

X-ray microtomography

David Haberthür

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

Grüessech mitenang!

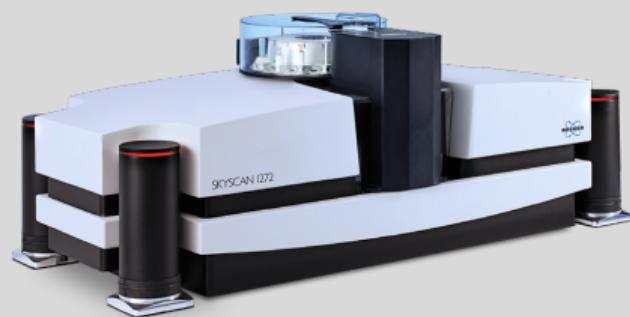
- David Haberthür
 - Physicist by trade
 - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
 - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
 - Post-Doc II: μ CT group, Institute of Anatomy, University of Bern, Switzerland.

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

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.
(Correlative imaging pipelines, museum/ collection material)

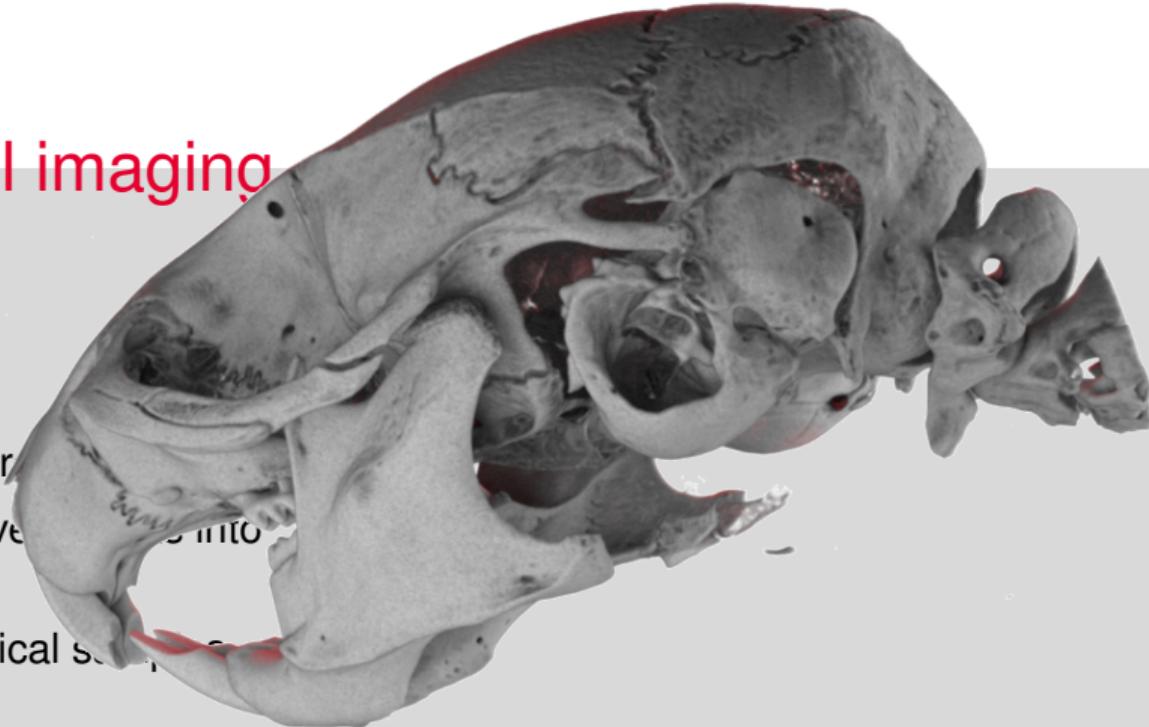
Biomedical imaging

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



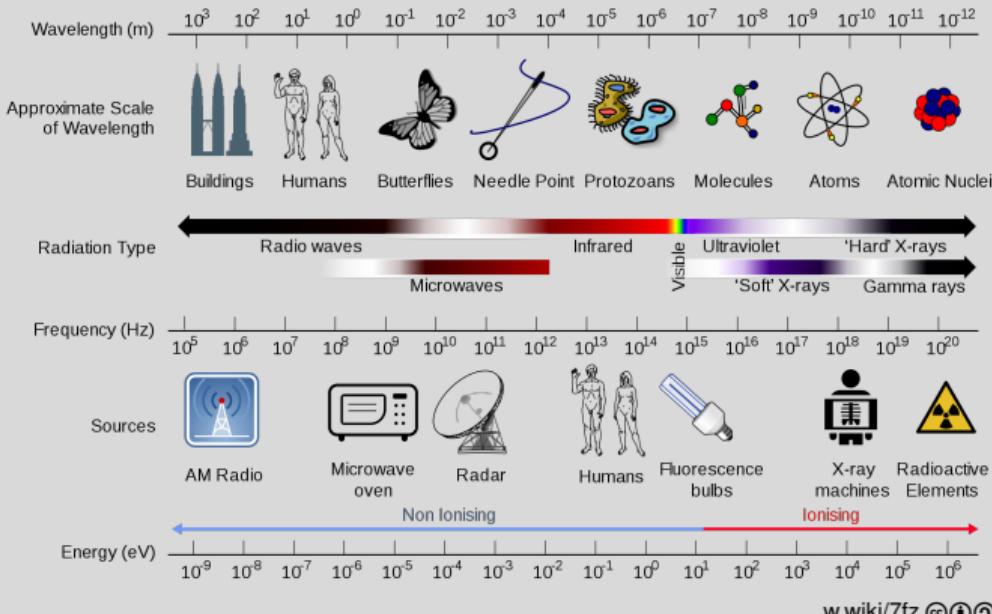
w.wiki/7g4

Biomedical imaging

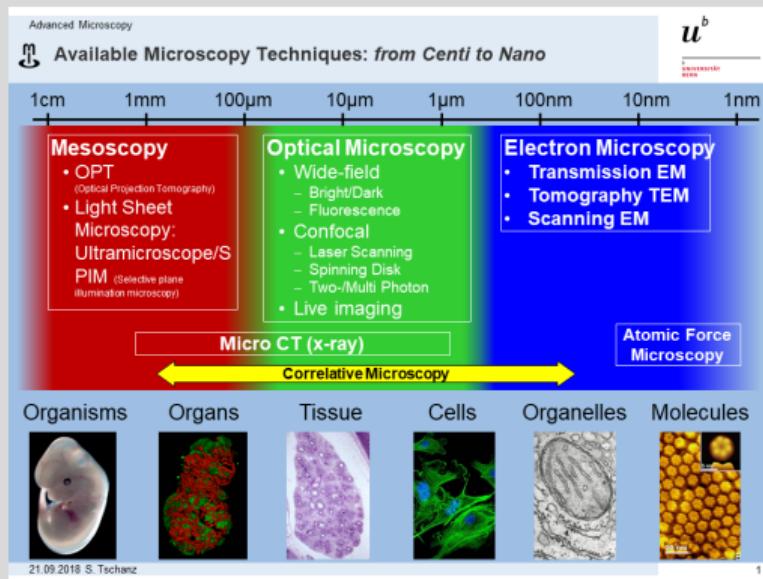


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Wavelength & Scale



Wavelength & Scale

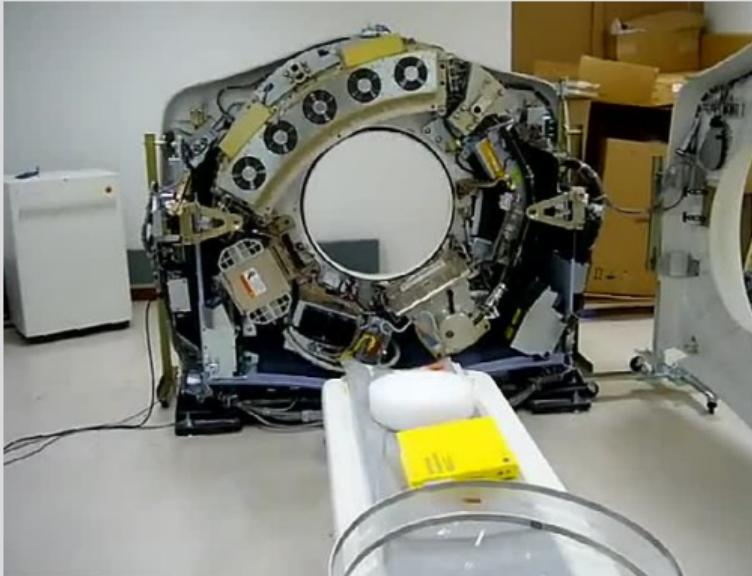


Stefan Tschanz, with permission

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

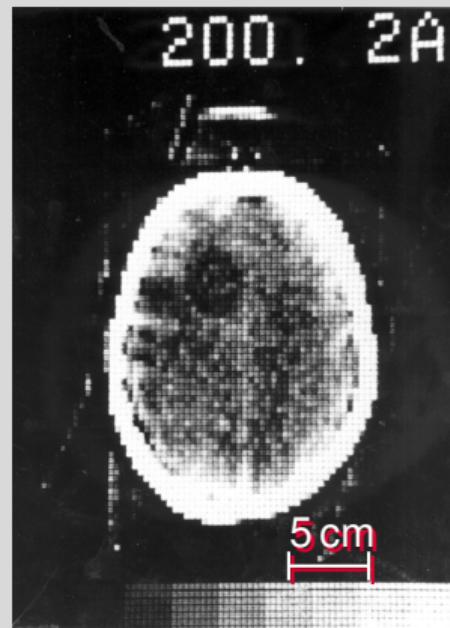
CT-Scanner



youtu.be/2CWpZKuy-NE

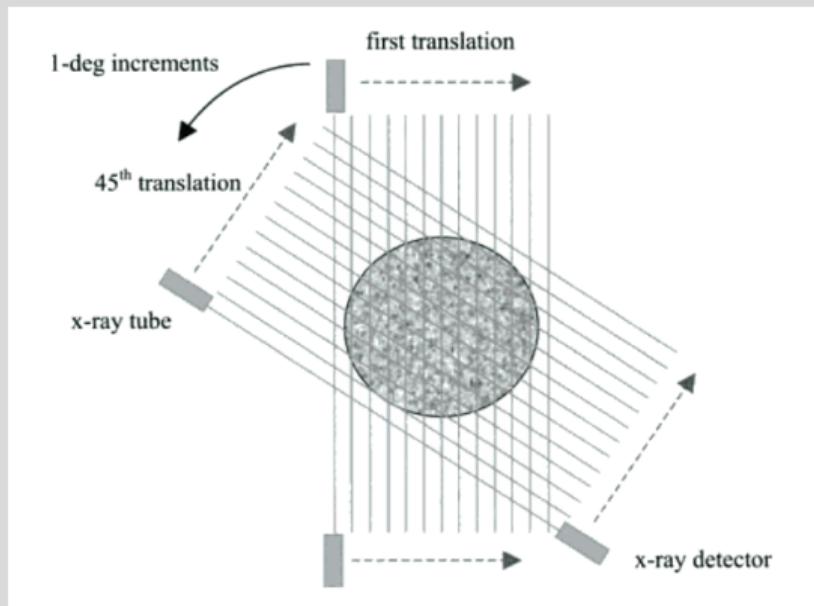
CT History

- 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]



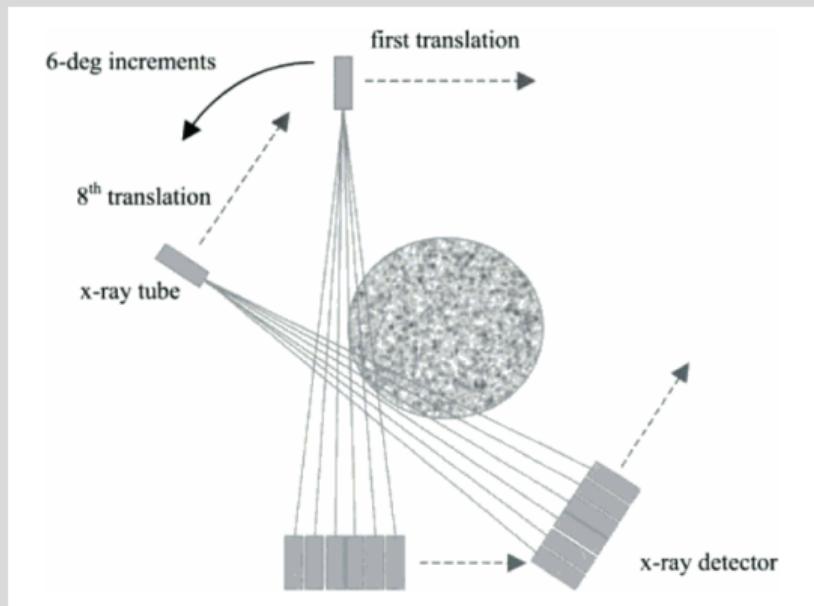
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- CT scanner generations: First



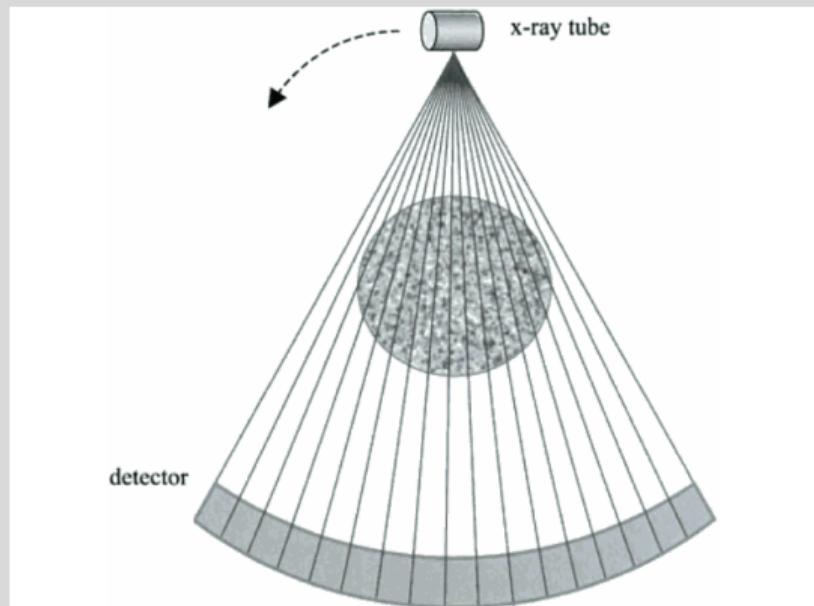
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- CT scanner generations: First, second and third



μ CT History

- Scanning gemstones [Lin2019] X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [24.1, 2], and high-resolution x-ray computed tomography (i. e., microtomography or microCT) was first reported in the 1980s [24.3–11]. MicroCT systems were developed in response to the need to nondestructively examine materials at higher resolution than provided by conventional CT imaging. Dr. Lee

X-ray interaction

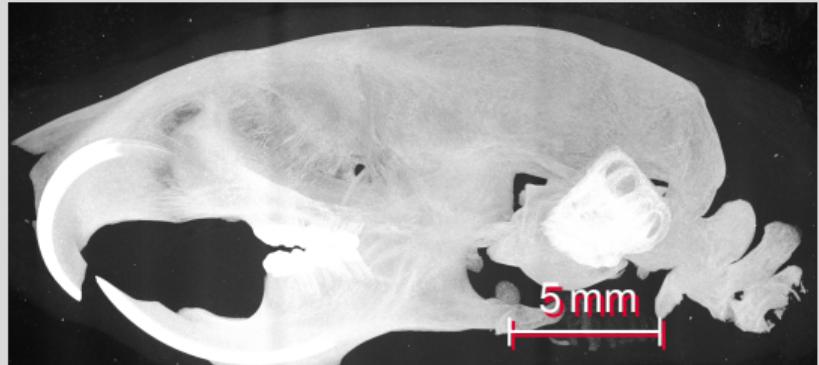
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([15])
 - Photoelectric absorption (τ) is strongly dependent on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

Composition of biological tissues

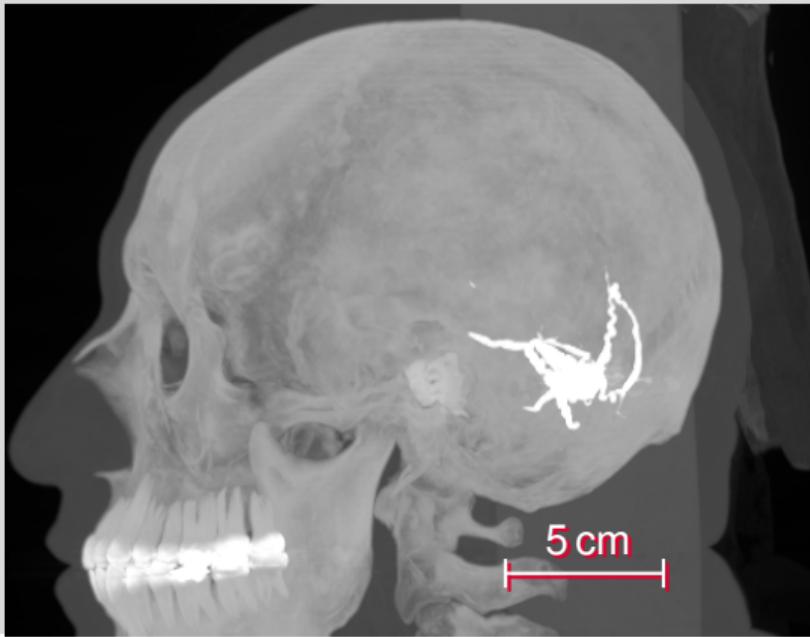
Tissue: content by mass percentage

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

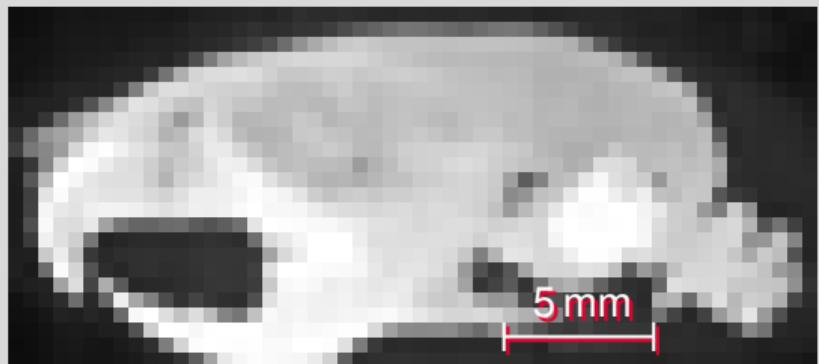
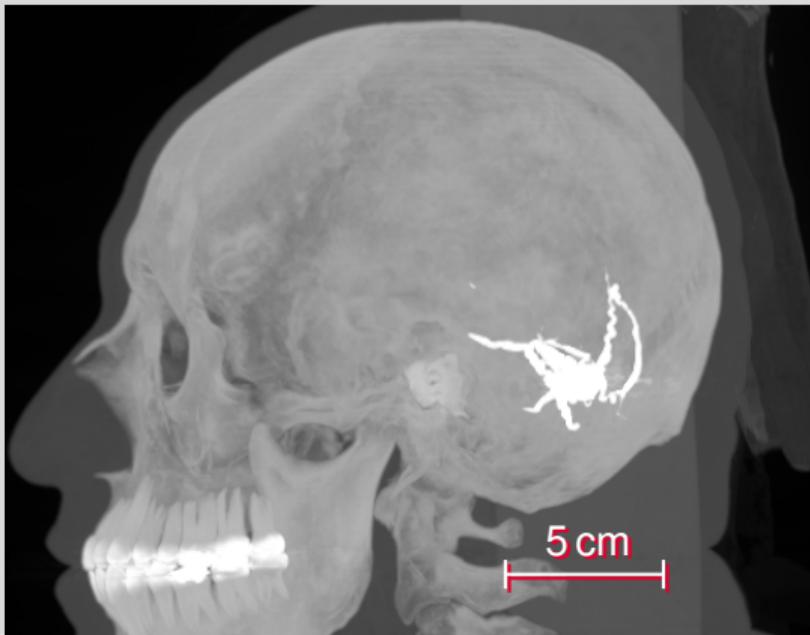
Why μ CT?



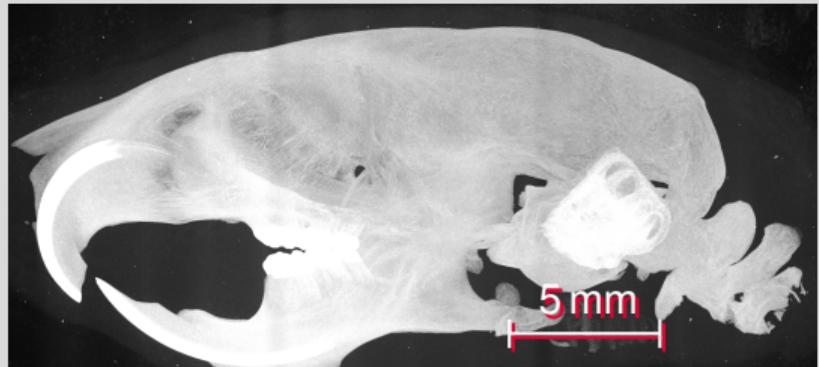
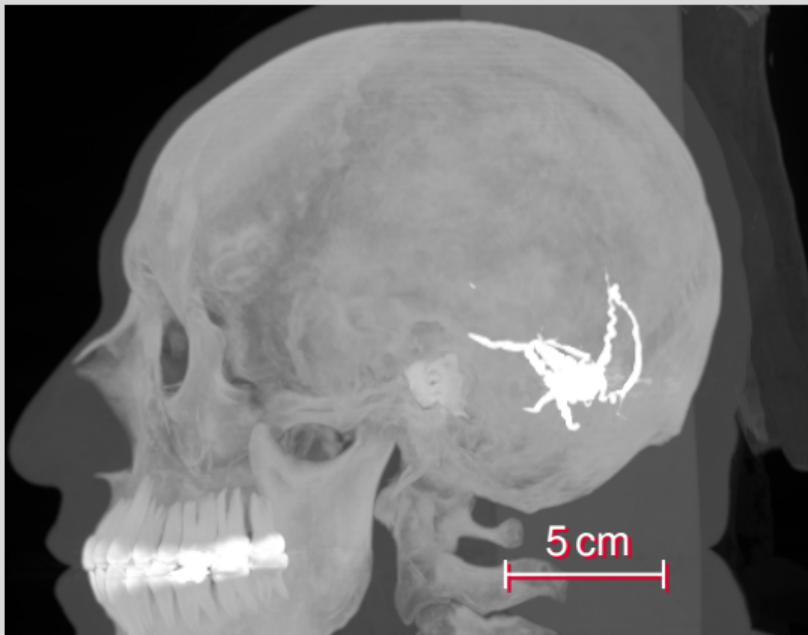
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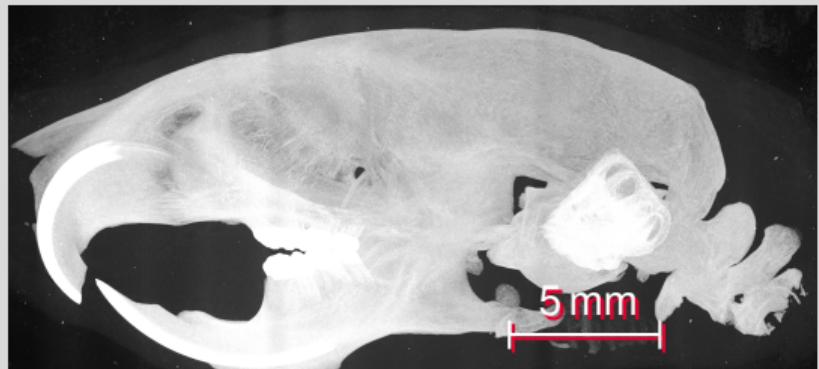
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Why μ CT?



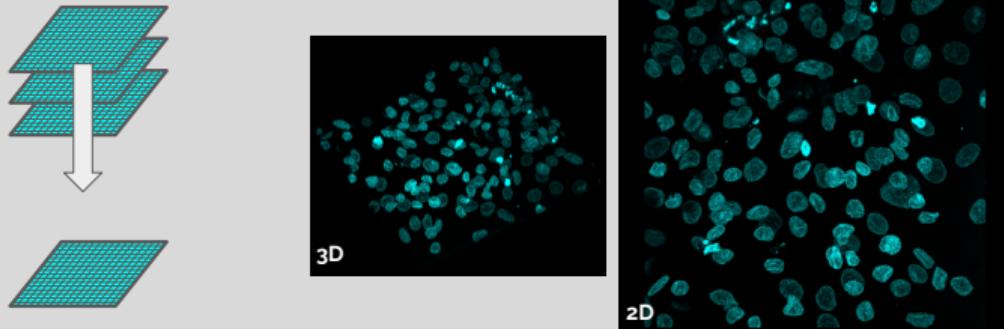
From [17], Subject C3L-02465



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



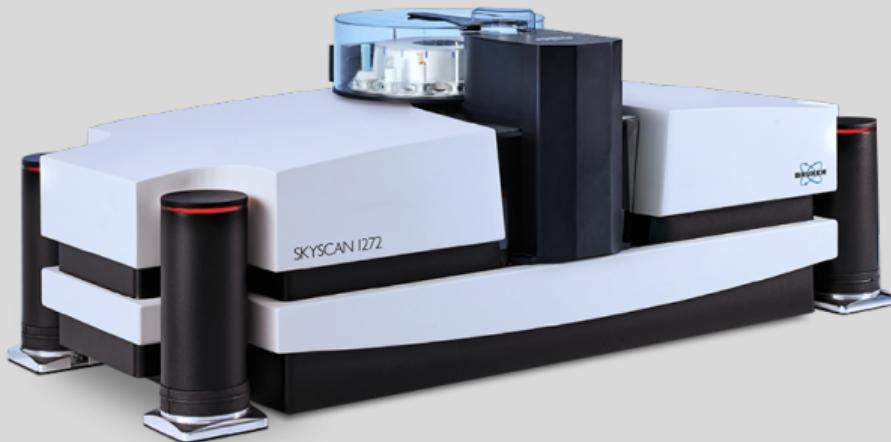
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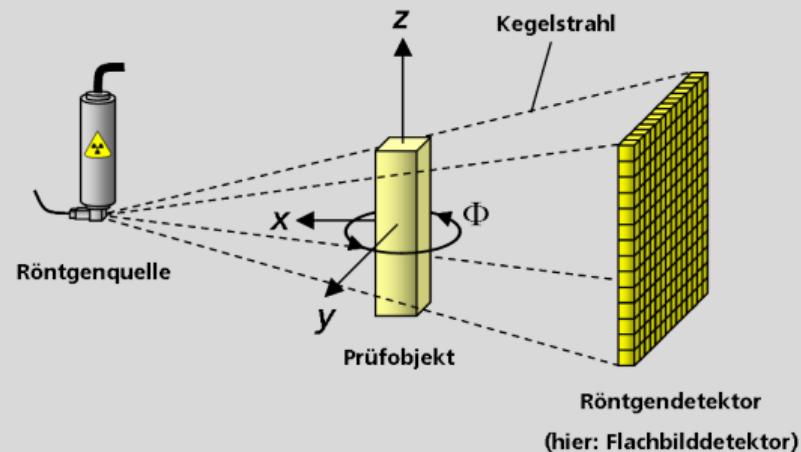


flic.kr/p/7Xhk2Y

What is happening?

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

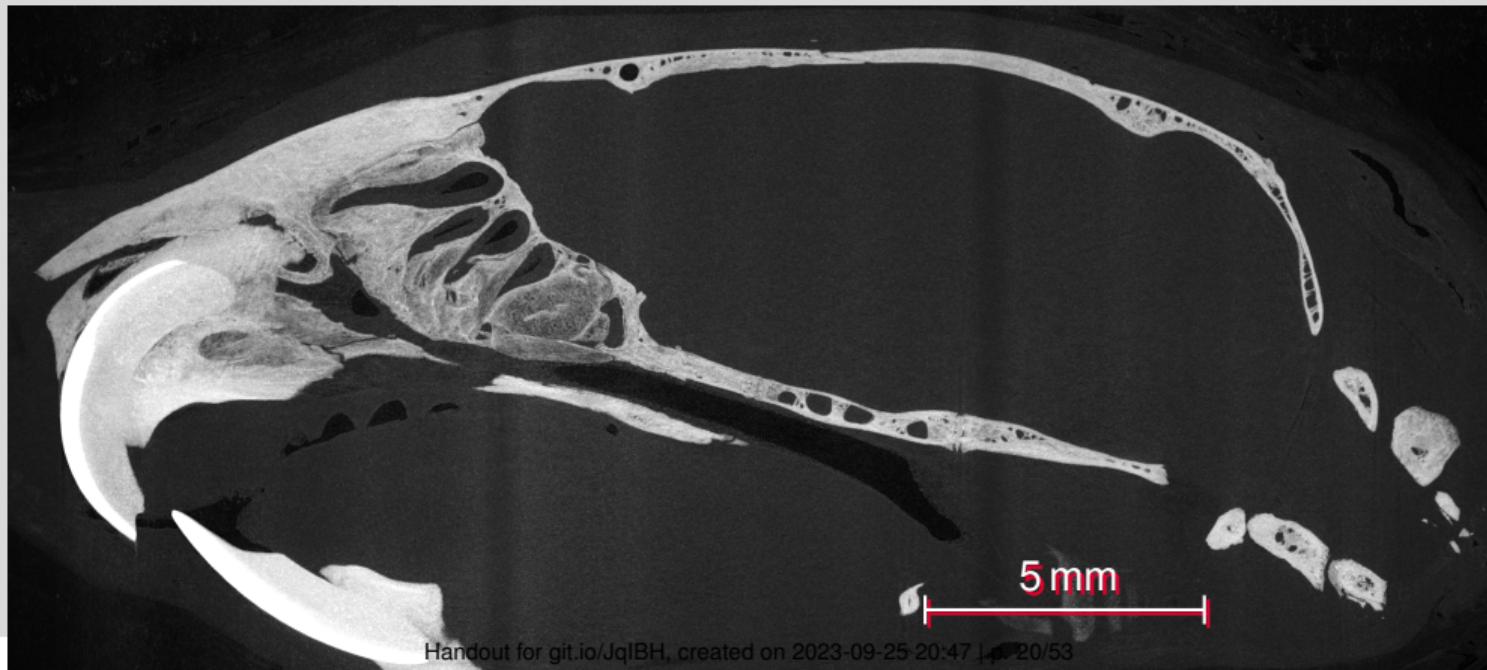
- an x-ray source
- a sample
- a detector



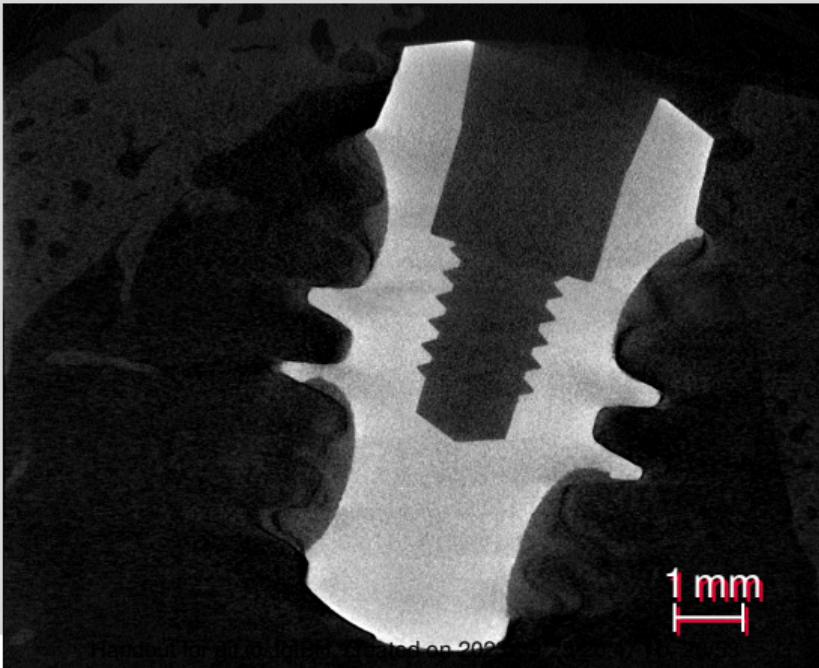
w.wiki/7g3

Machinery

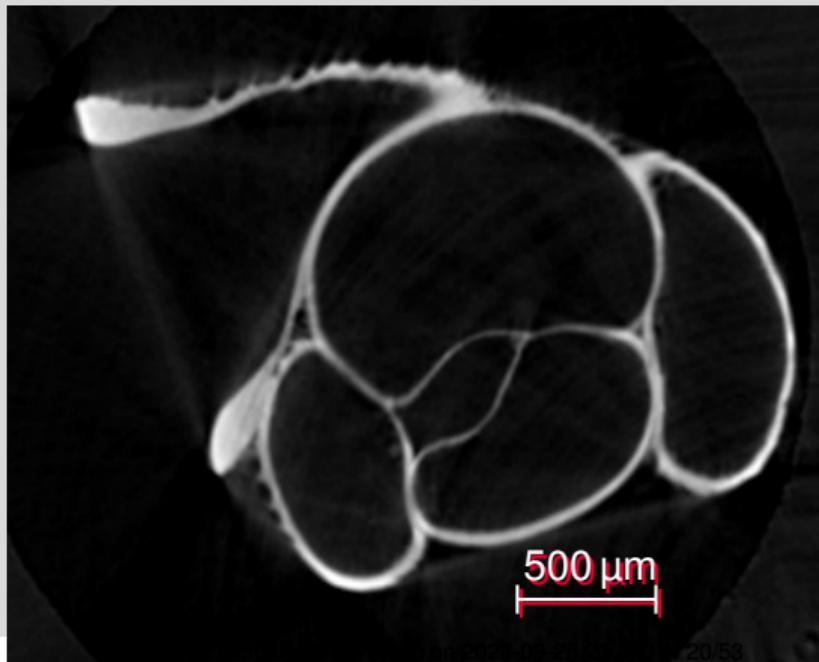
Examples



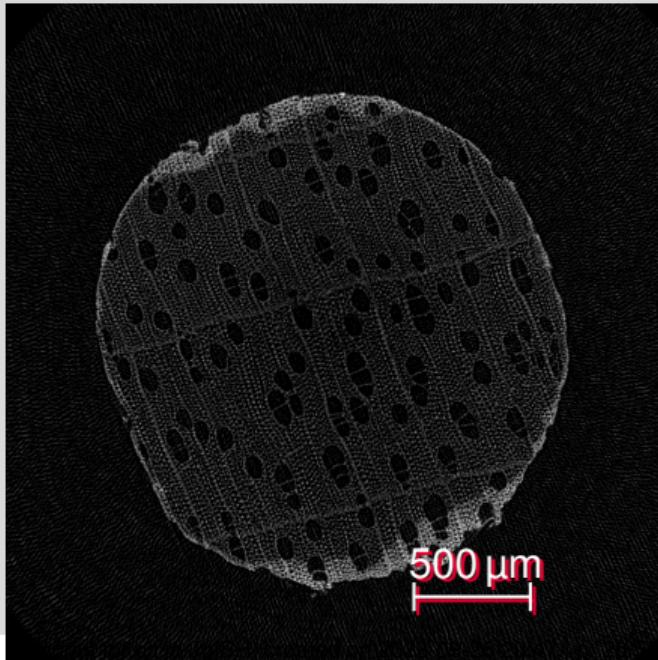
Examples



Examples



Examples



Examples



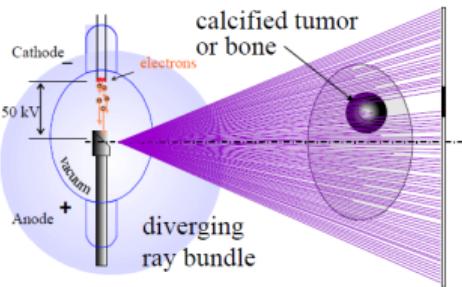
Preparation

- Study design
- Sample preparation

Projections

X-ray generation and contrast

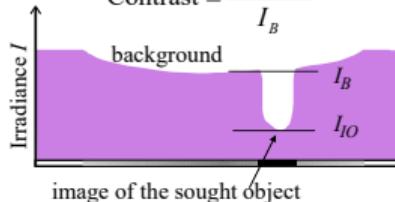
X-ray tube:
nearly point like
photon source



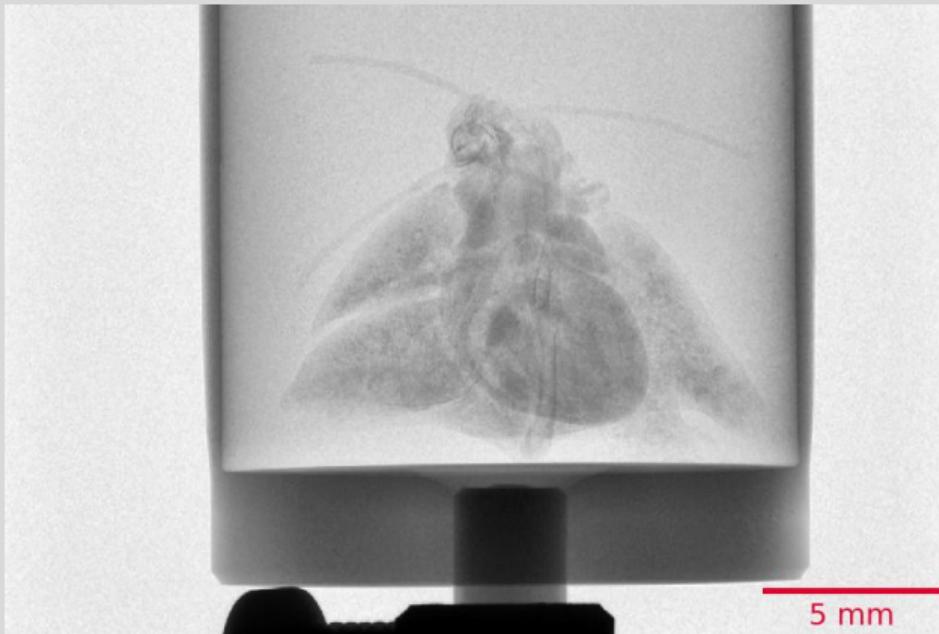
Contrast is given by
absorption of intensity I

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

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



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

- 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/g9rj
 - [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

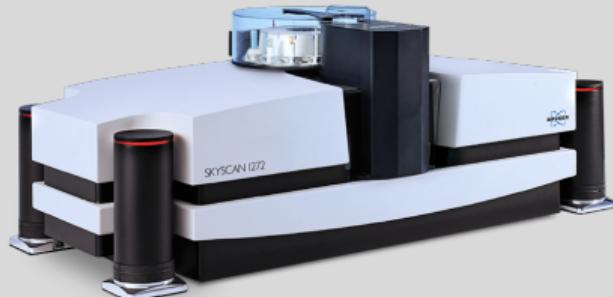
How?

- 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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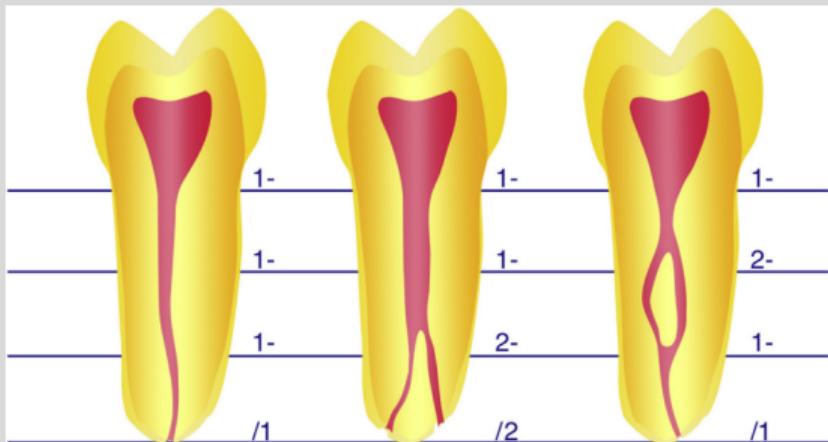
Sample changer on the SkyScan 1272

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?

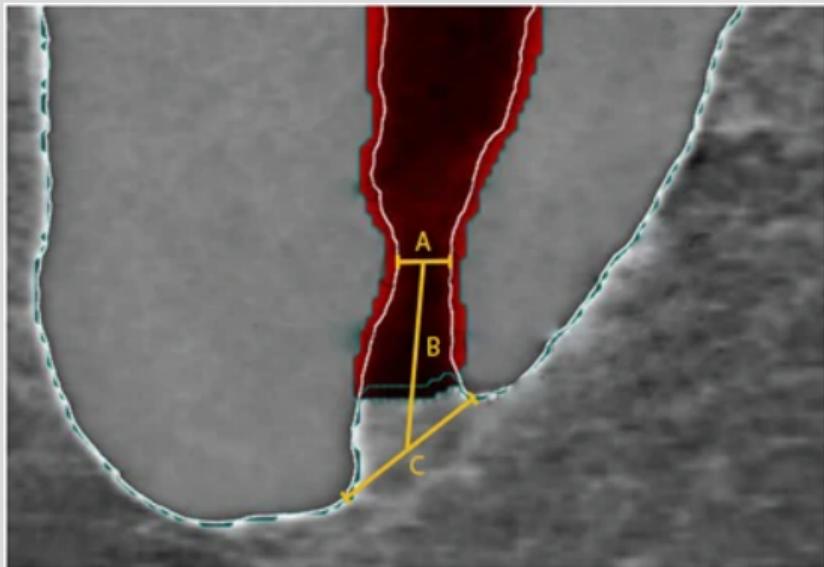
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From [21], Fig. 2

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From [23], Fig. 1

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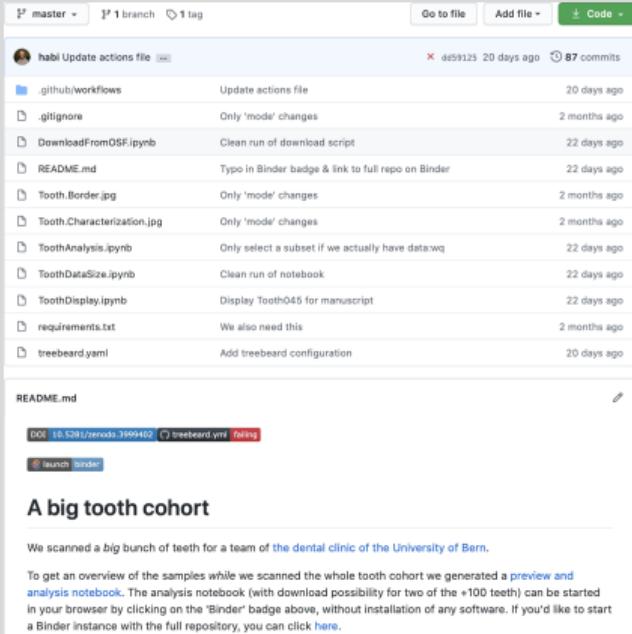


18.44 Kartę PEKA, Zarząd Transportu Miejskiego zachęca do wymiany KOM-kart na Po

gph.is/2nqkple

How?

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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 commits from a user named 'habi'.

Commit	Message	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 'README.md' file with the following content:

```
DOE 10.5281/zenodo.3999402 treebeard.yaml failing
```

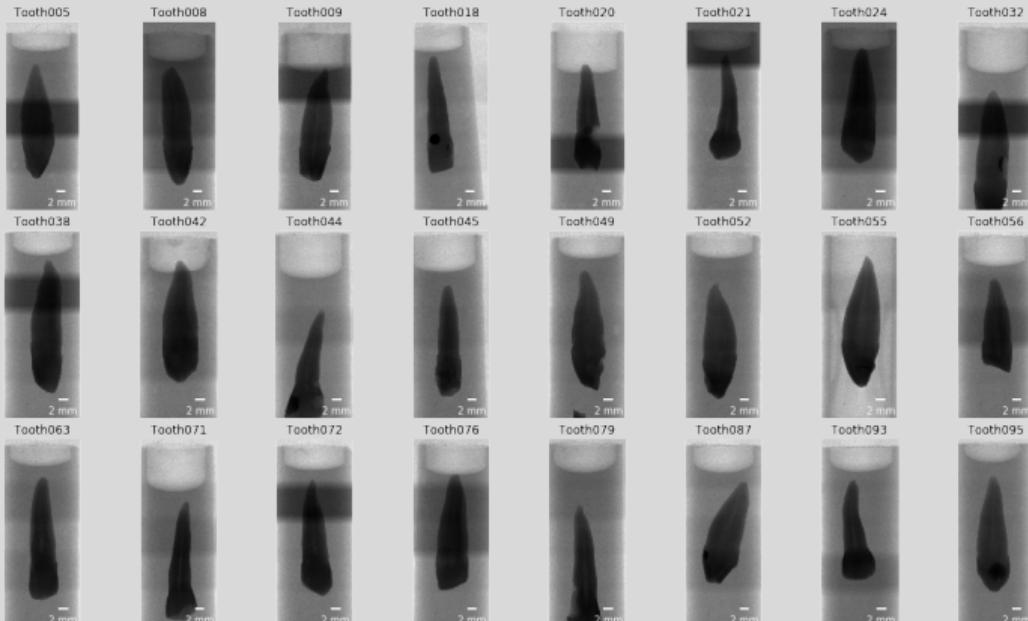
[Launch Binder](#)

A big tooth cohort

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

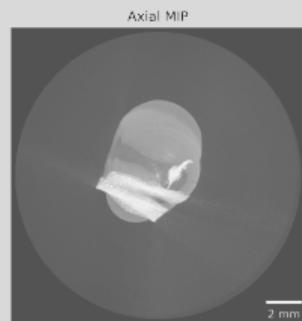
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of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

μ CT imaging



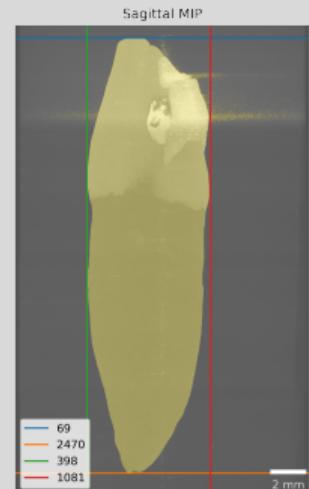
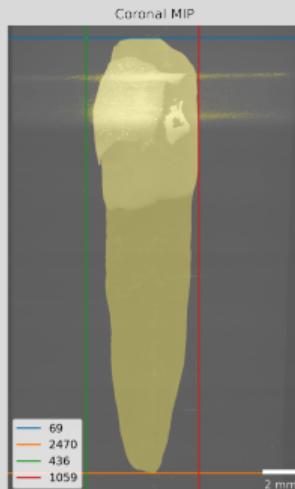
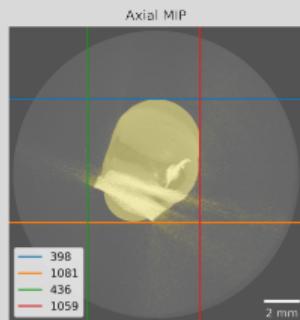
Dataset cropping

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



Dataset cropping

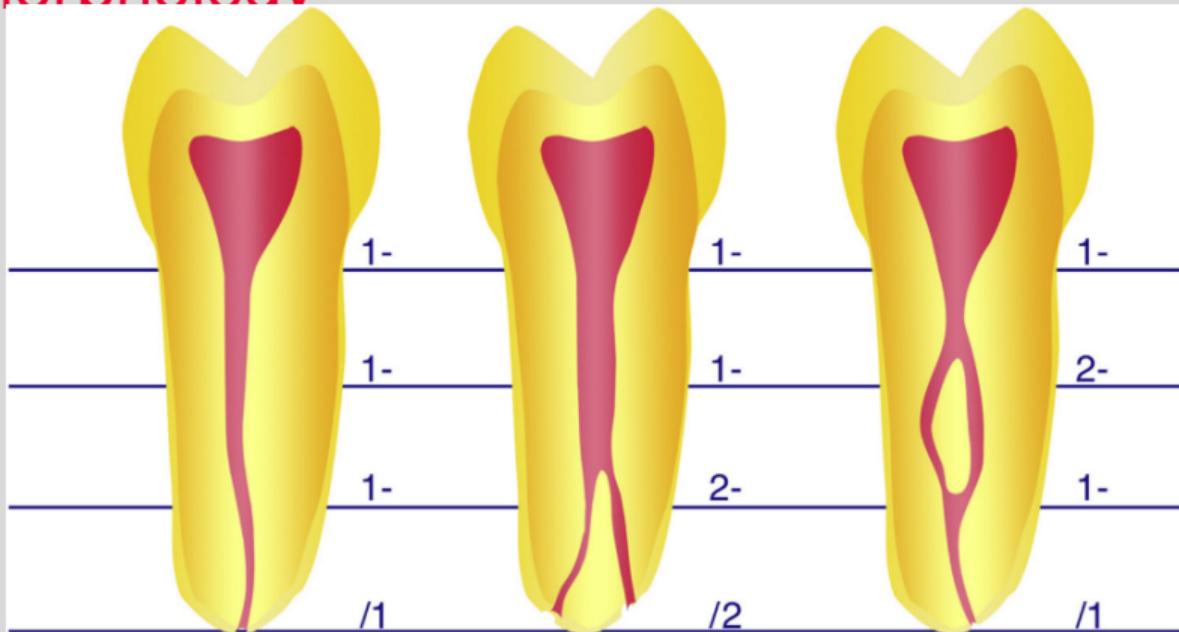
- Full datasets: 326 GB
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Tooth morphology

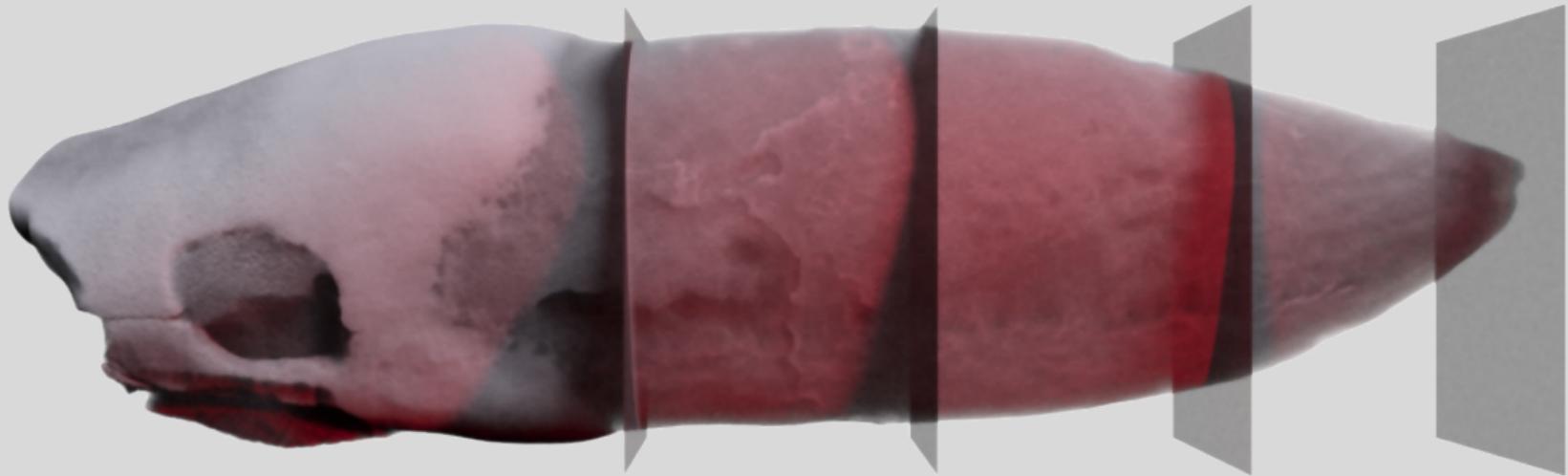


Tooth morphology

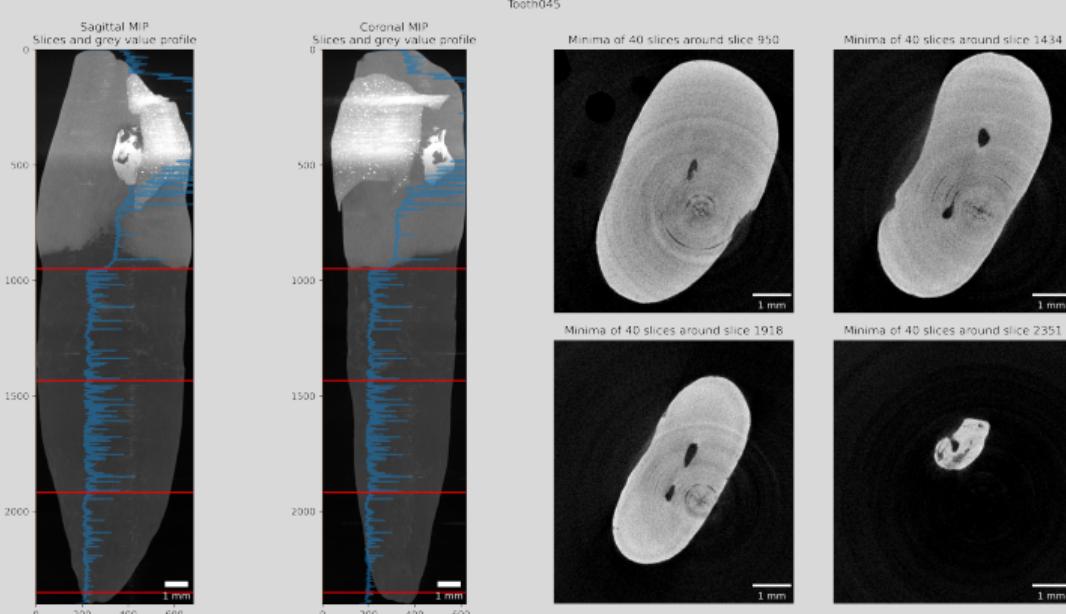


From [21], Fig. 2

Tooth morphology

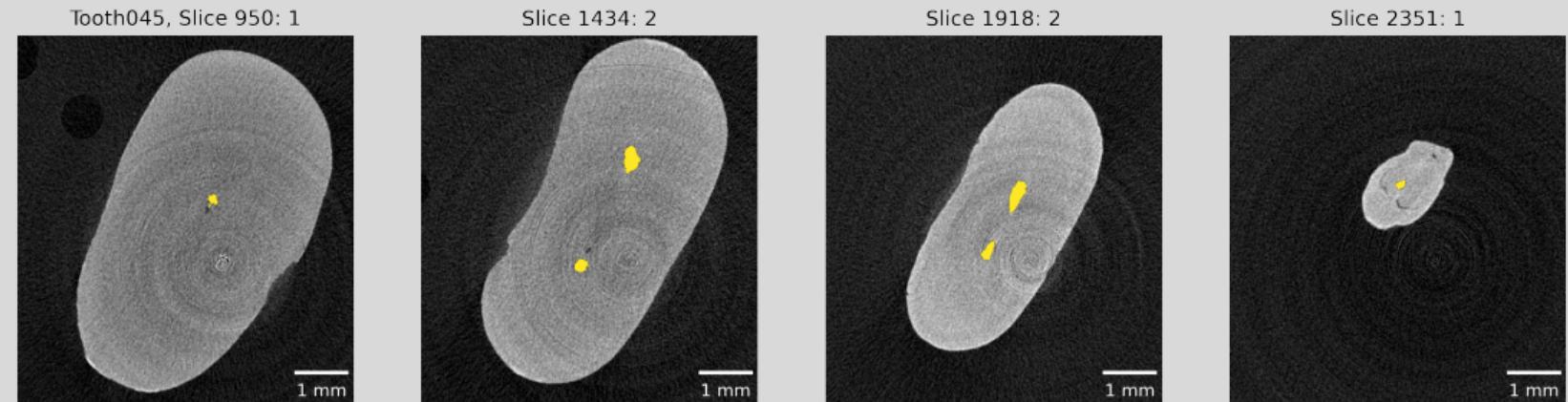


Detection of enamel-dentin border



Detection of enamel-dentin border

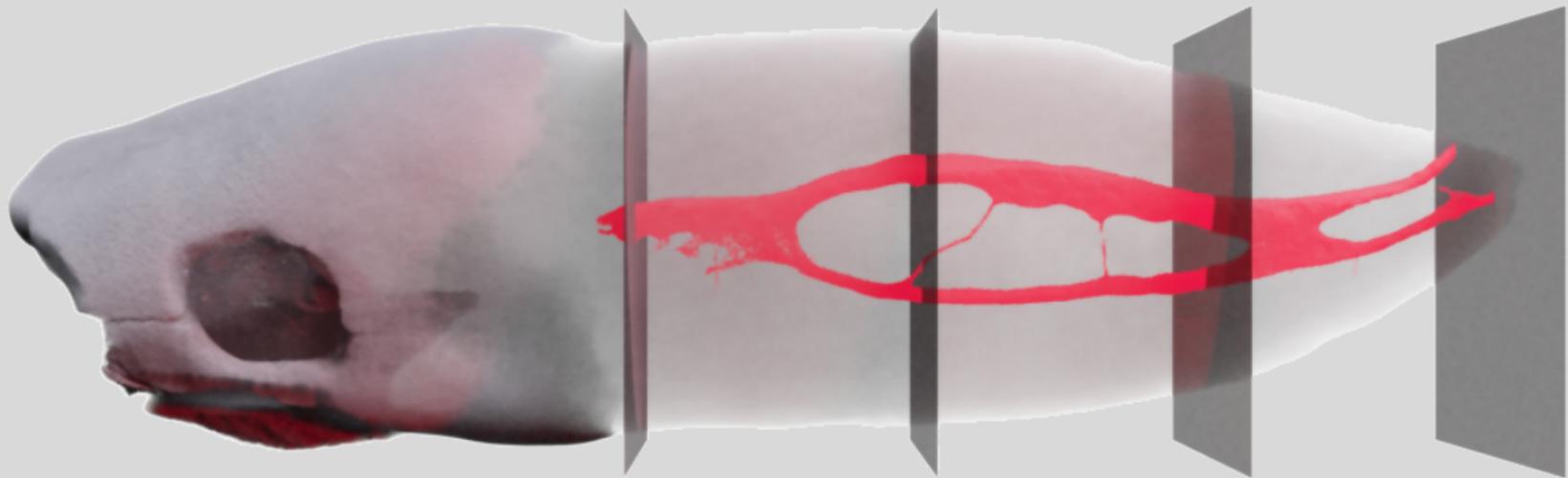
Tooth045



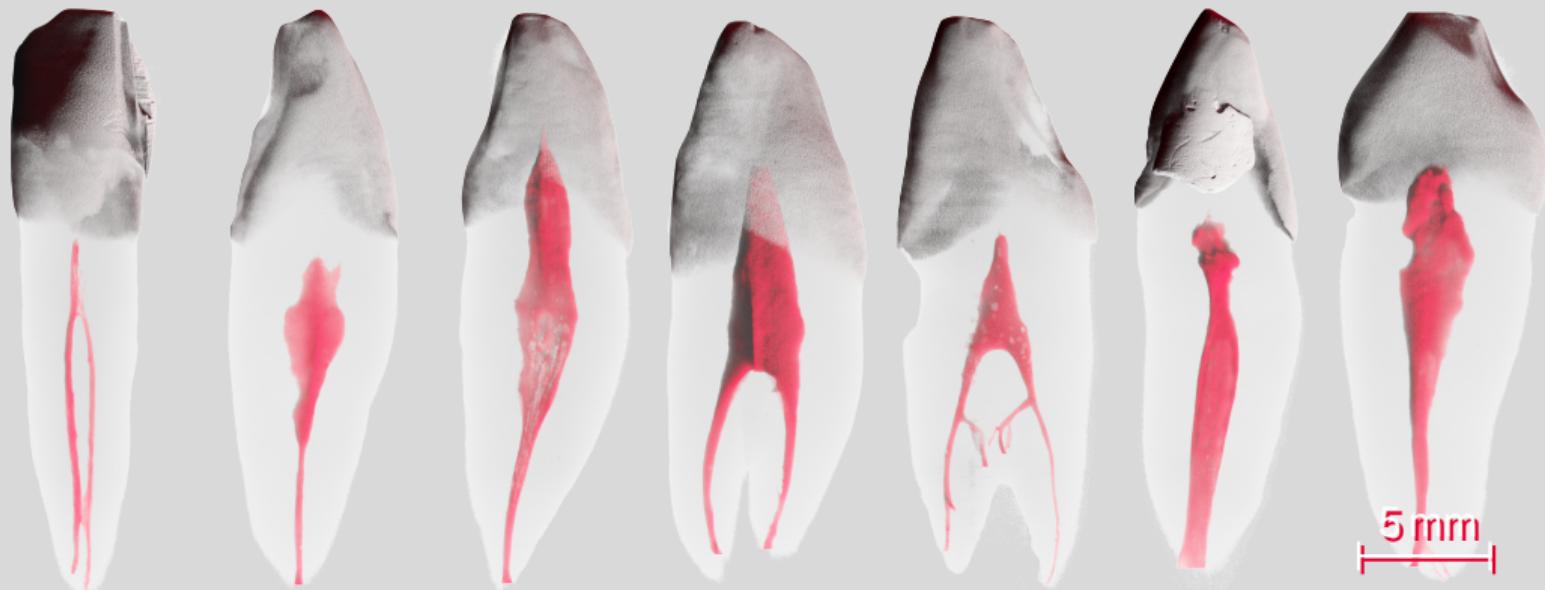
Outcome root canal configuration classification

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
		1-2-1/1	1
	Lingual	1-1-1/1	2
		1-1-1/2	1

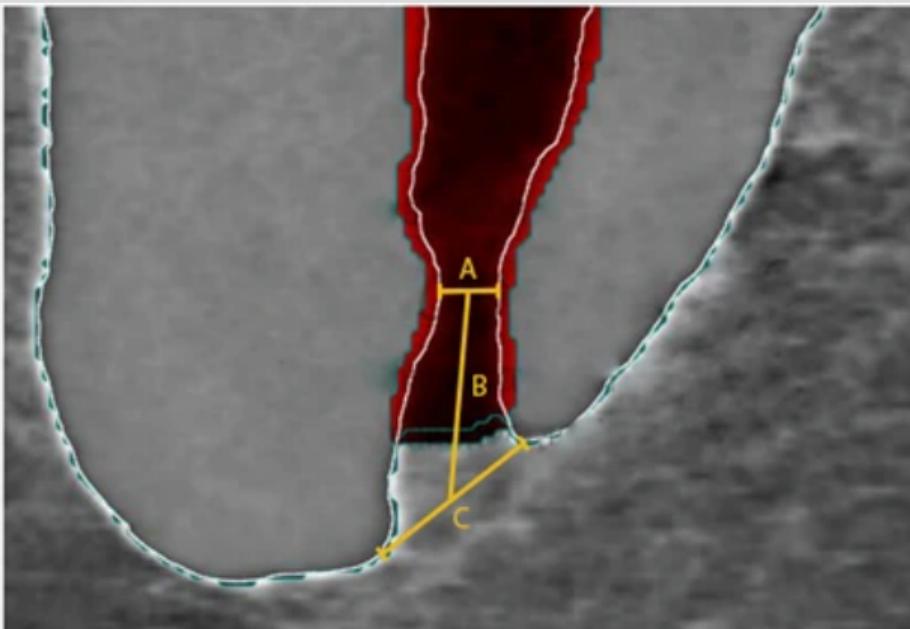
Extraction of root canal space



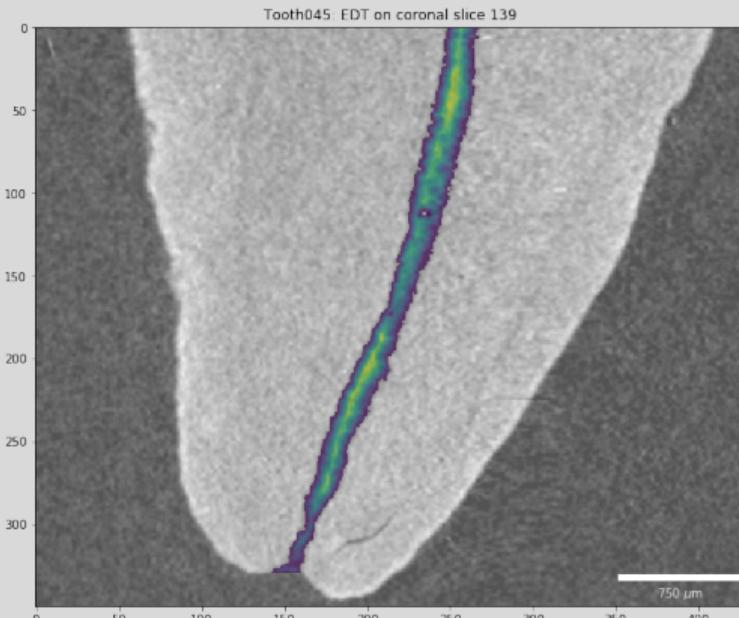
Results of root canal space extraction



Analysis of the physiological foramen geometry



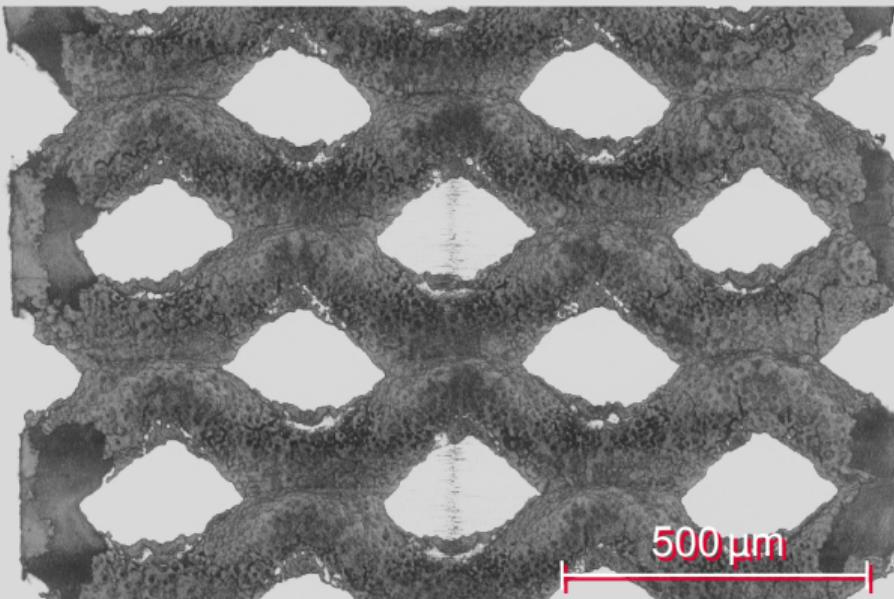
Analysis of the physiological foramen geometry



Conclusion ZMK

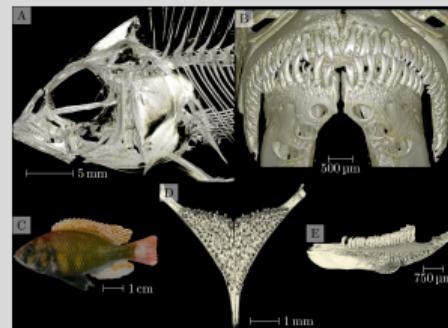
- Efficient use of time, e. g. more teeth does not mean more (human) work
- Reproducible analysis with *free and open-source* software, usable by *anyone*
- Objective analysis, e. g. no operator bias

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])



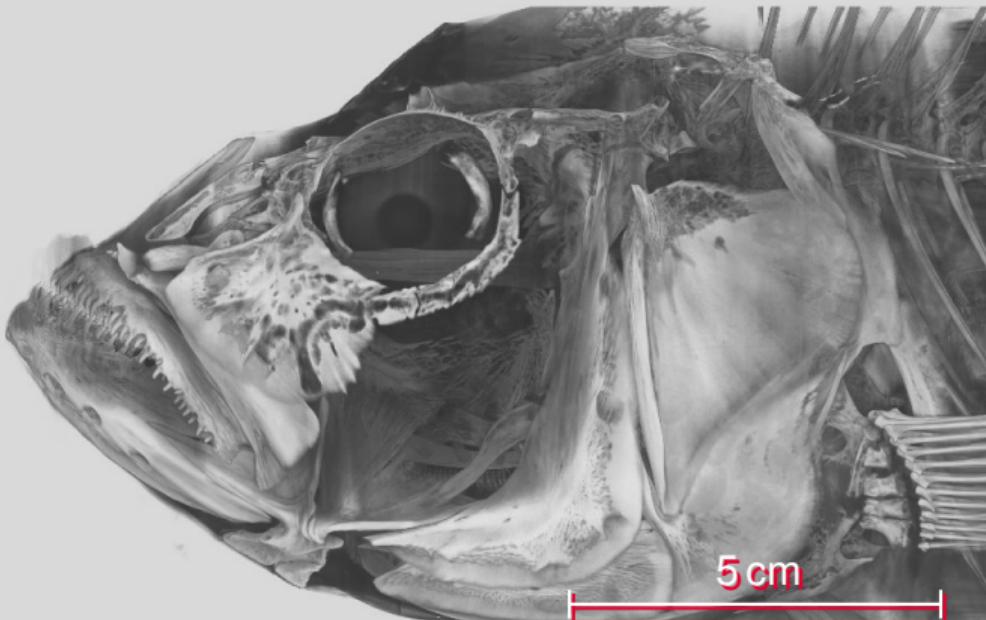
[a] Haberthür, David et al. DOI: 10.1101/2023.03.30.534917.

Cichlids

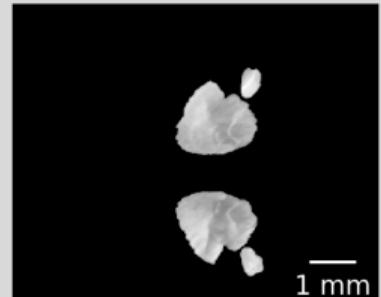
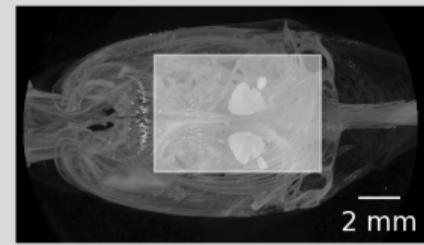
- Institute of Ecology and Evolution
 - Ole Seehausen
 - Marcel Häsler
 - Mikki Law
 - Kassandra Ford
- 150 Lake Victoria fishes (+300 tomographic scans)
- Morphological description of oral and pharyngeal jaws
- PCA of skull structure

```
Scanner=SkyScan2214
Instrument S/N=20C18017
Software Version=1.7
Camera Type=FlatPanel: 2...50um, 140mm max.FOV
Filename Prefix=20um_FP_spiral_test
Number Of Files= 1104
Number Of Rows= 1944
Number Of Columns= 3072
Source Voltage (kV)= 80
Source Current (uA)= 200
Image Pixel Size (um)=30.000008
Exposure (ms)=445
Rotation Step (deg)=0.200
Flat Field Correction=ON
Filter=Al 1mm
Study Date and Time=12 Feb 2021 15h:44m:15s
Estimated Scan duration=0h:50m:56s
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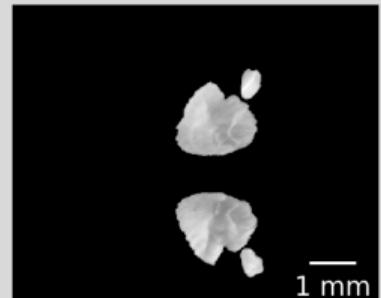
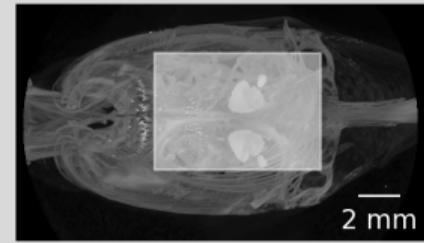
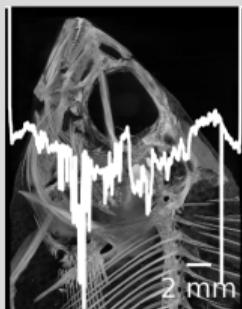
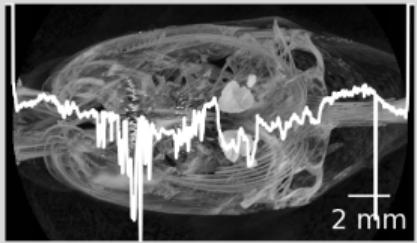
Cichlids



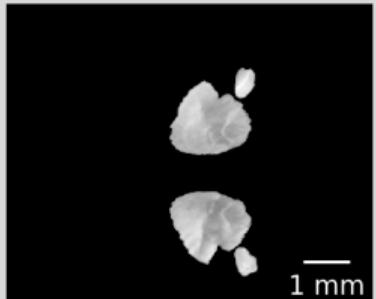
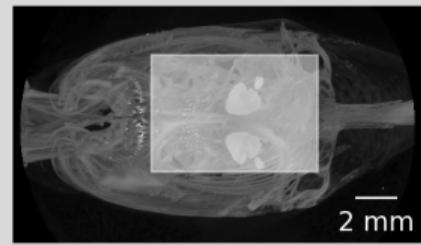
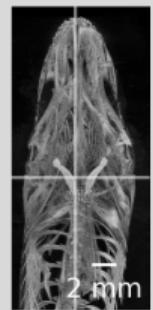
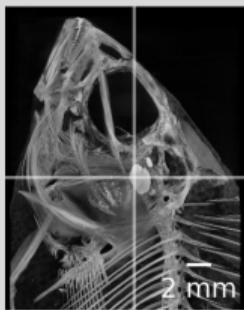
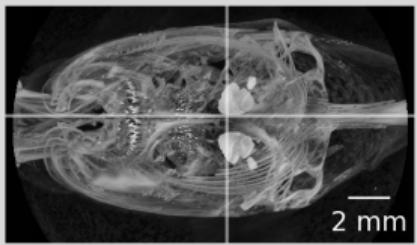
Data wrangling by example: Cichlids



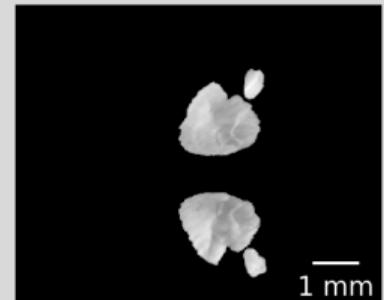
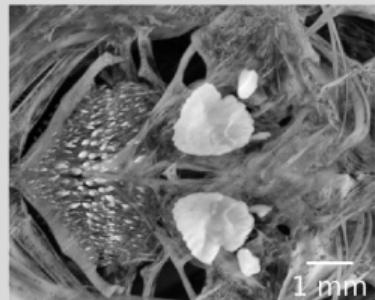
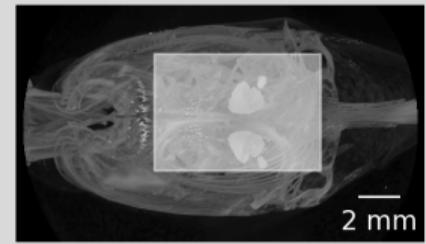
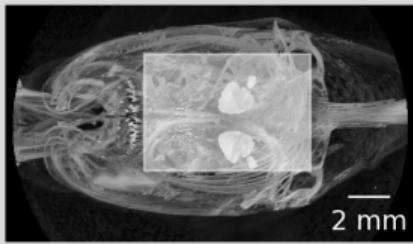
Data wrangling by example: Cichlids



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 \LaTeX with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
 - Complete source code: git.io/fjpP7
 - The \LaTeX 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: haberthuer@ana.unibe.ch

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

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