

X-ray microtomography

David Haberthür

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

Grüessech mitenang!

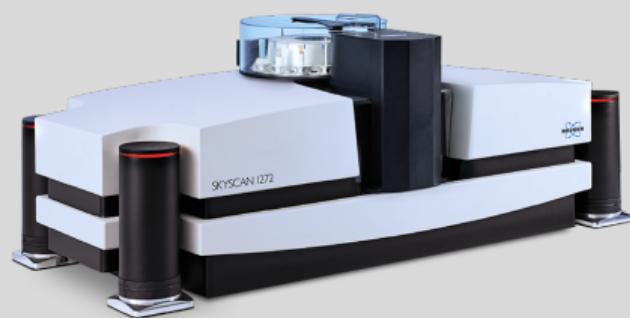
- David Haberthür
 - Physicist by trade
 - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
 - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
 - Post-Doc II: μ CT group, Institute of Anatomy, University of Bern, Switzerland.

Grüessech from the μ CT-group



μ 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]



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Contents

Overview

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Interaction of x-rays with matter

Tomography today

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

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Overview

Materials & Methods

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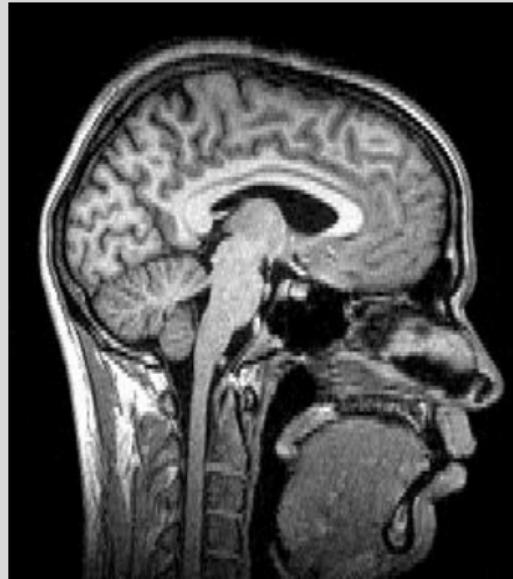
Metal foam analysis

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

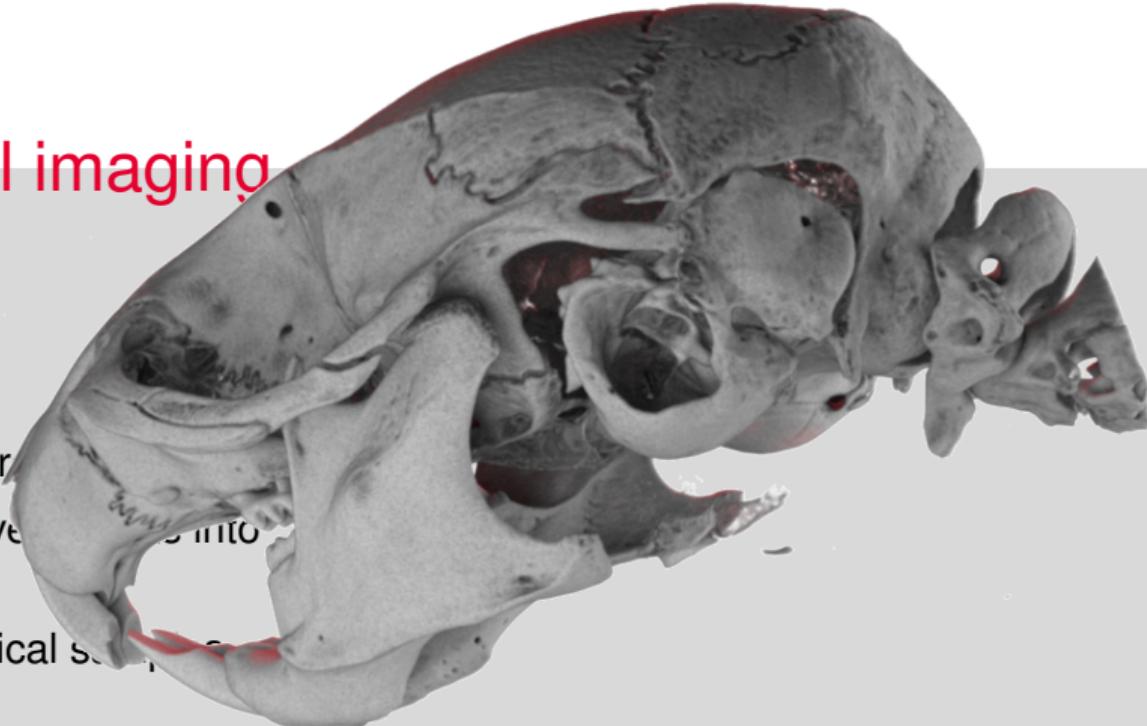
Biomedical imaging

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



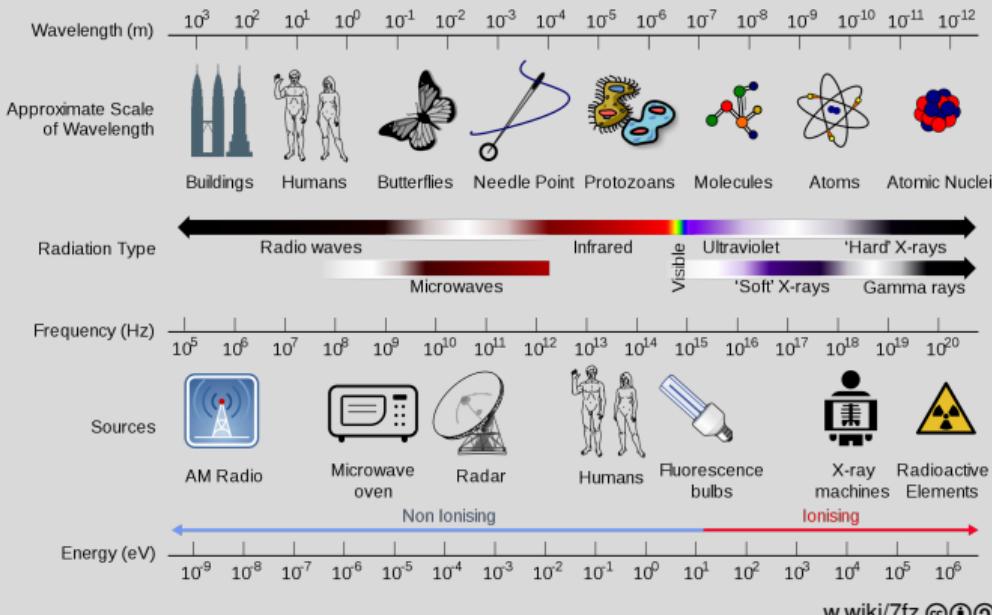
w.wiki/7g4 CC BY NC SA

Biomedical imaging

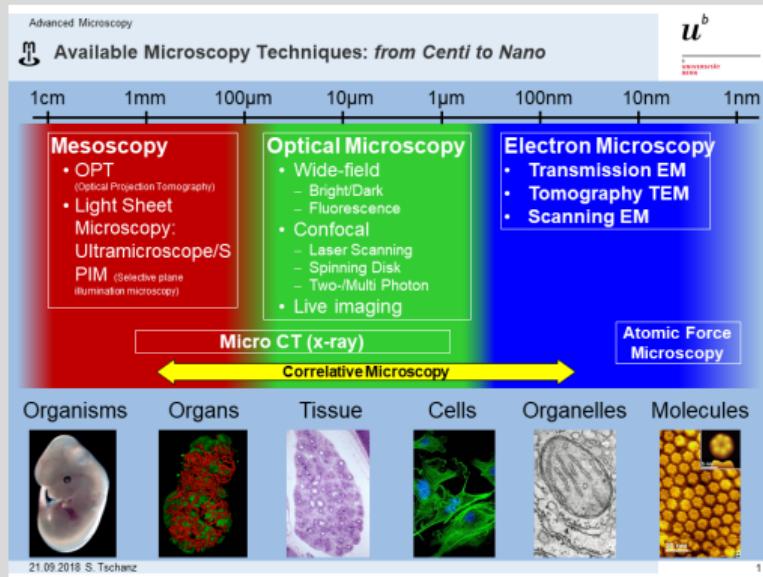


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Wavelength & Scale



Wavelength & Scale

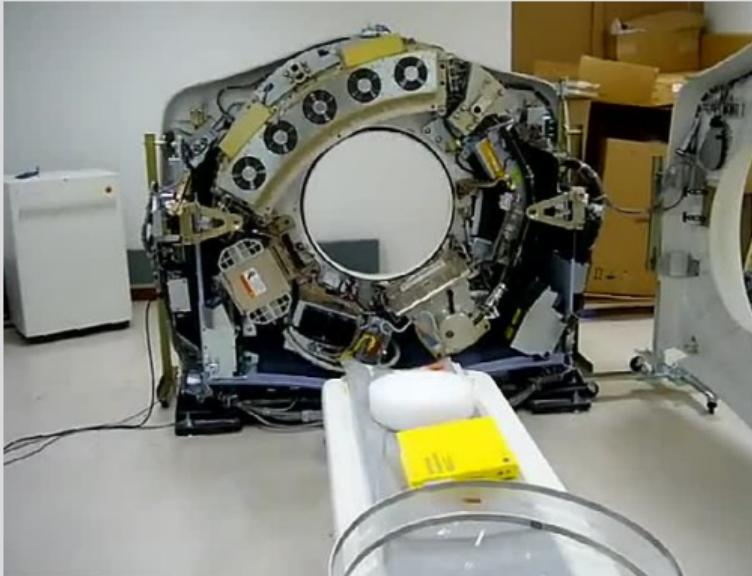


Stefan Tschanz, with permission

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

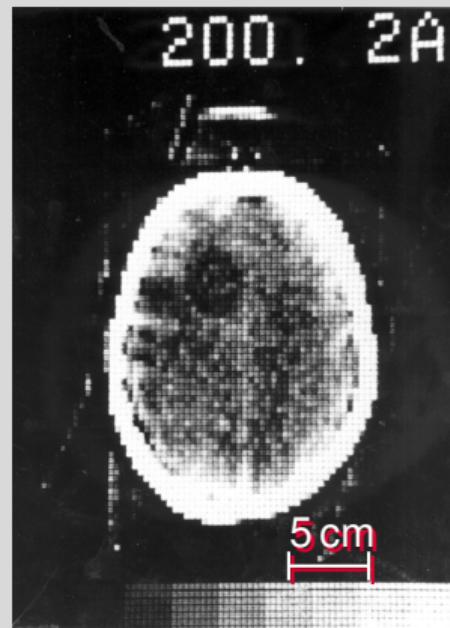
CT-Scanner



youtu.be/2CWpZKuy-NE

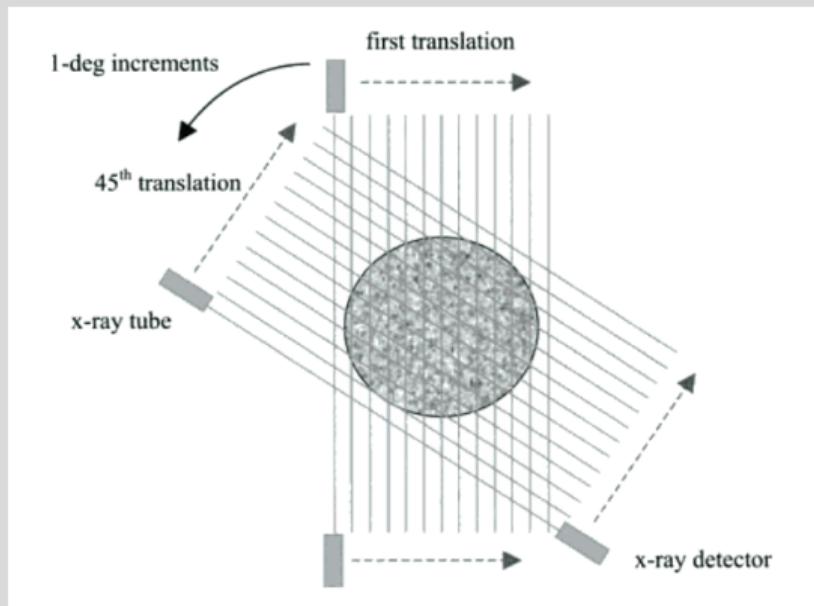
CT History

- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
 - 1976: Hounsfield worked on first clinical scanner [13]
 - Nice overview by Hsieh [14]



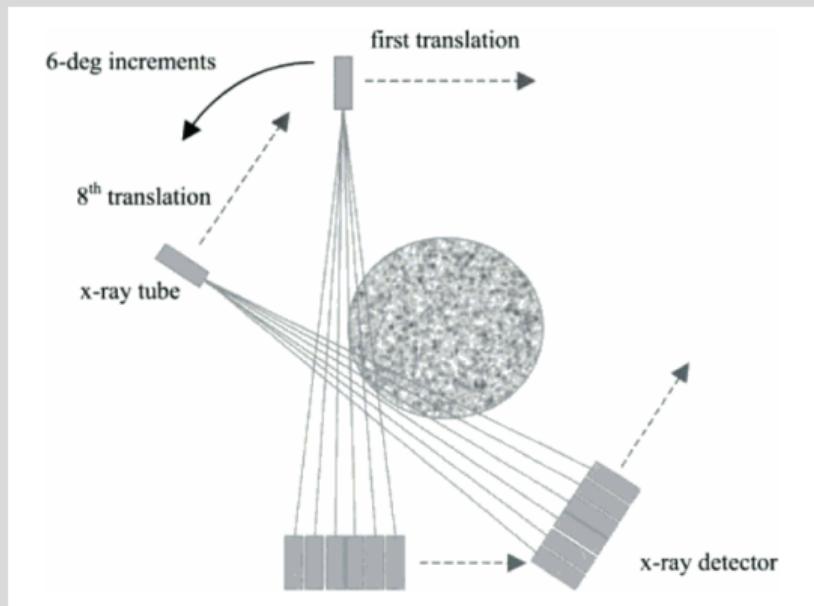
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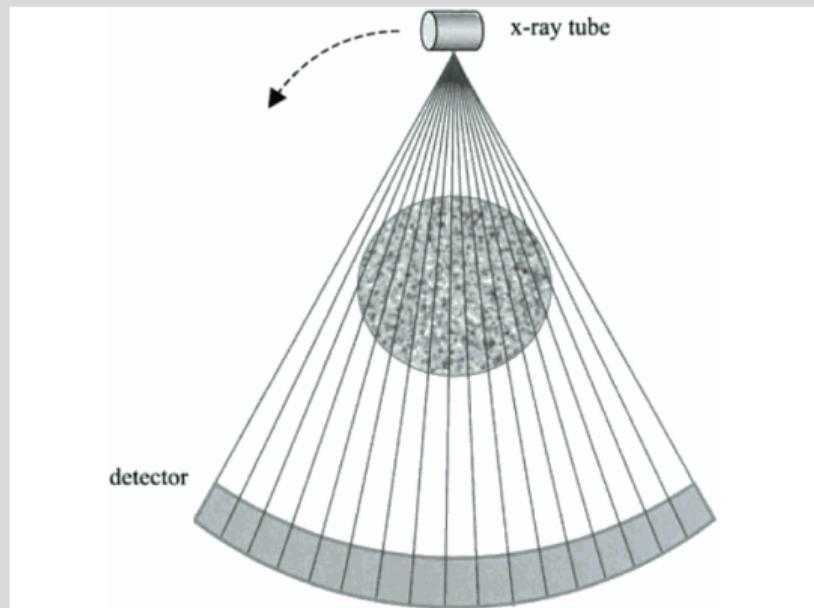
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- CT scanner generations: First, second and third



μ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [**Lin2019**]
- μ 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

μ 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

μ CT History III

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

X-ray interaction

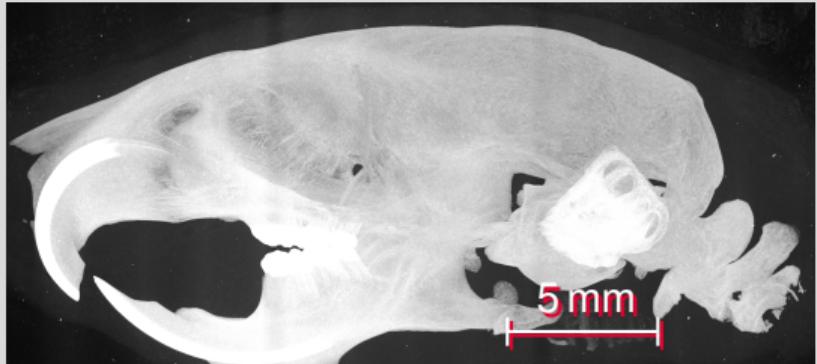
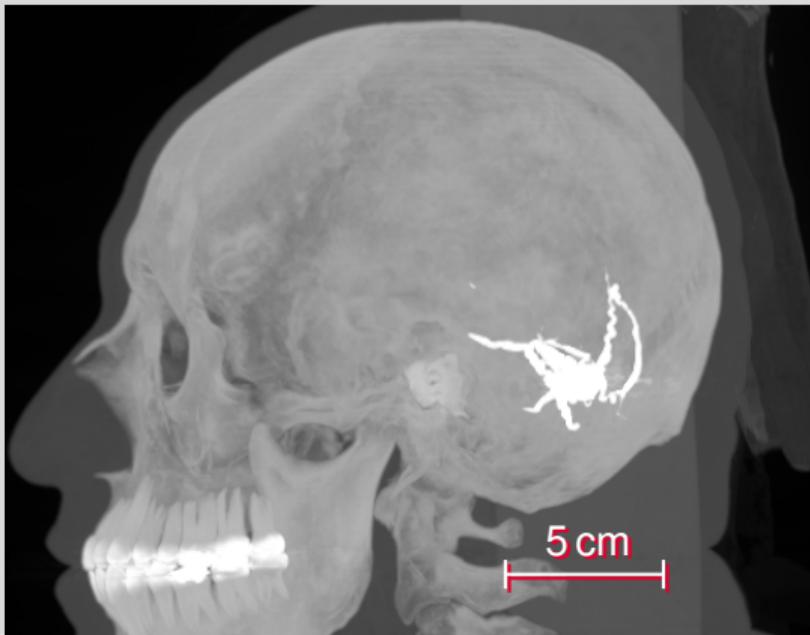
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([15])
 - Photoelectric absorption (τ) is strongly dependent on 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

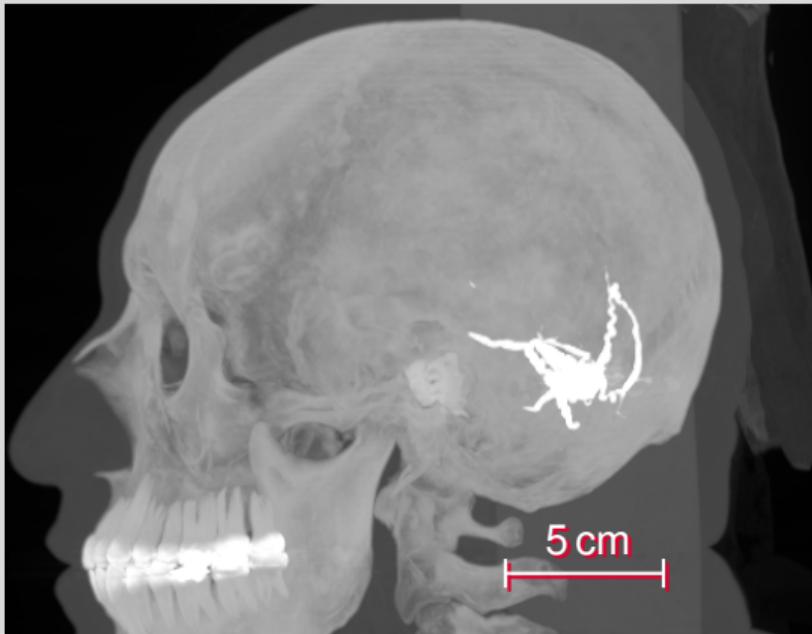
Tissue: content by mass percentage

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

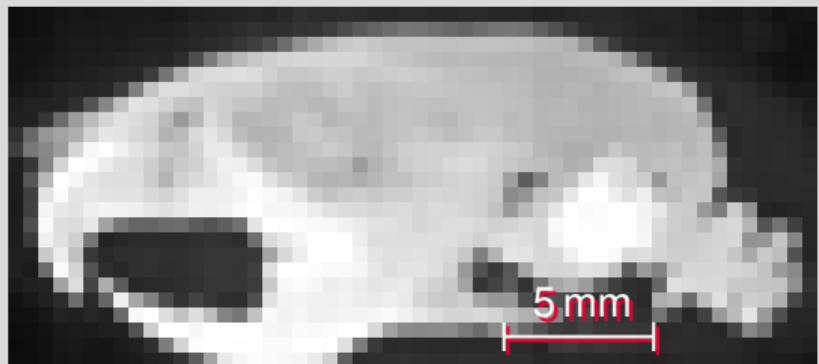
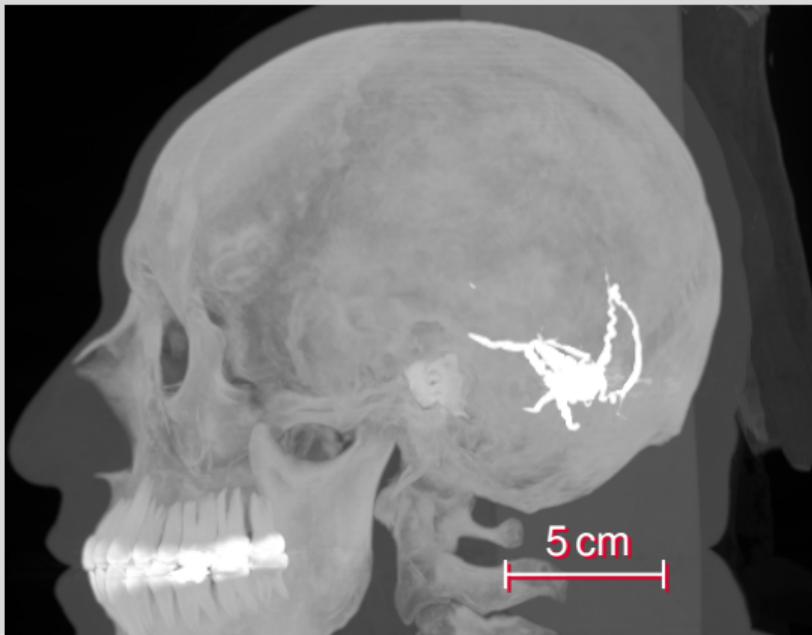
Why μ CT?



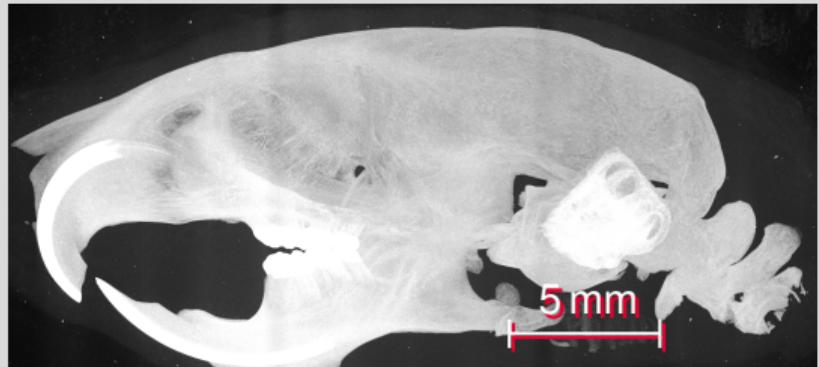
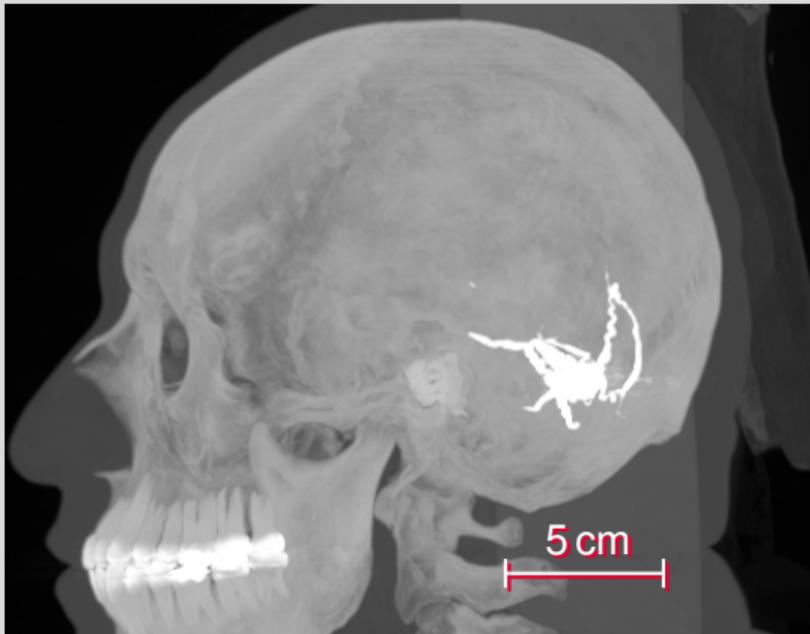
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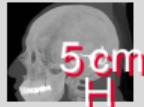
Why μ CT?



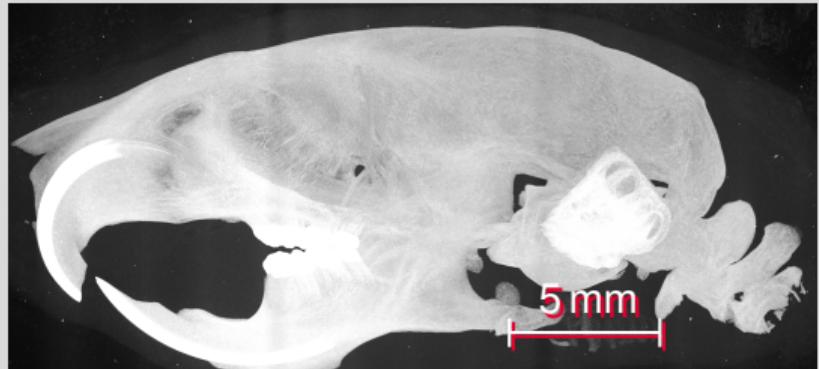
Why μ CT?



Why μ CT?



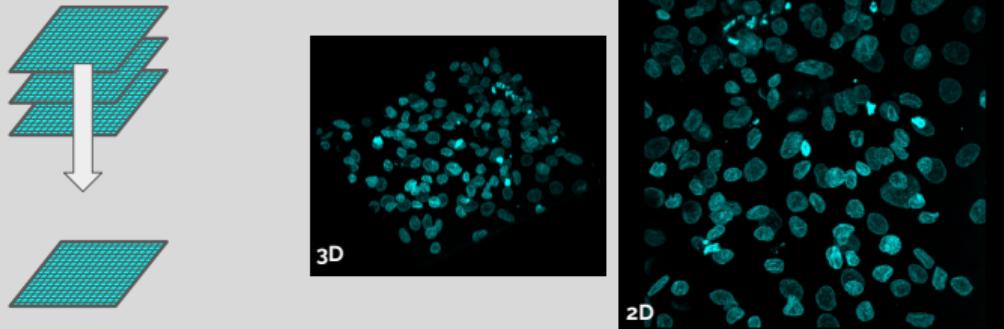
From [17], Subject *C3L-02465*



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 μm (*in vivo*) or 0.5 μm (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



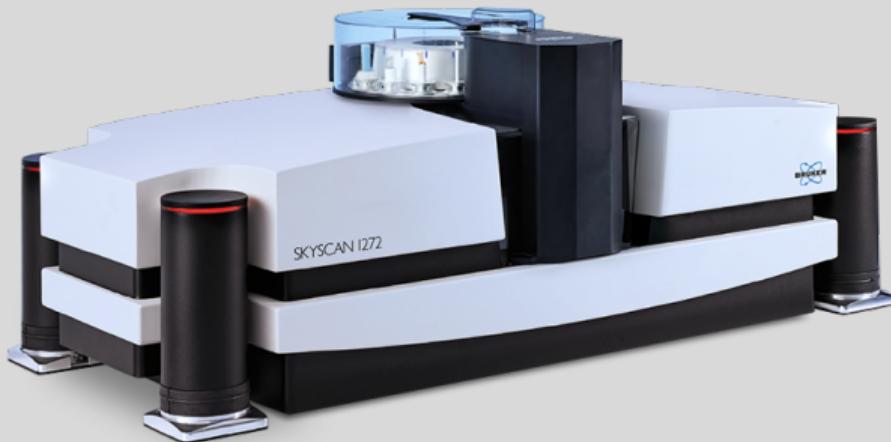
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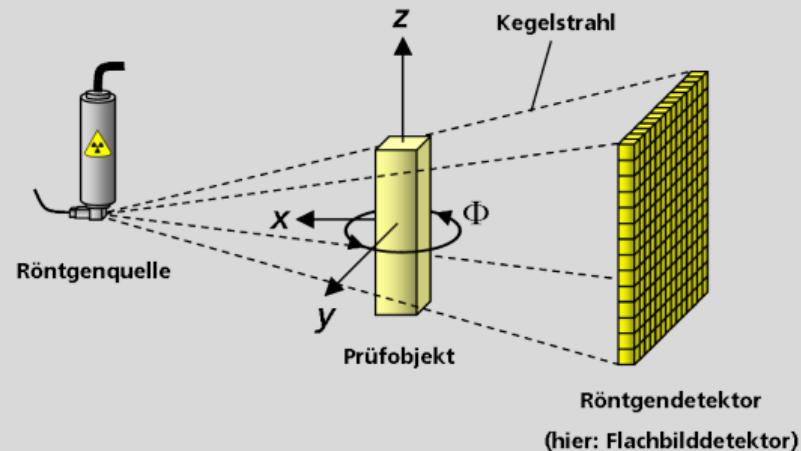


flic.kr/p/7Xhk2Y ☺ ⓘ ⓘ

What is happening?

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

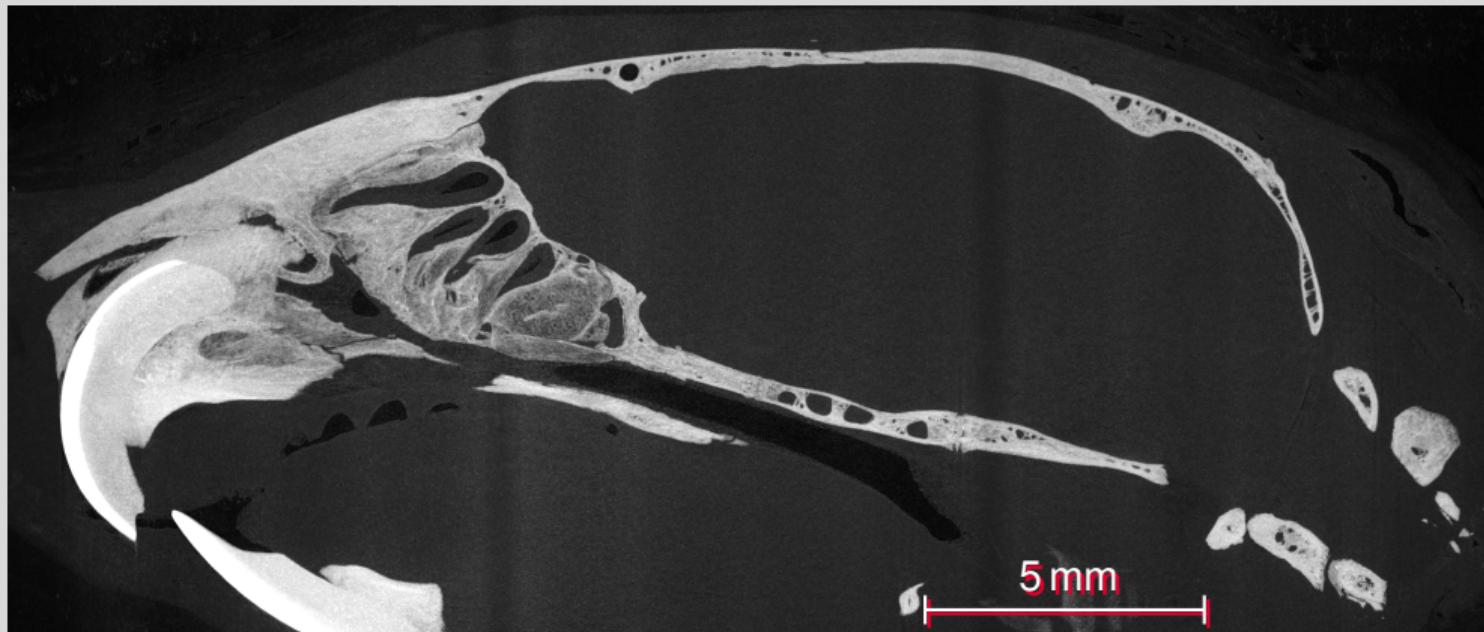
- an x-ray source
- a sample
- a detector



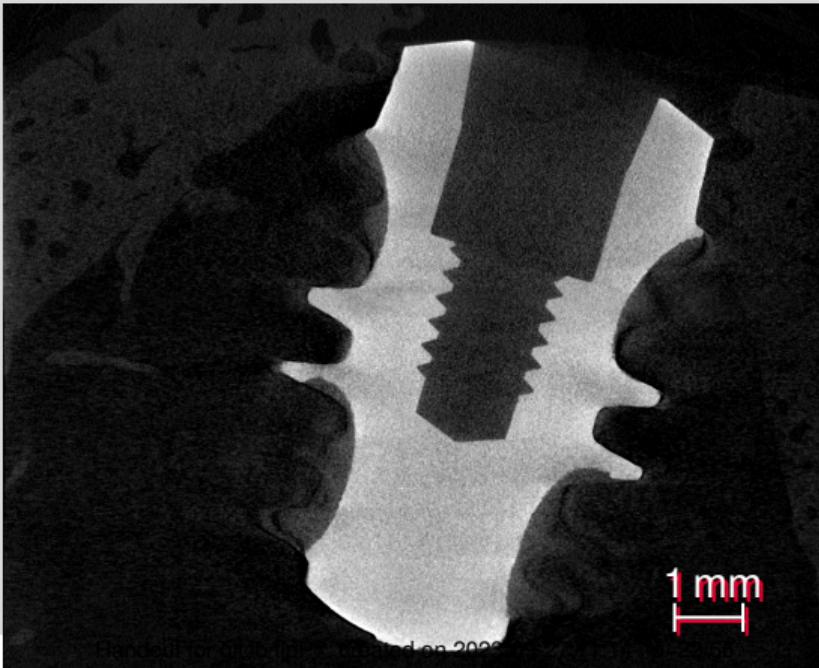
w.wiki/7g3

Machinery

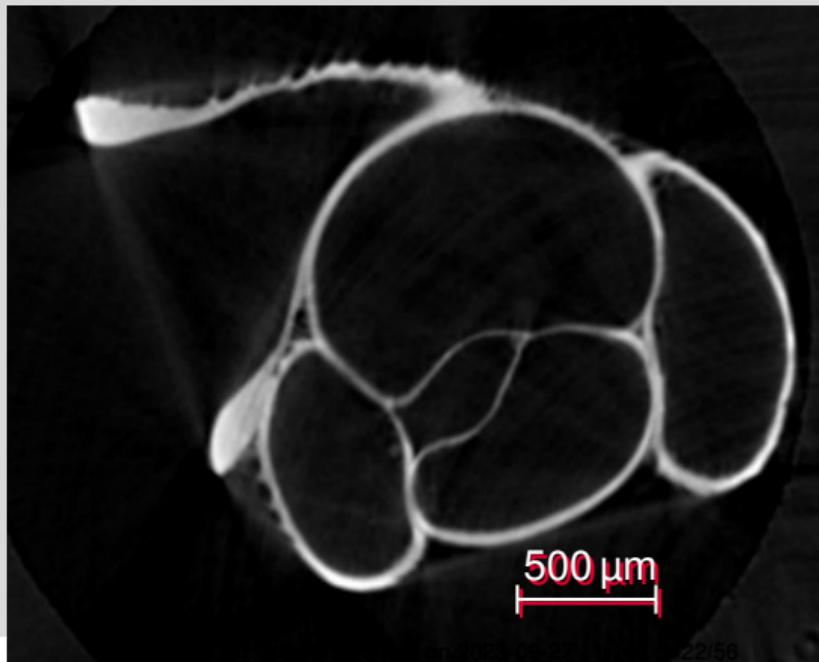
Examples



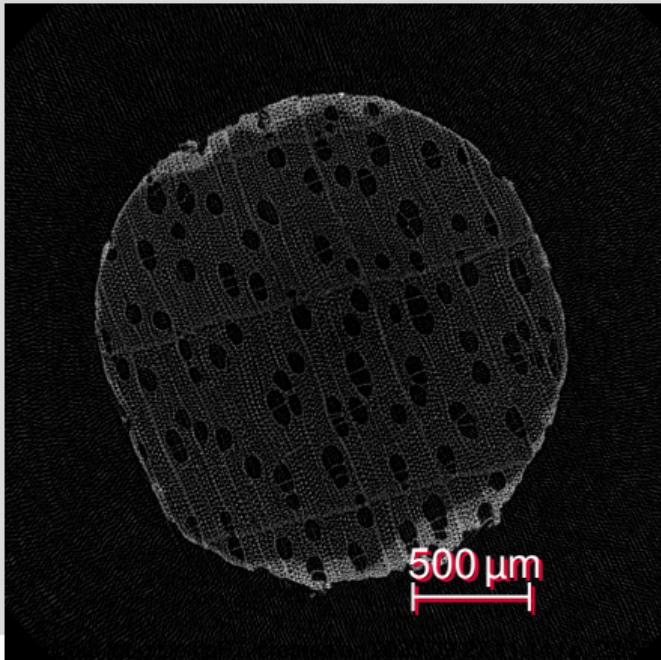
Examples



Examples



Examples



Examples



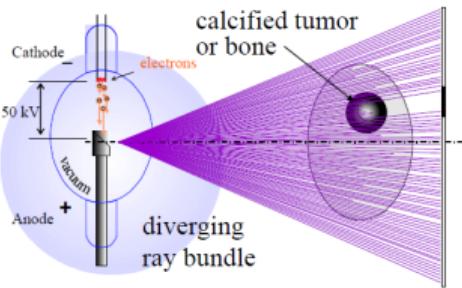
Preparation

- Study design
- Sample preparation

Projections

X-ray generation and contrast

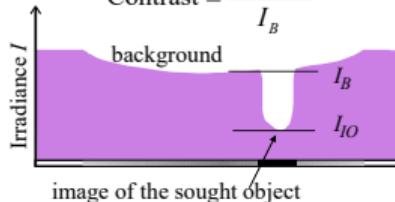
X-ray tube:
nearly point like
photon source



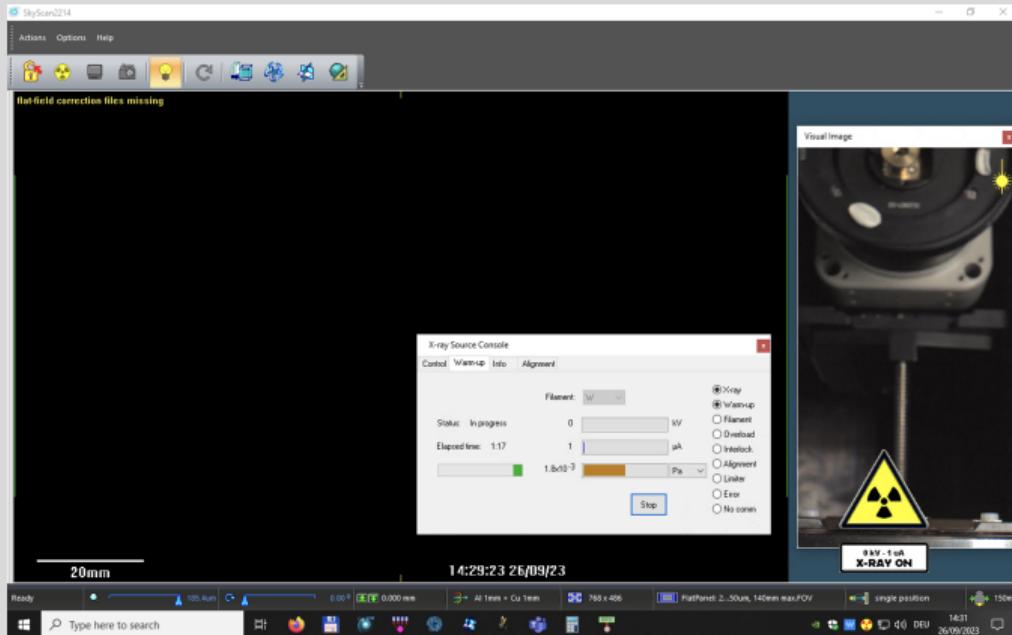
Contrast is given by
absorption of intensity I

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

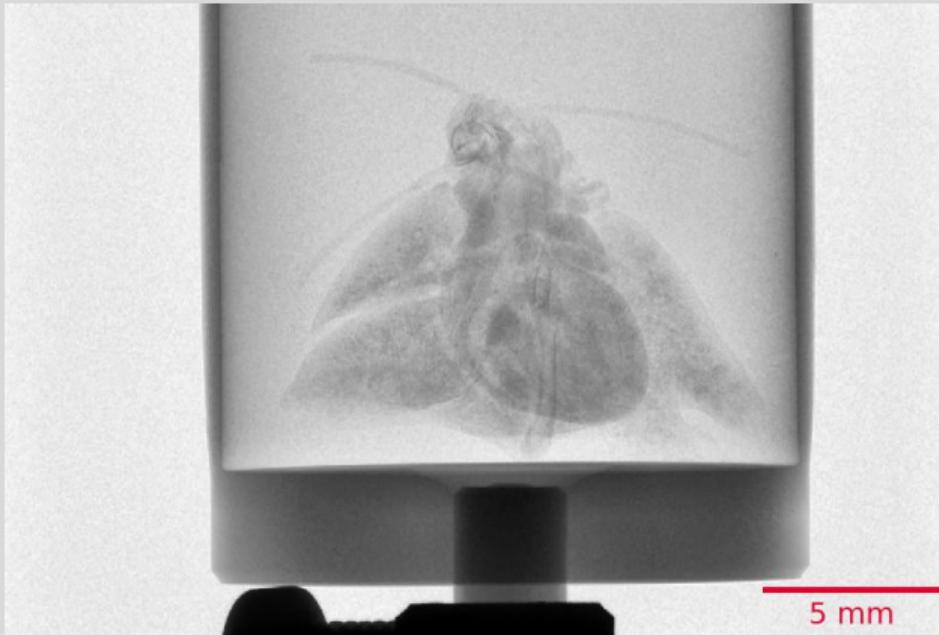
$$\text{Contrast} = \frac{I_{IO} - I_B}{I_B}$$



Projection acquisition



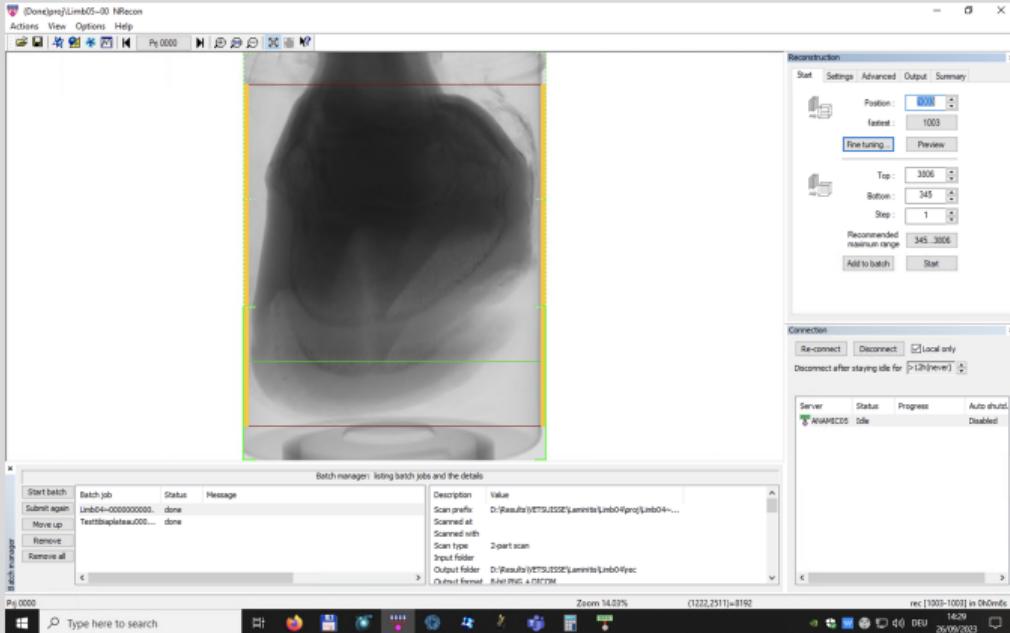
Projections



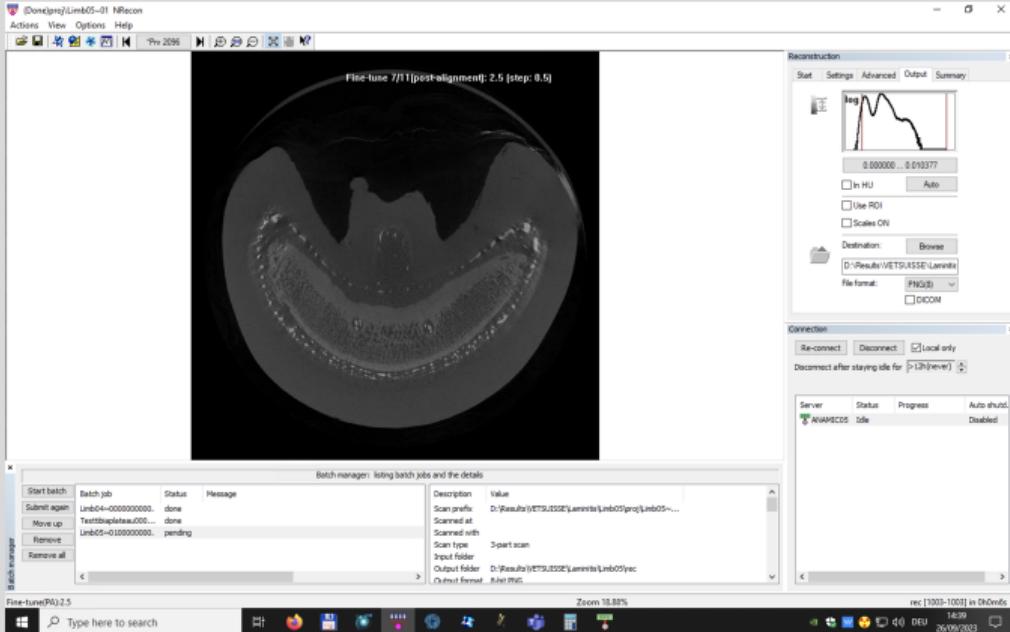
Projections

- 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

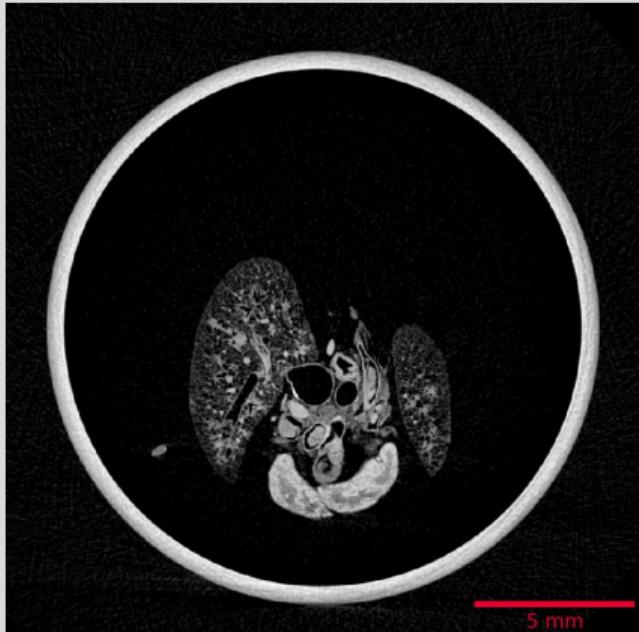
Reconstructions



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
- Scientific reports
- Two papers:
 - [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

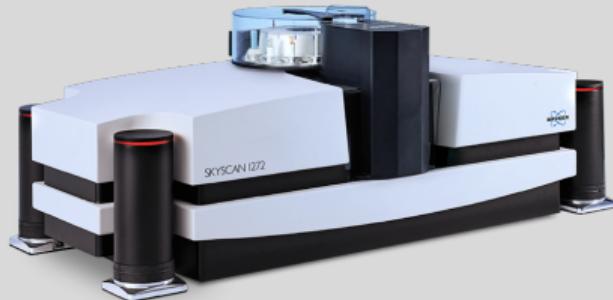
How?

- 104 extracted human permanent mandibular canines
- μ 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!



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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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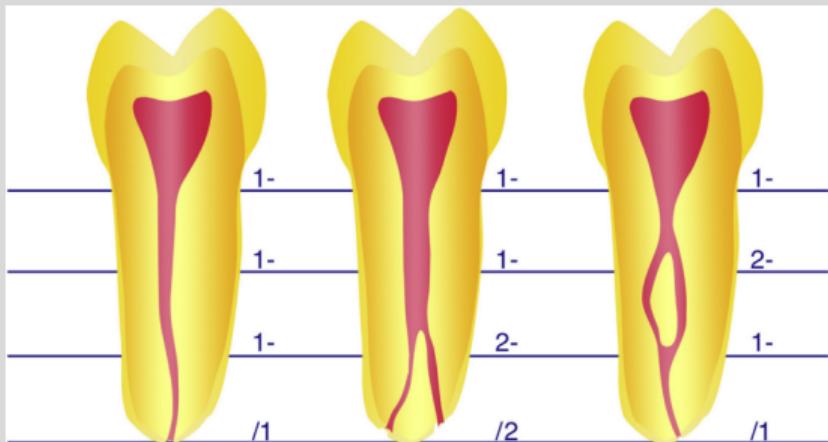
Sample changer on the SkyScan 1272

In total:

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

How?

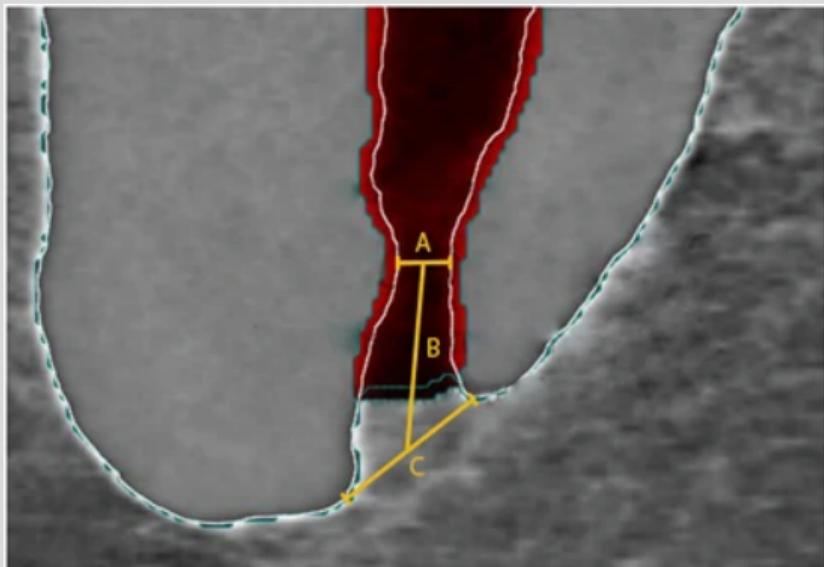
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From [21], Fig. 2

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From [23], Fig. 1

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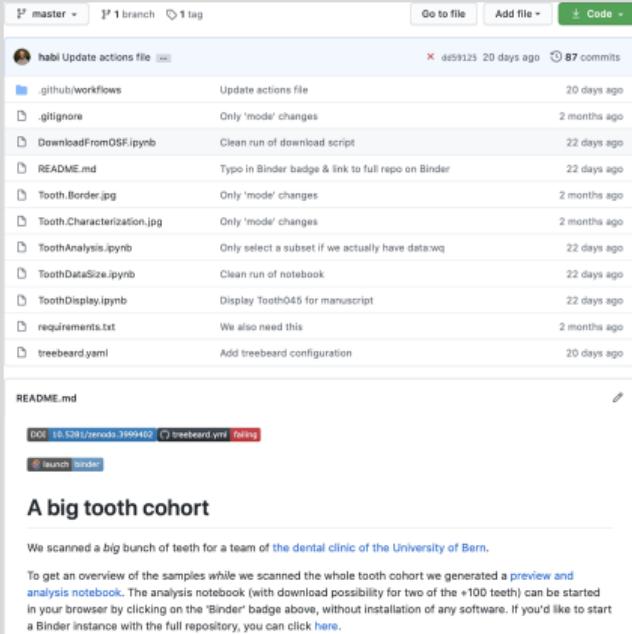
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gph.is/2nqkple

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The screenshot shows a GitHub repository page for a project named "habi". The repository has 87 commits, the most recent being a update to the actions file. The commits are listed with their commit message, date, and a "Code" button. Below the commits is a "README.md" file which contains a DOI link, a "treebeard.yaml" file status indicator, and a "launch binder" button. A section titled "A big tooth cohort" describes the dataset, stating it includes 104 teeth from the dental clinic of the University of Bern. It also provides links to a preview and analysis notebook.

master · 1 branch · 1 tag · Go to file · Add file · Code

habi · Update actions file · dd59125 · 20 days ago · 87 commits

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

README.md

DOI: 10.5281/zenodo.3999402 · treebeard.yaml · failing

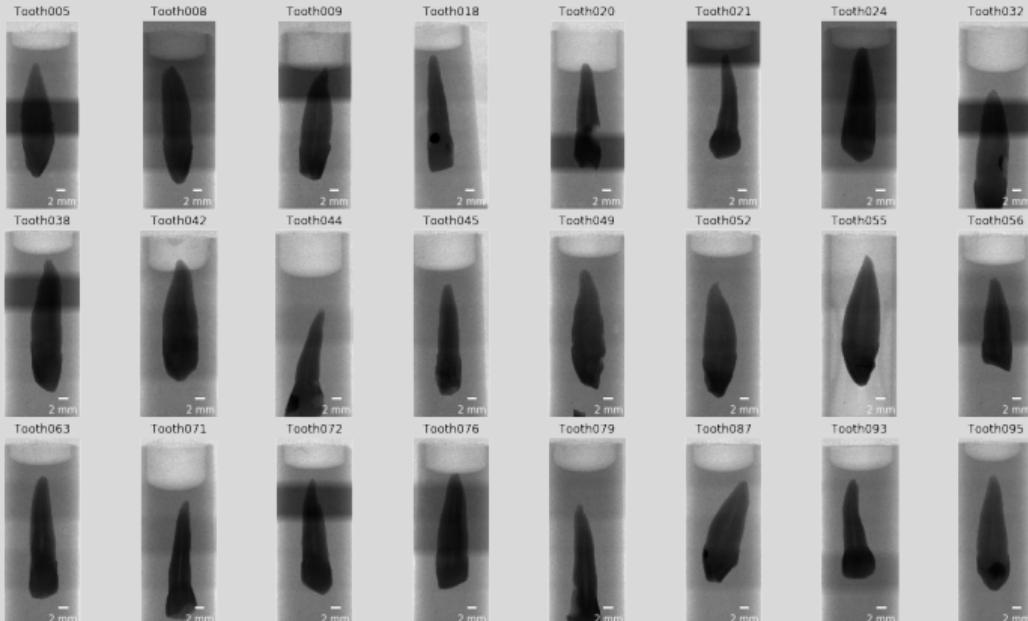
launch binder

A big tooth cohort

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

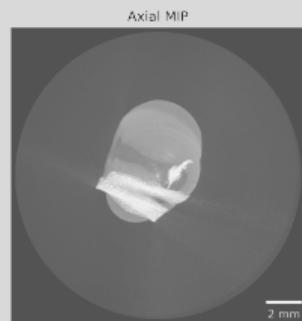
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of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

μ CT imaging



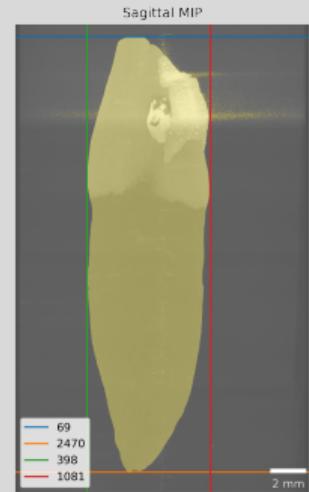
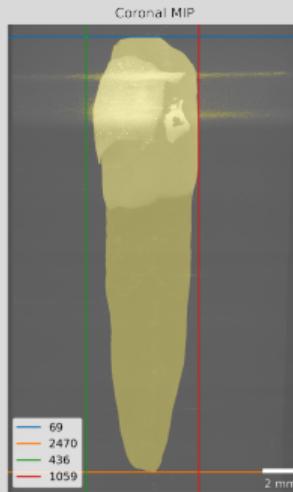
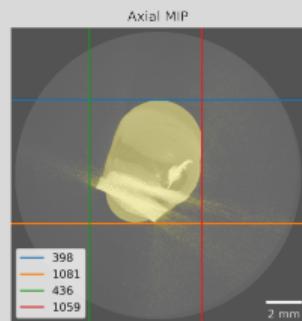
Dataset cropping

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

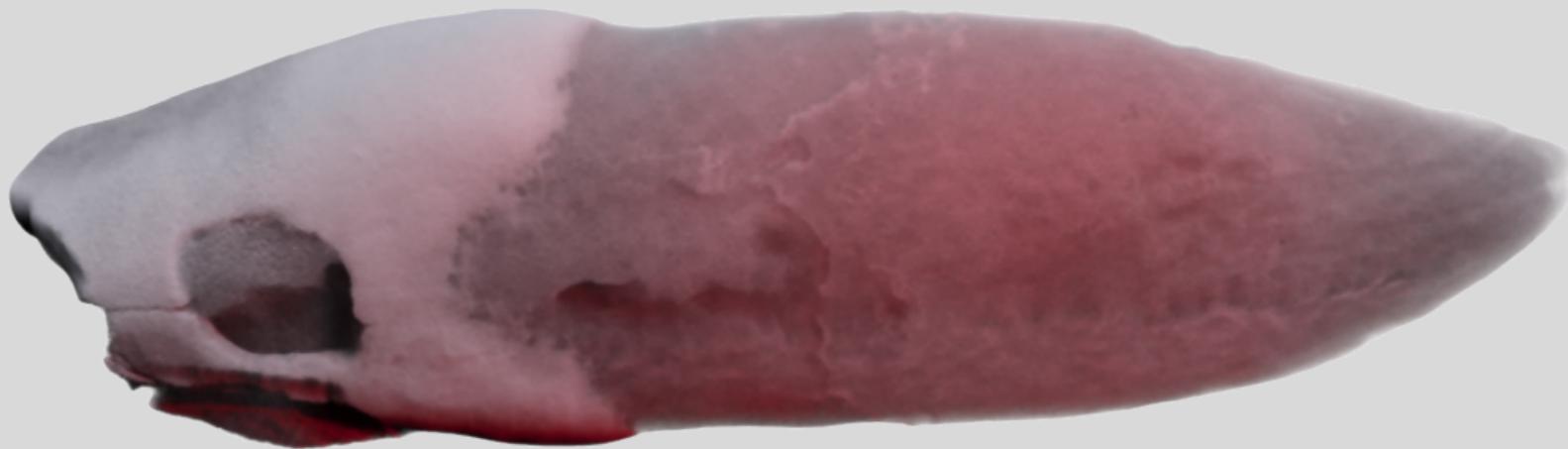


Dataset cropping

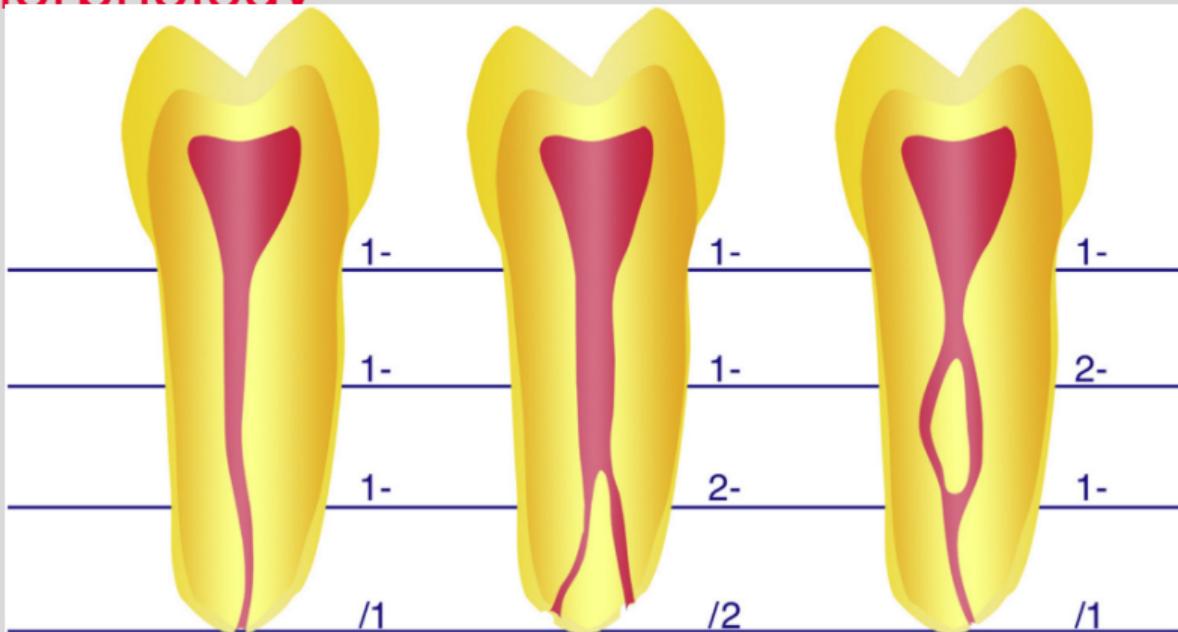
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Tooth morphology

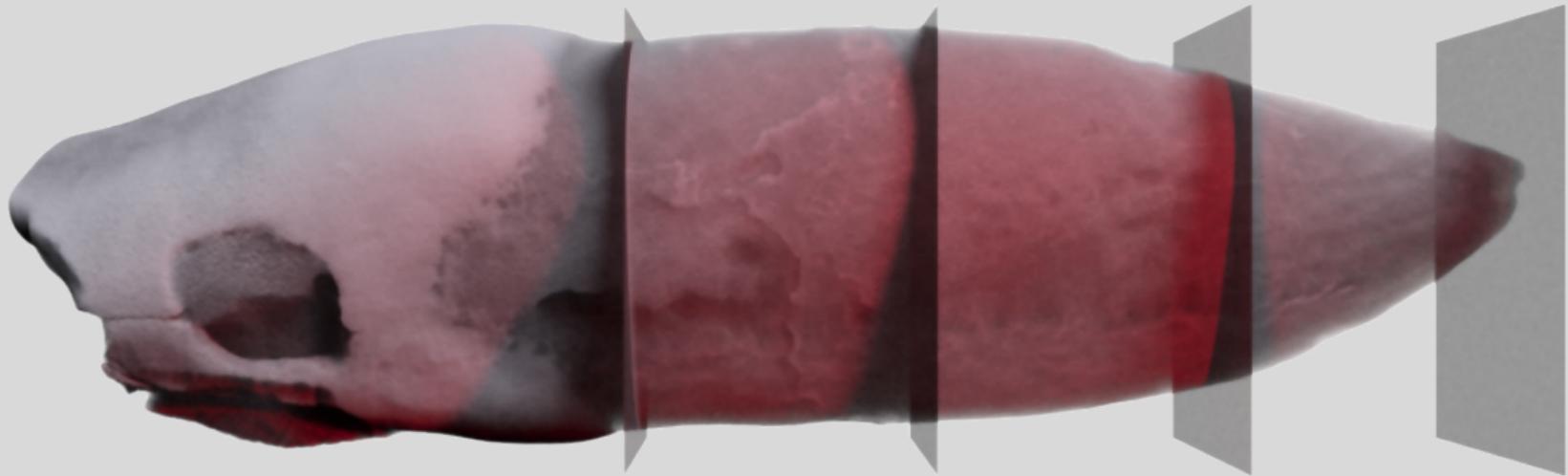


Tooth morphology

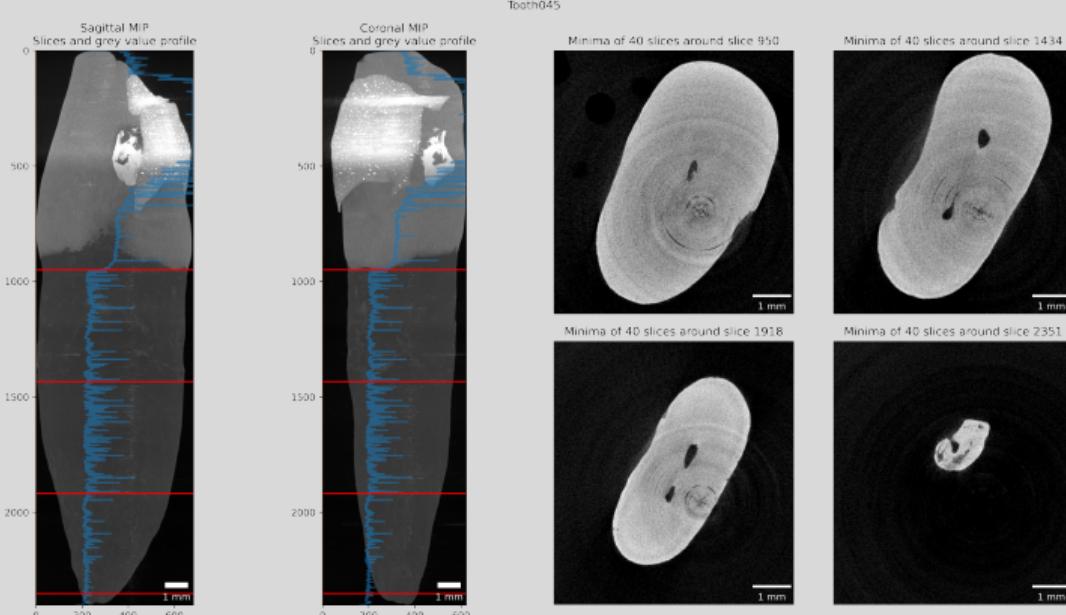


From [21], Fig. 2

Tooth morphology

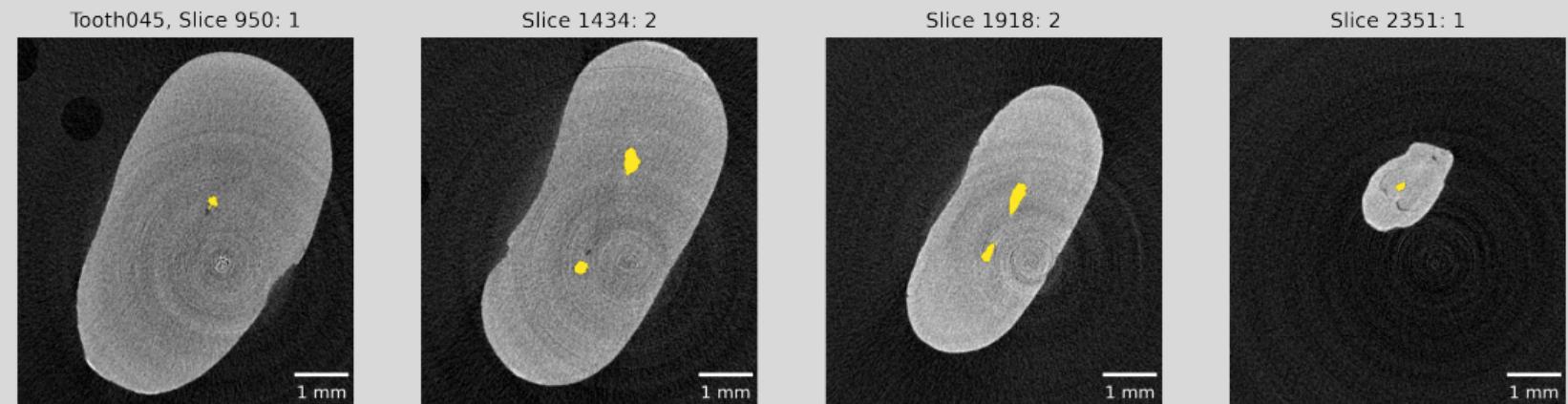


Detection of enamel-dentin border



Detection of enamel-dentin border

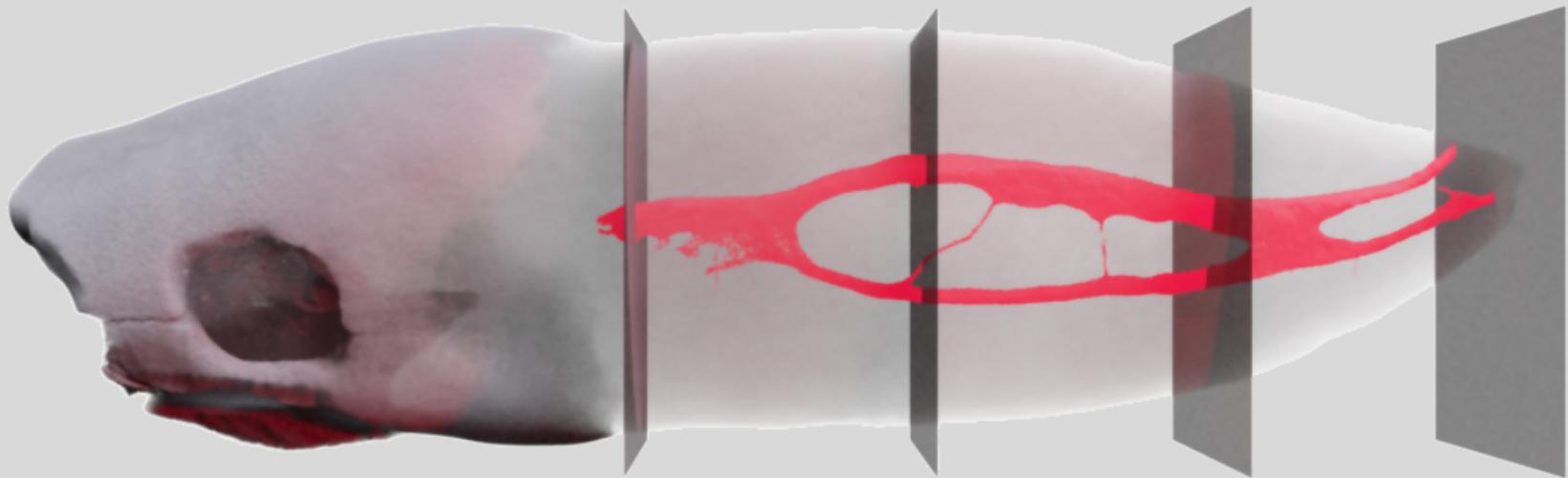
Tooth045



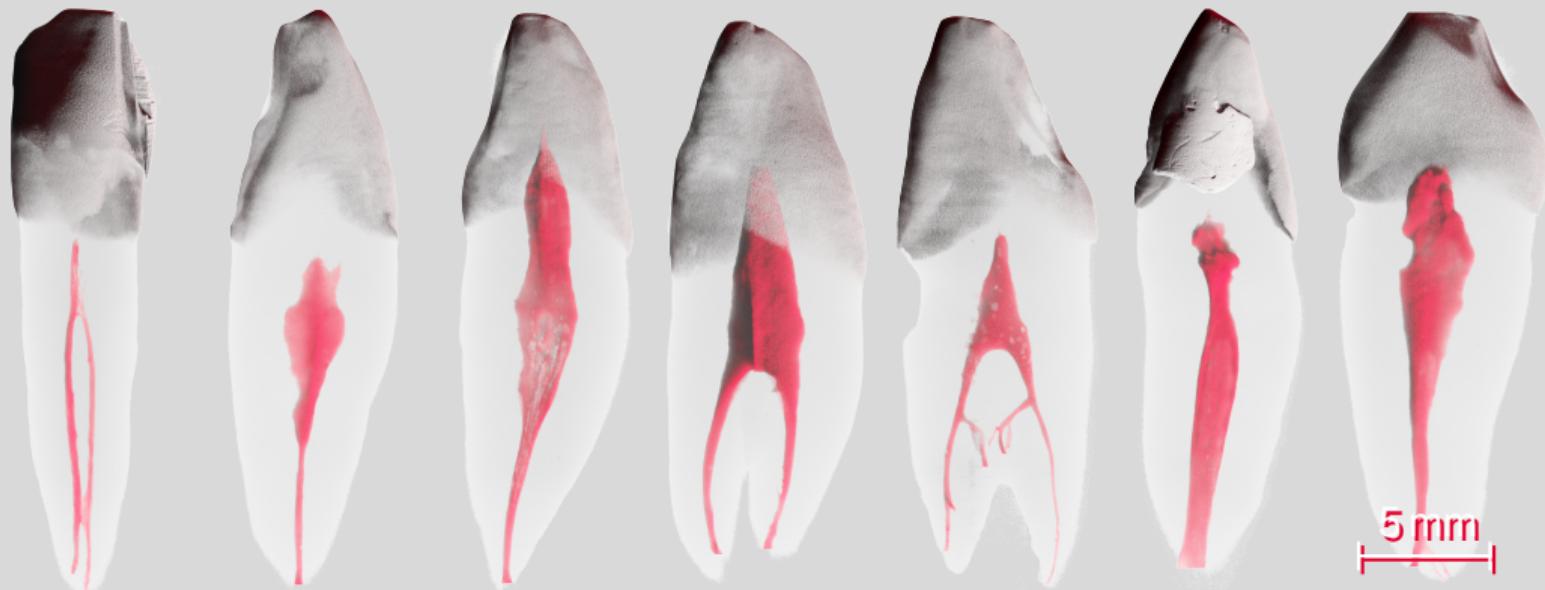
Outcome root canal configuration classification

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

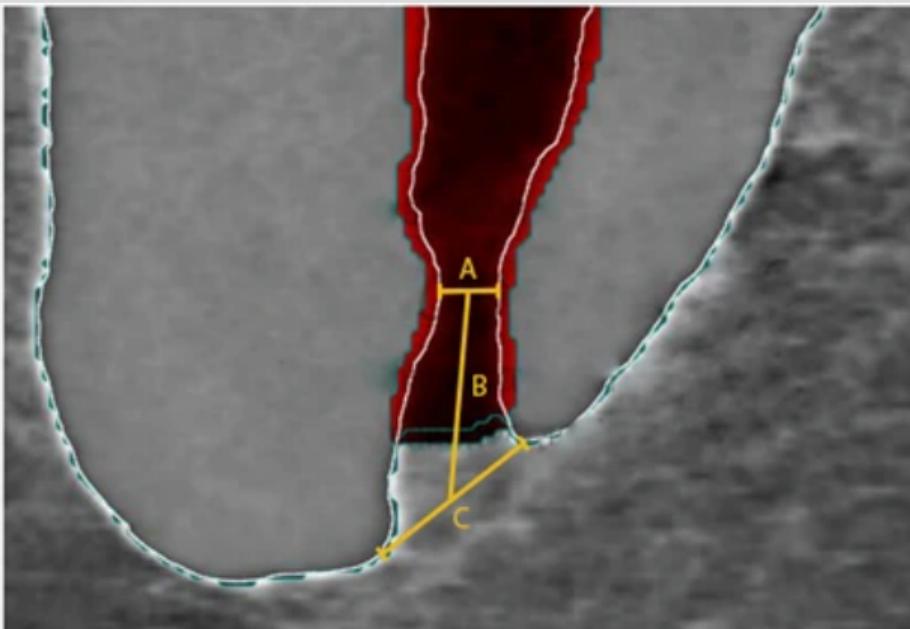
Extraction of root canal space



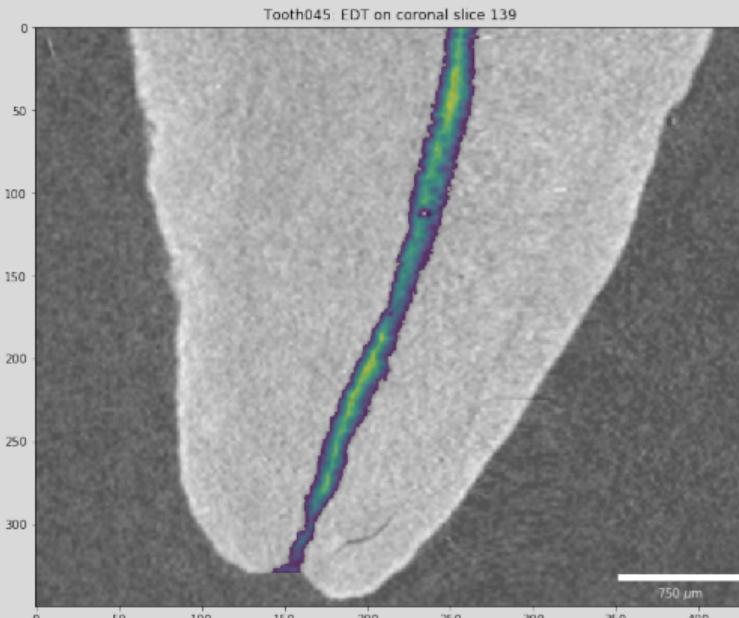
Results of root canal space extraction



Analysis of the physiological foramen geometry



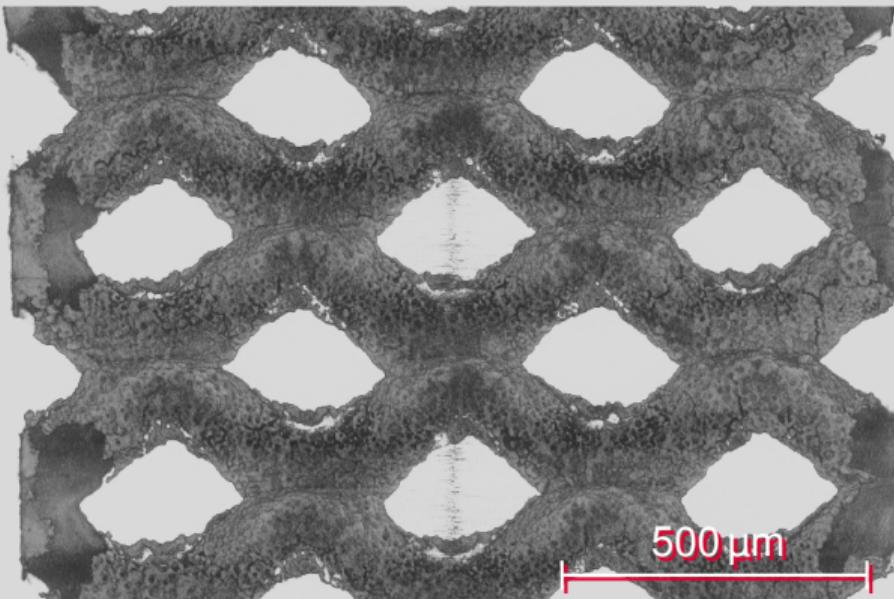
Analysis of the physiological foramen geometry



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

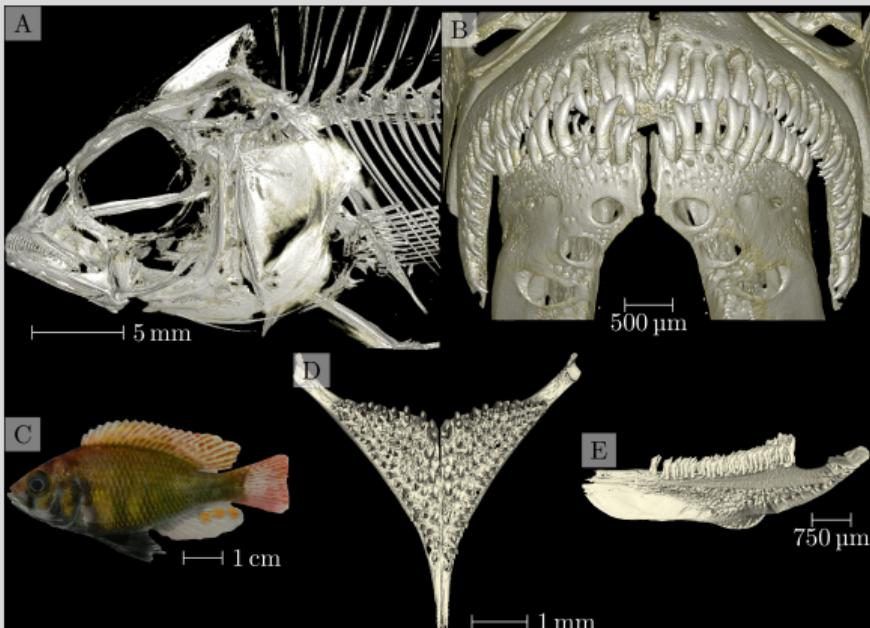
Metal foam



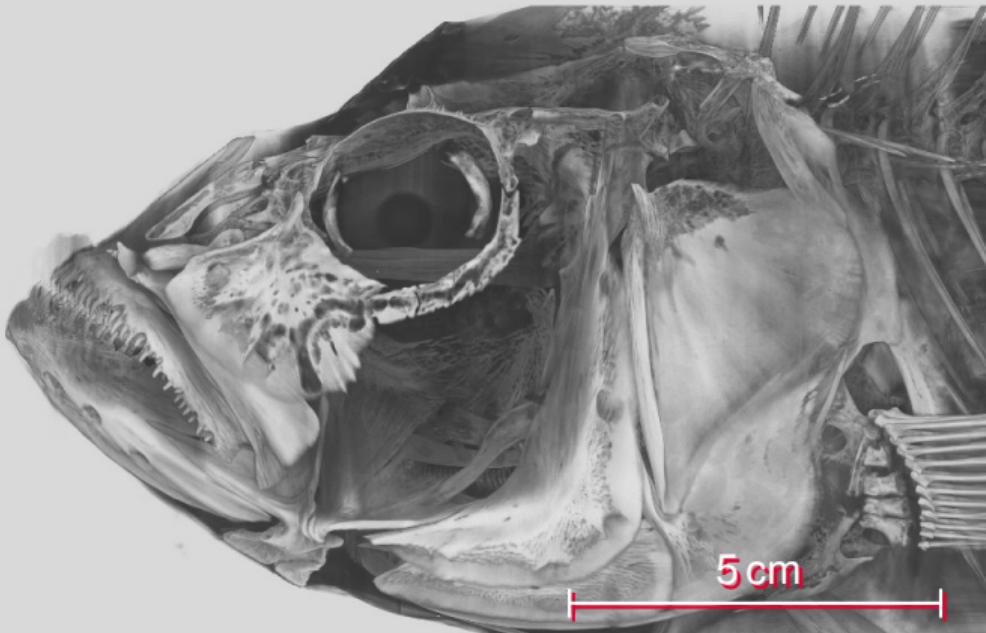
Data wrangling by example: Cichlids

- 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.



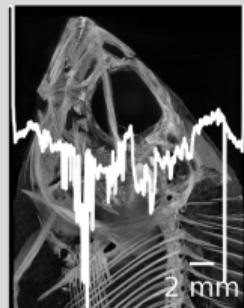
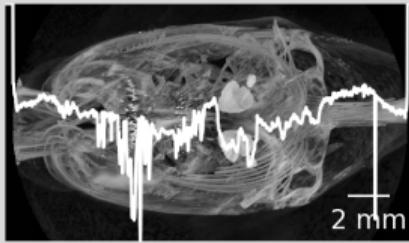
Cichlids



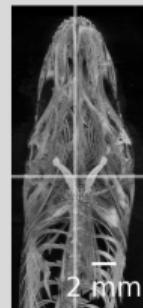
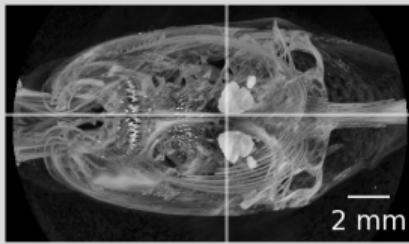
Data wrangling by example: Cichlids



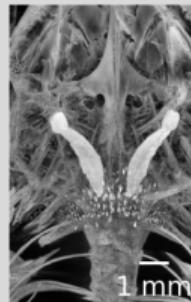
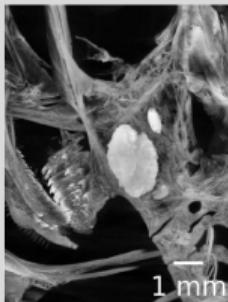
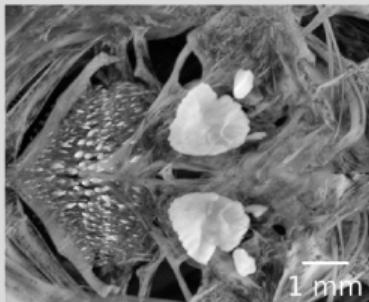
Data wrangling by example: Cichlids



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^AT_EX with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
 - Complete source code: git.io/fjpP7
 - The L^AT_EX code is automatically compiled with a GitHub action [1] to a (handout) PDF which you can access here: git.io/JeQxO
- Did you spot an error?
 - File an issue: git.io/fjpPb
 - Submit a pull request: git.io/fjpPN
 - Send me an email: david.haberthuer@unibe.ch

[1] Details on how this works are specified in a small test repository here: git.io/JeOOj

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