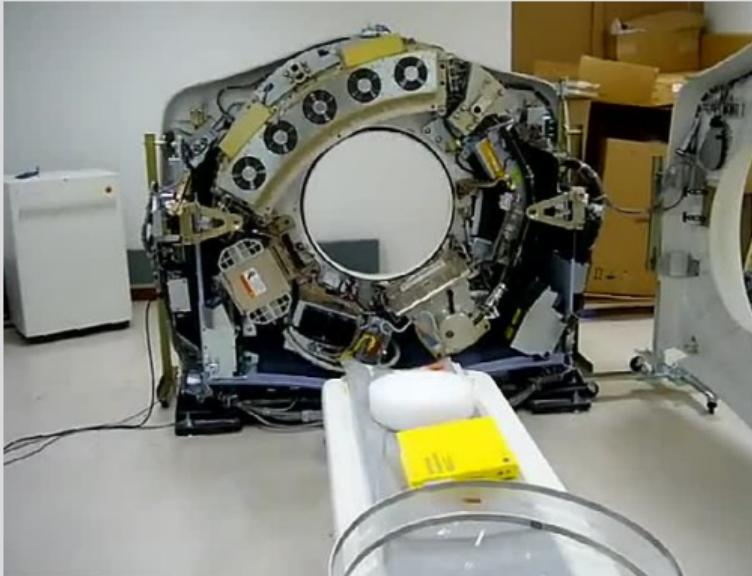


Stefan Tschanz, with permission

# 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

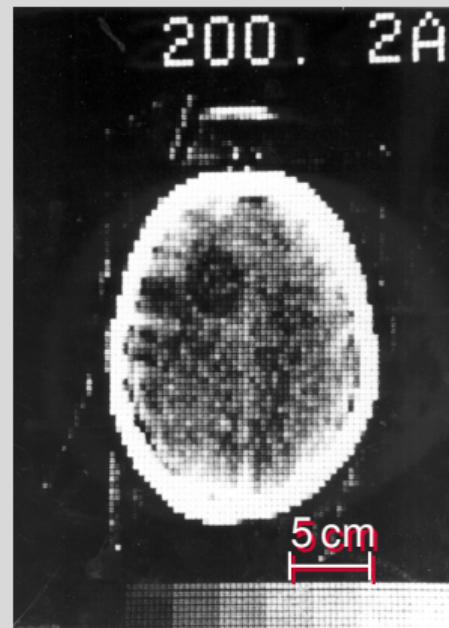
# CT-Scanner



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

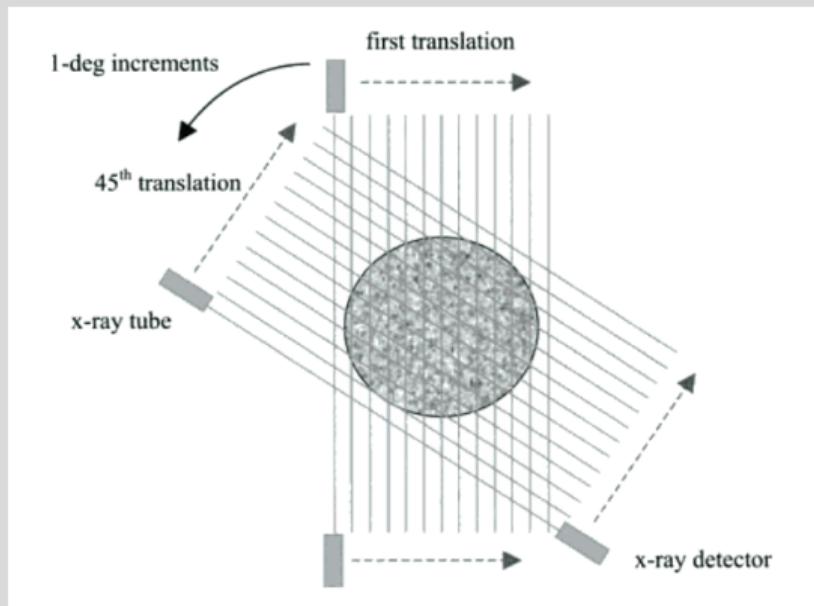
## CT History

- Long history
  - 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]
  - Nice overview by Hsieh [14]



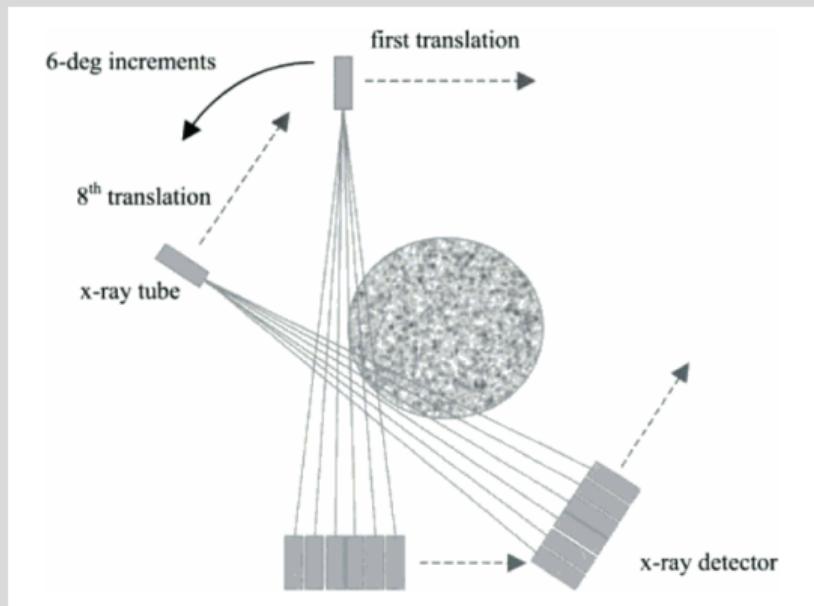
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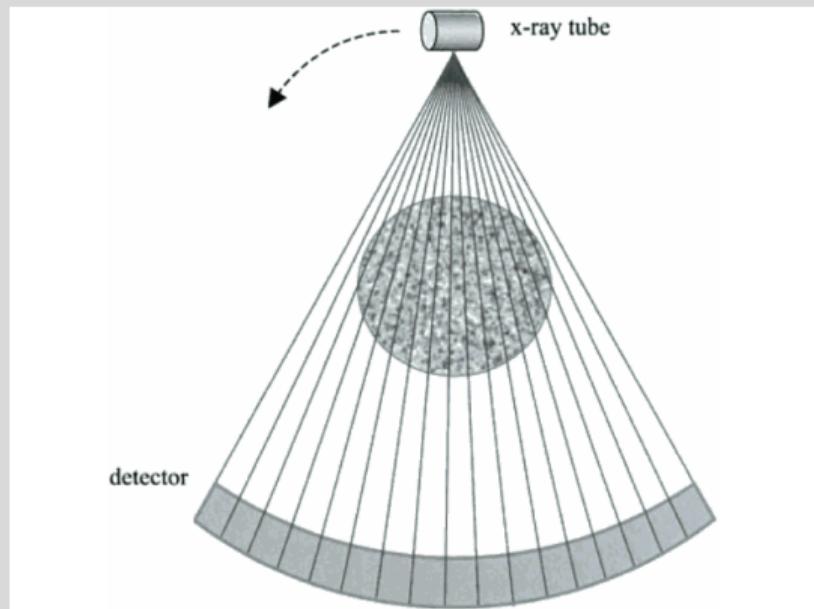
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- CT scanner generations: First, second and third



# $\mu$ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [**Lin2019**]
- $\mu$ CT was first reported in the 1980s, for scanning gemstones
- Lee Feldkamp [**Feldkamp1984**] 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

## $\mu$ CT History II

- Met with scientists at Henry Ford Hospital and University of Michigan interested in understanding the relationship between the microstructure and biomechanical function of trabecular bone to study osteoporotic fractures [**Feldkamp 1983**]
- CT scanners in medical diagnostics, beginning in the early 1970s
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Today: Nondestructive imaging for 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

## $\mu$ CT History III

- Since the 1990s,  $\mu$ CT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
- $\approx$ 2500  $\mu$ CT systems are in use worldwide with over 1000 publications annually

# X-ray interaction

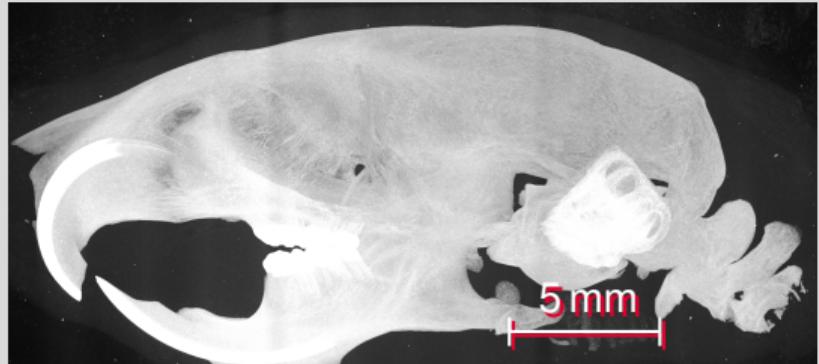
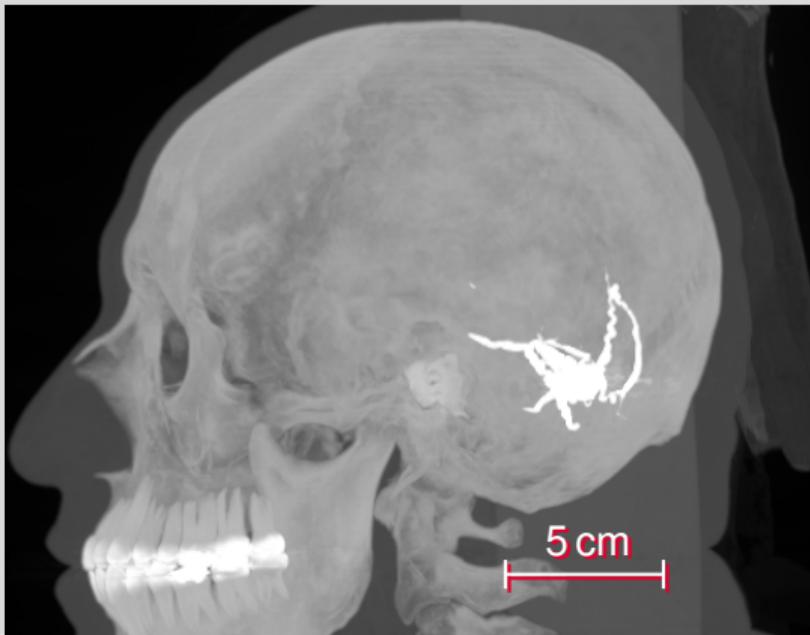
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([15])
  - Photoelectric absorption ( $\tau$ ) is strongly dependent 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 [16, i. e. Beer-Lamberts law]:  $I(t) = I_0 e^{-\alpha z}$

# Composition of biological tissues

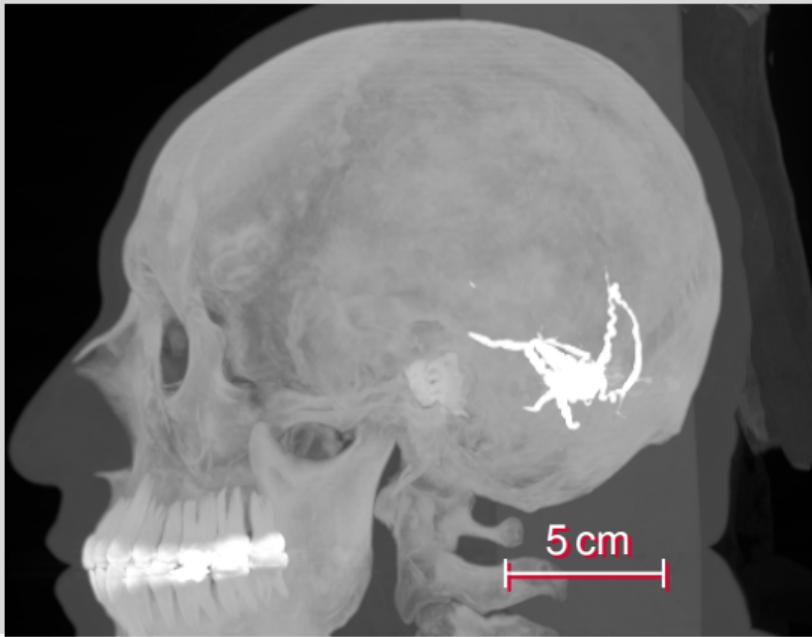
Tissue: content by mass percentage

Element	H	C	N	O	Na	P	S	Cl	K	Ca
Atomic number	1	6	7	8	11	15	16	17	19	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

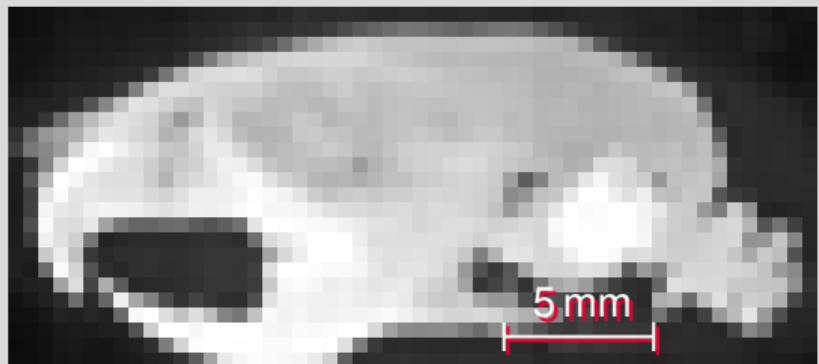
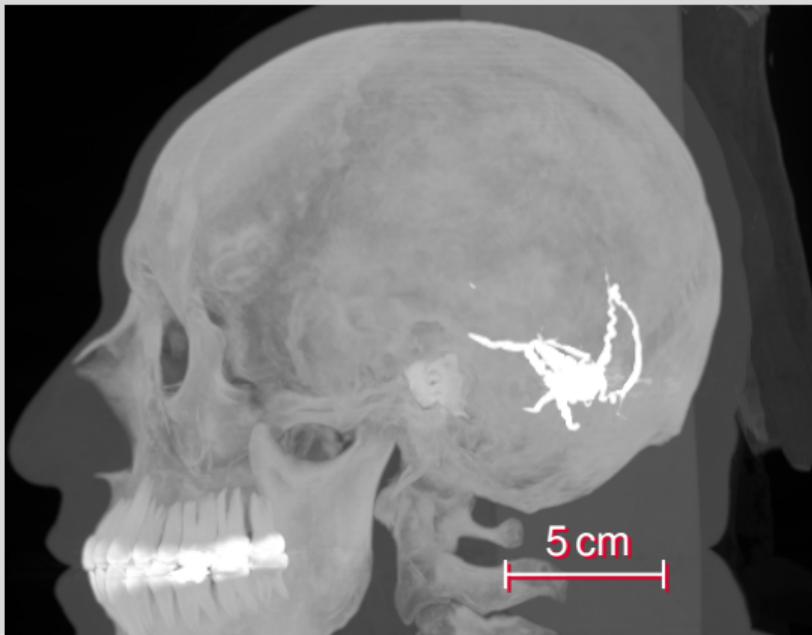
# Why $\mu$ CT?



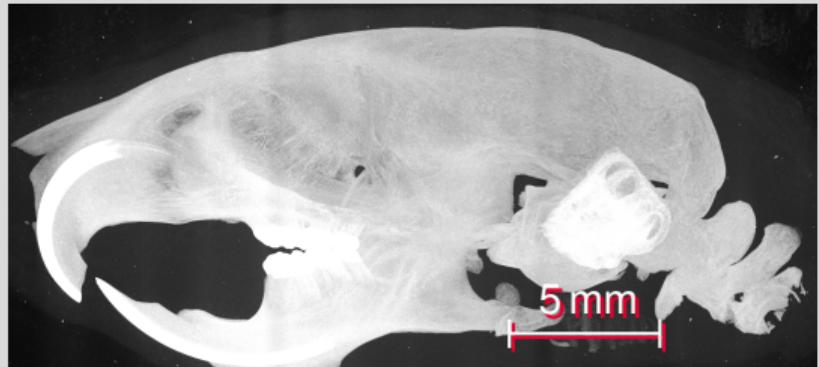
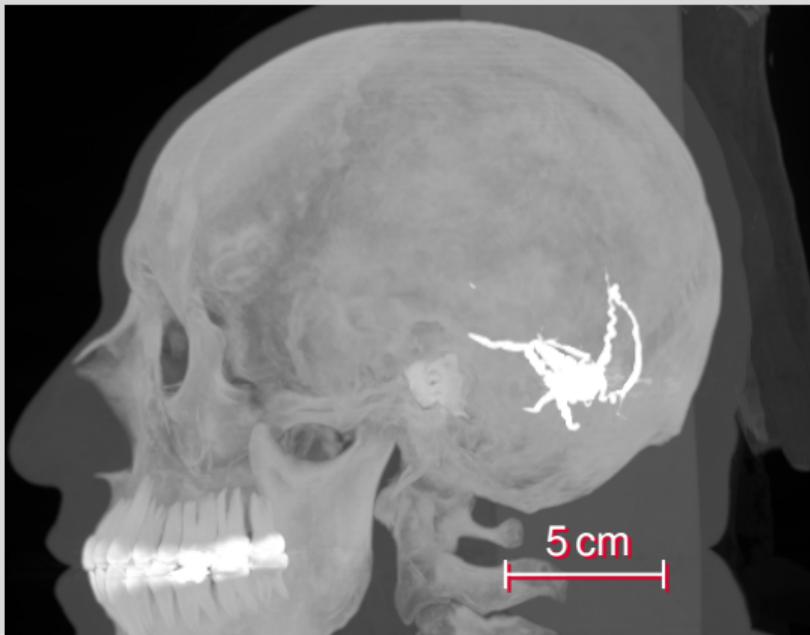
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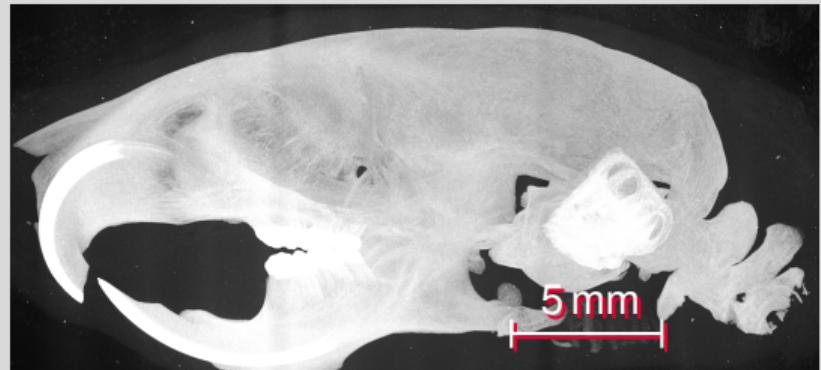
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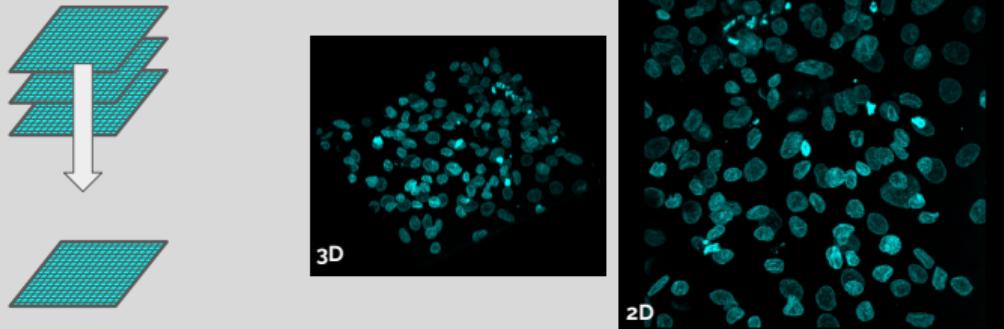
From [17], Subject *C3L-02465*



# 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.



# 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



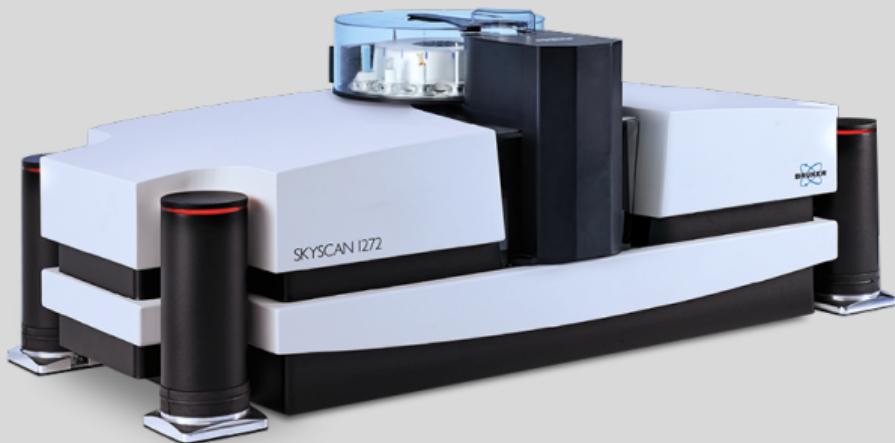
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[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

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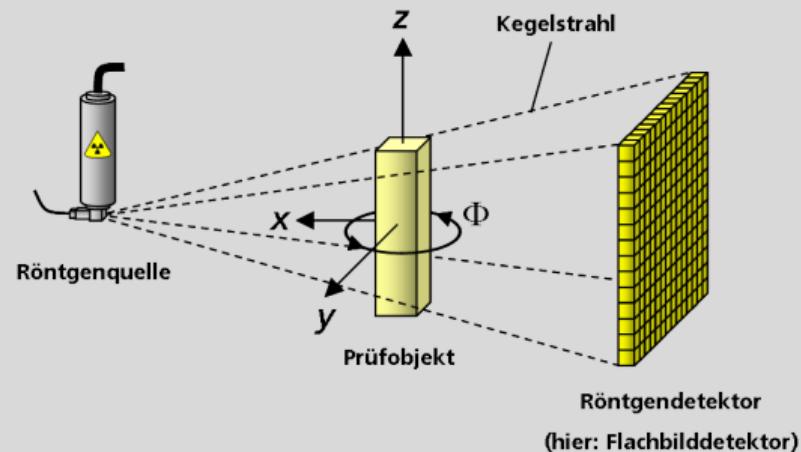


flic.kr/p/7Xhk2Y

# What is happening?

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

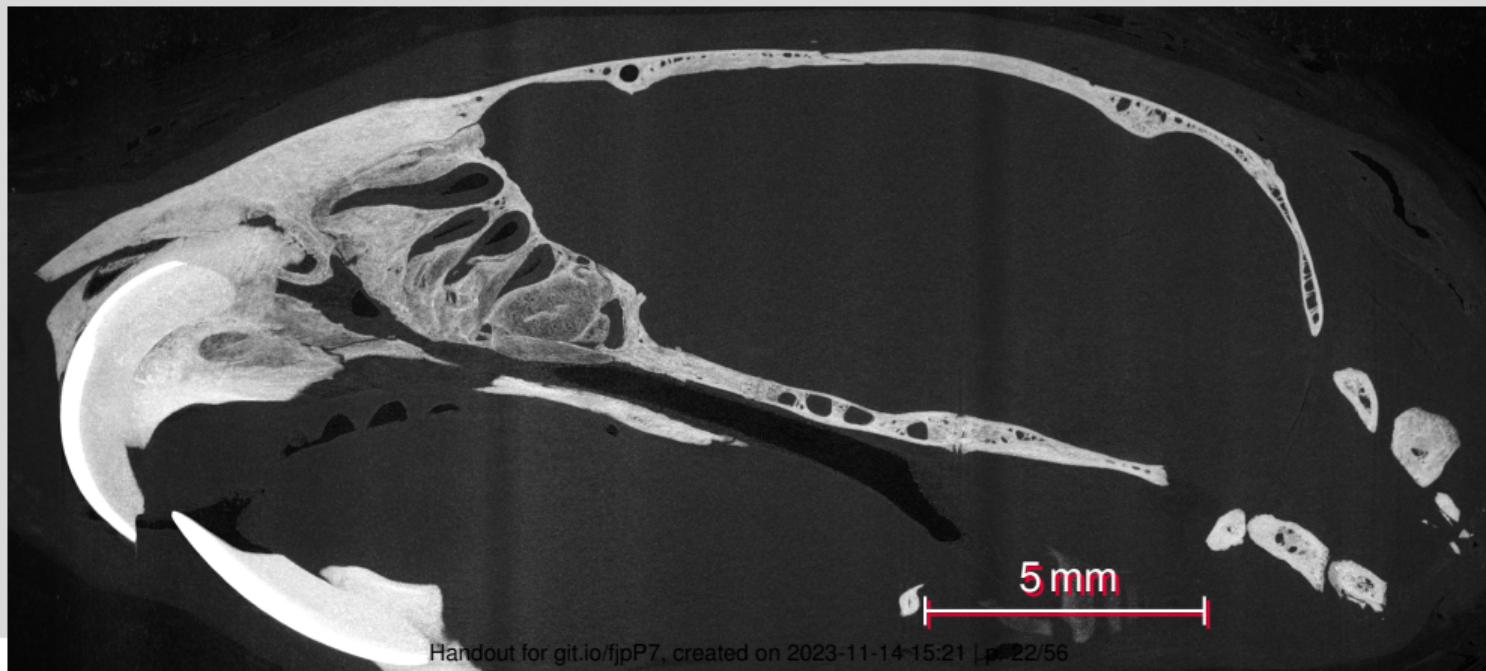
- an x-ray source
- a sample
- a detector



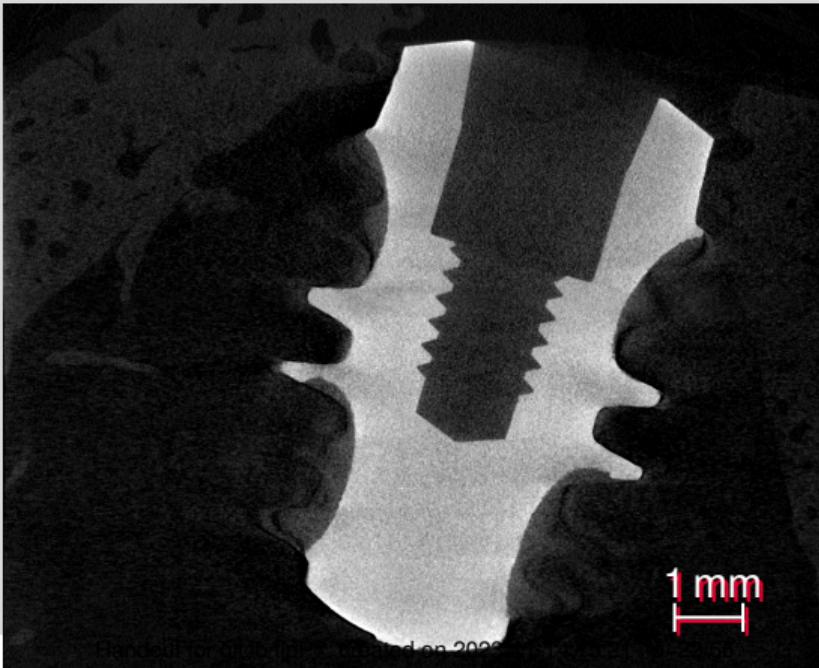
w.wiki/7g3

# Machinery

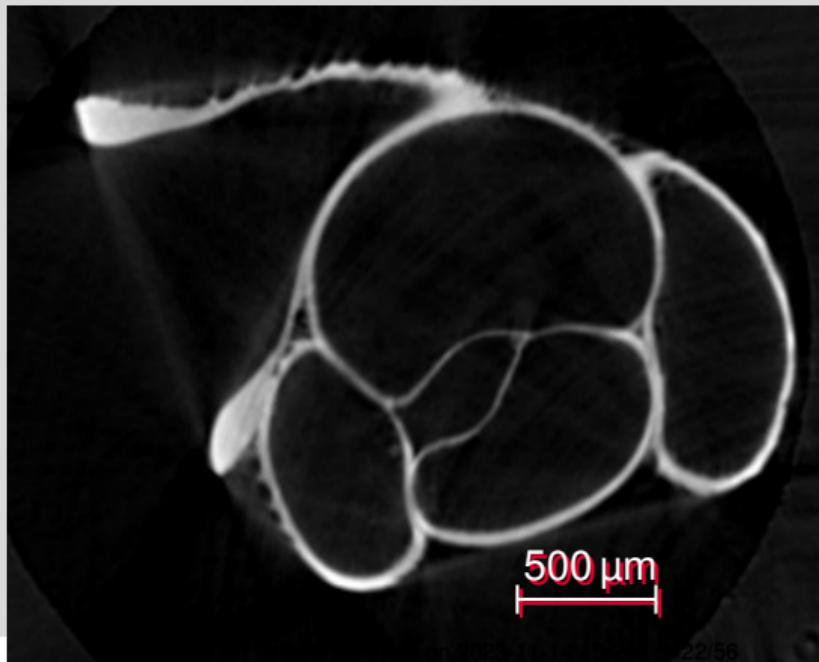
## Examples



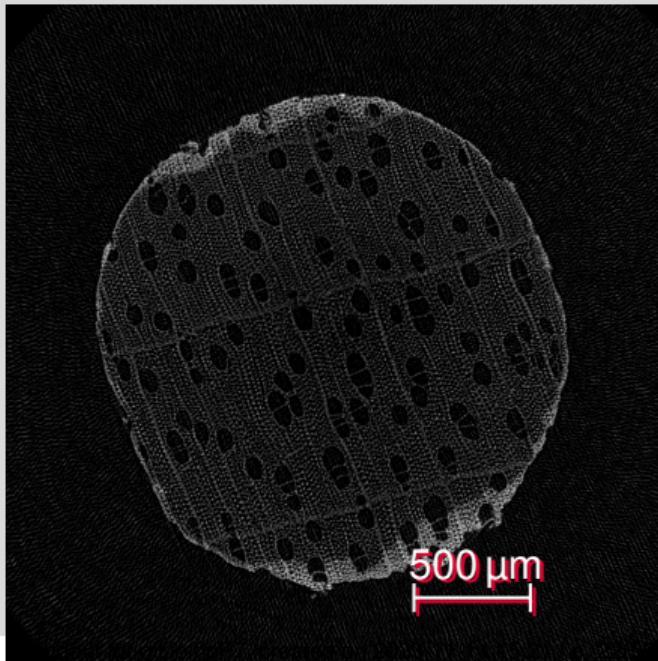
## Examples



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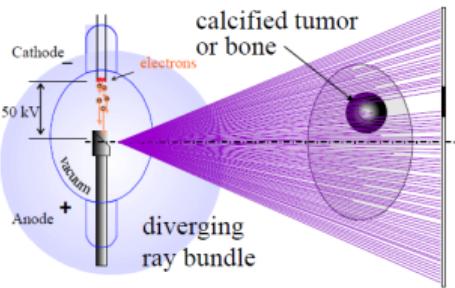
# Preparation

- Study design
- Sample preparation

# Projections

## X-ray generation and contrast

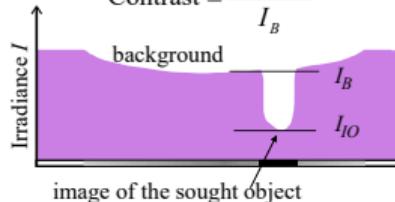
X-ray tube:  
nearly point like  
photon source



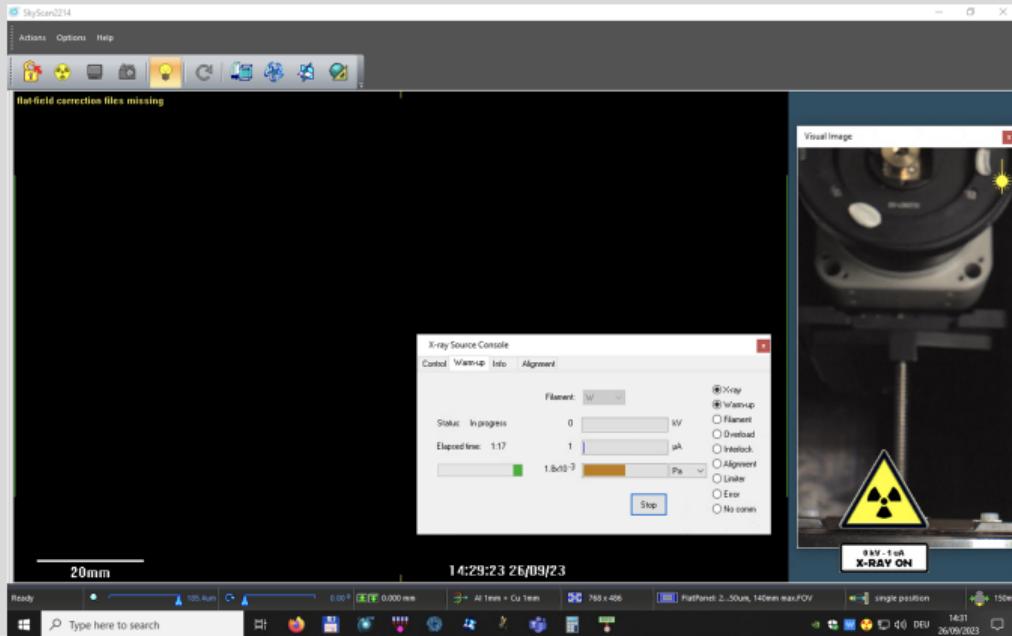
Contrast is given by  
absorption of intensity I

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

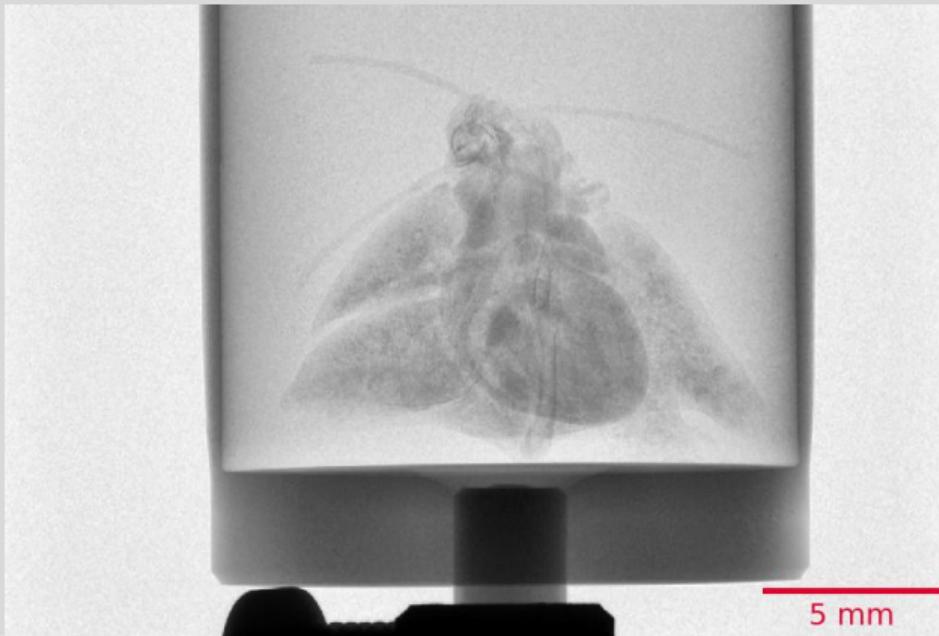
$$\text{Contrast} = \frac{I_{IO} - I_B}{I_B}$$



# Projection acquisition



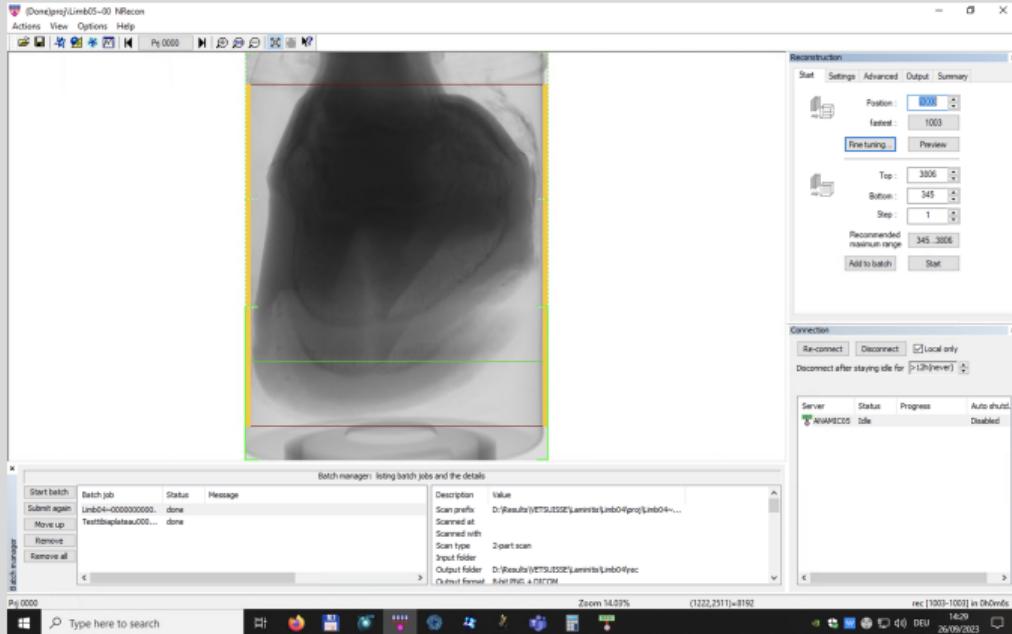
# Projections



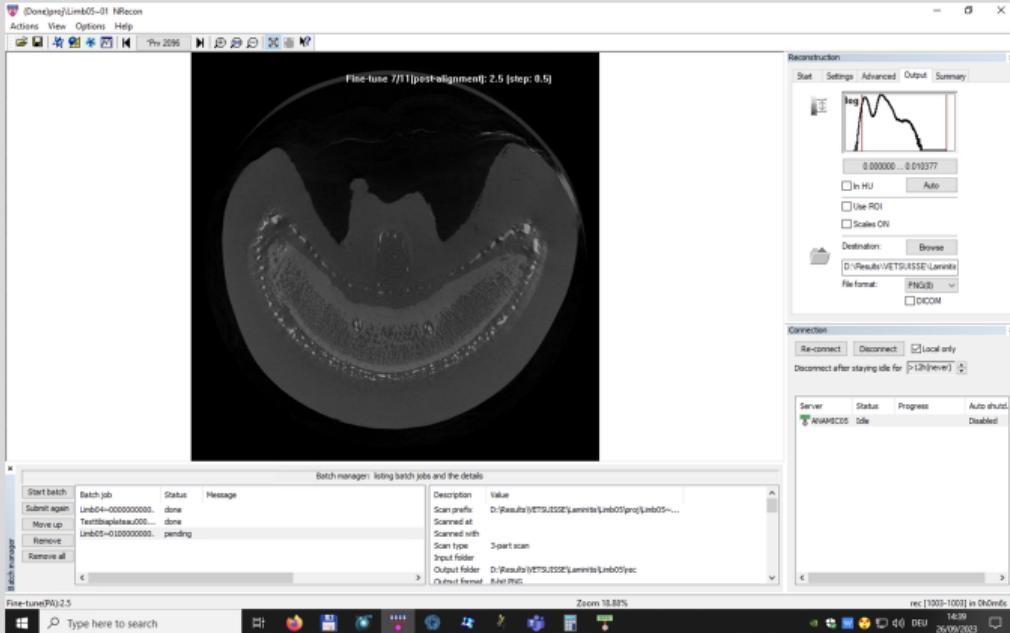
# Projections

- 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

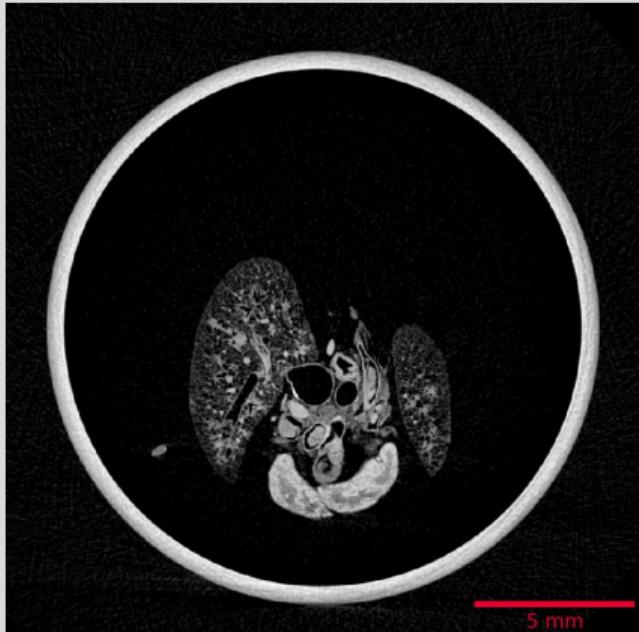
# Reconstructions



# Reconstructions



# 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

## Visualization



# Visualization

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

# What to use?

- ImageJ/Fiji [18]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  -  in Jupyter [19]
  - 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:

- Number of publications
- Structure of dentin
- Two publications:
  - [10], BMC Oral Health, doi.org/g9rP
  - [20], Scientific Reports, doi.org/g7r8



# 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 [19])
- Two publications:
  - [10], BMC Oral Health, doi.org/gjpw2d
  - [20], Scientific Reports, doi.org/g7r8

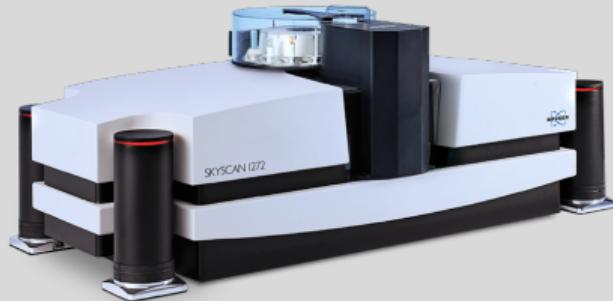
# How?

- 104 extracted human permanent mandibular canines
- $\mu$ CT imaging
- Root canal configuration, according to Briseño-Marroquín et al. [21]
- *Reproducible* analysis [22], e. g. you can click a button to double-check or recalculate the results yourself!



# How?

- 104 extracted human permanent mandibular canines
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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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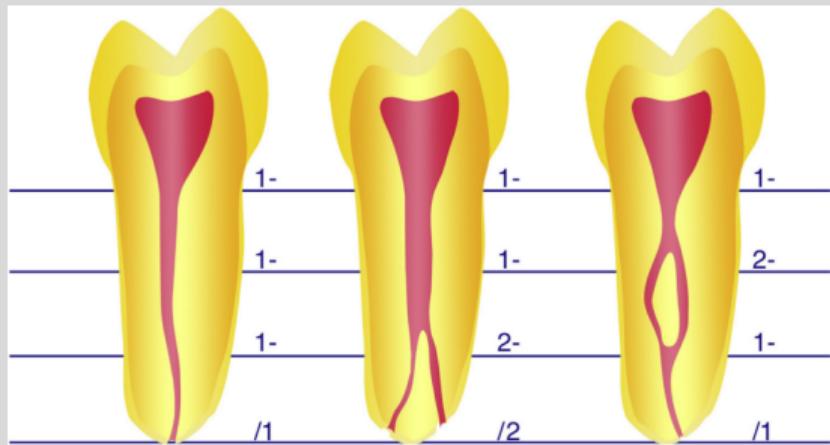
*Sample changer on the SkyScan 1272*

In total:

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

# How?

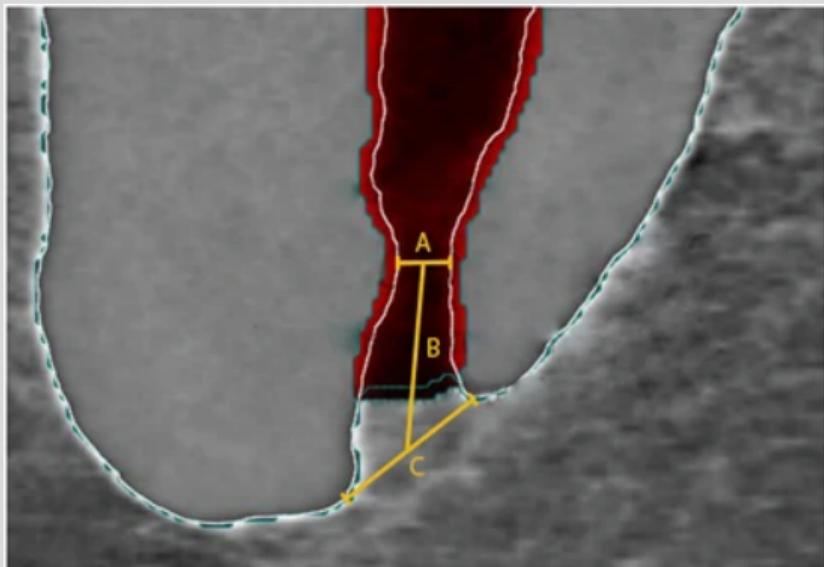
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From [21], Fig. 2

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From [23], Fig. 1

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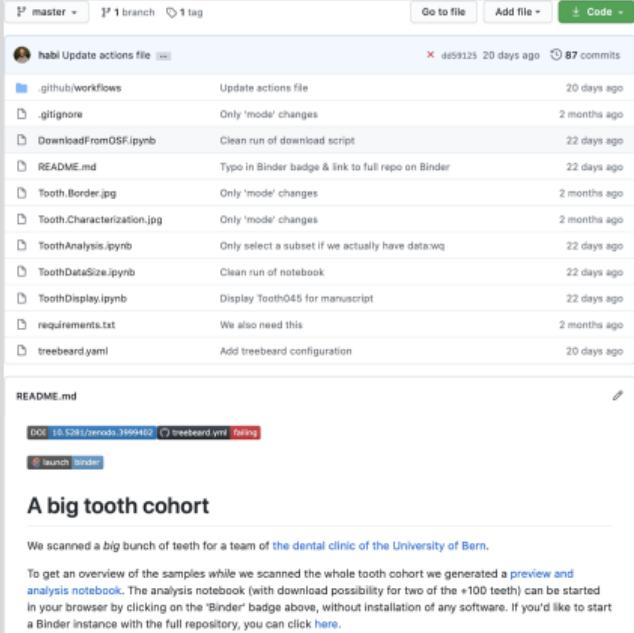
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[gph.is/2nqkple](https://gph.is/2nqkple)

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The screenshot shows a GitHub repository page for a user named 'habi'. The repository has 87 commits. The commits are listed below:

Commit	Description	Date
Update actions file	Update actions file	20 days ago
github/workflows	Only 'mode' changes	2 months ago
.gitignore	Clean run of download script	22 days ago
DownloadFromOSF.ipynb	Typo in Binder badge & link to full repo on Binder	22 days ago
README.md	Only 'mode' changes	2 months 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

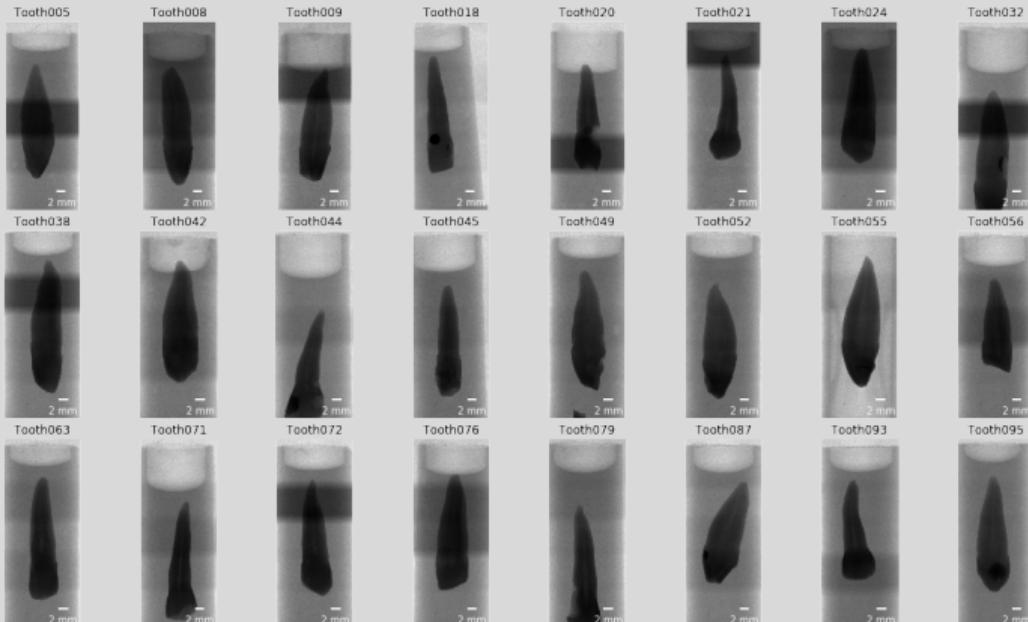
Below the commits is a file named README.md. At the bottom of the page, there is a note about a big tooth cohort and a link to a preview and analysis notebook.

A big tooth cohort

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

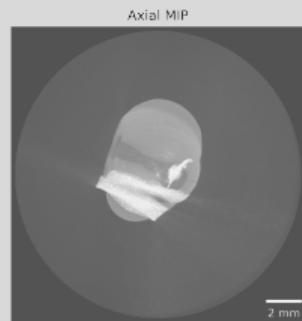
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of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

# µCT imaging



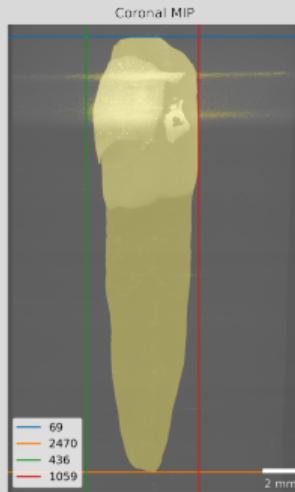
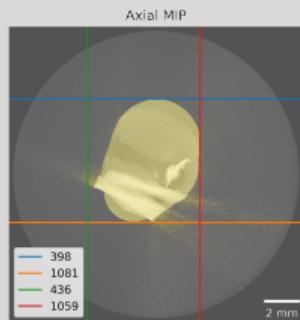
# Dataset cropping

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

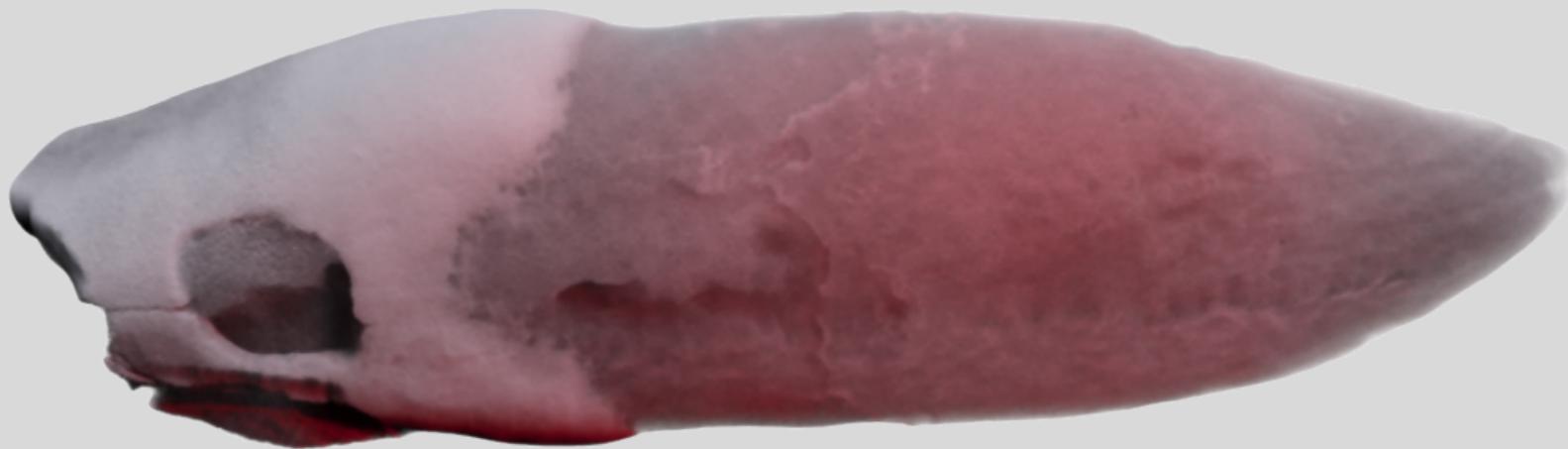


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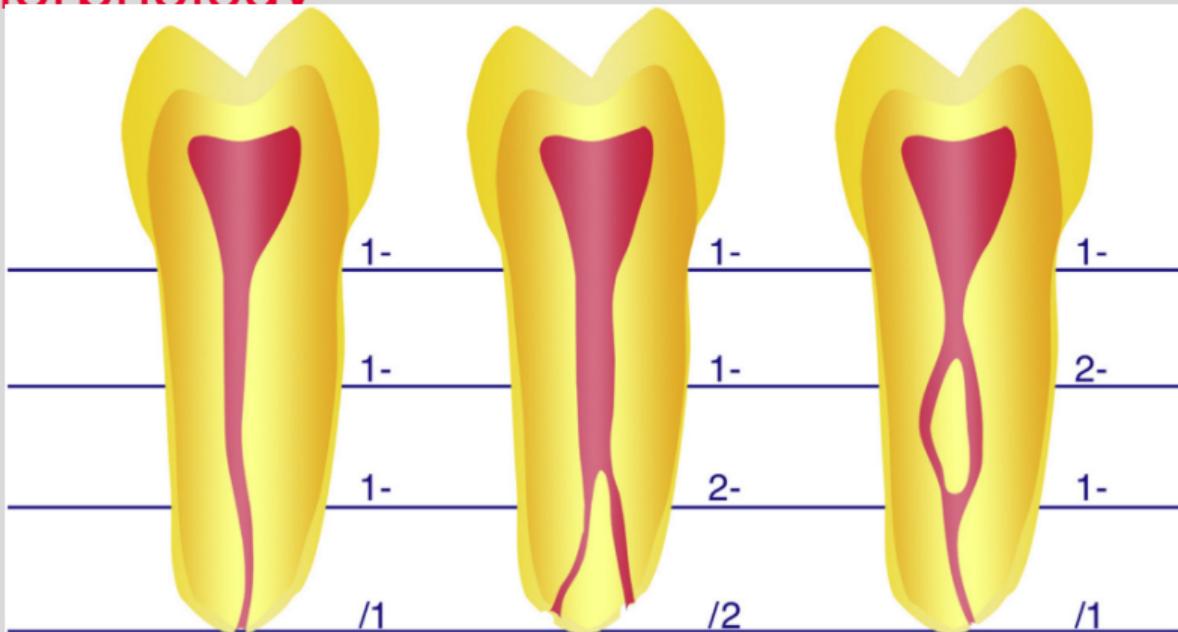
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## Tooth morphology

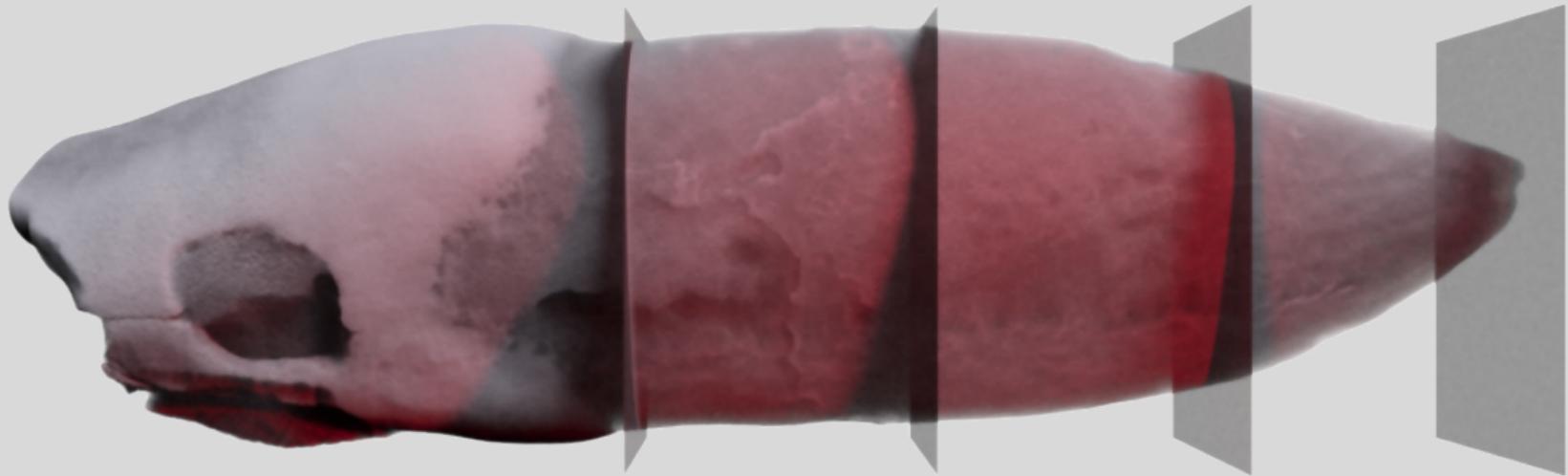


## Tooth morphology

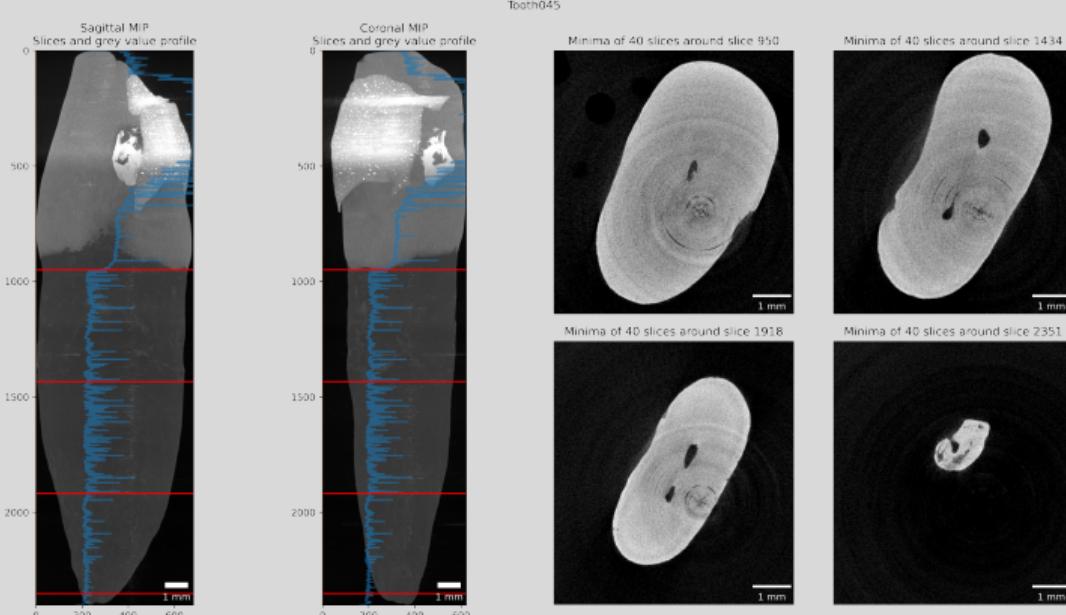


From [21], Fig. 2

# Tooth morphology

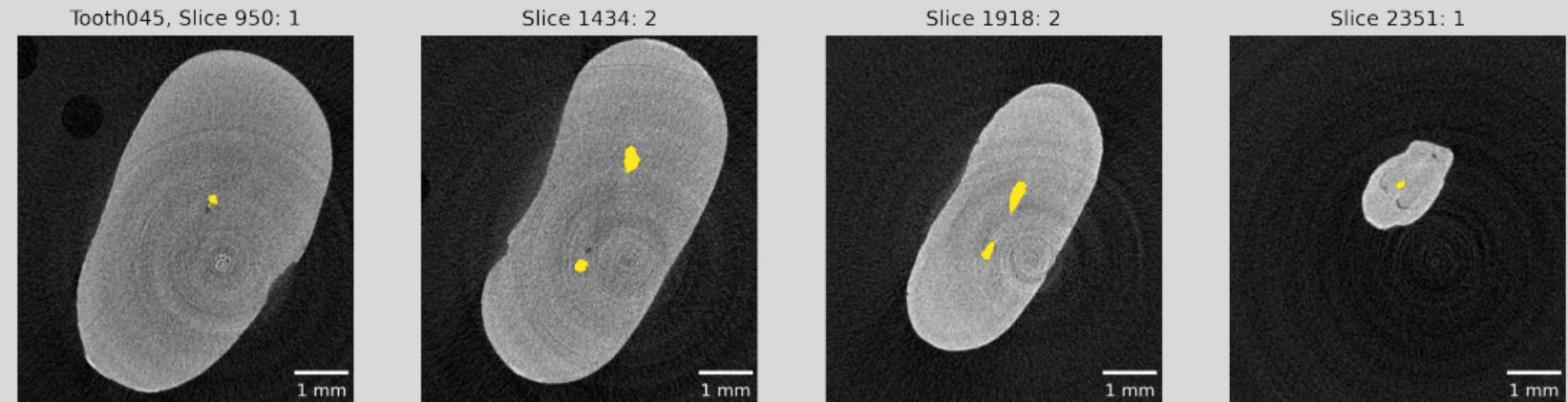


# Detection of enamel-dentin border



# Detection of enamel-dentin border

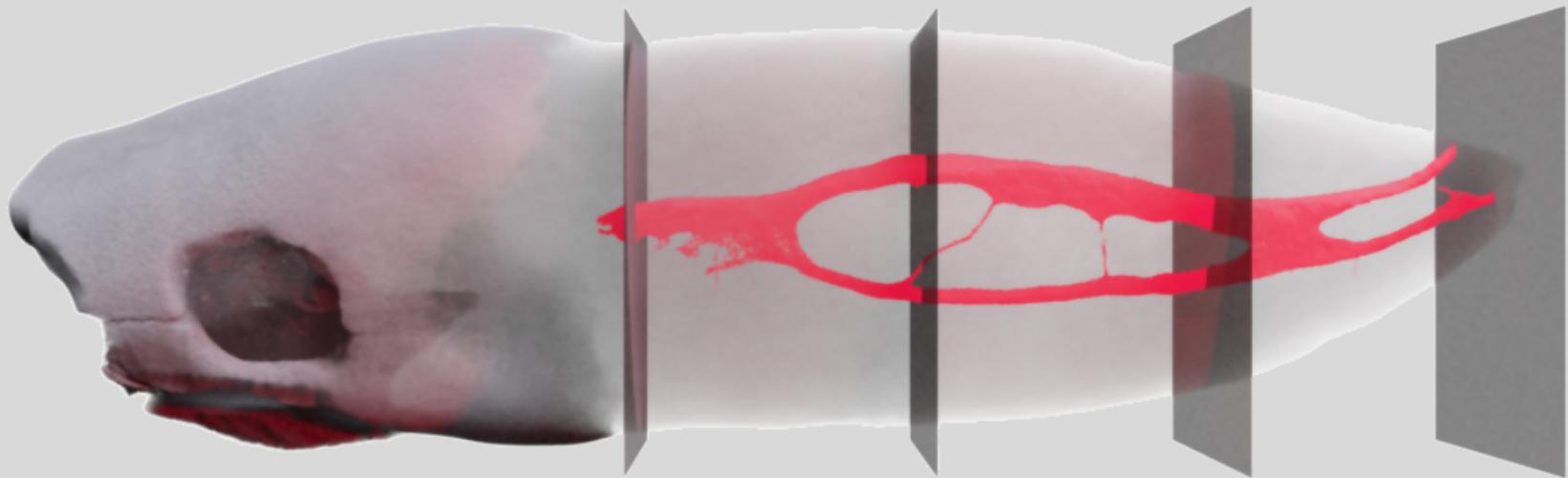
Tooth045



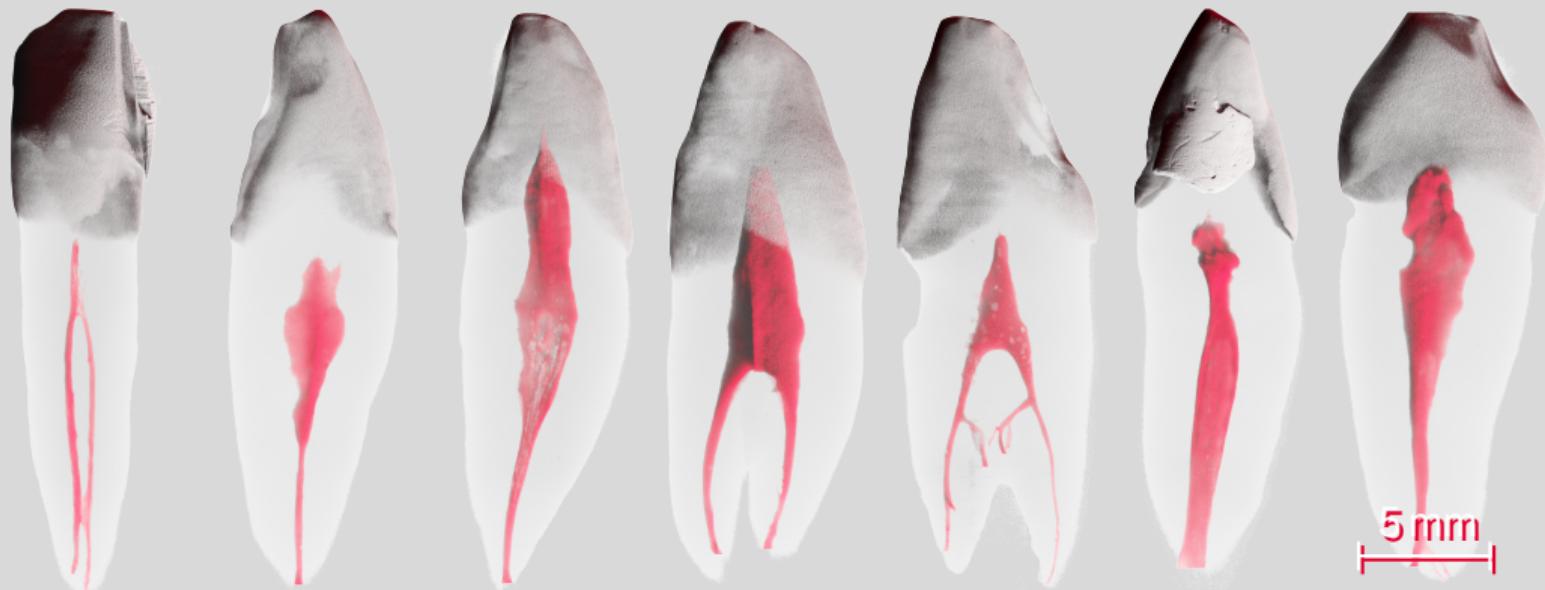
# Outcome root canal configuration classification

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

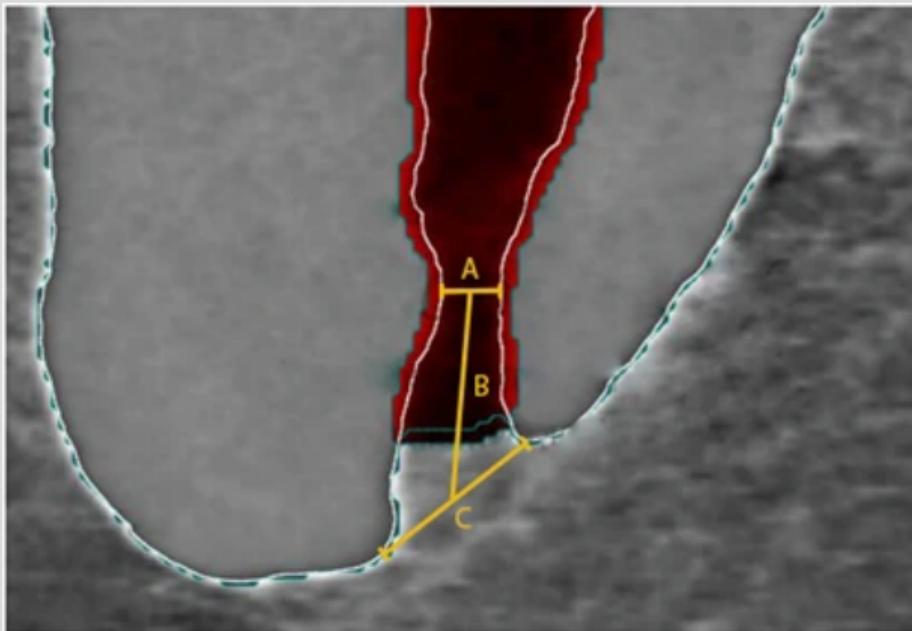
## Extraction of root canal space



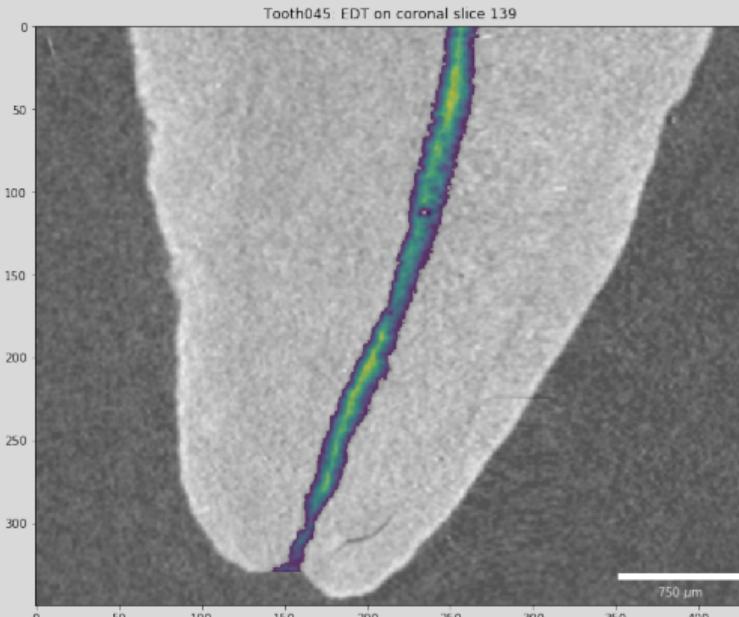
## Results of root canal space extraction



# Analysis of the physiological foramen geometry



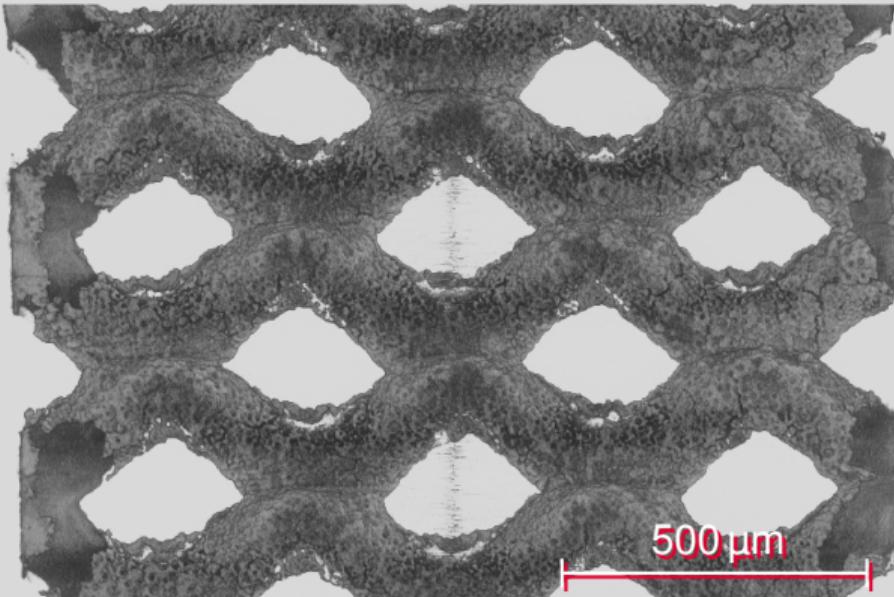
# Analysis of the physiological foramen geometry



# 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

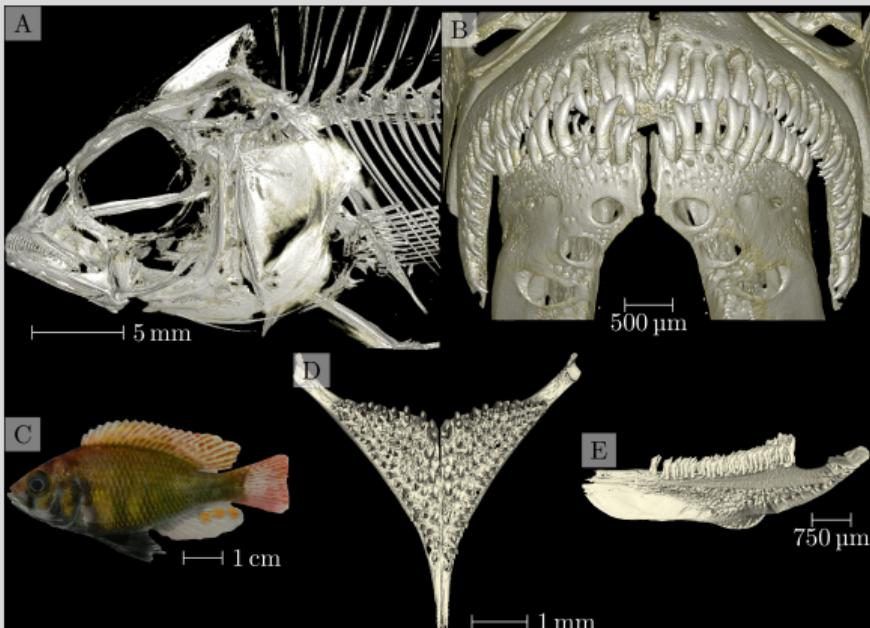
# Metal foam



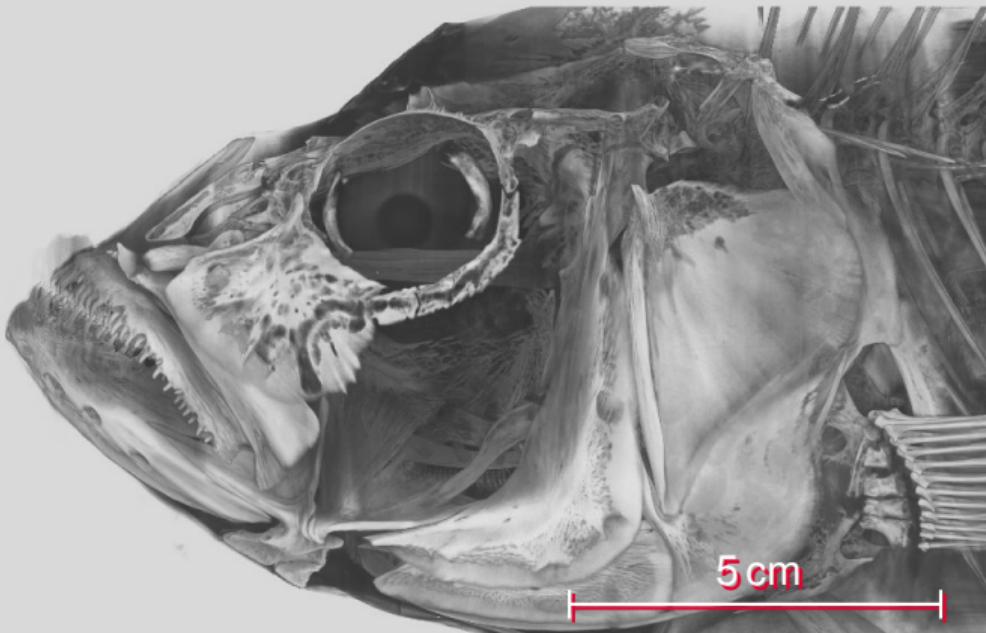
## Data wrangling by example: Cichlids

- 372 tomographic scans of 133 different Cichlids, from 6–18 cm [a]
- 9.8 TB of projection images, 1.5 TB of reconstructions
- Reproducible and automated dataset wrangling, checking and image analysis ( in Jupyter [19])

[a] Haberthür, David et al. DOI: 10.1101/2023.03.30.534917.



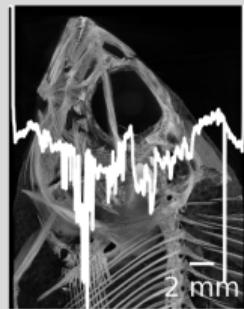
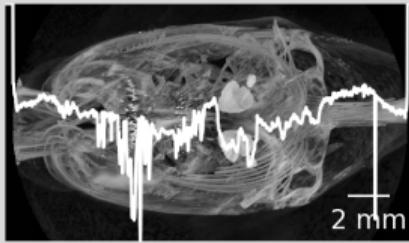
# Cichlids



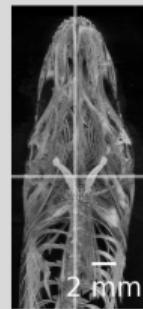
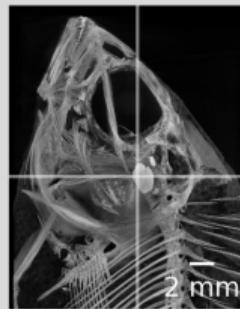
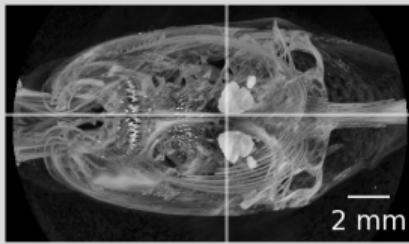
## Data wrangling by example: Cichlids



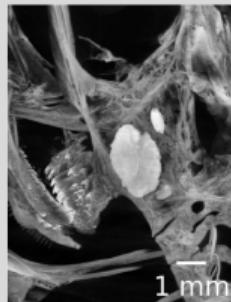
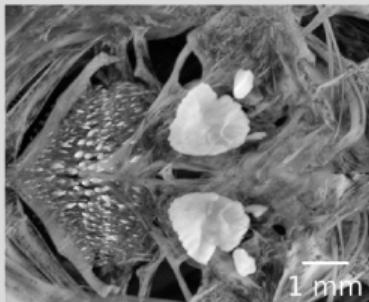
## Data wrangling by example: Cichlids



## Data wrangling by example: Cichlids



## Data wrangling by example: Cichlids



# Thanks!

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

# Colophon

- This BEAMER presentation was crafted in L<sup>A</sup>T<sub>E</sub>X 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 L<sup>A</sup>T<sub>E</sub>X code is automatically compiled with a GitHub action [1] 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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[1] Details on how this works are specified in a small test repository here: [git.io/JeOOj](https://git.io/JeOOj)

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