X-ray microtomography

David Haberthür

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

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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.

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Grüessech from the µCT-group

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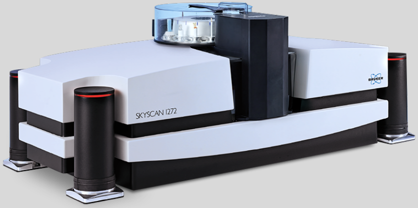
µCT-group

- 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

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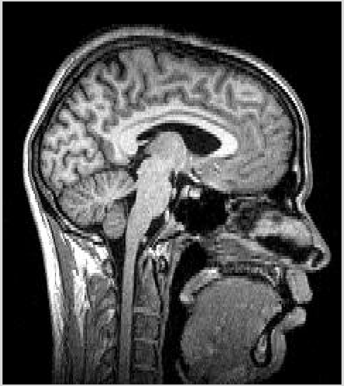
µCT

- 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



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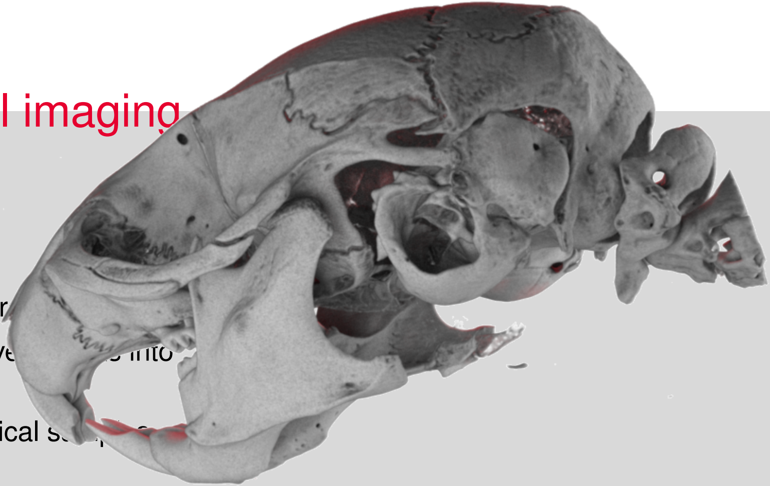
Biomedical imaging



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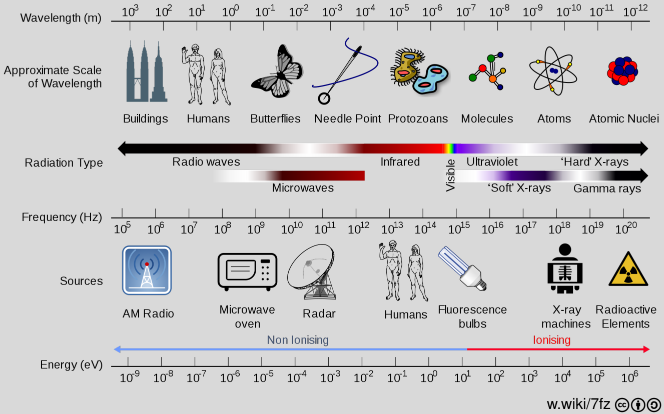
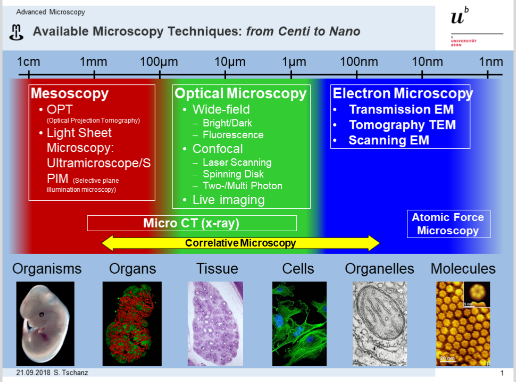
Wavelength & Scale



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

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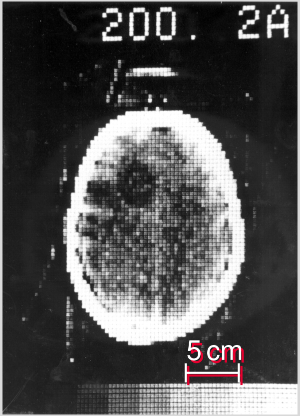
CT History

- 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]



From [**Beckmann2006**], Figure 5

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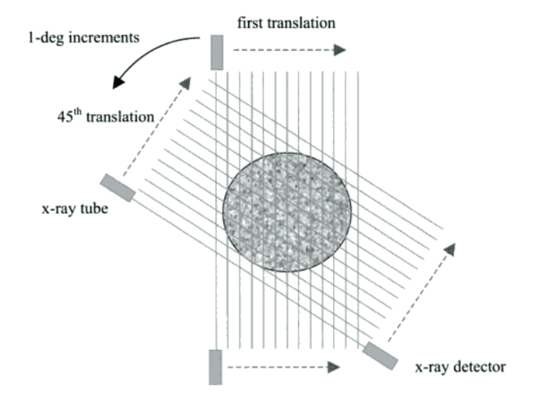
- CT scanner generations: First



From [14], Figure 1.12

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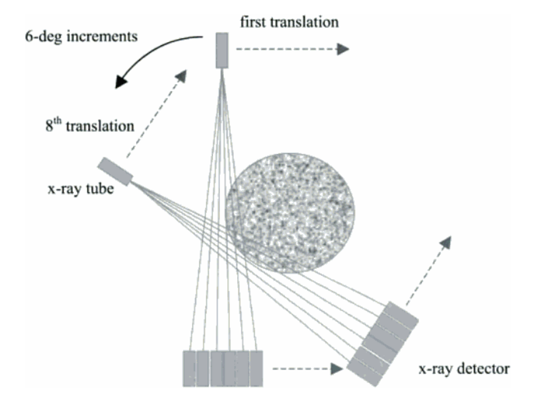
- CT scanner generations: First, second



From [14], Figure 1.13

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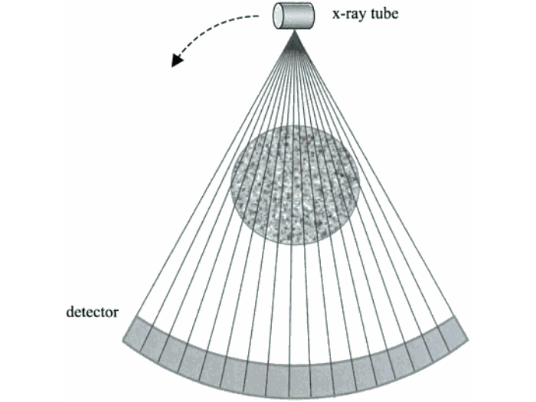

From [14], Figure 1.14

µCT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [**Lin2019**]
- µCT 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 stagers, and an image intensifier at Ford Motor Company's Scientific Research Laboratory to nondestructively detect damage in ceramic manufactured automobile parts

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µCT 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 [**Feldkamp1983**]
- 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

µCT History III

- Since the 1990s, µCT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
- $\approx$2500 µCT 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]: $I(t) = I_0 \, e^{-\alpha z}$

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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

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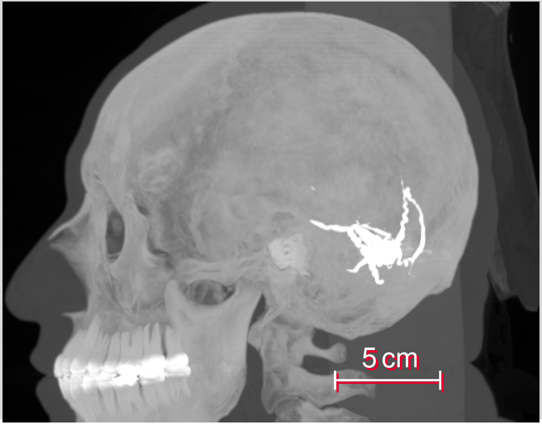
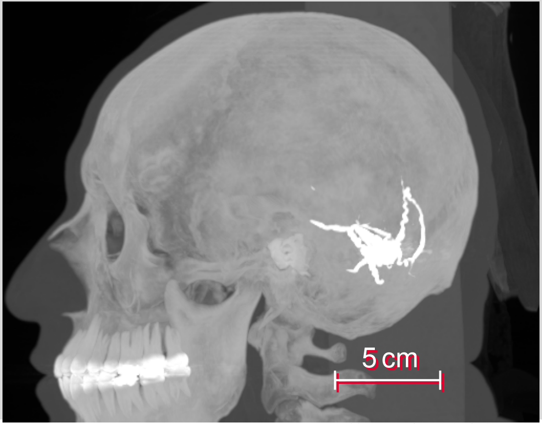
Why µCT?



From [17], Subject C3L-02465

Why µCT?



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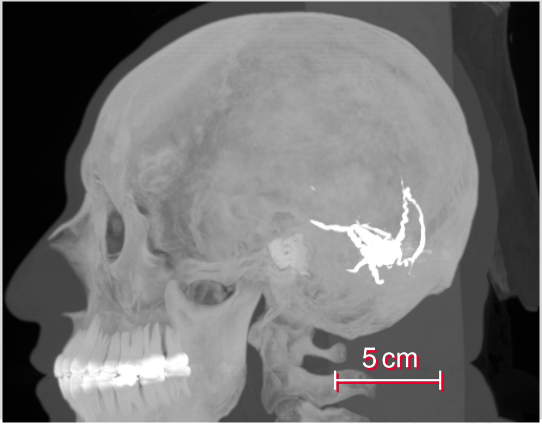

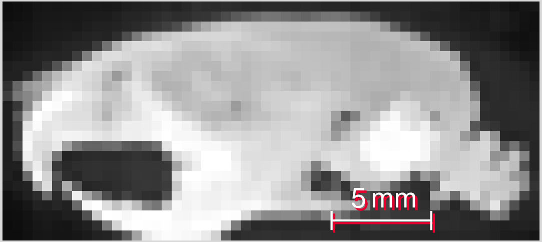

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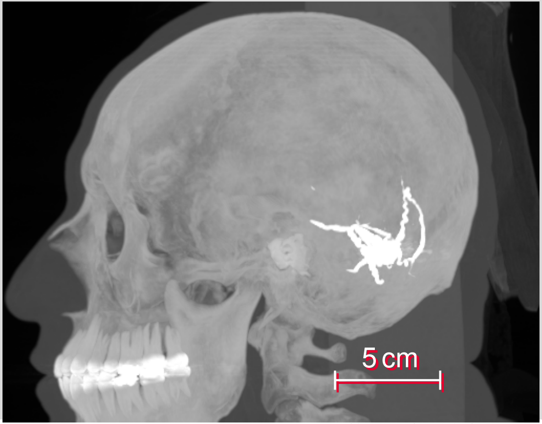

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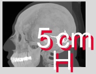

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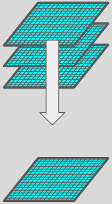
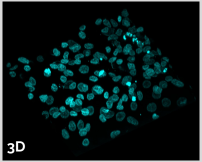
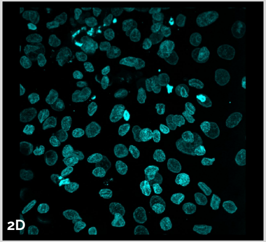
Maximum intensity projection

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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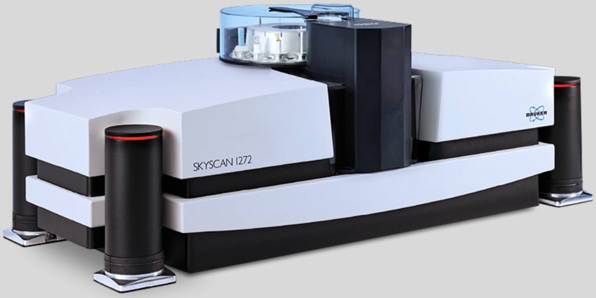

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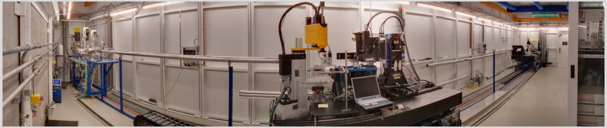

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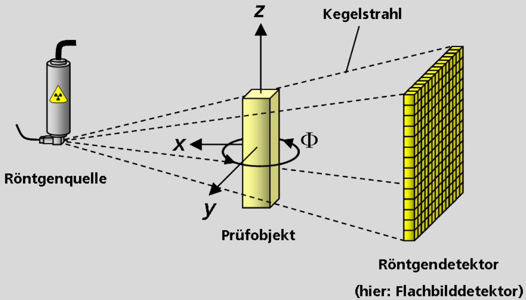
What is happening?

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

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



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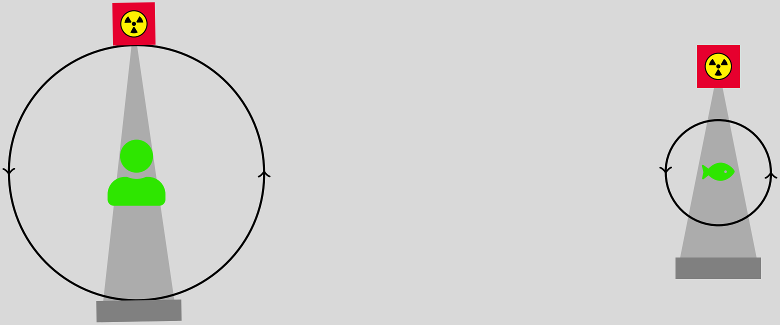

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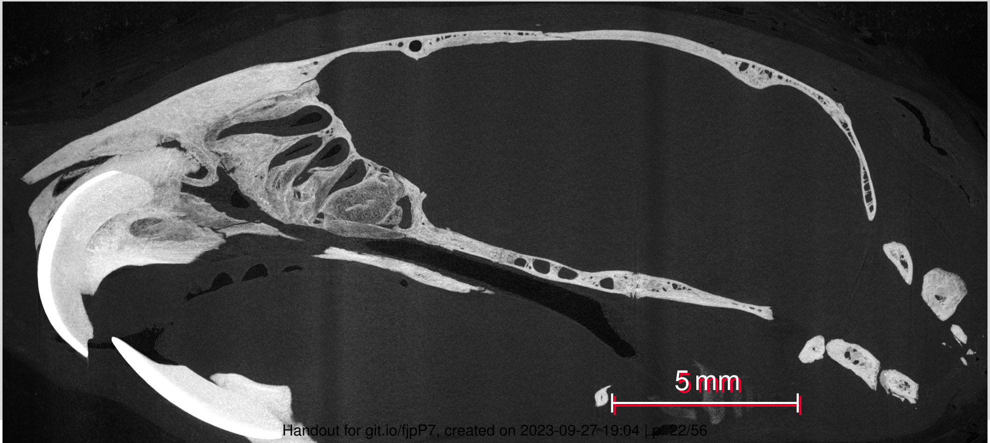
Examples



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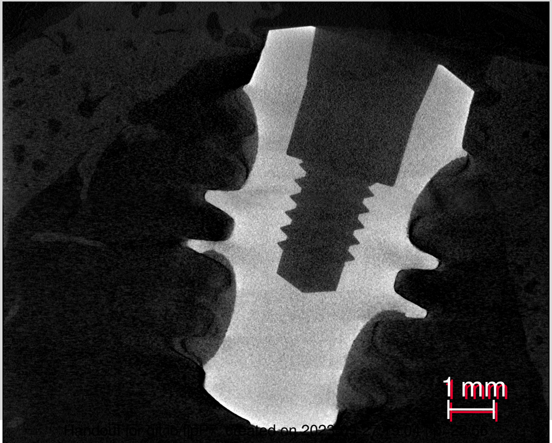
Examples



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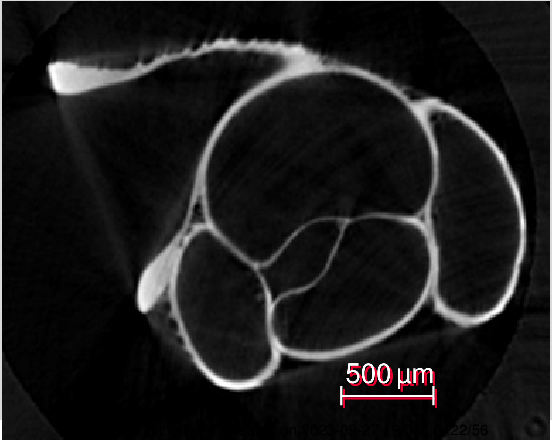
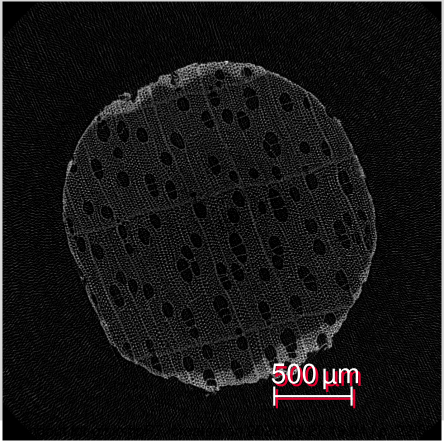


Examples



Examples

Preparation

- Study design
- Sample preparation

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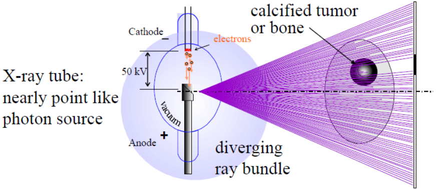
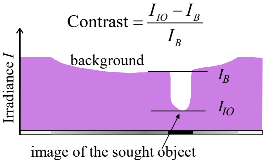
Projections

X-ray generation and contrast

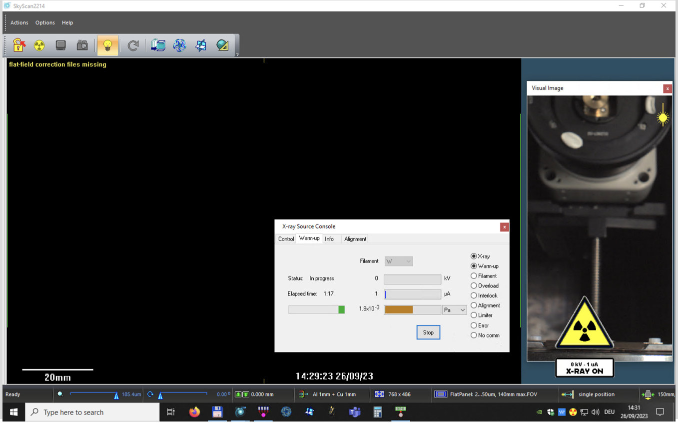


Contrast is given by absorption of intensity I

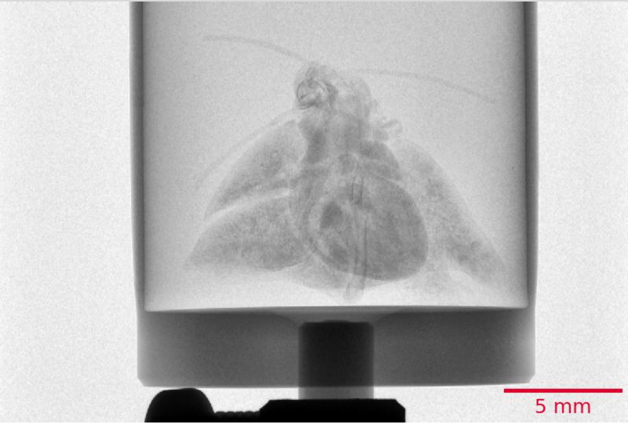
Note that contrast is negative X-ray shadowgraphy is a bright field technique



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

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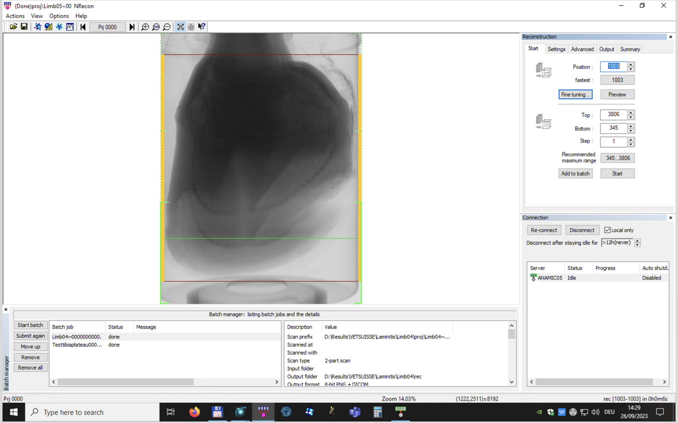
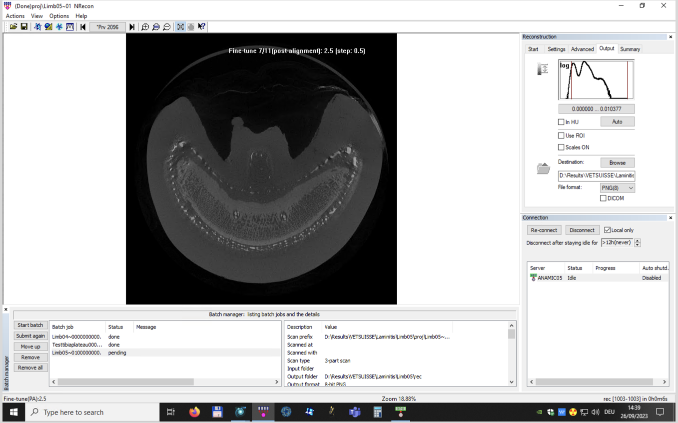
Reconstructions



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

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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

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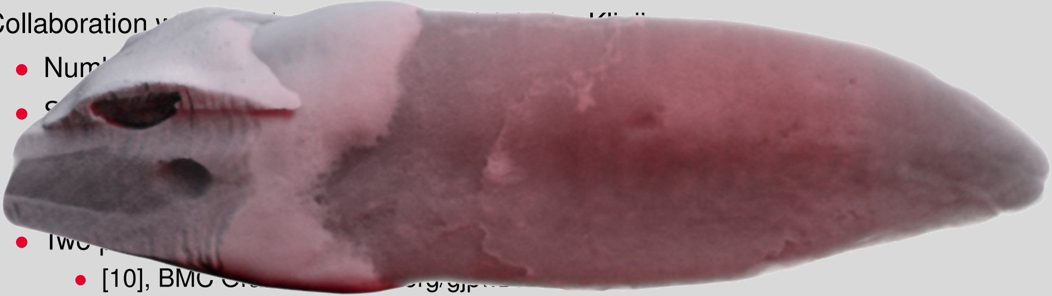
Internal morphology of human teeth

Collaboration w

- Num
- S
- Two
  - [10], BMC
  - [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
- µCT 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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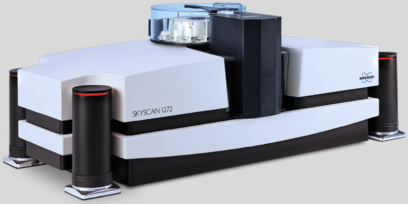

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```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files=  482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)=  80
Source Current (uA)= 125
Image Pixel Size (um)=9.999986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

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Sample changer on the SkyScan 1272

In total:

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

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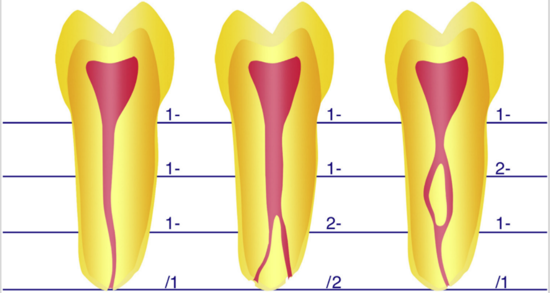

From [21], Fig. 2

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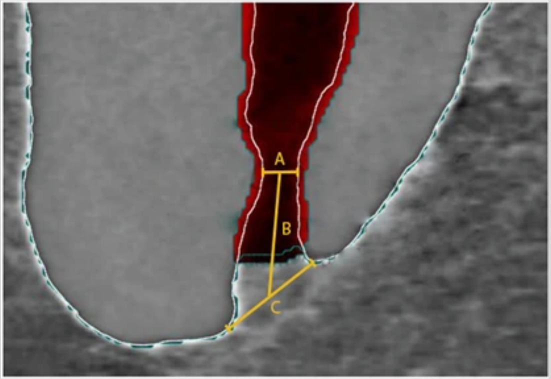

From [23], Fig. 1

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gph.is/2nqkple

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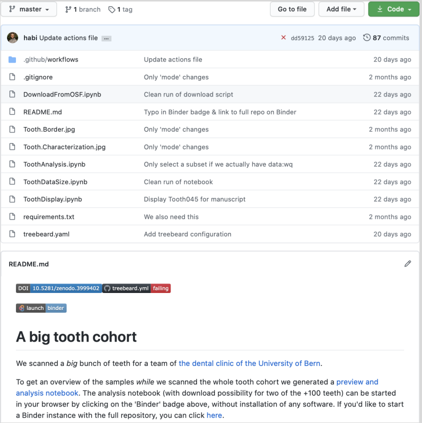
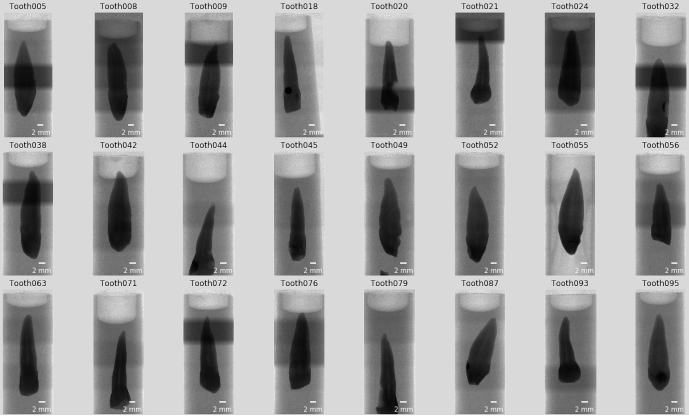


µCT imaging


Tooth005
Tooth008
Tooth009
Tooth018
Tooth020
Tooth021
Tooth024
Tooth032
Tooth038
Tooth042
Tooth044
Tooth045
Tooth049
Tooth052
Tooth055
Tooth056
Tooth063
Tooth071
Tooth072
Tooth076
Tooth079
Tooth087
Tooth093
Tooth095
2 mm

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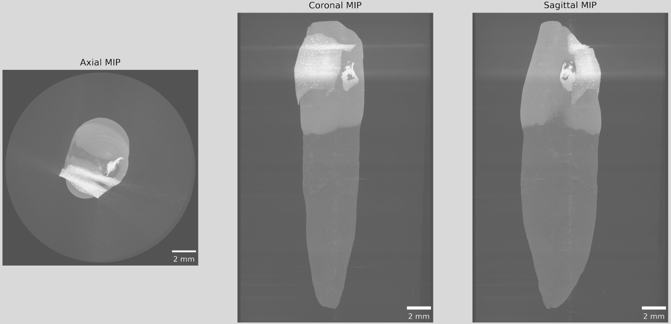
Dataset cropping

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



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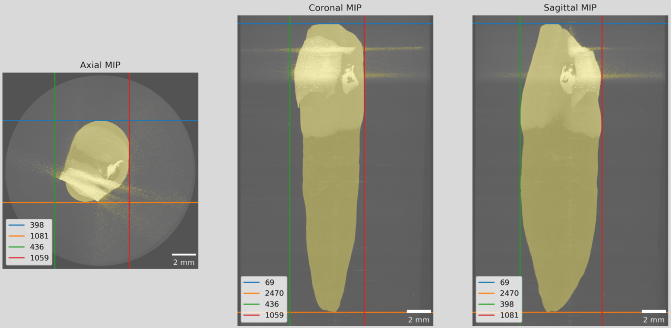
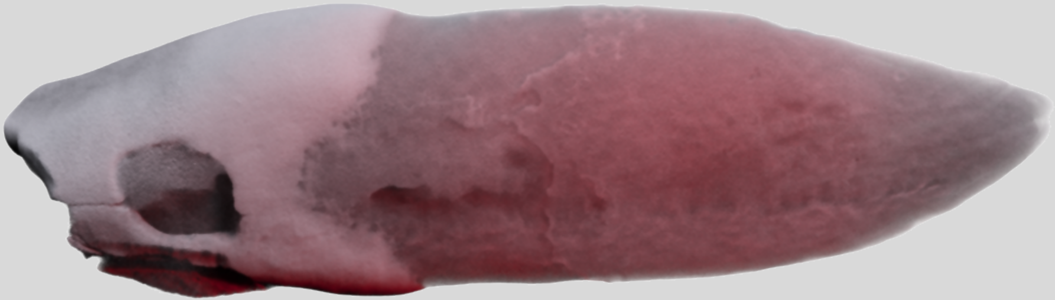
- Full datasets: 326 GB
- Cropped datasets: 115 GB



Tooth morphology



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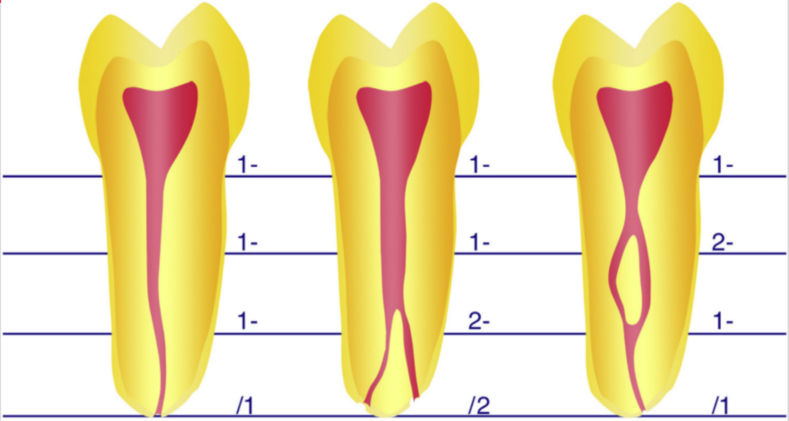
Tooth morphology



From [21], Fig. 2

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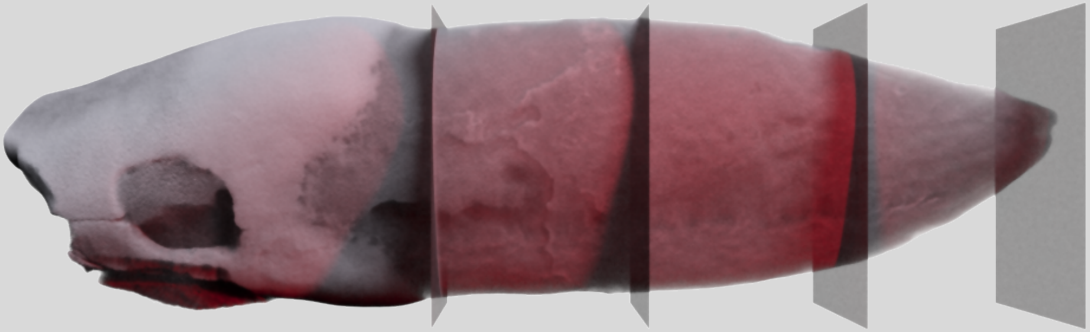
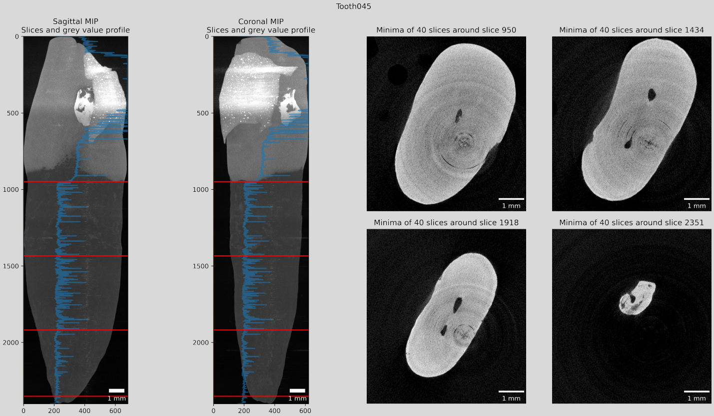
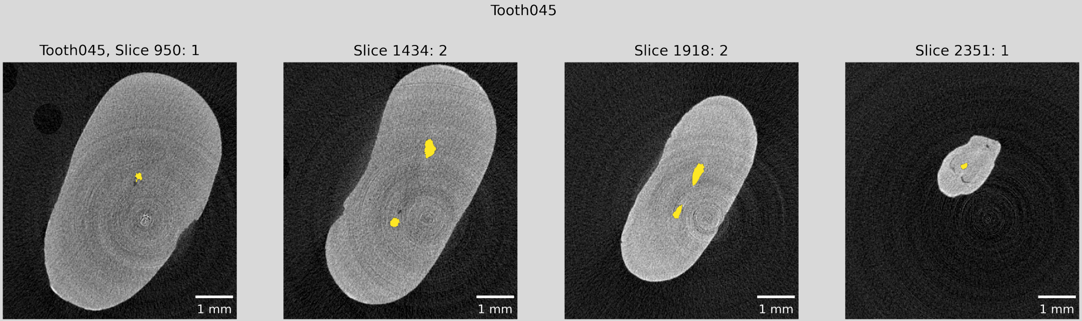
Tooth morphology



Detection of enamel-dentin border



Detection of enamel-dentin border



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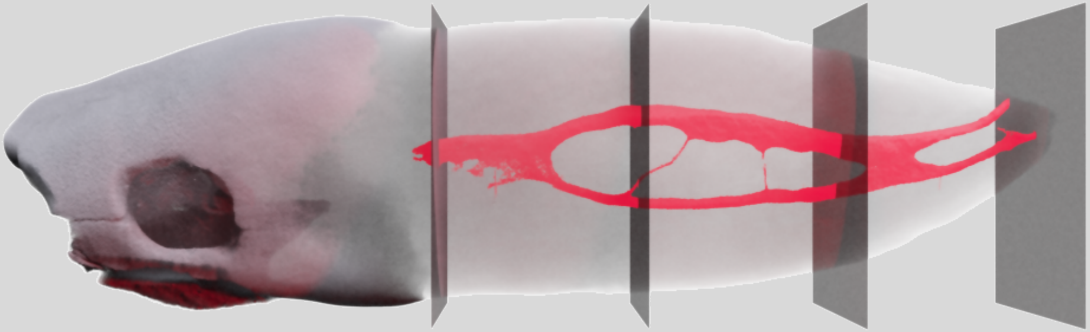
Outcome root canal configuration classification

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



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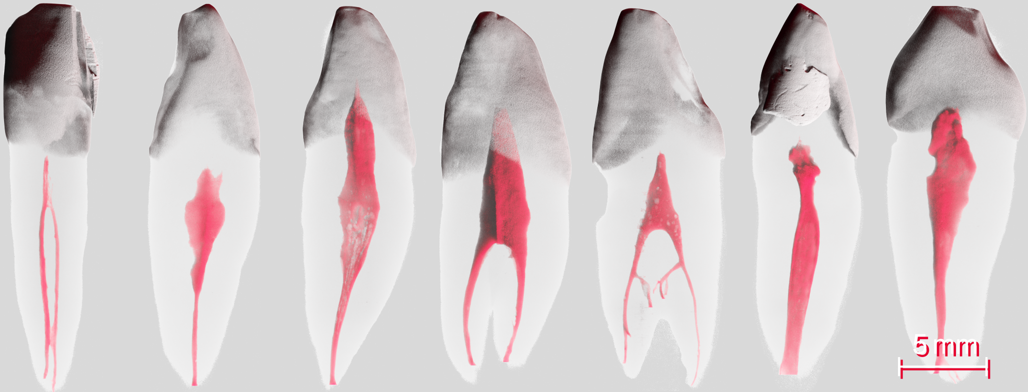
Results of root canal space extraction



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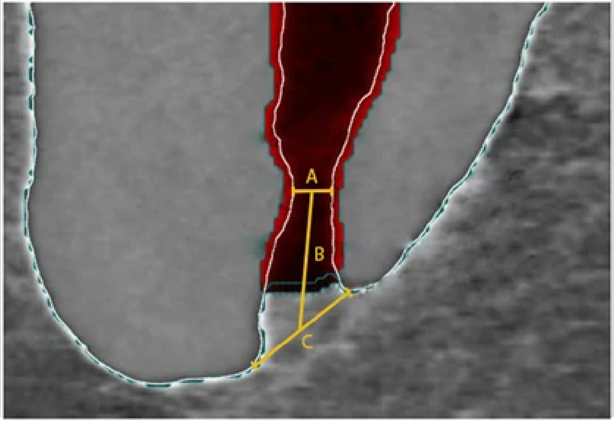
Analysis of the physiological foramen geometry



From [23], Fig. 1

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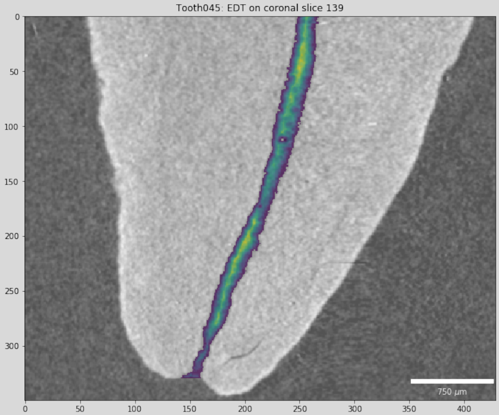
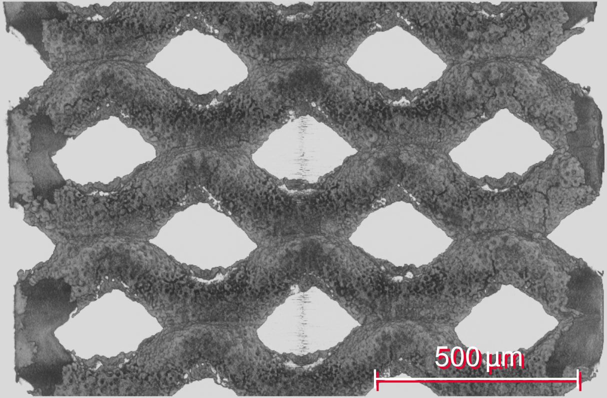
Analysis of the physiological foramen geometry



Conclusion ZMK

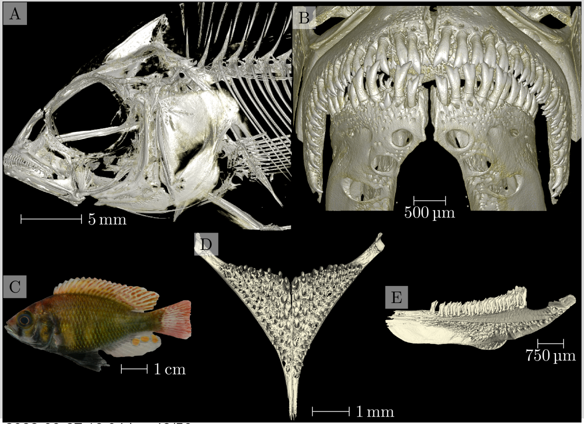
- Efficient use of time, e. g. more teeth does not mean more (human) work
- Reproducible analysis with *free and open-source* software, usable by *anyone*
- Objective analysis, e. g. no operator bias

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.

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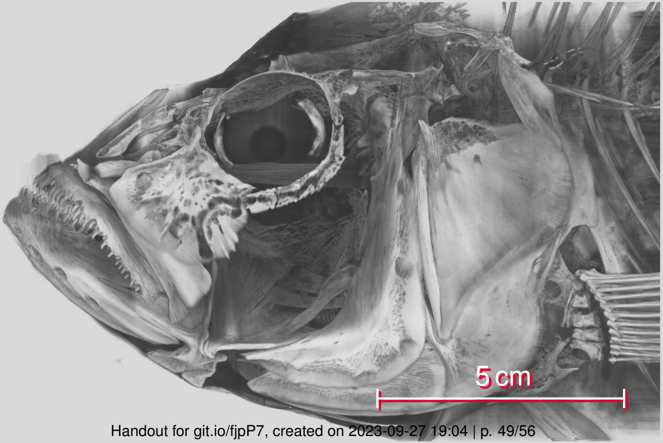
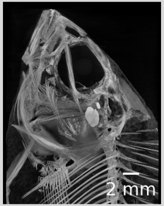
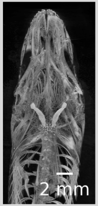
Cichlids

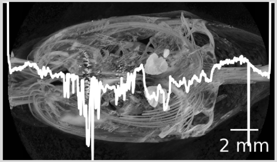


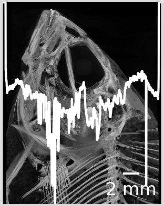
Data wrangling by example: Cichlids

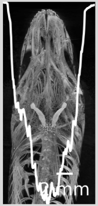




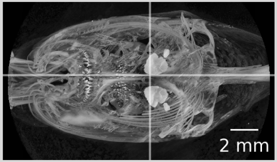
Data wrangling by example: Cichlids

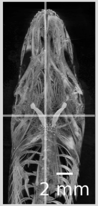






Data wrangling by example: Cichlids





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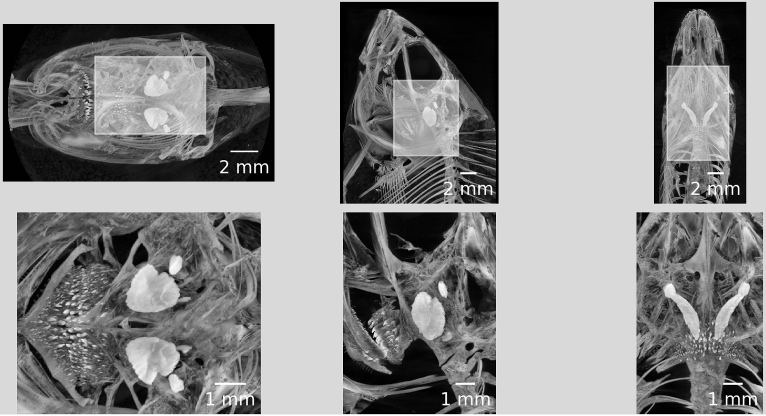
Data wrangling by example: Cichlids



Thanks!

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

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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: 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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[20] Thomas Gerhard Wolf et al. "Internal Morphology of 101 Mandibular Canines of a Swiss-German Population by Means of Micro-CT: An Ex Vivo Study". DOI: 10.1038/s41598-021-00758-w.

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