

u^b

b

**UNIVERSITY
OF BERN**

u^b X-ray microtomography

485018-HS2024-0: Advanced Course II Ultraprecision Engineering

David Haberthür

Institute of Anatomy, September 25, 2024

u^b 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^b

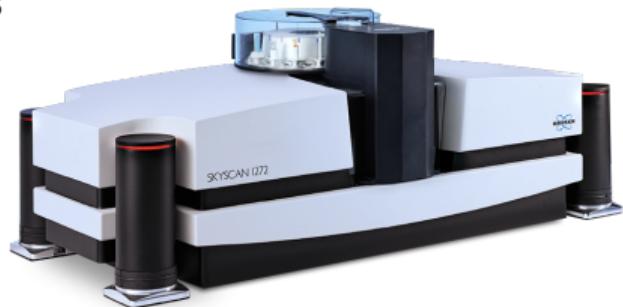
Grüessech from the μ CT group



David.Haberthuer@unibe.ch Ruslan.Hlushchuk@unibe.ch Oleksiy.Khoma@unibe.ch

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]



bruker.com/skyscan1272

u^b

Contents

Overview & Imaging methods

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

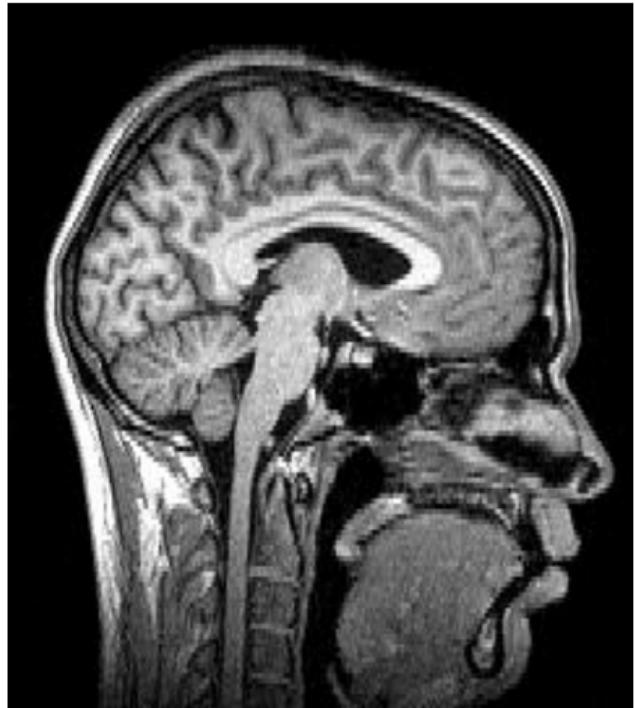
μ CT^b

- 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

u^b

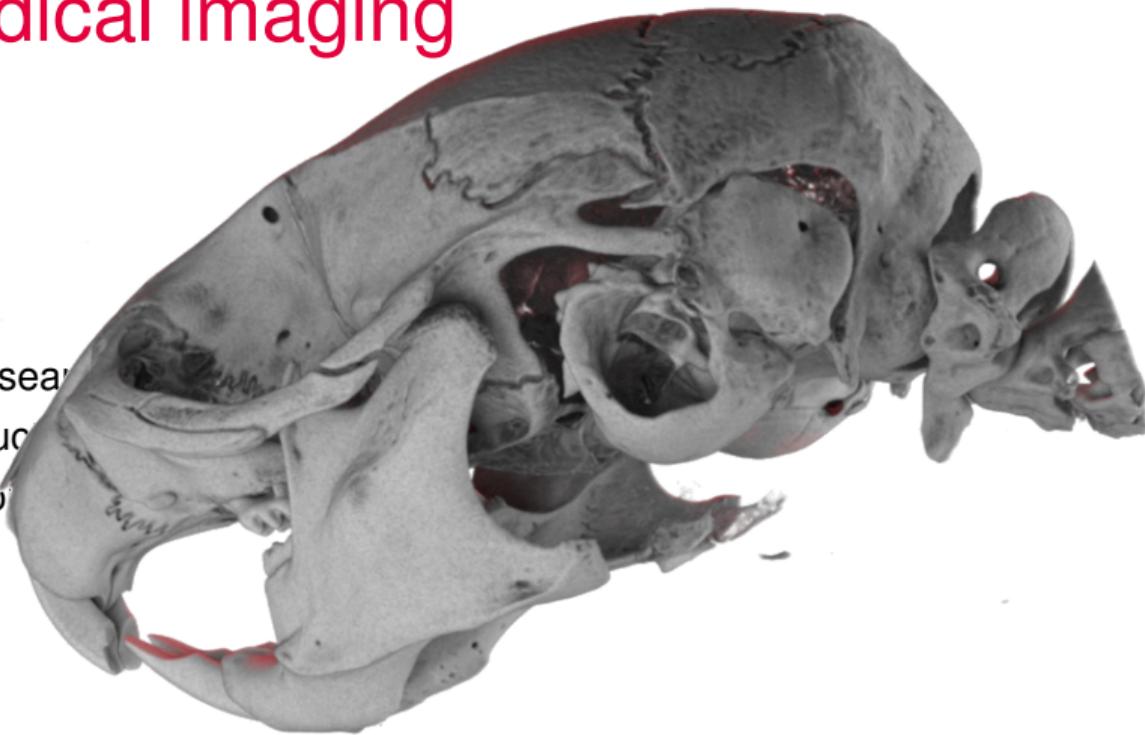
Biomedical imaging

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



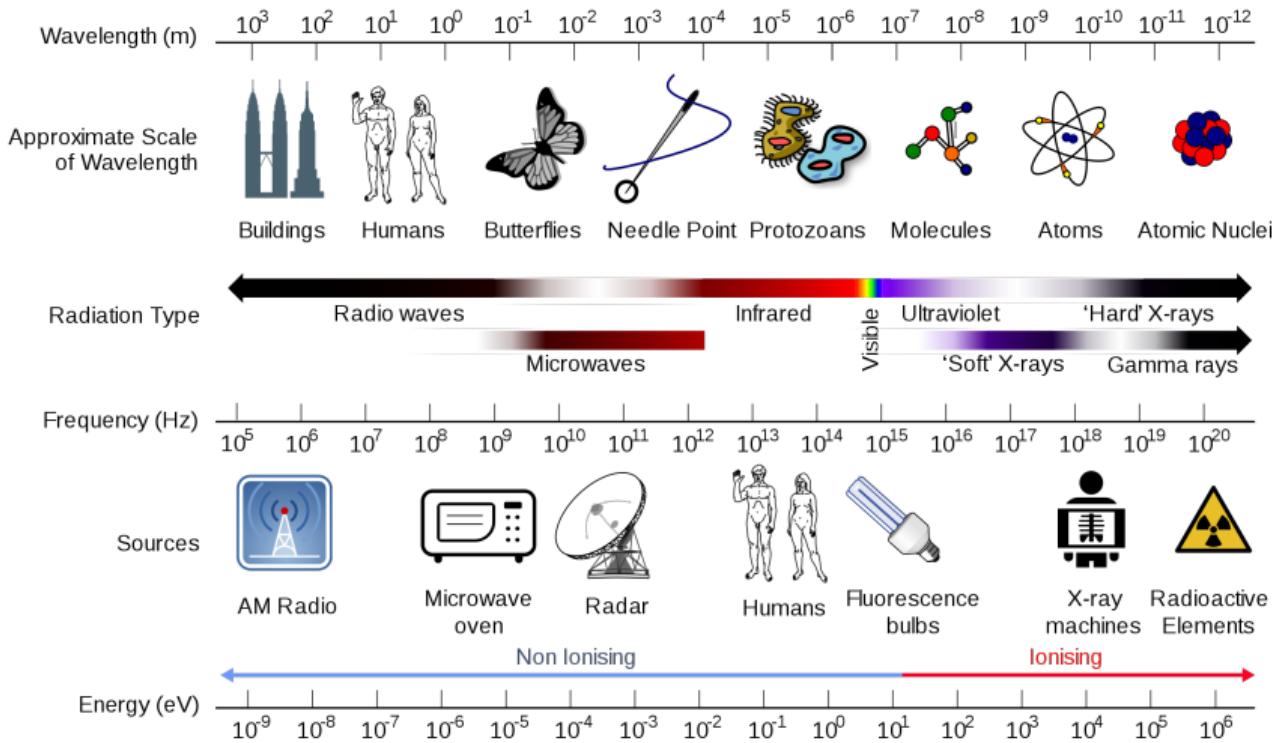
w.wiki/7g4

Biomedical imaging



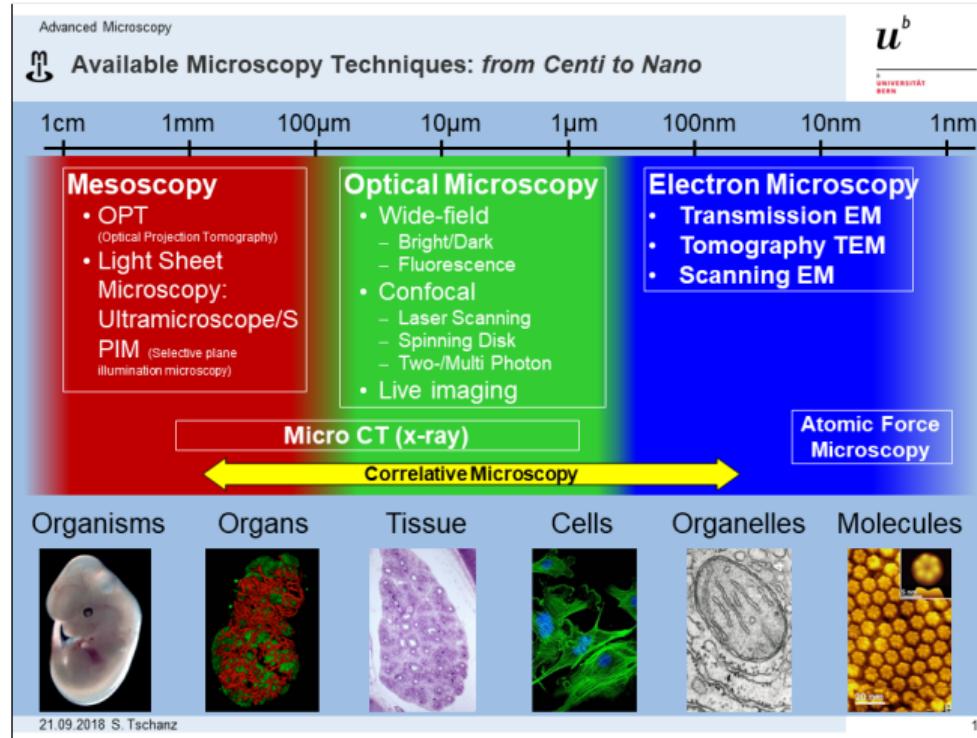
- Medical research
- Non-destructive
- (Small) Biology

u^b Wavelengths & Scales



w.wiki/7fz

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

u^b

CT-Scanner



youtu.be/2CWpZKuy-NE

u^b

CT History

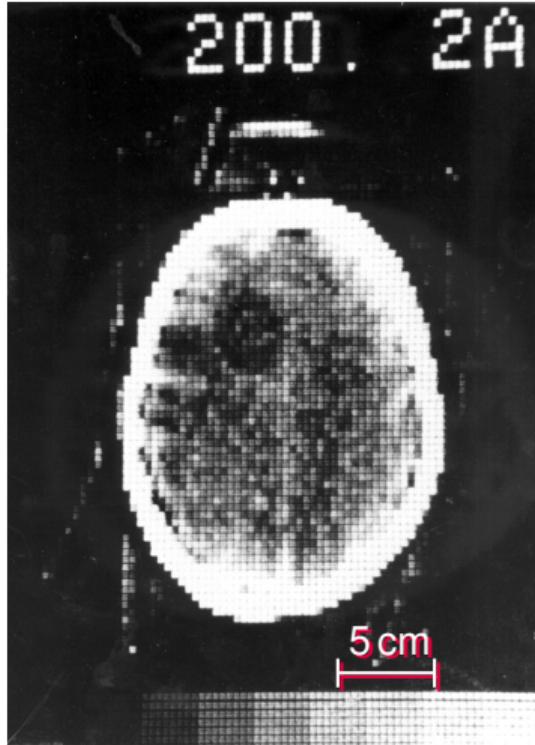
- 1895: Wilhelm Conrad Röntgen discovers X-rays



w.wiki/BHAN

CT History

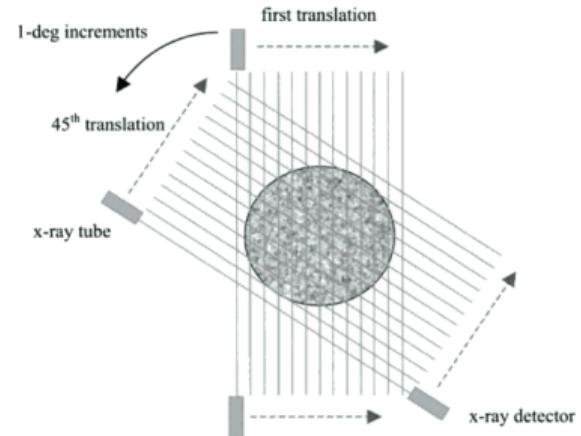
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]



From [14], Figure 5

CT History

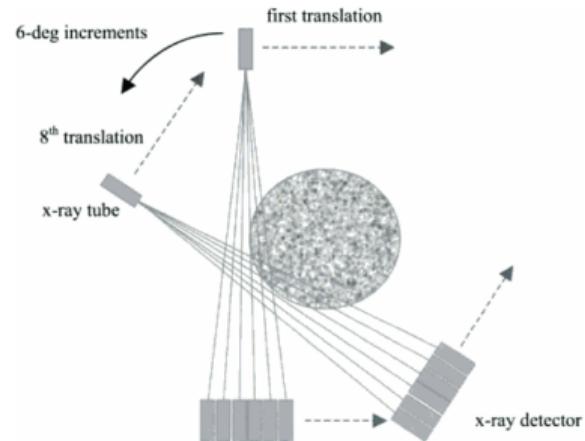
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]
- CT scanner generations
 - First generation



From [15], Figure 1.12

CT History

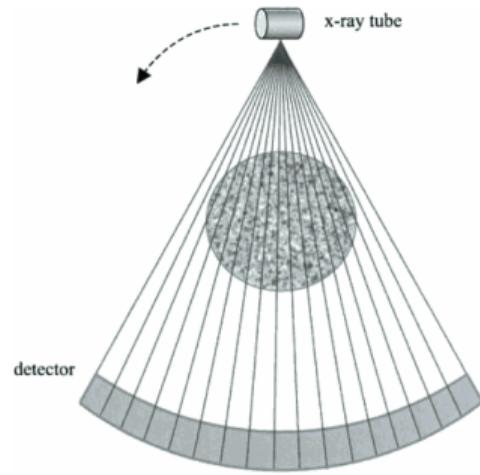
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]
- CT scanner generations
 - First generation
 - Second generation



From [15], Figure 1.13

CT History

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



From [15], Figure 1.14

μ CT History

- 1984: Lee Feldkamp [16] developed an early laboratory microCT system at Ford Motor Company's Scientific Research Laboratory (to nondestructively detect damage in ceramic automobile parts). Then collaborated with scientists at Henry Ford Hospital and University of Michigan interested in understanding bone microstructure [17] and osteoarthritis [18].
- 1987: The first commercially available μ CT scanner was developed by SkyScan (now Bruker)
- Today: μ CT is widely used for non-destructive imaging in
 - 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, μ CT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
 - Paleontology and Archaeology: study of fossils and archaeological artifacts
- \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.” ([19])
 - 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: $\lambda' - \lambda = \frac{\hbar}{m_e c} (1 - \cos \theta)$
- Lowering x-ray energy increases contrast
- X-ray penetration decreases exponentially with sample thickness [20, i. e. Beer-Lambers law]: $I(t) = I_0 e^{-\alpha z}$

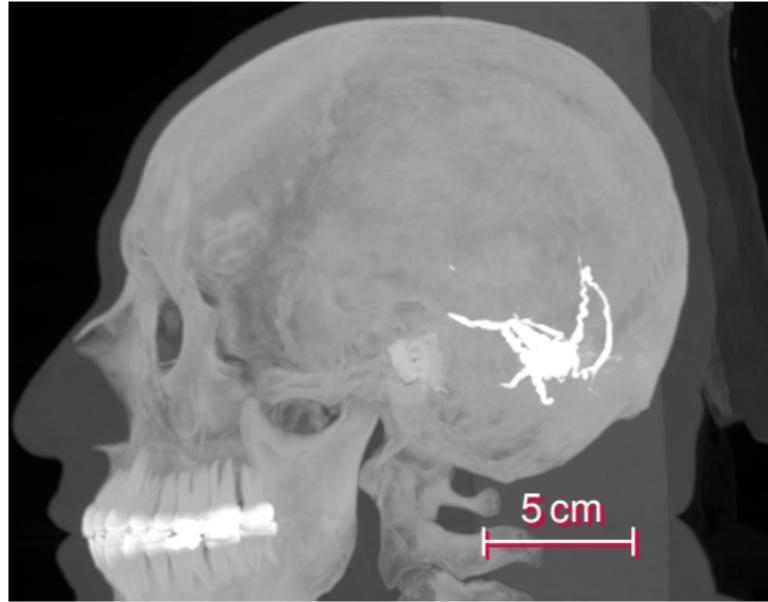
Composition of biological tissues

Tissue: content by mass percentage

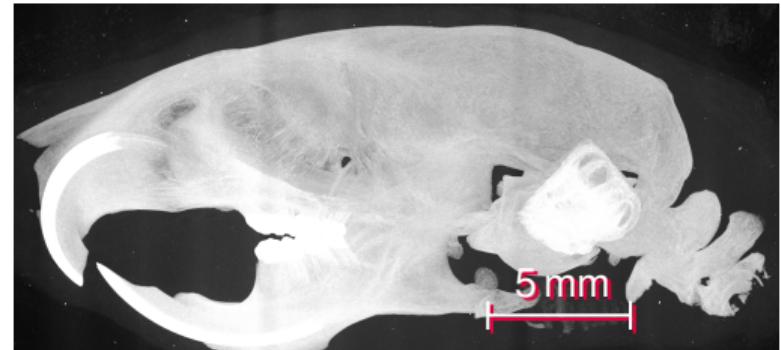
Element Atomic number	H 1	C 6	N 7	O 8	Na 11	P 15	S 16	Cl 17	K 19	Ca 20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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

u^b

Why μ CT?



From [21], Subject C3L-02465



u^b

Why μ CT?

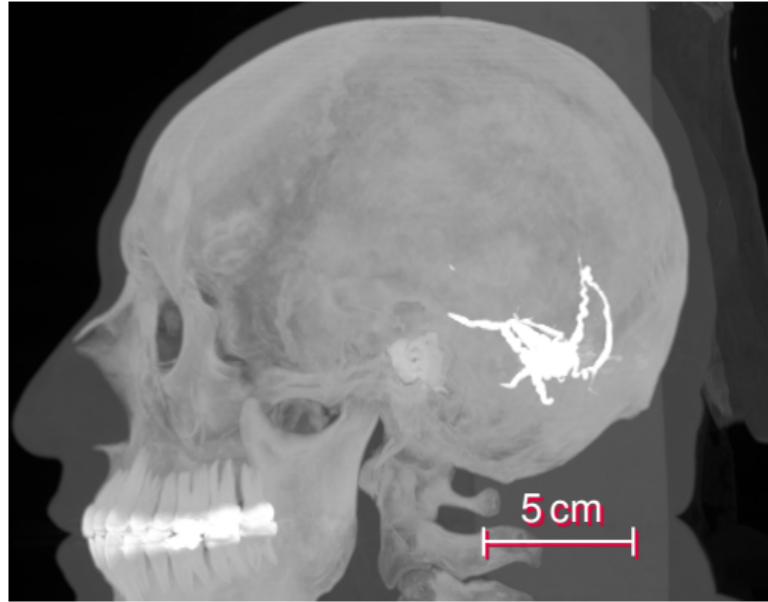


From [21], Subject C3L-02465

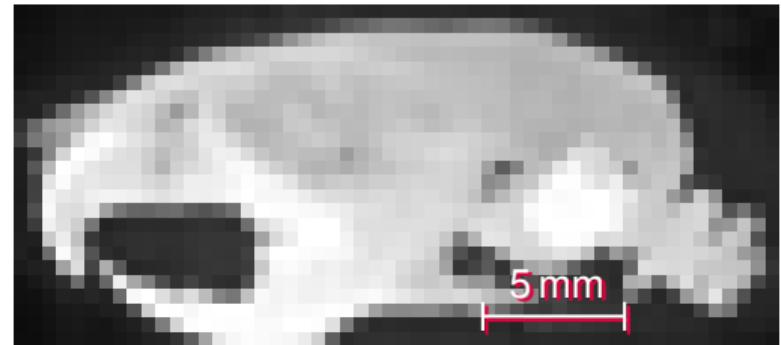


u^b

Why μ CT?

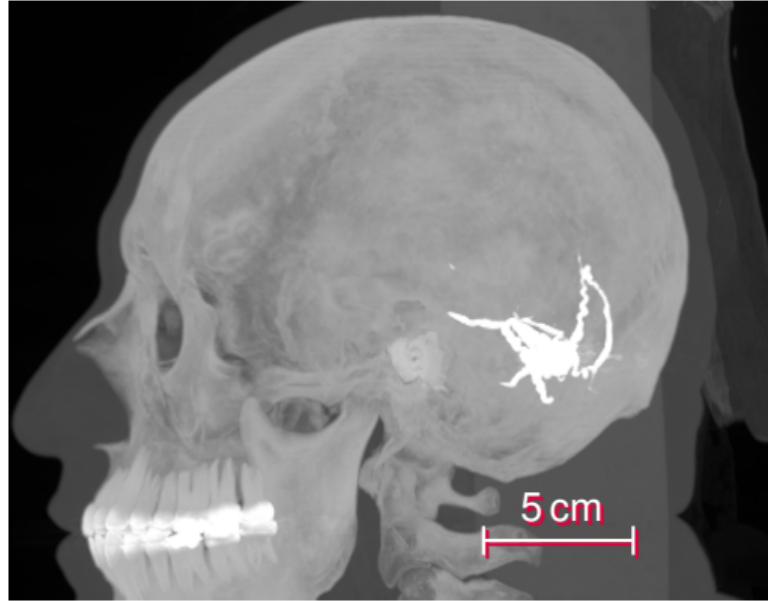


From [21], Subject C3L-02465

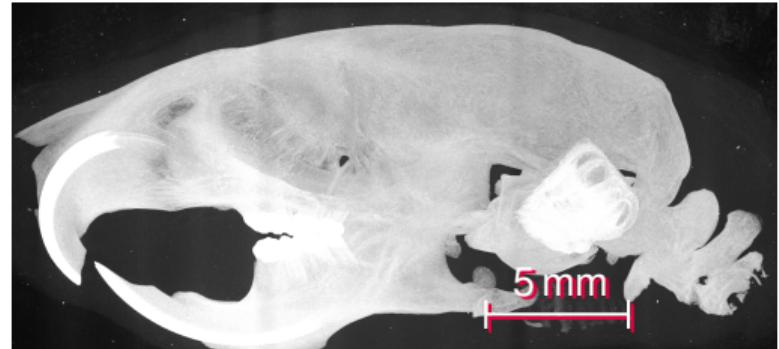


u^b

Why μ CT?

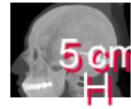


From [21], Subject C3L-02465

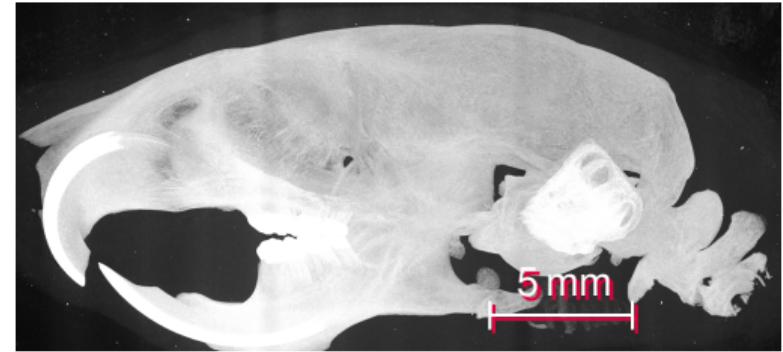


u^b

Why μ CT?



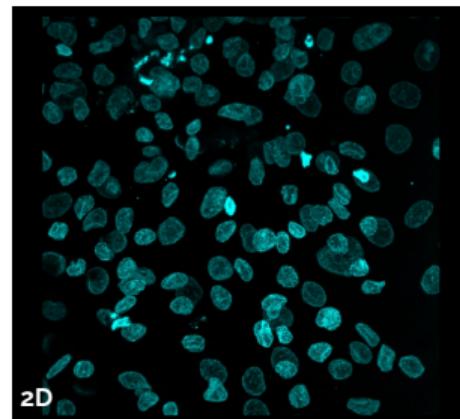
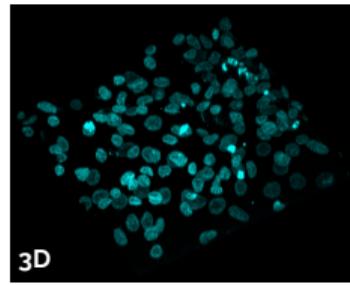
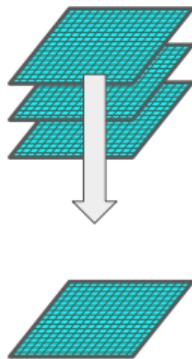
From [21], Subject C3L-02465



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



flic.kr/p/D4rbom

Machinery

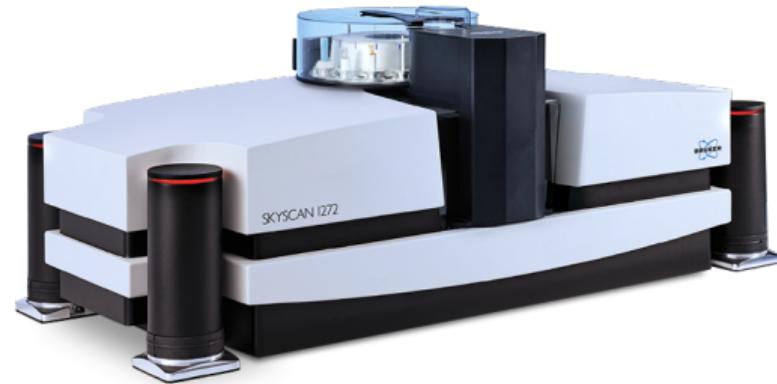
- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around 7 μm (*in vivo*)
 - Voxel size around 0.5 μm (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



flic.kr/p/fpTrGu @@

Machinery

- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around 7 μm (*in vivo*)
 - Voxel size around 0.5 μm (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



bruker.com/skyscan1272

Machinery

- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around 7 μm (*in vivo*)
 - Voxel size around 0.5 μm (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



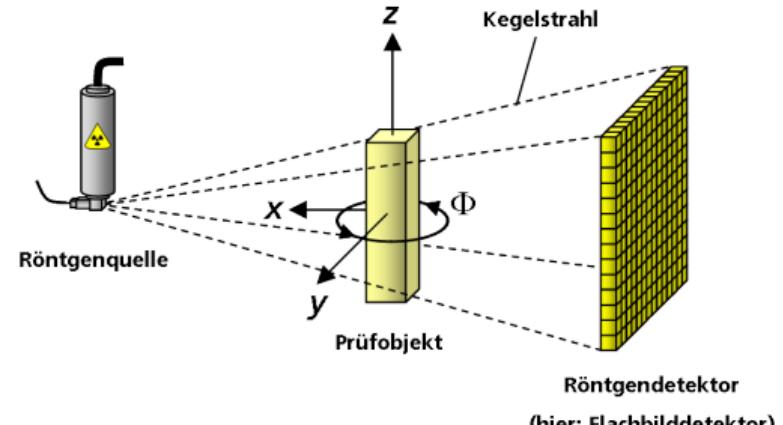
flic.kr/p/7Xhk2Y

u^b

What is happening?

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

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



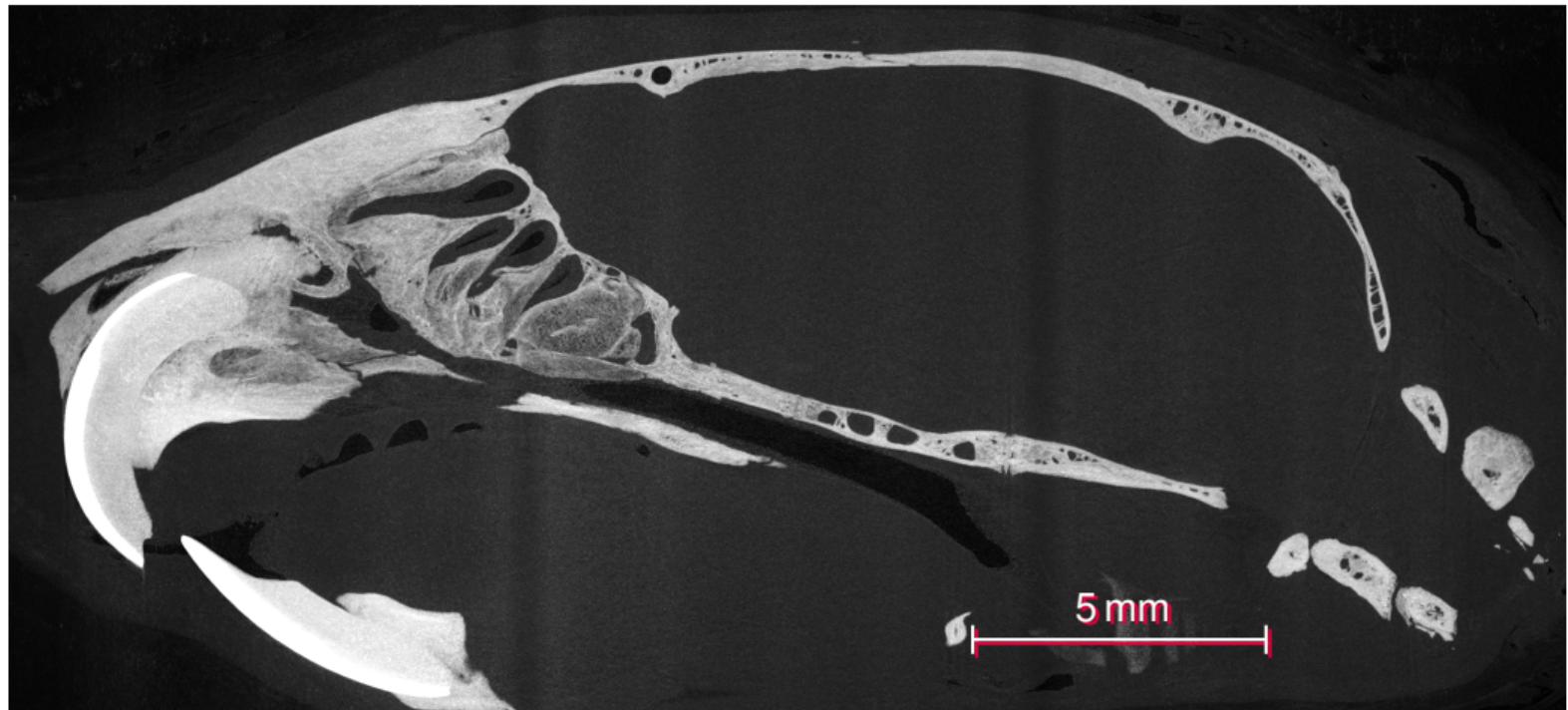
w.wiki/7g3 @①②

u^b

Machinery

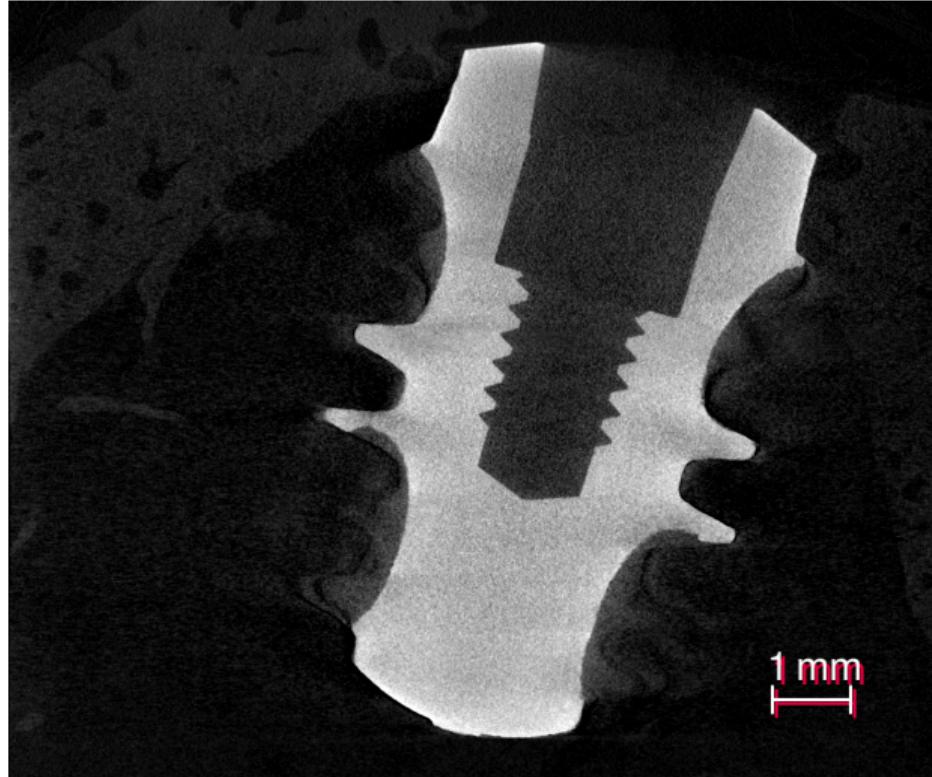
u^b

Examples



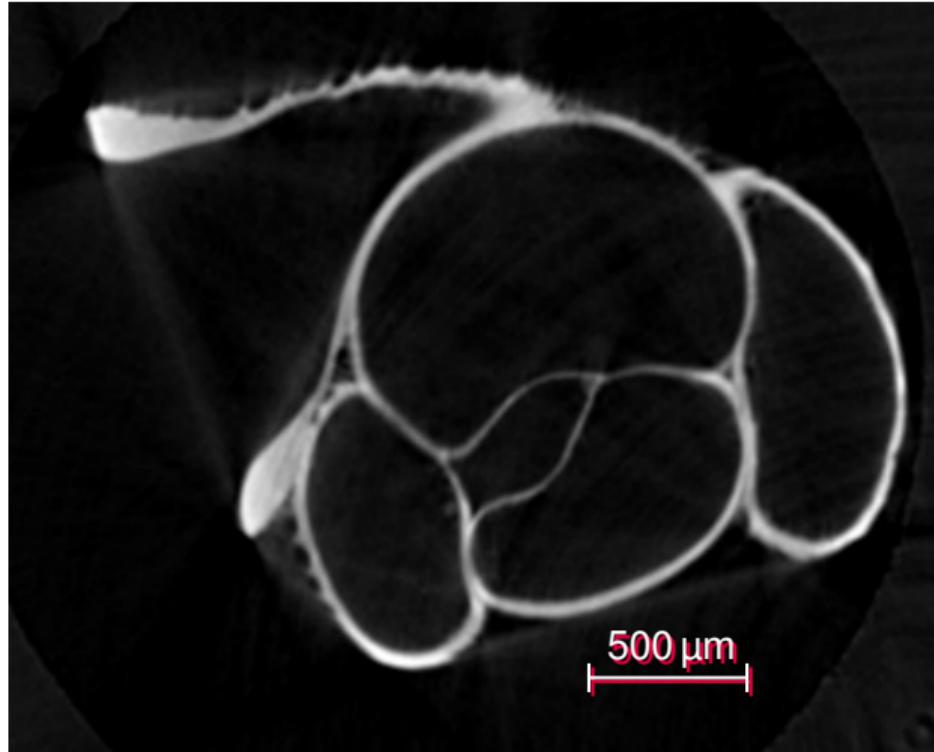
u^b

Examples



u^b

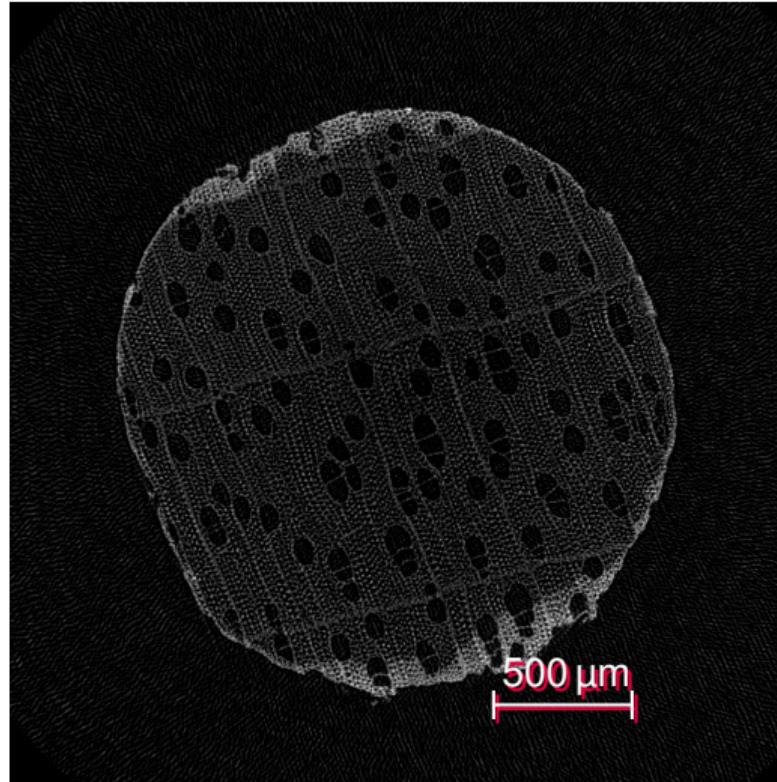
Examples



From [8], *Diancta phoenix*

u^b

Examples



u^b

Examples

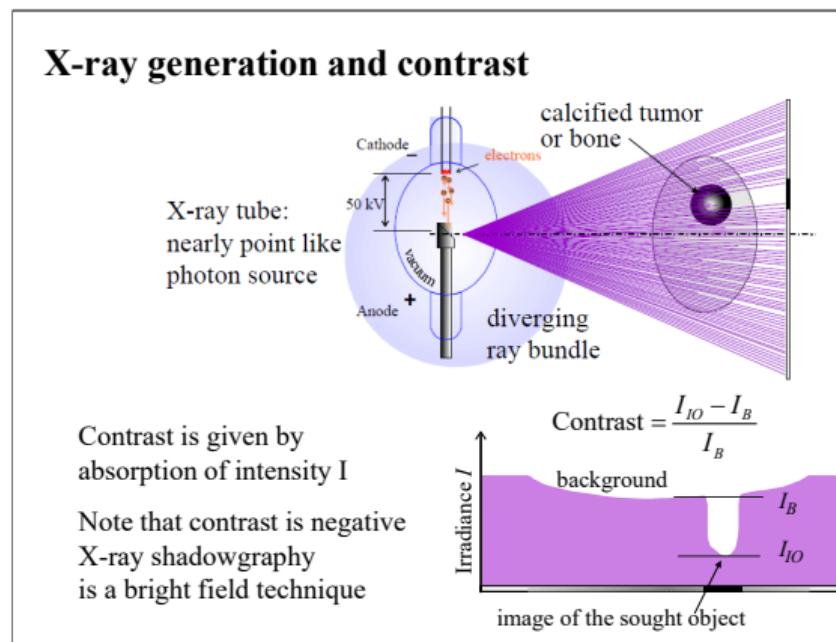


u^b

Preparation

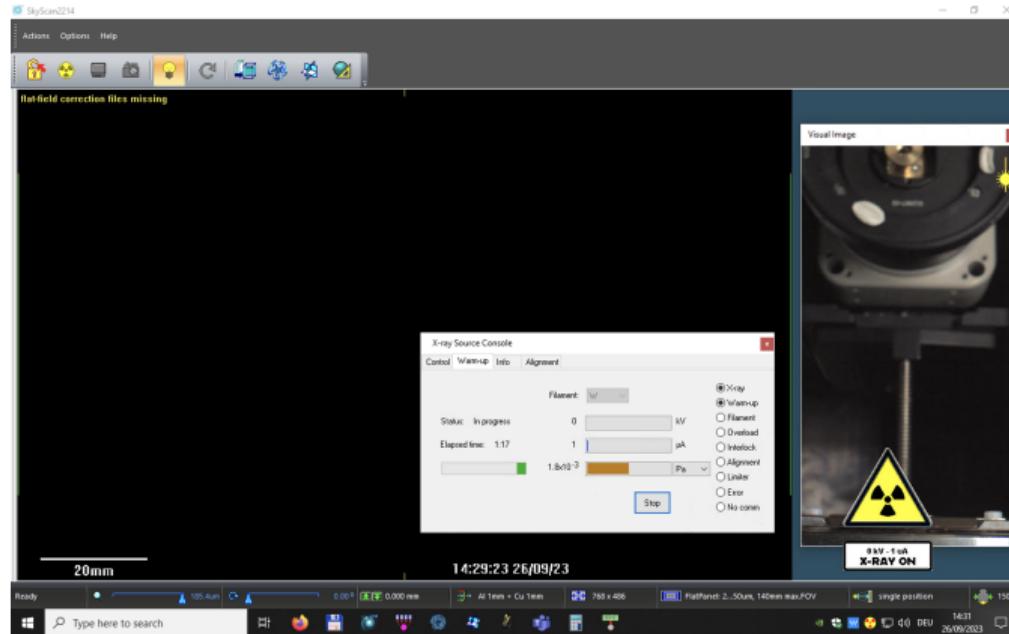
- Study design
- Sample preparation

Projections



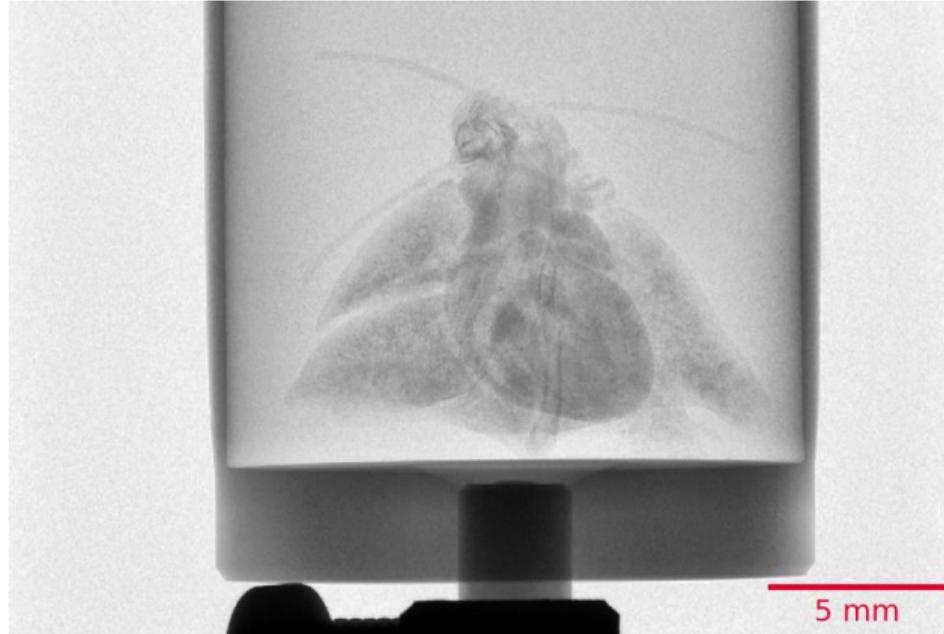
Contrast, Magnification and Resolution—Laws of Physics for Microscopists (1, 2022) by Martin Frenz, Slide 21

u^b Projection acquisition



u^b

Projections

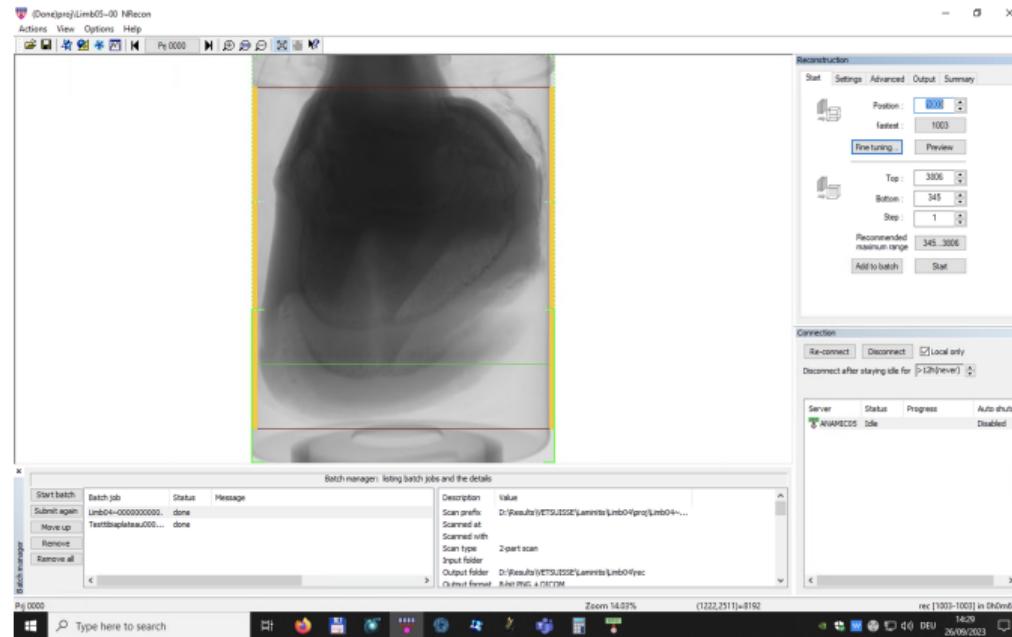


Projections

- 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

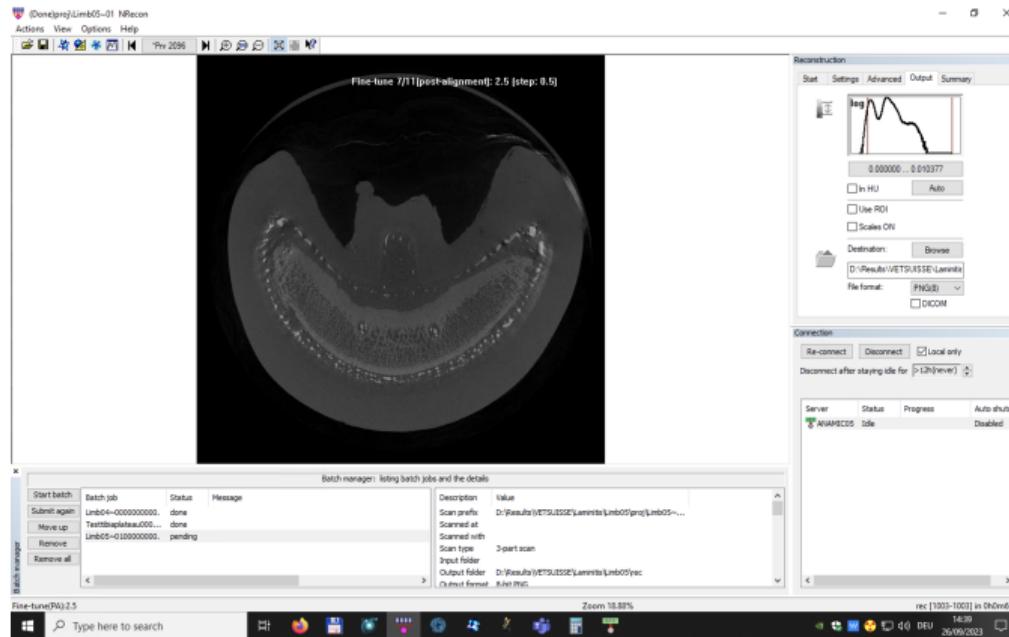
u^b

Reconstructions



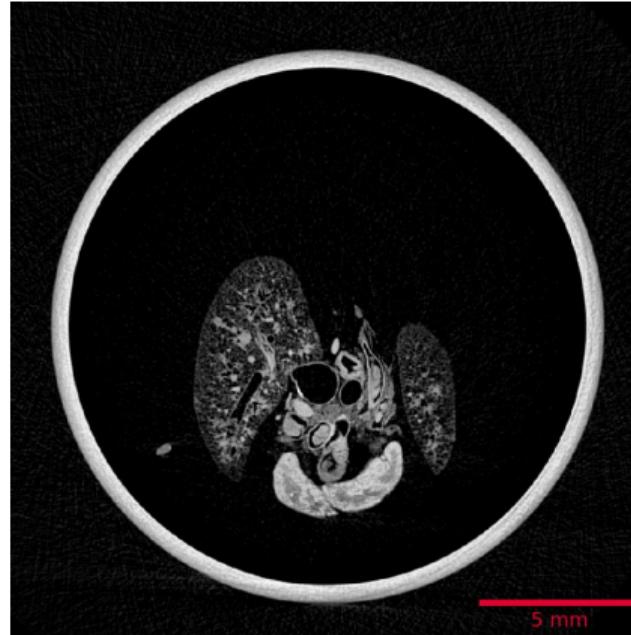
u^b

Reconstructions



u^b

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
- Cone beam reconstruction [16]
- Corrections (beam hardening, etc.)
- Writing to stack

u^b

Visualization



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

u^b

What to use?

- ImageJ/Fiji [22]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
 -  in Jupyter [23]
 - **git**
 - Script all your things!
 - Data repositories; i. e. sharing is caring!

Quantitative data

- Pretty images are nice to have, but science is quantitative data
- Segmentation
- Characterization

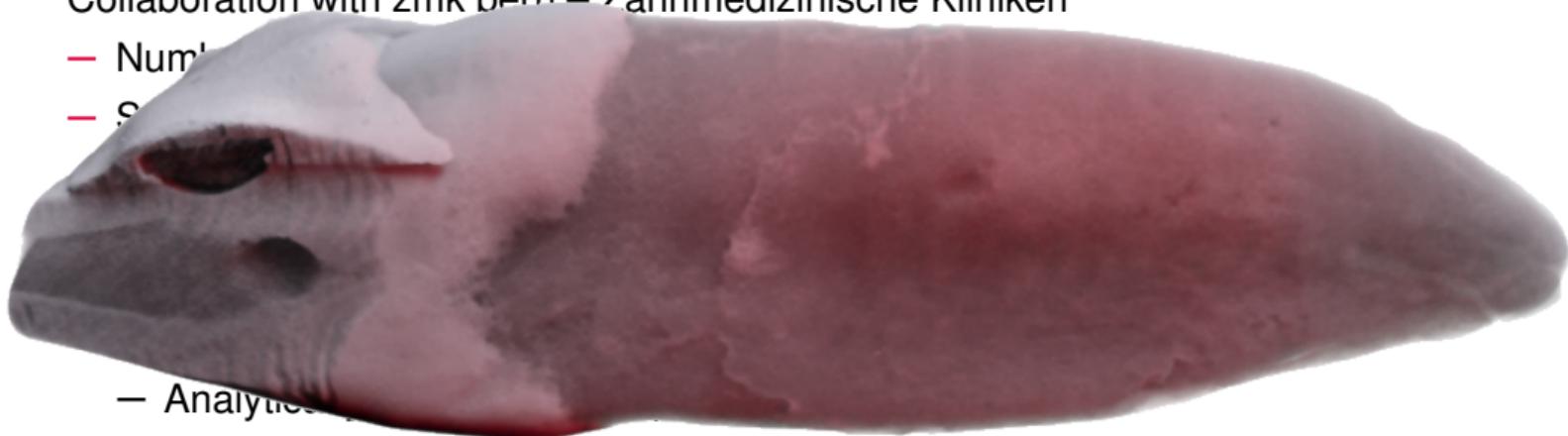
u^b

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number of teeth
- Structure

- Analytics



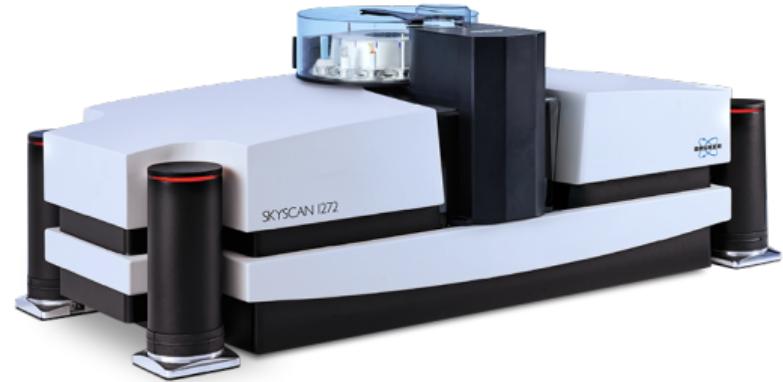
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!



How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!



bruker.com/skyscan1272

- 104 extracted human permanent mandibular canines
- µCT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!

```
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
```

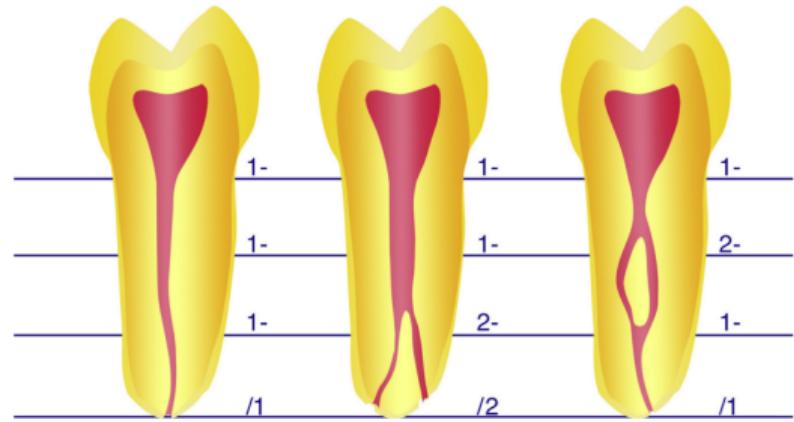
- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!

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

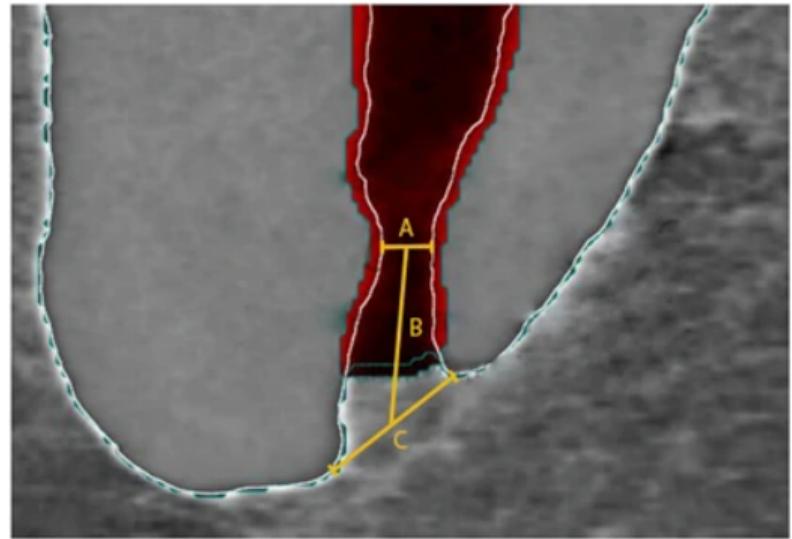
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!



How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!



From [27], Fig. 1

How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e. g. you can click a button to double-check or recalculate the results yourself!



gph.is/2nqkple

u^b

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [25]
- *Reproducible* analysis [26], e.g. you can click a button to double-check or recalculate the results yourself!

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 in the '.github/workflows' directory, each with a commit history:

File	Description	Last Commit
.github/workflows/Update actions file	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 this is a 'README.md' page with the following content:

READEME.md

DOI: <https://doi.org/10.5281/zenodo.3999402> treebeard.yaml failing

[launch Binder](#)

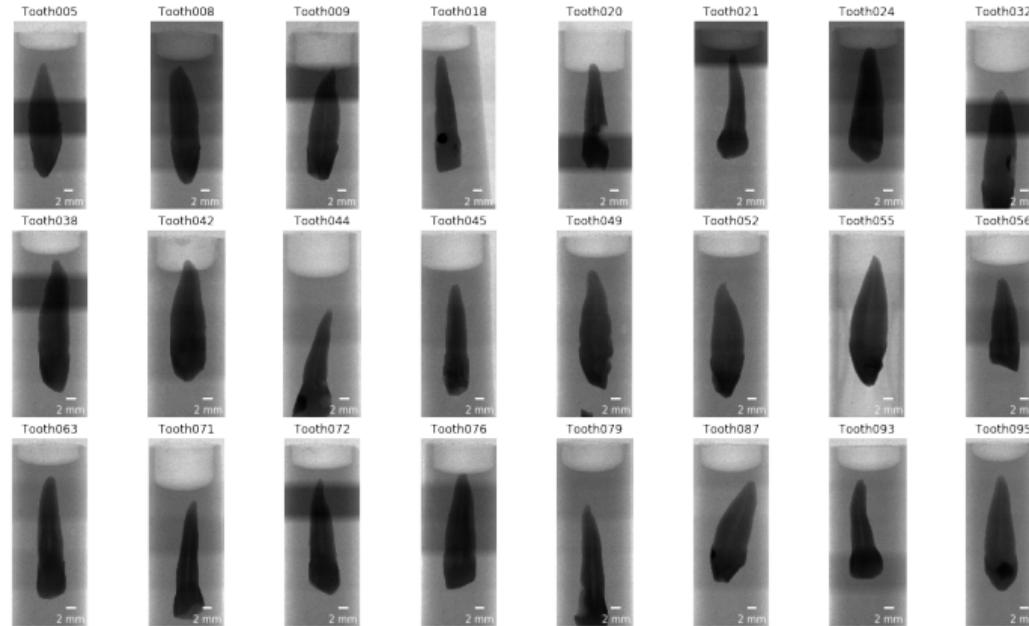
A big tooth cohort

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 b

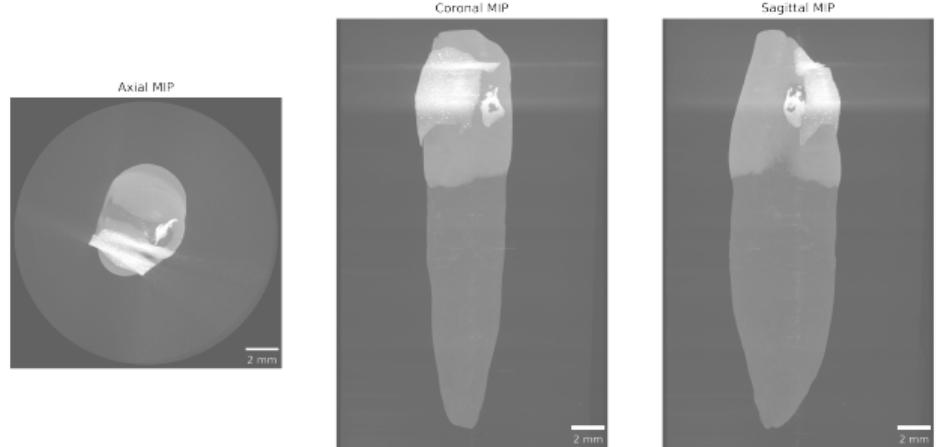
μ CT imaging



u^b

Dataset cropping

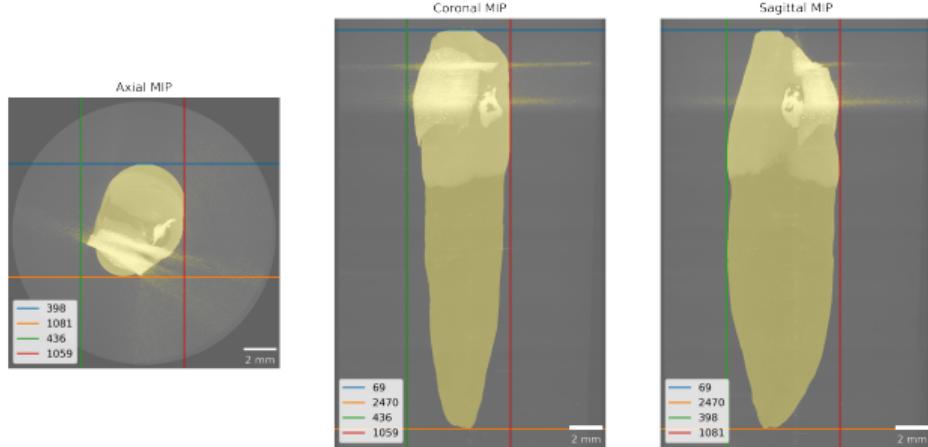
- Full datasets: 326 GB
- Cropped datasets: 115 GB



u^b

Dataset cropping

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



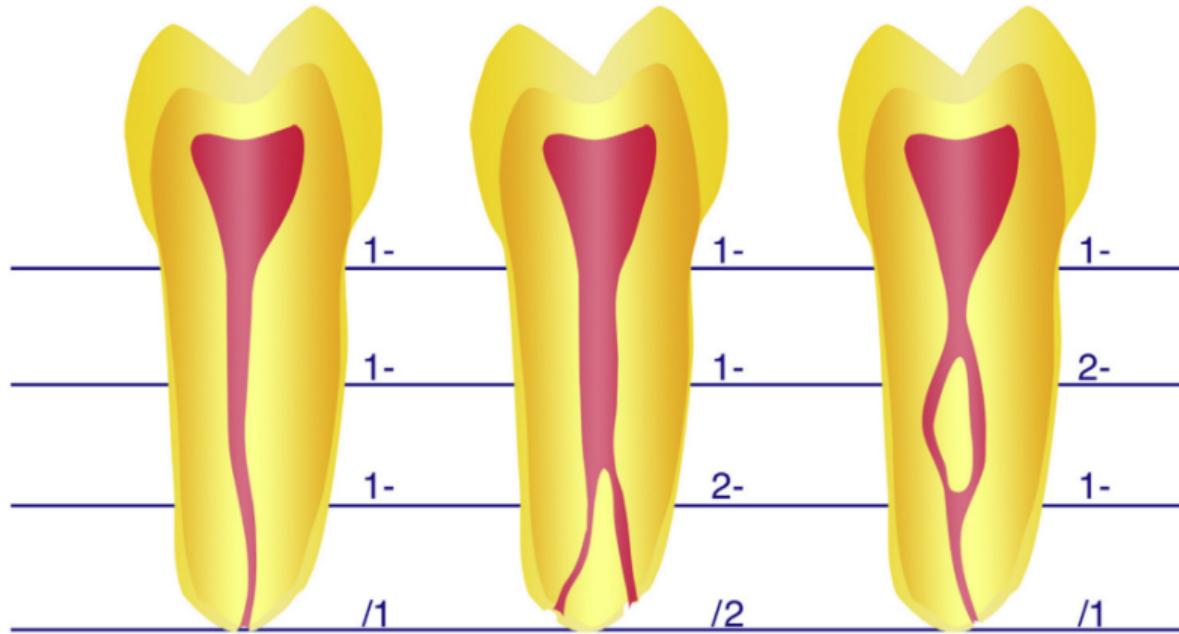
u^b

Tooth morphology



u^b

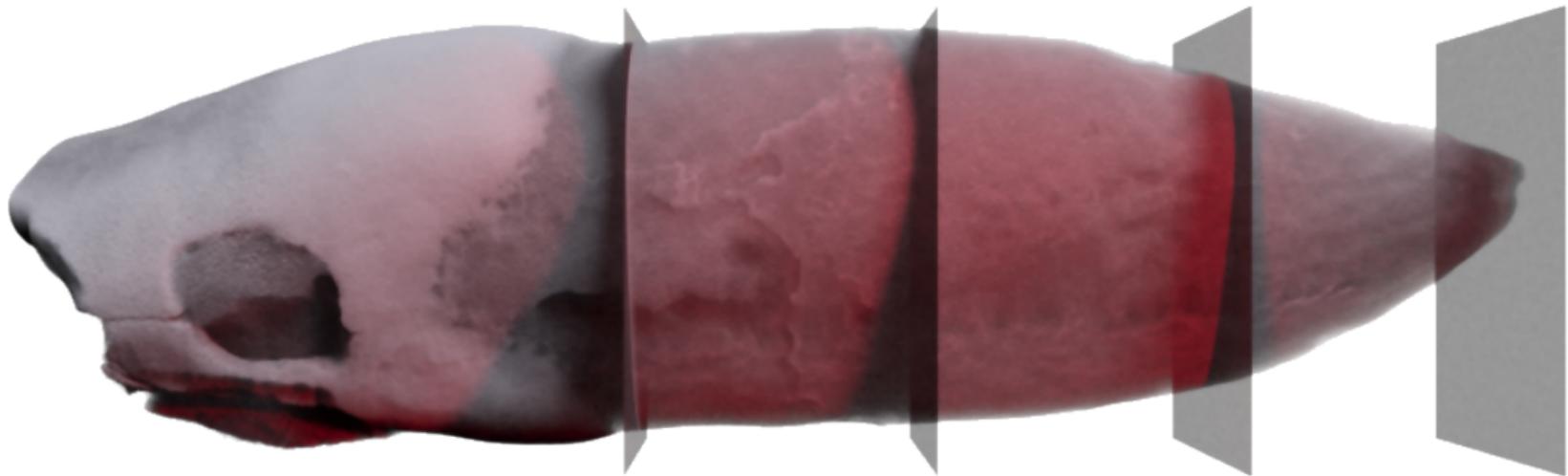
Tooth morphology



From [25], Fig. 2

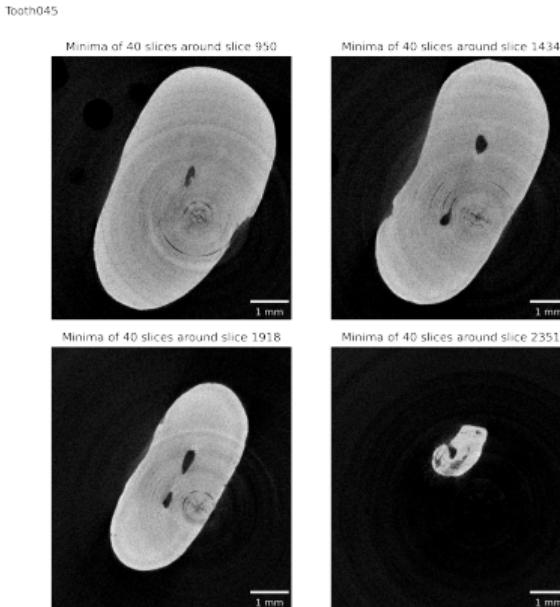
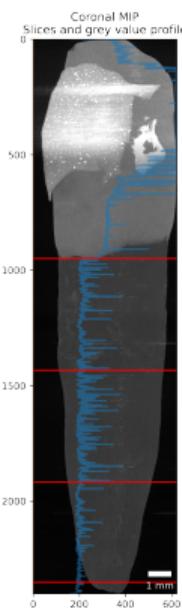
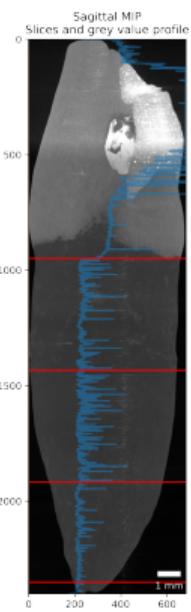
u^b

Tooth morphology



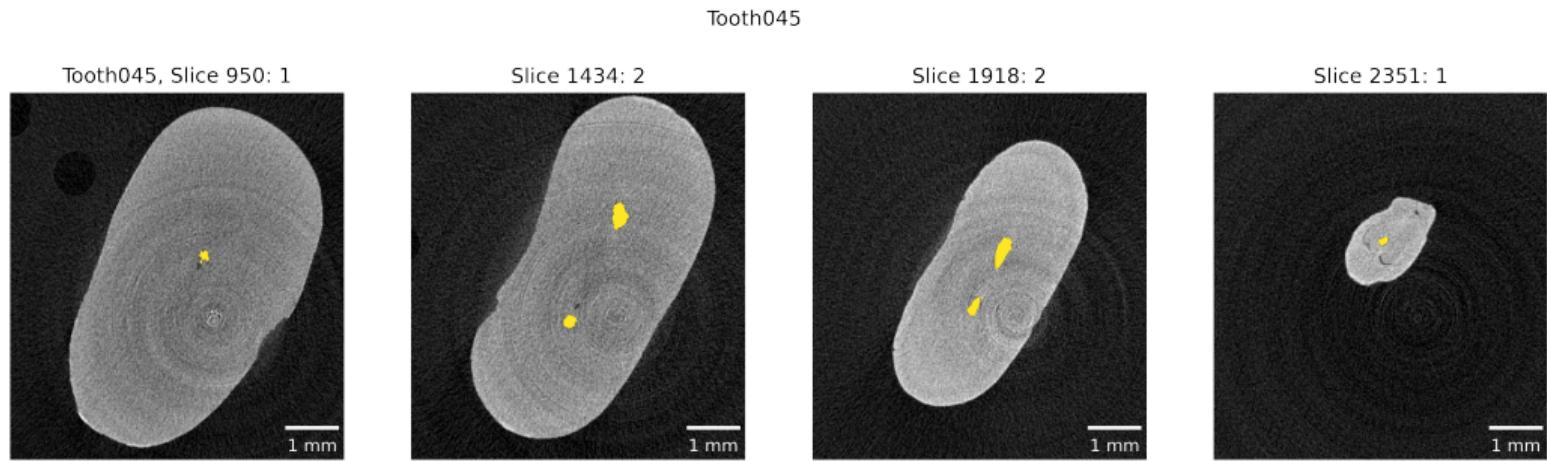
u^b

Detection of enamel-dentin border



u^b

Detection of enamel-dentin border



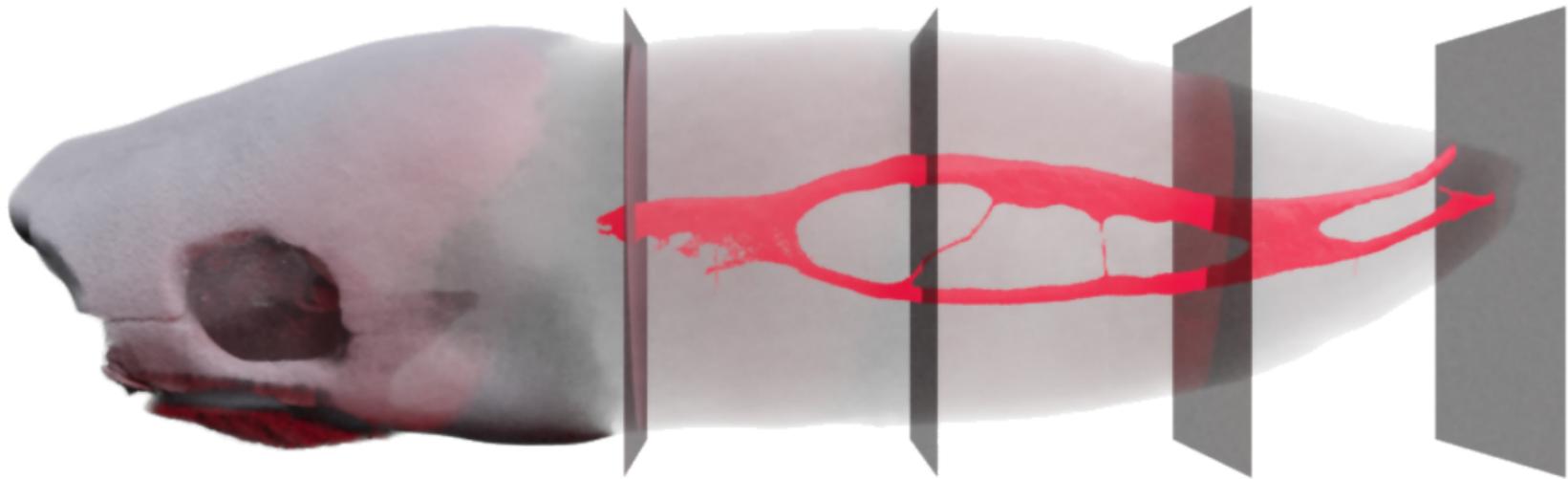
u^b

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

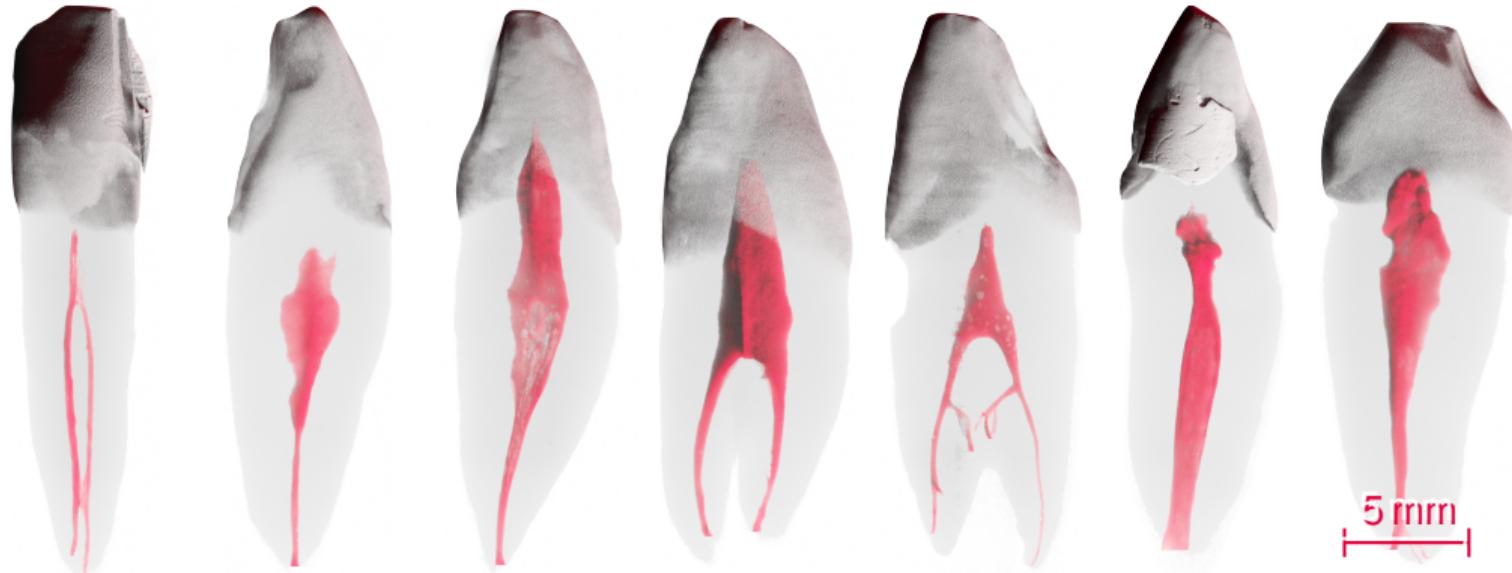
u^b

Extraction of root canal space



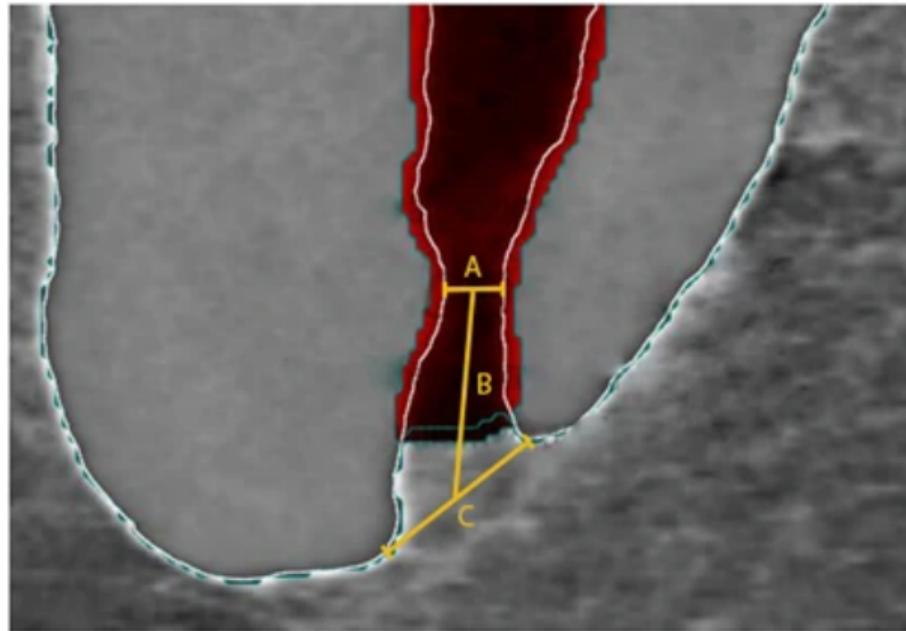
u^b

Results of root canal space extraction



u^b

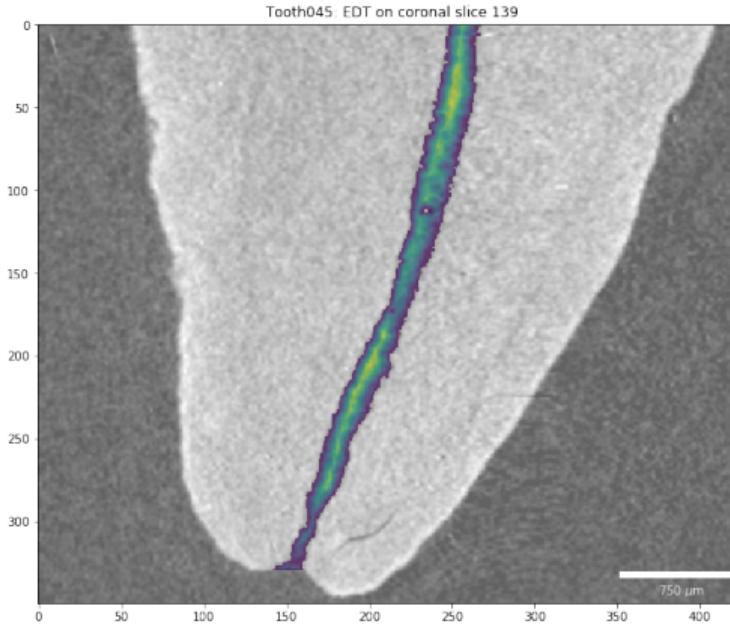
Physiological foramen geometry



From [27], Fig. 1

u^b

Physiological foramen geometry

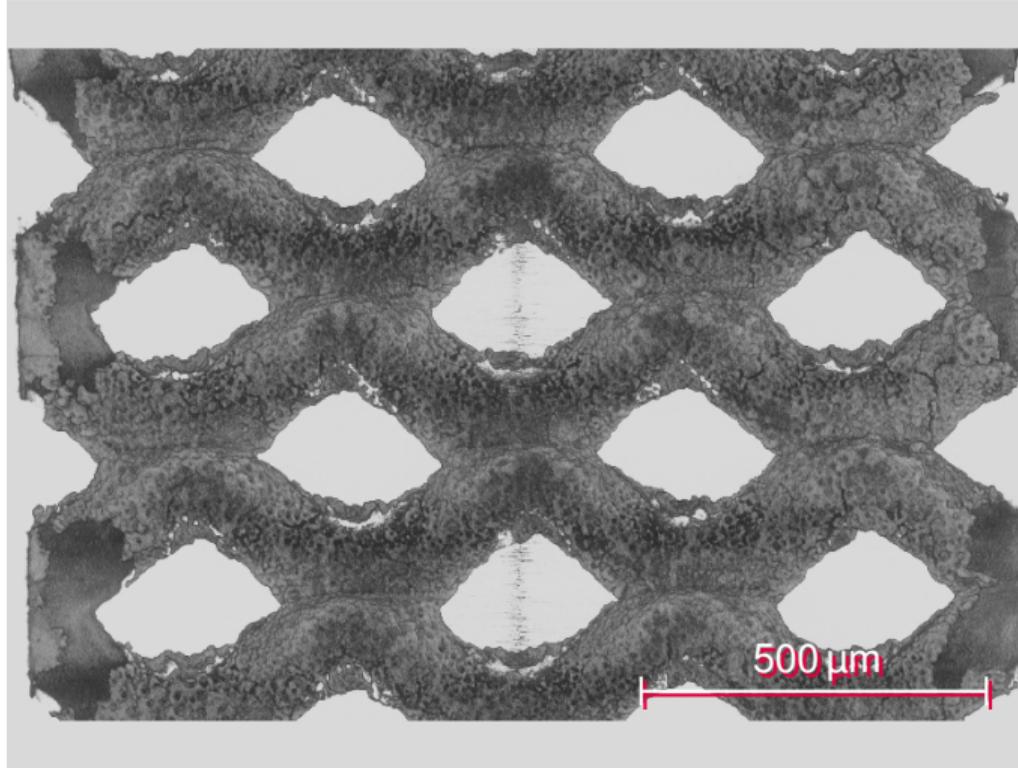


Conclusion ZMK

- 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



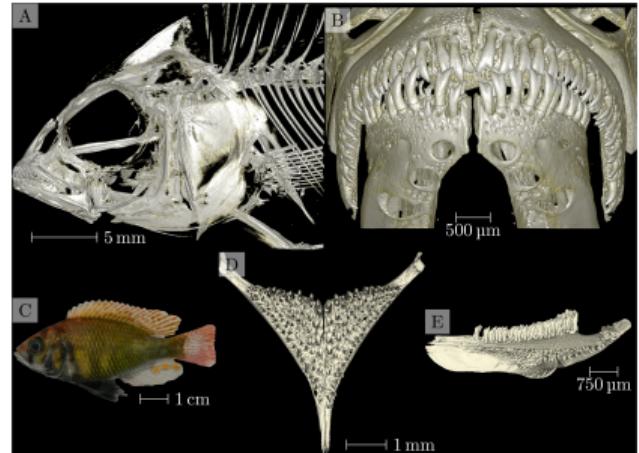
Etienne Berner NanoElectroCatalysis Group

u^b

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

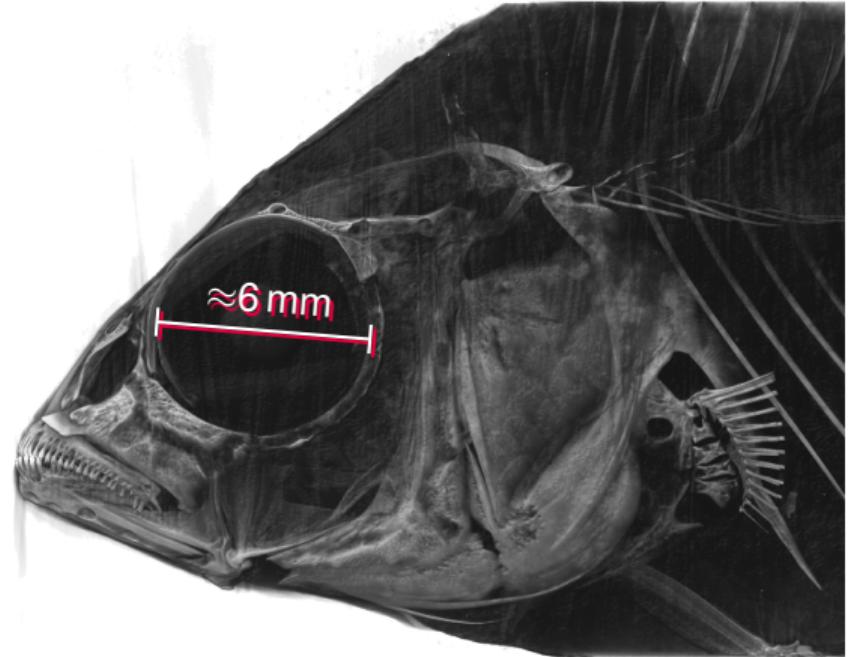


DOI:gsst8t, Fig. 1

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

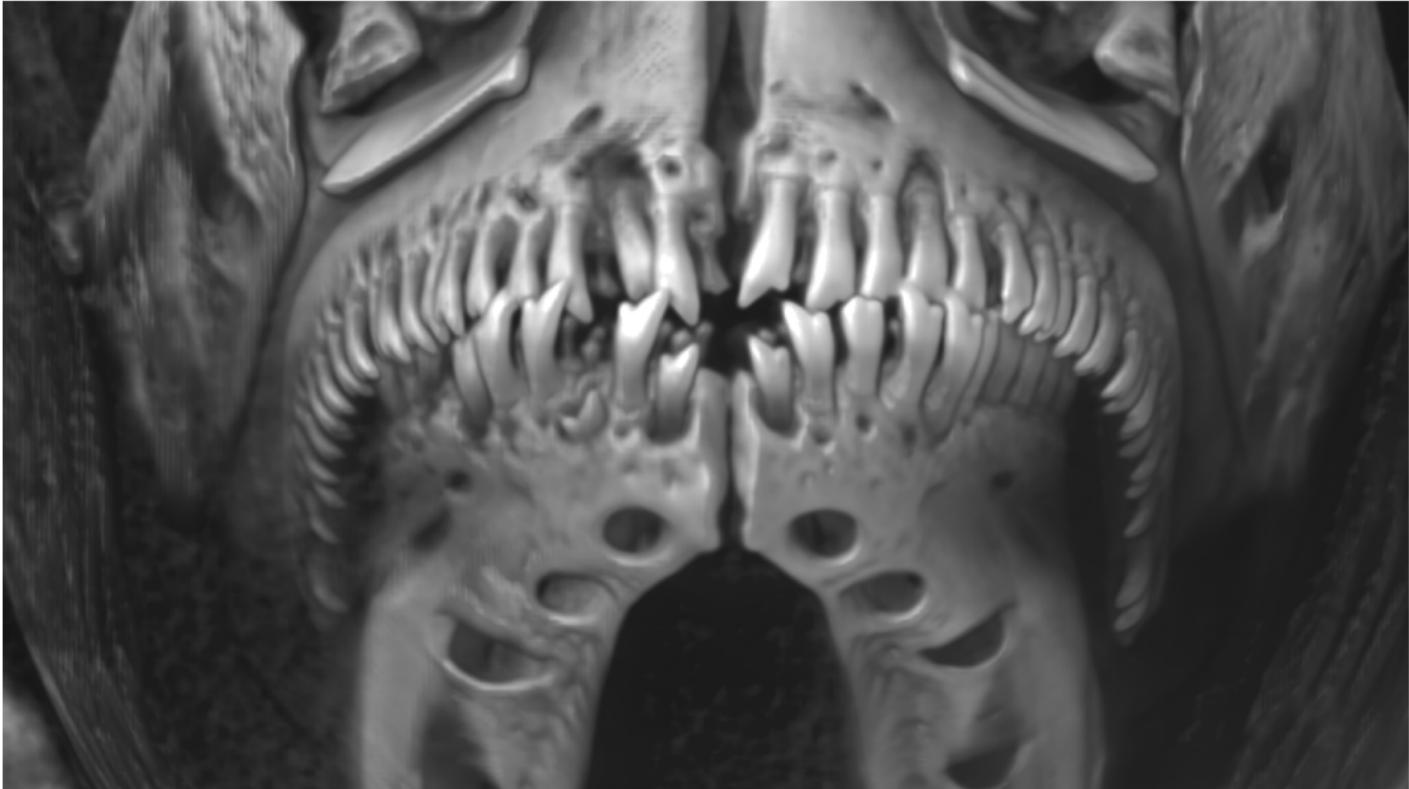
u^b

Visualization of cichlid head



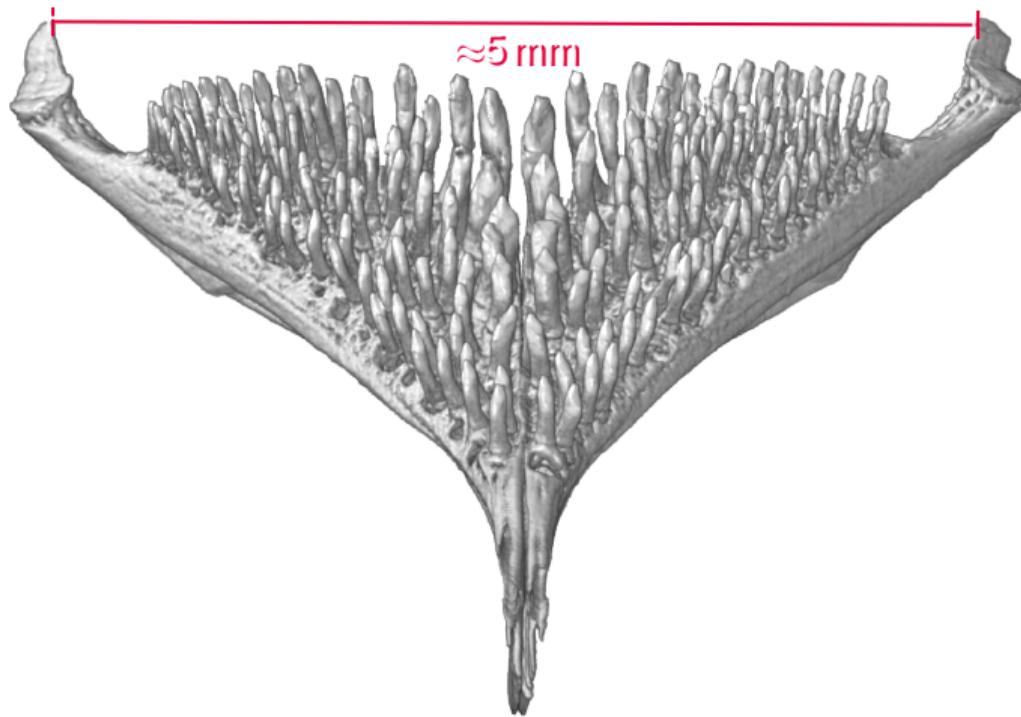
u^b

Visualization of cichlid head



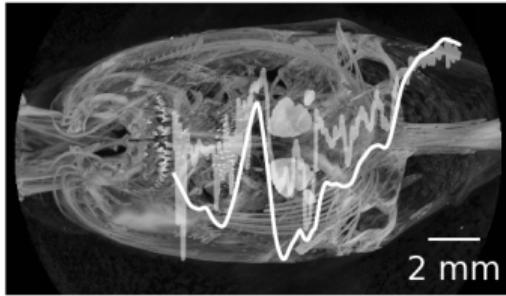
u^b

Visualization of segmented pharyngeal jaw



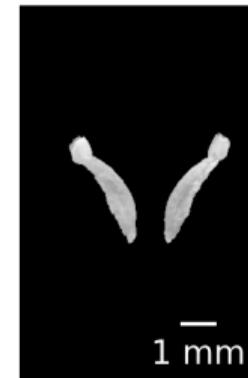
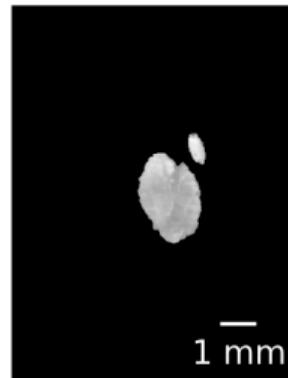
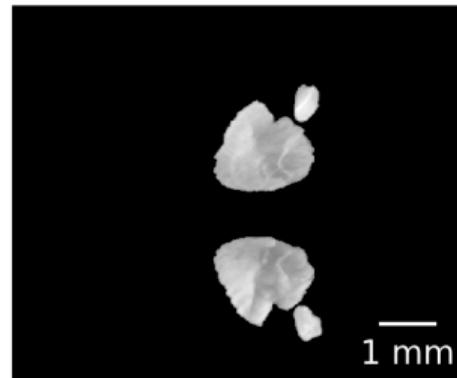
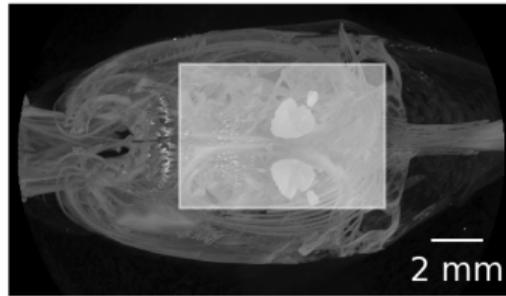
u^b

Data wrangling by example: Cichlids



u^b

Data wrangling by example: Cichlids



u^b

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 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

References I

- [1] R. Hlushchuk *et al.*, “Cutting-edge microangio-CT: New dimensions in vascular imaging and kidney morphometry”, Mar. 2018. DOI: 10.1152/ajprenal.00099.2017.
- [2] H. Nording *et al.*, “The C5a/C5a receptor 1 axis controls tissue neovascularization through CXCL4 release from platelets”, Dec. 2021. DOI: 10.1038/s41467-021-23499-w.
- [3] R. Hlushchuk *et al.*, “Innovative high-resolution microCT imaging of animal brain vasculature”, Oct. 2020. DOI: 10.1007/s00429-020-02158-8.
- [4] T. Wüthrich *et al.*, “Development of vascularized nerve scaffold using perfusion-decellularization and recellularization”, Aug. 2020. DOI: 10.1016/j.msec.2020.111311.
- [5] C. Zubler *et al.*, “The anatomical reliability of the superficial circumflex iliac artery perforator (SCIP) flap”, Mar. 2021. DOI: 10.1016/j.aanat.2020.151624.
- [6] M. Messerli *et al.*, “Adaptation mechanism of the adult zebrafish respiratory organ to endurance training”, Feb. 2020. DOI: 10.1371/journal.pone.0228333.
- [7] V. Trappetti *et al.*, “Synchrotron Microbeam Radiotherapy for the treatment of lung carcinoma: A pre-clinical study”, Aug. 2021. DOI: 10.1016/j.ijrobp.2021.07.1717.
- [8] E. Bochud *et al.*, “A new Diancta species of the family Diplommatinidae (Cyclophoroidea) from Vanua Levu Island, Fiji”, Nov. 2021. DOI: 10.3897/zookeys.1073.73241.

References II

- [9] S. Halm *et al.*, "Micro-CT imaging of Thiel-embalmed and iodine-stained human temporal bone for 3D modeling.", 2021. DOI: 10.1186/s40463-021-00522-0.
- [10] D. Haberthür *et al.*, "Automated segmentation and description of the internal morphology of human permanent teeth by means of micro-CT.", Apr. 2021. DOI: gjpw2d.
- [11] D. Haberthür *et al.*, "Microtomographic investigation of a large corpus of cichlids.", Sep. 2023. DOI: gsst8t.
- [12] A. M. Cormack., "Representation of a Function by Its Line Integrals, with Some Radiological Applications.", Sep. 1963. DOI: 10.1063/1.1729798.
- [13] G. N. Hounsfield., "Historical notes on computerized axial tomography.", 1976.
- [14] E. C. Beckmann., "CT scanning the early days.", Jan. 2006. DOI: 10.1259/bjr/29444122.
- [15] J. Hsieh. *Computed Tomography: Principles, Design, Artifacts, and Recent Advances*. Society of Photo Optical, 2003.
- [16] L. A. Feldkamp *et al.*, "Practical cone-beam algorithm.", Jun. 1984. DOI: 10.1364/JOSAA.1.000612.
- [17] L. A. Feldkamp *et al.*, "The direct examination of three-dimensional bone architecture in vitro by computed tomography.", Feb. 1989. DOI: 10.1002/jbmr.5650040103.
- [18] M. W. Layton *et al.*, "Examination of subchondral bone architecture in experimental osteoarthritis by microscopic computed axial tomography.", Nov. 1988. DOI: 10.1002/art.1780311109.

References III

- [19] M. Hammer. *X-Ray Physics: X-Ray Interaction with Matter, X-Ray Contrast, and Dose*,
- [20] Wikipedia contributors. *Beer–Lambert law — Wikipedia, The Free Encyclopedia*, 2019.
- [21] K. Clark *et al.*, “The Cancer Imaging Archive (TCIA): Maintaining and Operating a Public Information Repository”, Dec. 2013. DOI: 10.1007/s10278-013-9622-7.
- [22] J. Schindelin *et al.*, “Fiji: An open-source platform for biological-image analysis.”, Jul. 2012. DOI: 10.1038/nmeth.2019.
- [23] T. Kluyver *et al.*, “Jupyter Notebooks – a publishing format for reproducible computational workflows.”, 2016. DOI: 10.3233/978-1-61499-649-1-87.
- [24] T. G. Wolf *et al.*, “Internal morphology of 101 mandibular canines of a Swiss-German population by means of micro-CT: An ex vivo study.”, 2021. DOI: g7r8.
- [25] B. Briseño-Marroquín *et al.*, “Root Canal Morphology and Configuration of 179 Maxillary First Molars by Means of Micro-computed Tomography: An Ex Vivo Study.”, Dec. 2015. DOI: 10.1016/j.joen.2015.09.007.
- [26] D. Haberthür., “Habi/zmk-tooth-cohort: Used for manuscript about method.”, Aug. 2020. DOI: 10.5281/ZENODO.3999402.
- [27] T. G. Wolf *et al.*, “Three-dimensional analysis of the physiological foramen geometry of maxillary and mandibular molars by means of micro-CT.”, Sep. 2017. DOI: 10.1038/ijos.2017.29.