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OF BERN

X-ray microtomography
485018-HS2024-0: Advanced Course II Ultraprecision Engineering

David Haberthür
Institute of Anatomy, September 25, 2024

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

Grüessech from the µCT group



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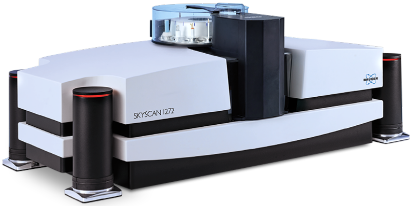
µCT-group

- 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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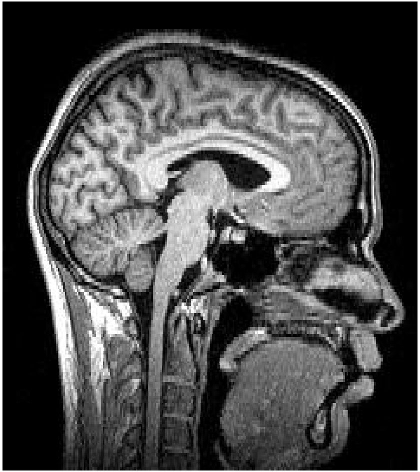
Contents

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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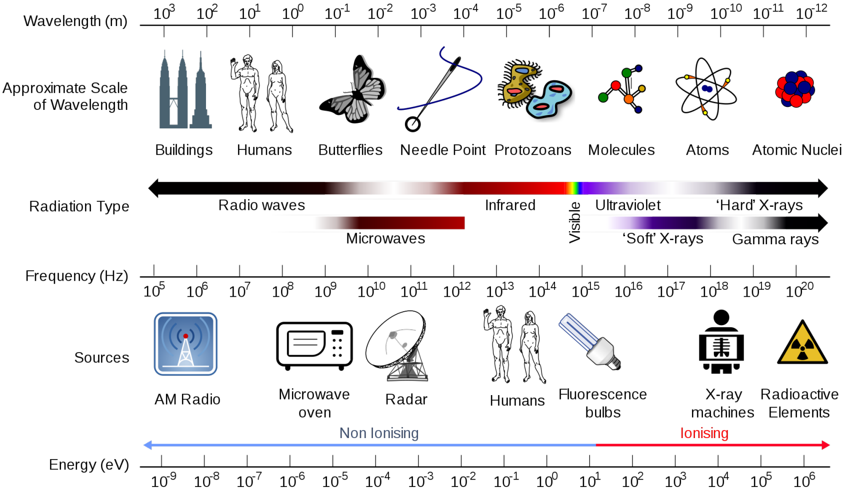
Biomedical imaging

- Medical resea
- Non-destruc
- (Small) Bio

Wavelengths & Scales



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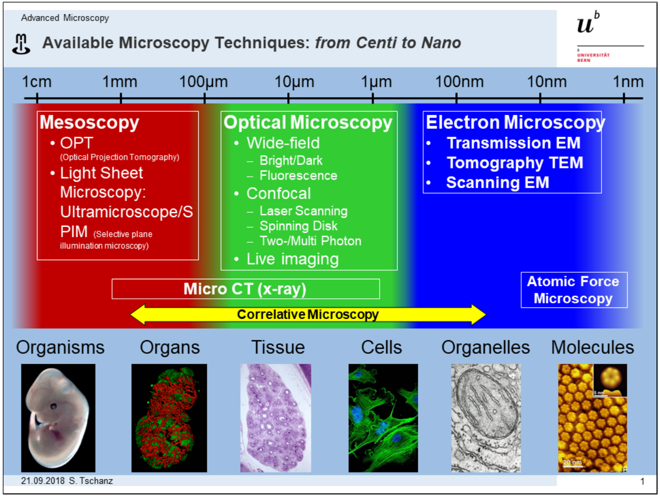

Stefan Tschanz, with permission

Imaging methods

- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy
  - *Analytical electron microscopy* by Dimitri
  - *SEM Grundlagen* by Sabine Kässmeyer and Ivana Jaric
  - *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan

CT-Scanner

youtu.be/2CWpZKuy-NE

CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays

w.wiki/BHAN

CT History

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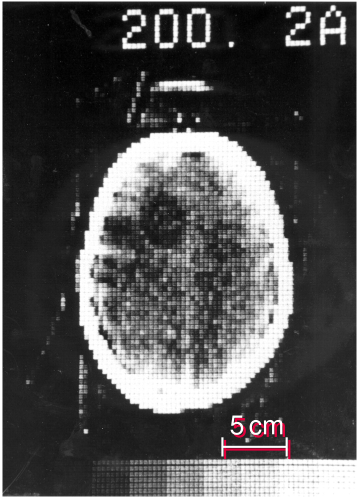
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]



From [14], Figure 5

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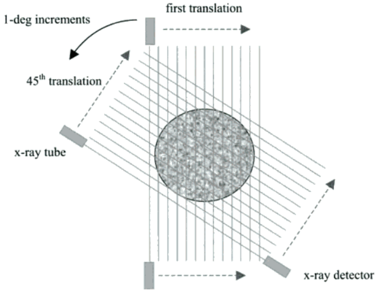
- CT scanner generations
  - First generation



From [15], Figure 1.12

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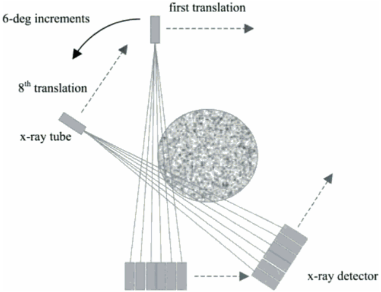
- CT scanner generations
  - First generation
  - Second generation



From [15], Figure 1.13

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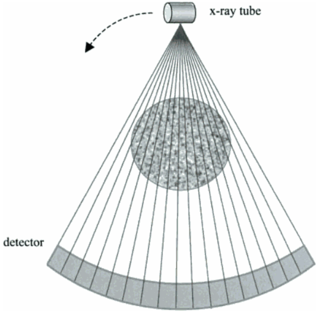
  - Third generation



From [15], Figure 1.14

µCT 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 µCT scanner was developed by SkyScan (now Bruker)
- Today: µCT 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, µCT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
  - Paleontology and Archaeology: study of fossils and archaeological artifacts
- ≈2500 µCT systems are in use worldwide with over 1000 publications annually

X-ray interaction

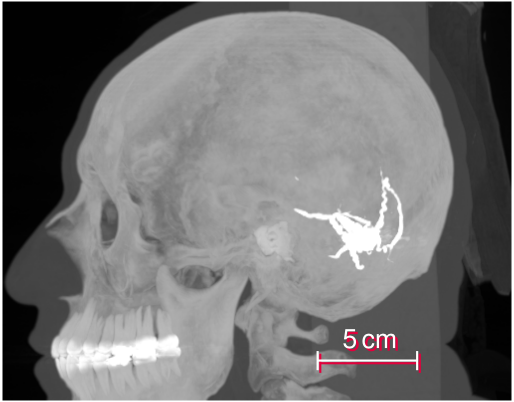
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([19])
  - Photoelectric absorption (τ) is strongly dependent on 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: $\lambda' - \lambda = \frac{h}{m_e c}(1 - \cos\theta)$
- Lowering x-ray energy increases contrast
- X-ray penetration decreases exponentially with sample thickness [20, i. e. Beer-Lamberts 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

Why µCT?



From [21], Subject *C3L-02465*

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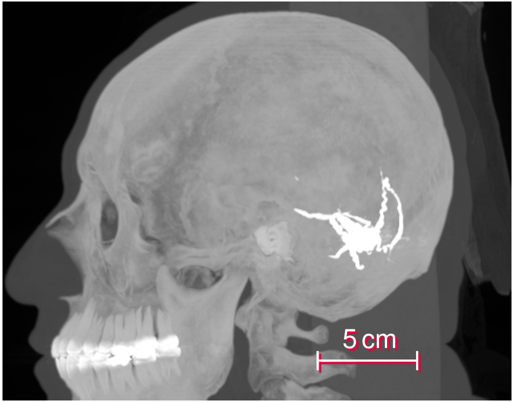

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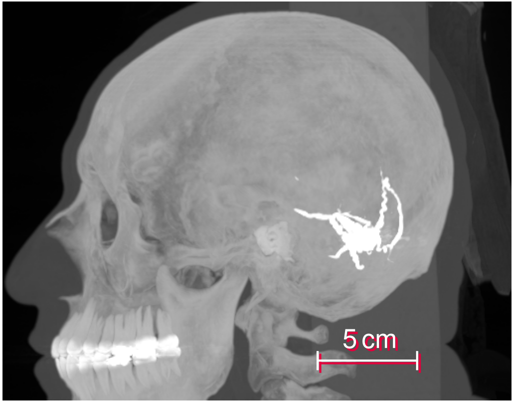

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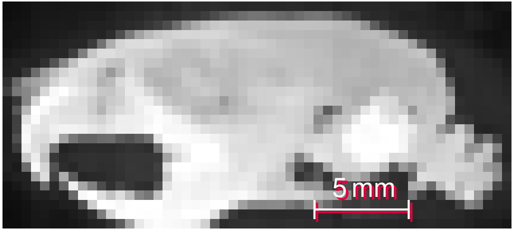

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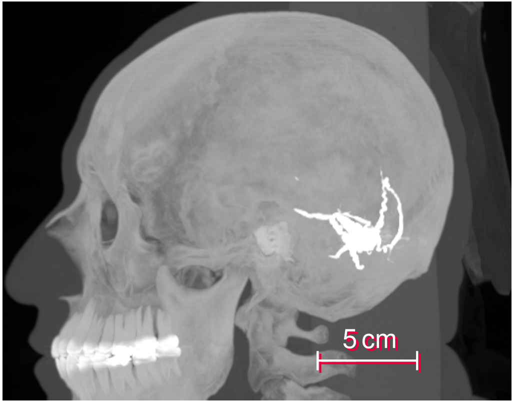

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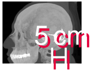

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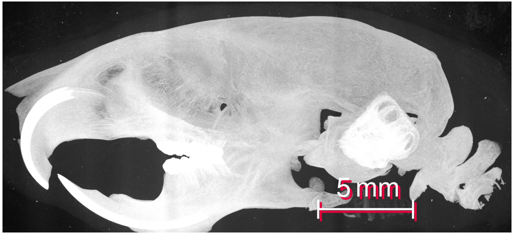

Maximum intensity projection

Projections

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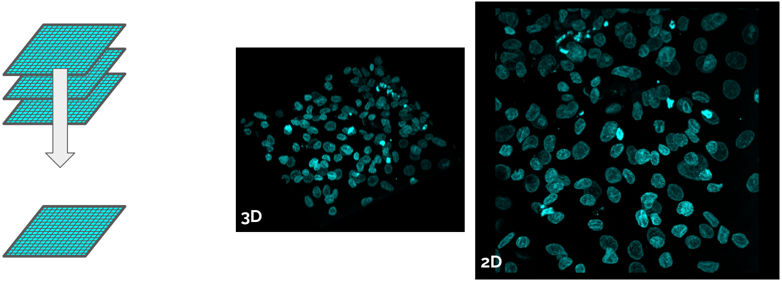
Reducing the dimensions of a dataset. For example projecting a volume (3D) to a surface by taking the maximum value across planes for each pixel.



Fundamentals of Digital Image Processing (2022) by Guillaume Witz, Slide 23

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

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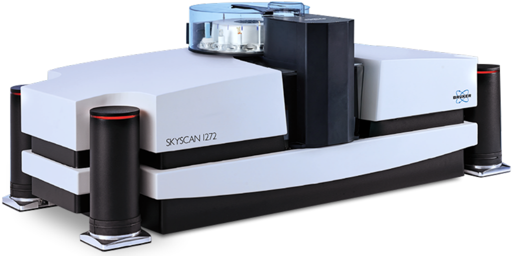

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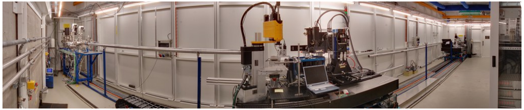
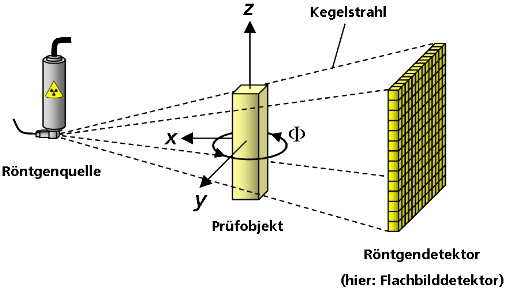


What is happening?

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

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



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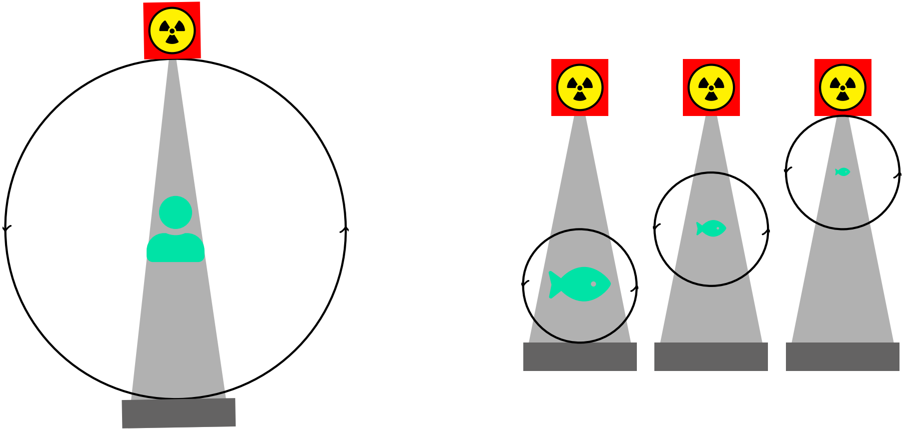
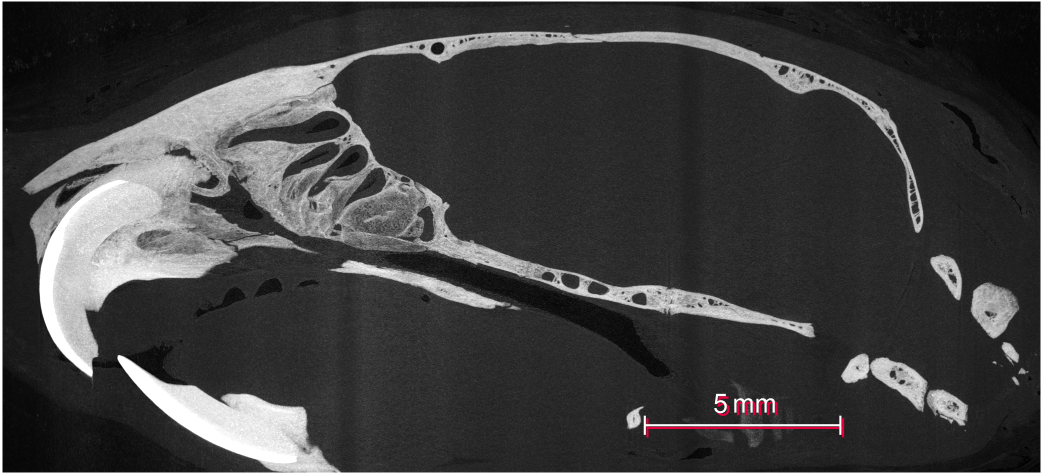
Machinery



Examples



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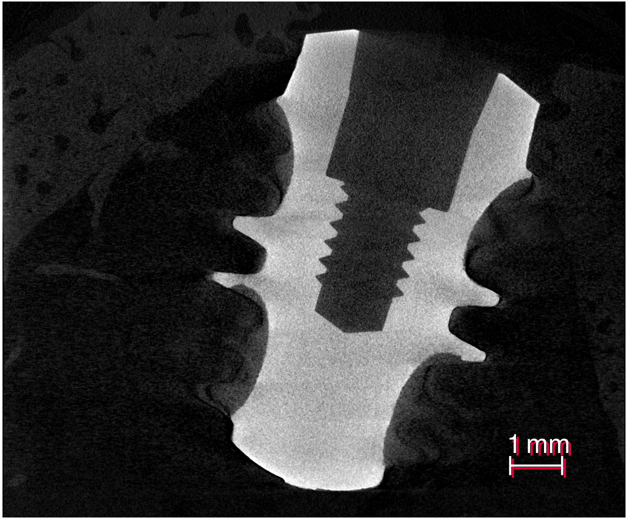

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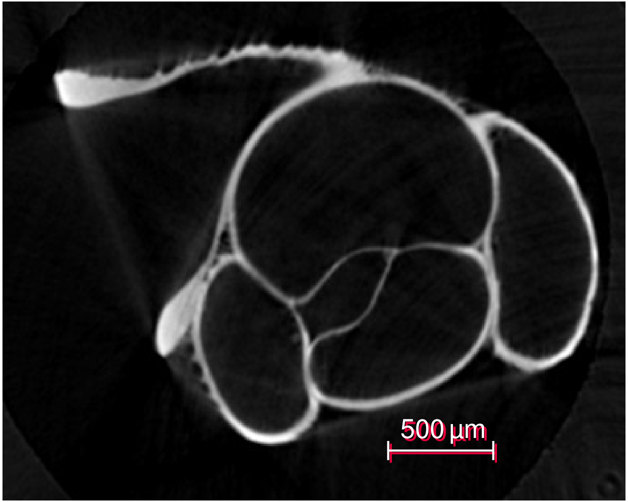
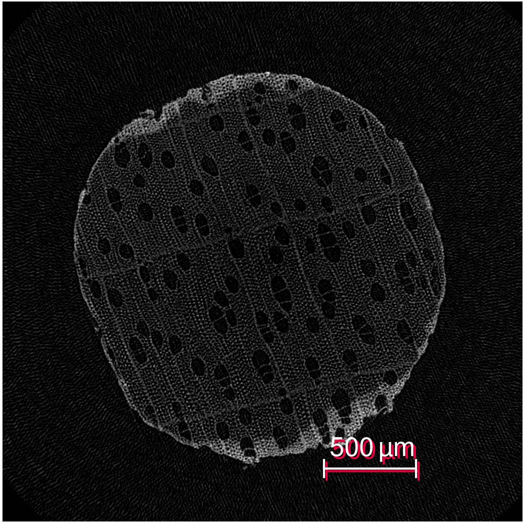


From [8], *Diancta phoenīx*

Examples



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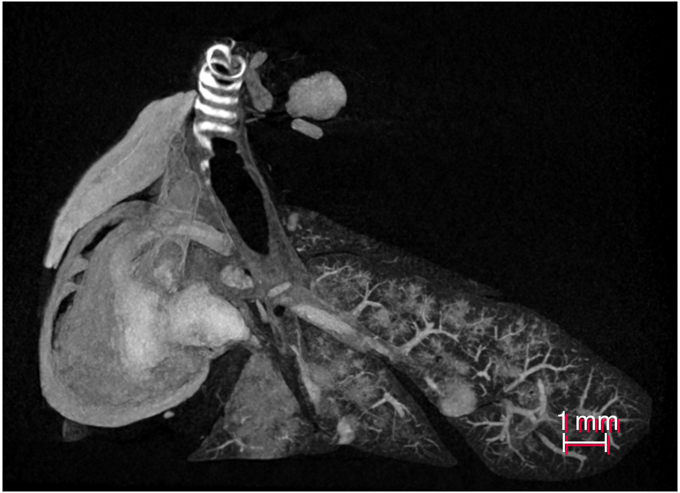
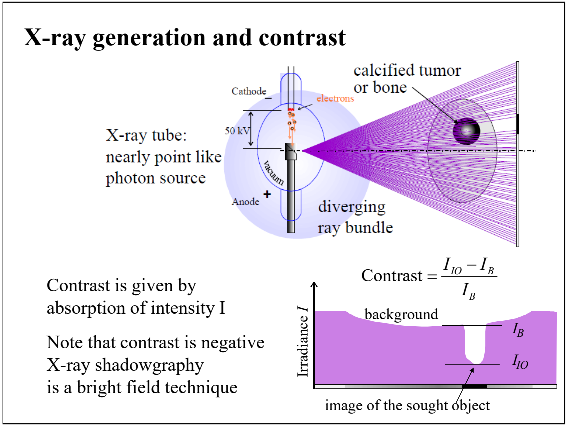


Preparation

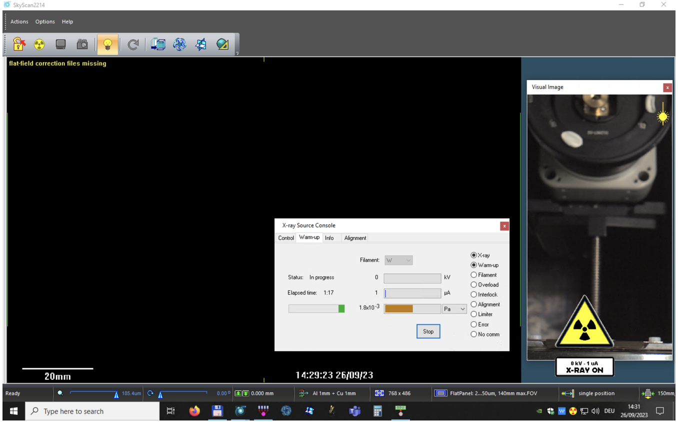
- Study design
- Sample preparation

Projections



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

Projection acquisition



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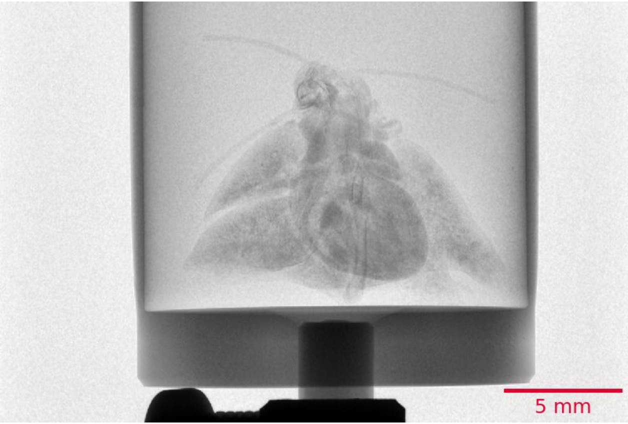
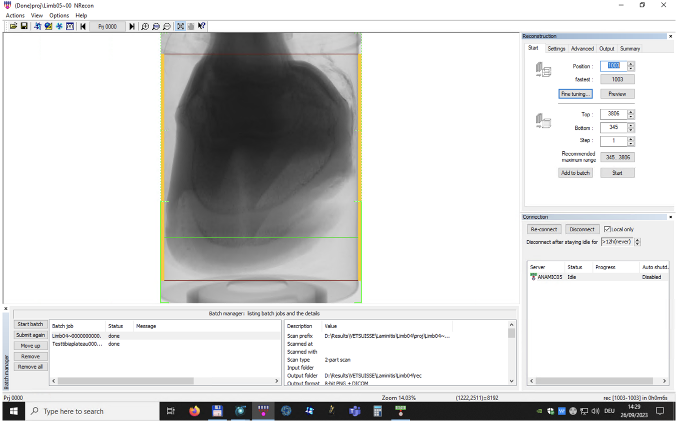


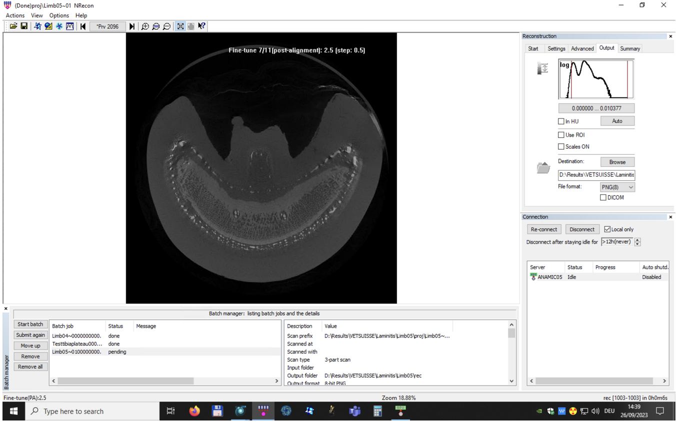
Projections

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

Visualization

Visualization

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

What to use?

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

Quantitative data

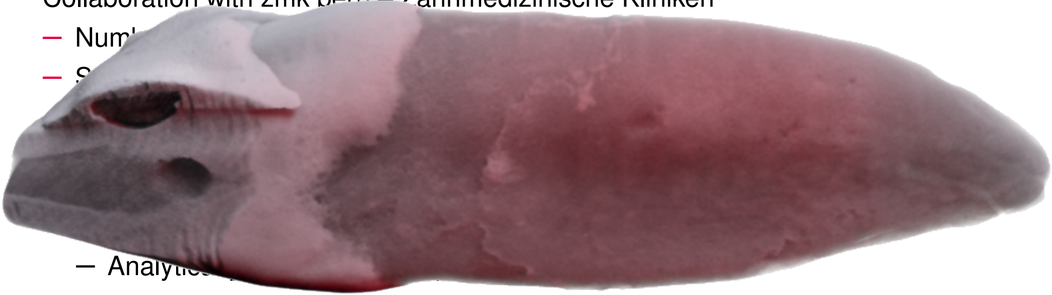
- Pretty images are nice to have, but science is quantitative data
- Segmentation
- Characterization

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

– Num

– S



– Analytic

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!

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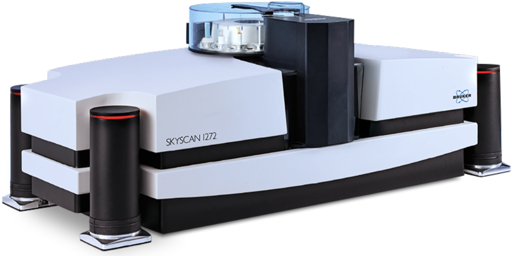

bruker.com/skyscan1272

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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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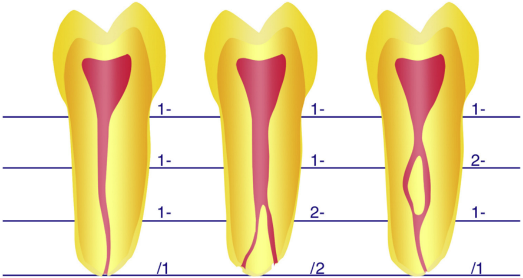

From [25], Fig. 2

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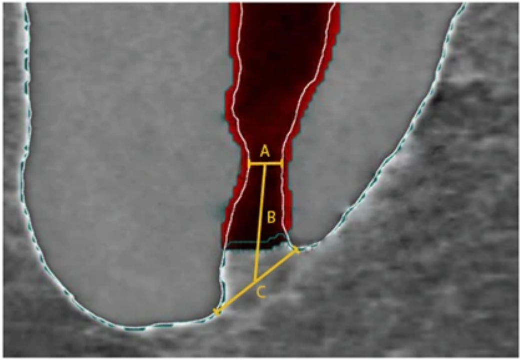

From [27], Fig. 1

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gph.is/2nqkple

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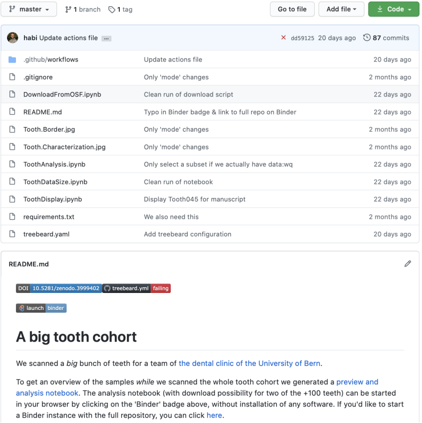
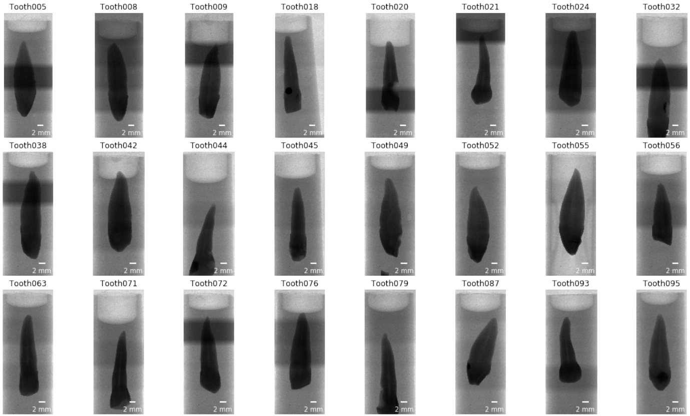


µCT imaging


Tooth005
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Tooth045
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Tooth052
Tooth055
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Tooth063
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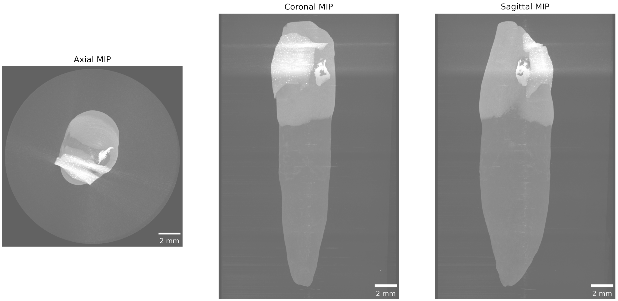
Tooth093
Tooth095
2 mm

Dataset cropping

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



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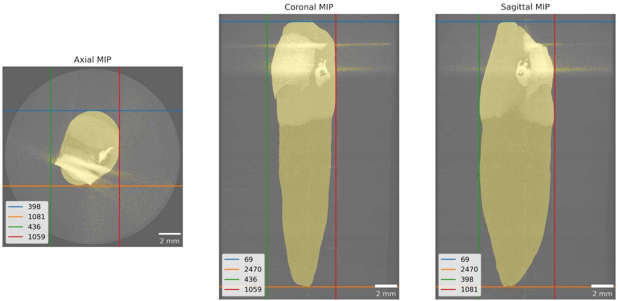
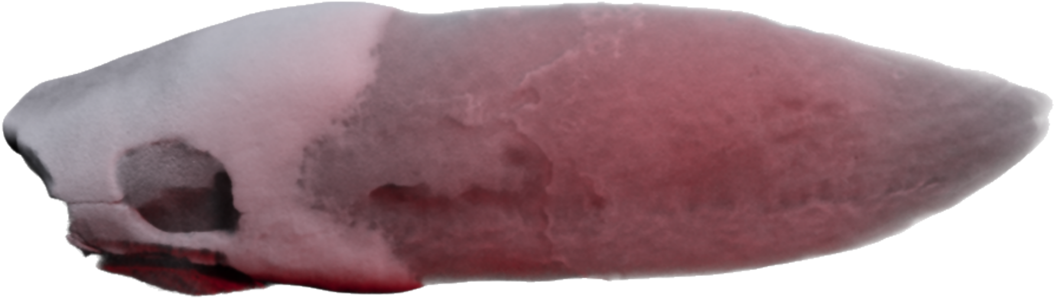


Tooth morphology



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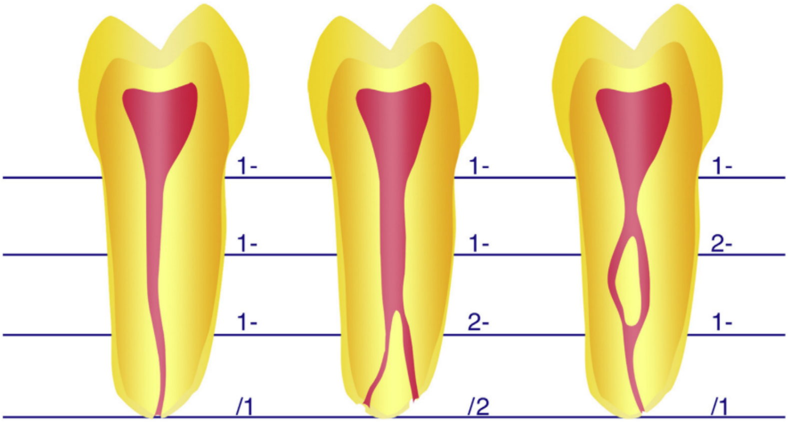
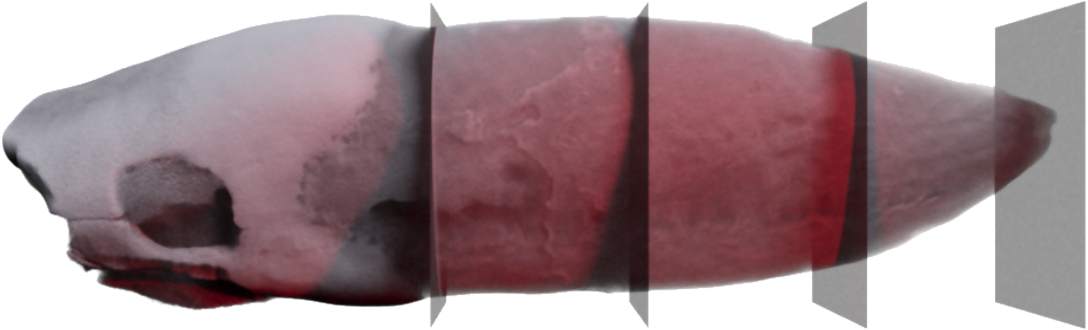
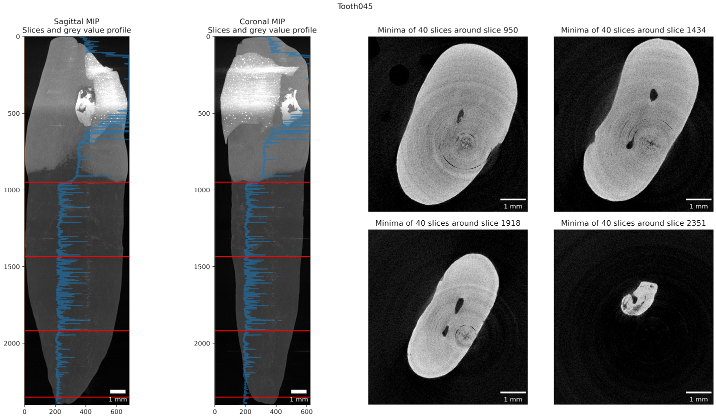


From [25], Fig. 2

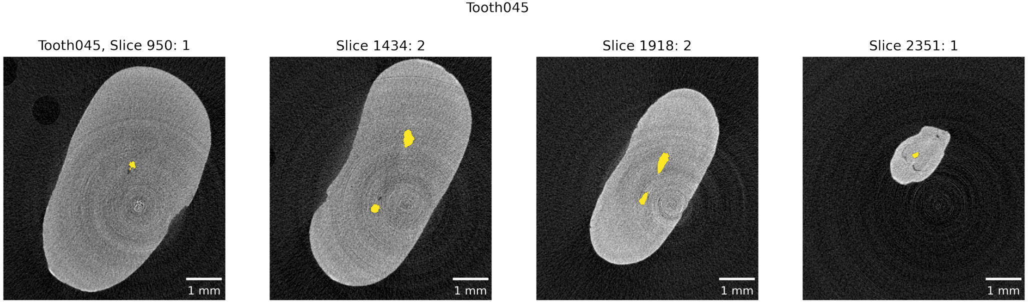
Tooth morphology



Detection of enamel-dentin border



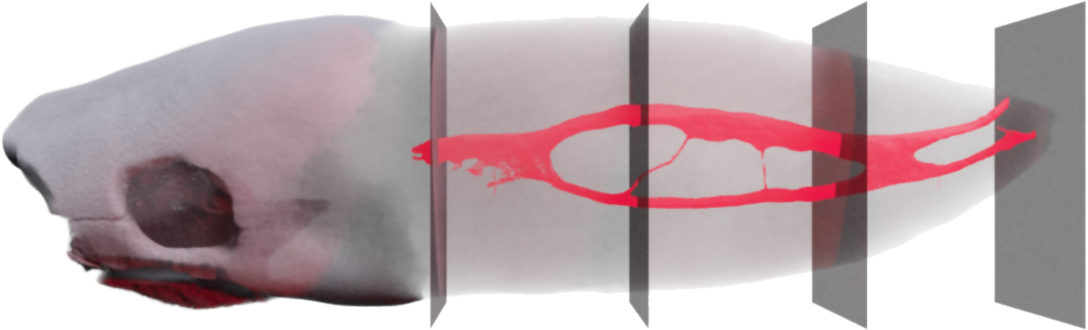
Detection of enamel-dentin border



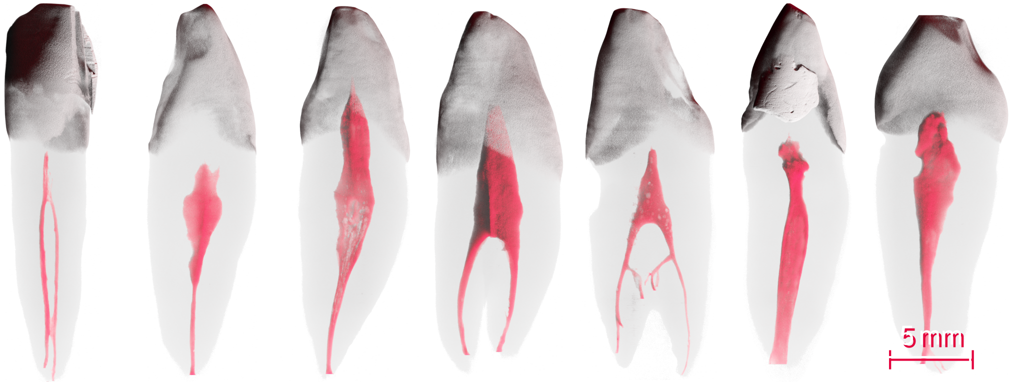
Outcome root canal configuration classification

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

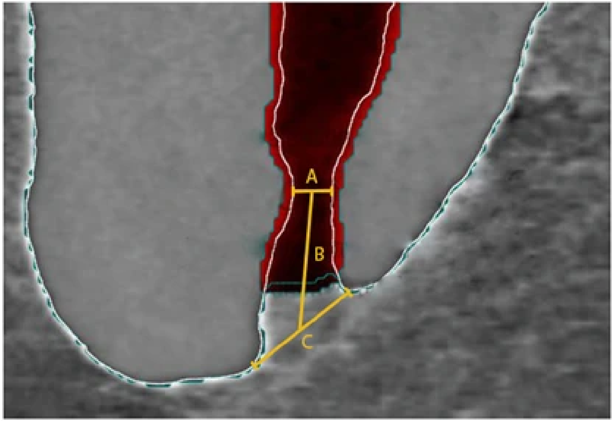
Extraction of root canal space



Results of root canal space extraction

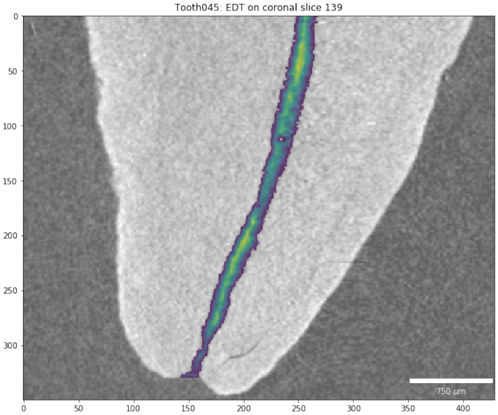


Physiological foramen geometry



From [27], Fig. 1

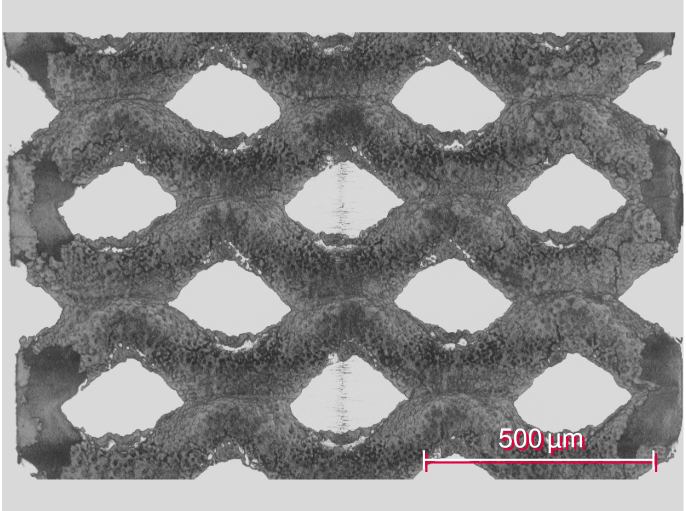
Physiological foramen geometry



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

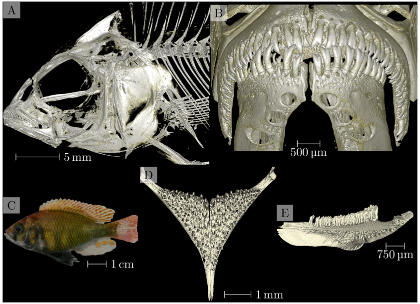


Etienne Berner NanoElectroCatalysis Group

Data wrangling by example: Cichlids

Collaboration with team of *Aquatic Ecology & Evolution*, from the Institute of Ecology and Evolution [11]

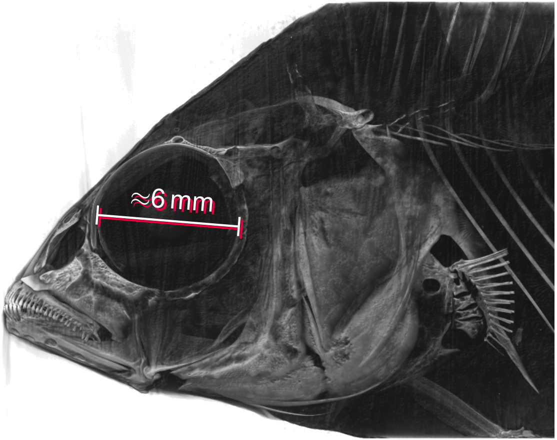
- 133 Cichlids from Lake Victoria, East Africa
  - Functional anatomy of the skulls and jaws
  - 6–18 cm in size
- 375 scans in total
  - Voxelsizes from 3.5–50 µm
  - 46 days of scanning time
  - 9.8 TB of raw data
  - 1.5 TB/+1 000 000 reconstructions



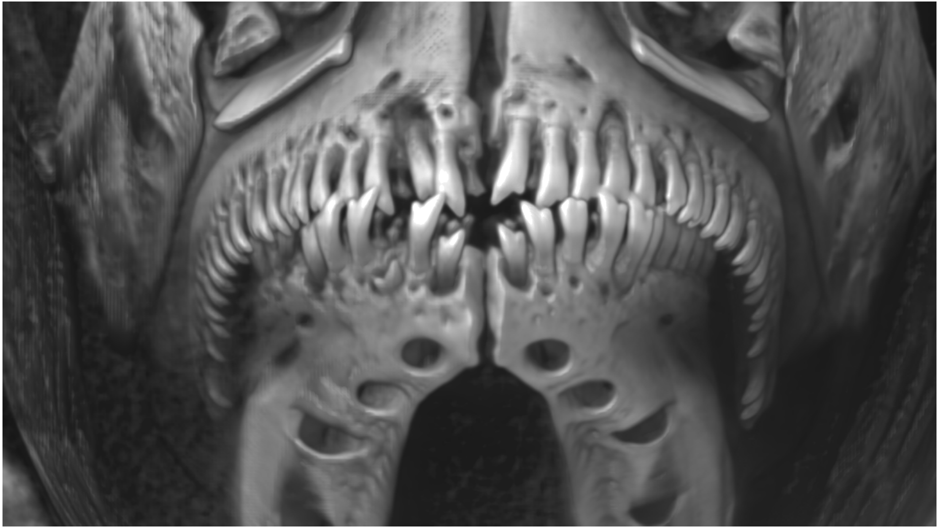
DOI:gsst8t, Fig. 1

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

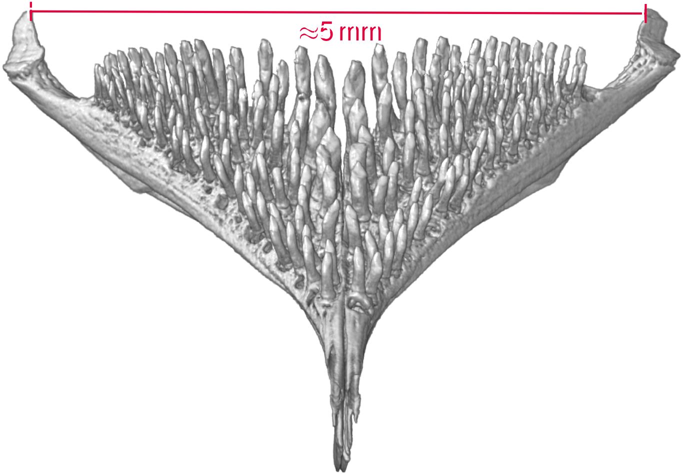
Visualization of cichlid head



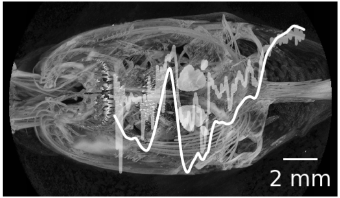
Visualization of cichlid head

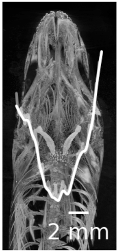


Visualization of segmented pharyngeal jaw

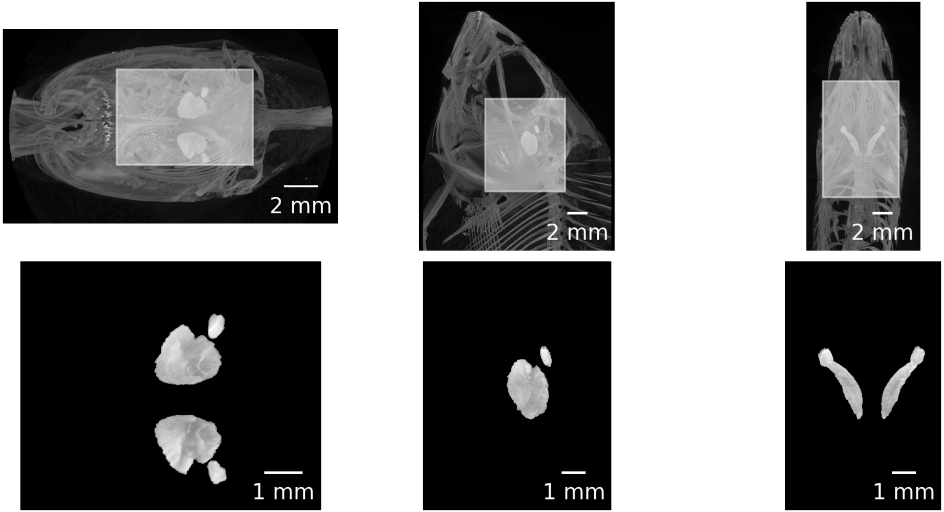


Data wrangling by example: Cichlids





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 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.