b
UNIVERSITY
OF BERN

X-ray microtomography

485018-HS2025-0: Advanced Course II Ultraprecision Engineering

David Haberthür
Institute of Anatomy, September 16, 2025

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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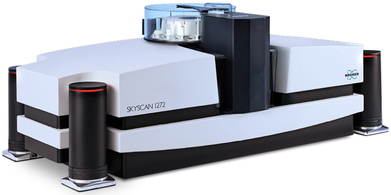
µCT-group

- 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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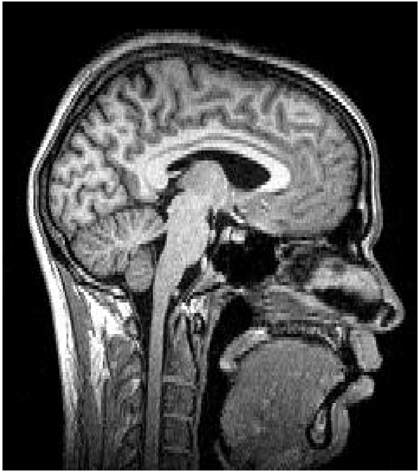
Contents

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
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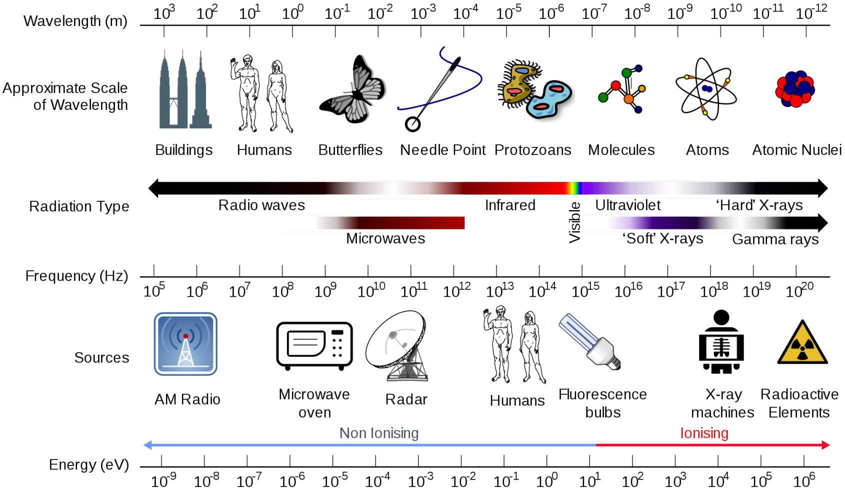
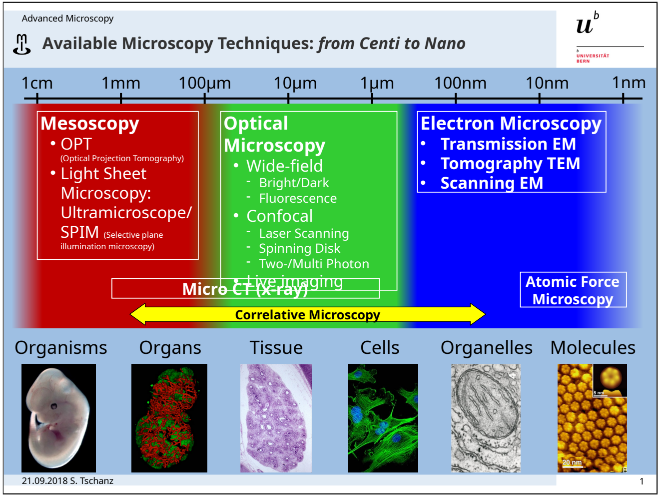
Wavelengths & Scales



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

CT-Scanner

youtu.be/2CWpZKuy-NE

CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays

w.wiki/BHAN

CT History

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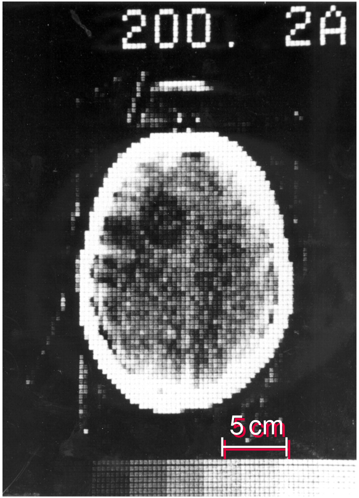
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]



From [14], Figure 5

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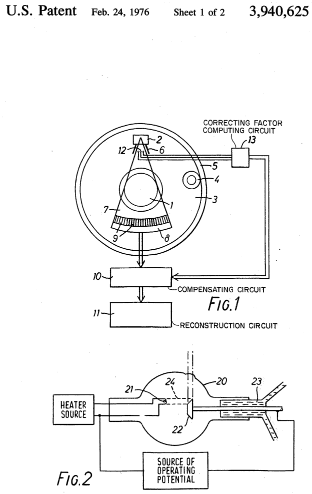

From [**US3940625A**], p. 2

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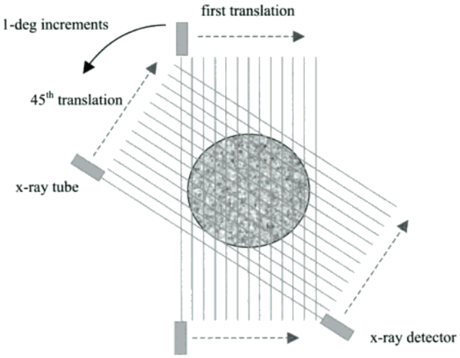
- CT scanner generations
  - First generation



From [15], Figure 1.12

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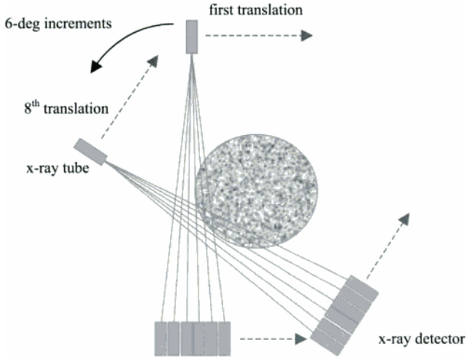
- CT scanner generations
  - First generation
  - Second generation



From [15], Figure 1.13

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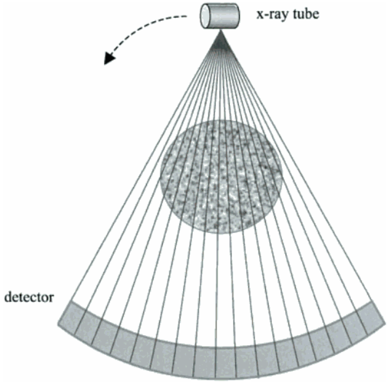
  - First generation
  - Second generation
  - Third generation



From [15], Figure 1.14

µCT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [**Lin2019**]
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp [16] developed an early laboratory microCT system 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
- Feldkamp 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**]

µCT History II

- µCT was first reported in the 1980s, for scanning gemstones
- Early 1990s: Manufacturers like SkyScan and Scanco Medical made µCT systems commercially available
- Today: Nondestructive imaging for quantifying the (micro)structure of (organic) materials
  - Mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
  - Teeth and their internal details
  - Tissues, small animals, and medical devices like stents and implants
  - Soft tissues and vasculature using radio-opaque contrast agents
  - Characterization of anatomical details in high resolution
- $\approx$2500 µCT systems are in use worldwide with over 1000 publications annually

X-ray interaction

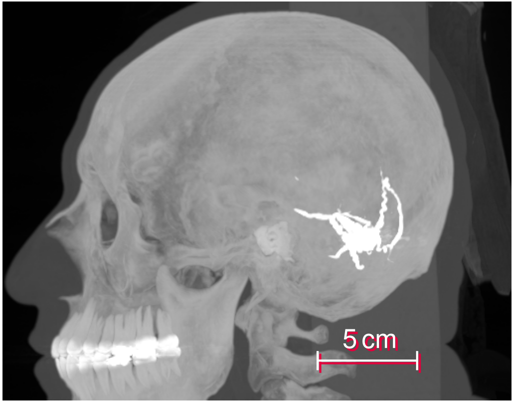
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([19])
  - Photoelectric absorption (τ) is strongly dependent on 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	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 µCT?



From [21], Subject *C3L-02465*

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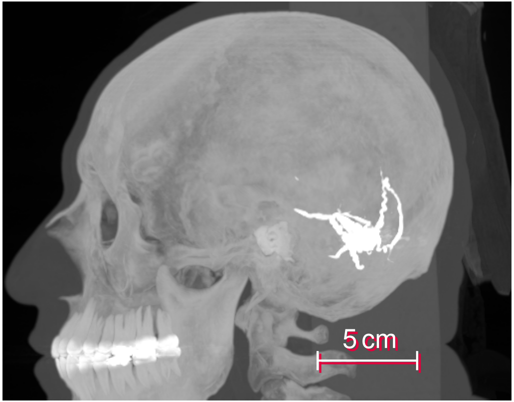

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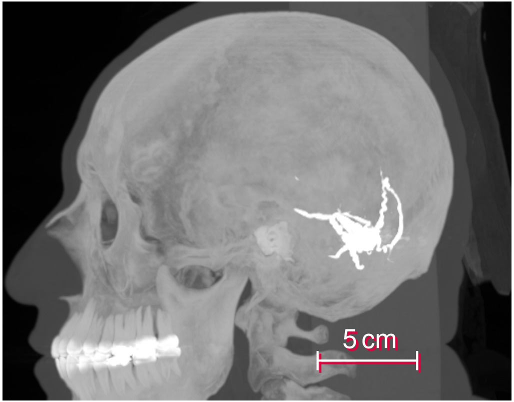

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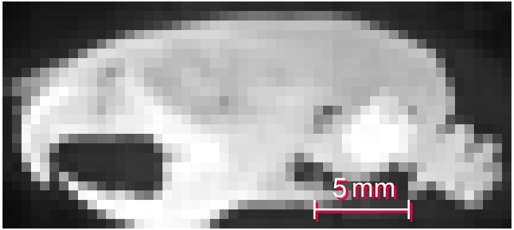

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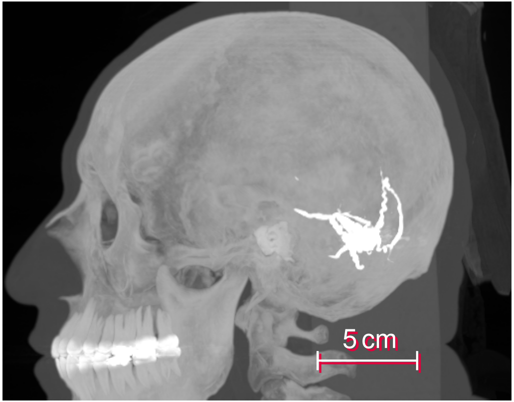

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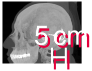

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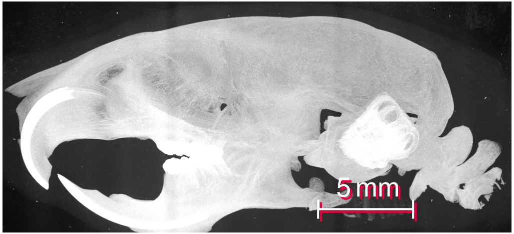
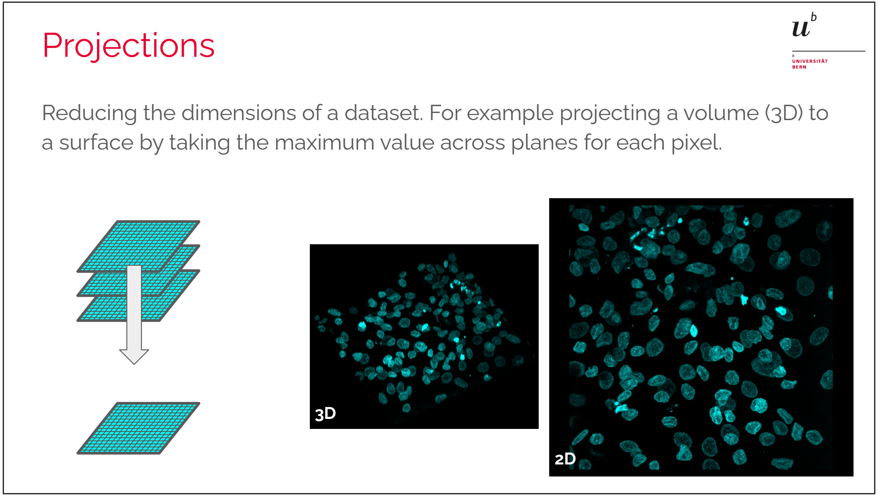


Maximum intensity projection



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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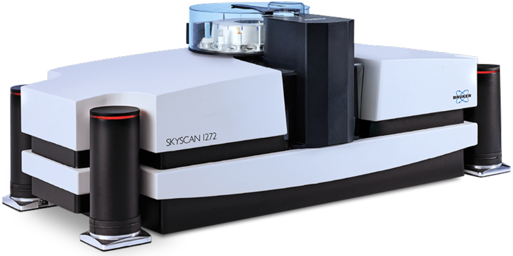

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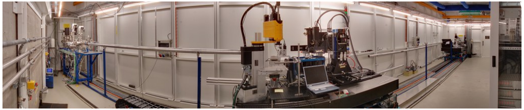

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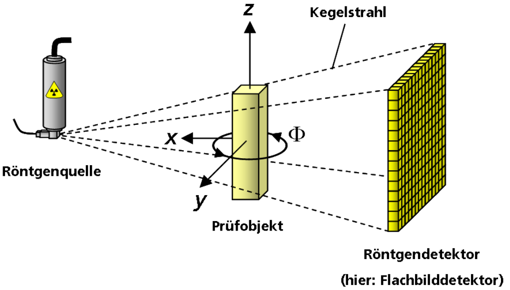
What is happening?

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

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



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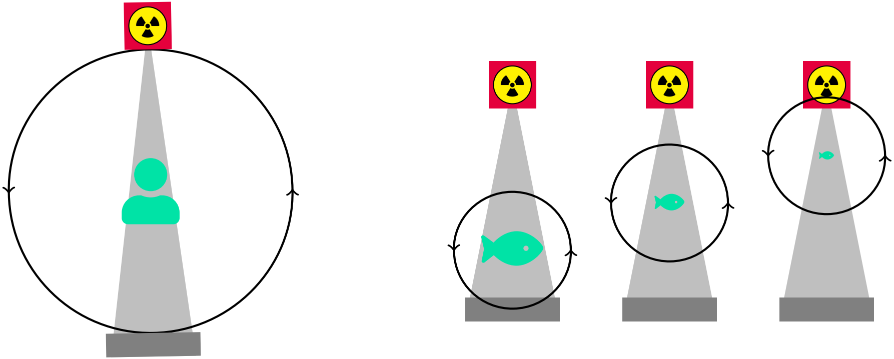
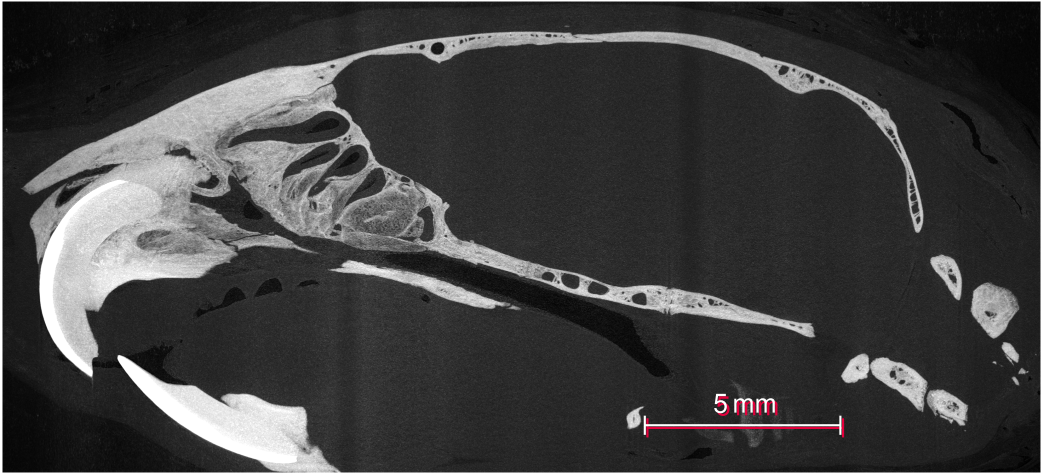
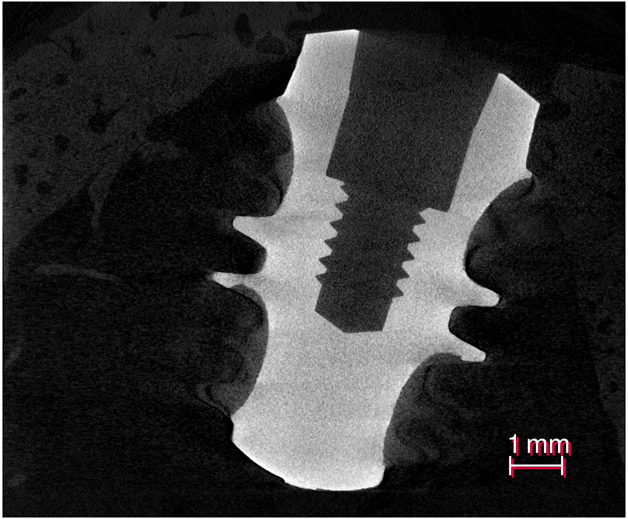
Machinery



Examples



Examples



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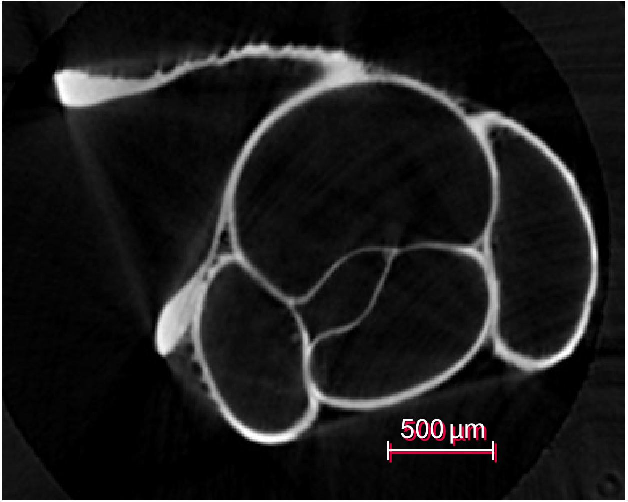
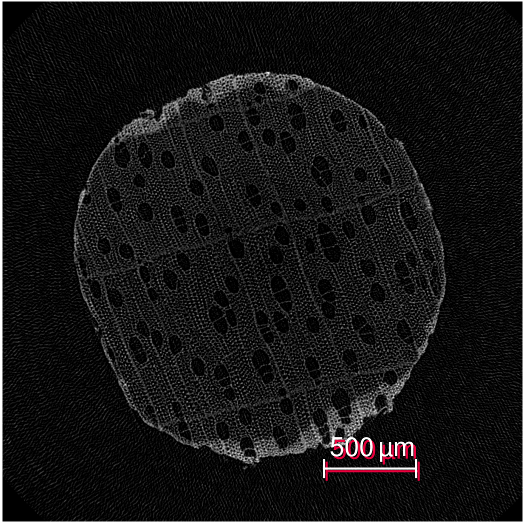


From [8], *Diancta phoenīx*

Examples



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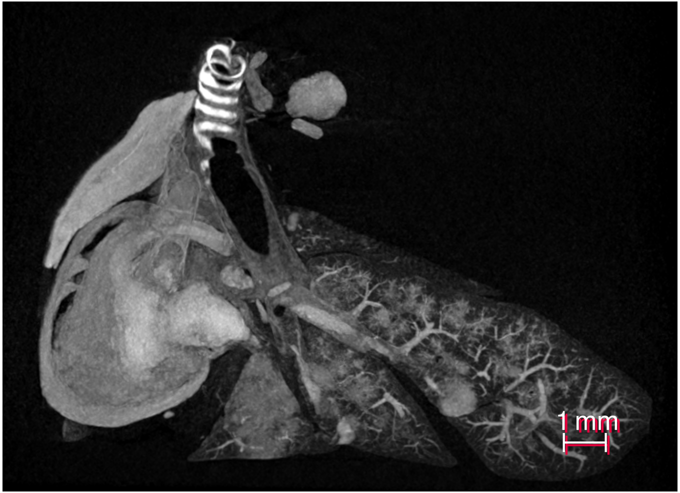
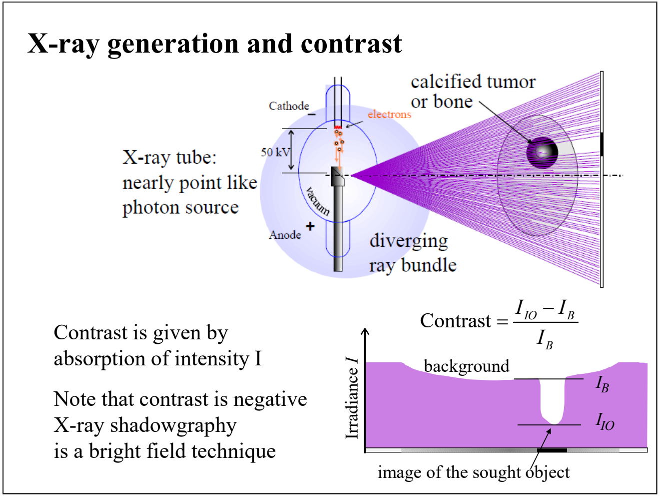


Preparation

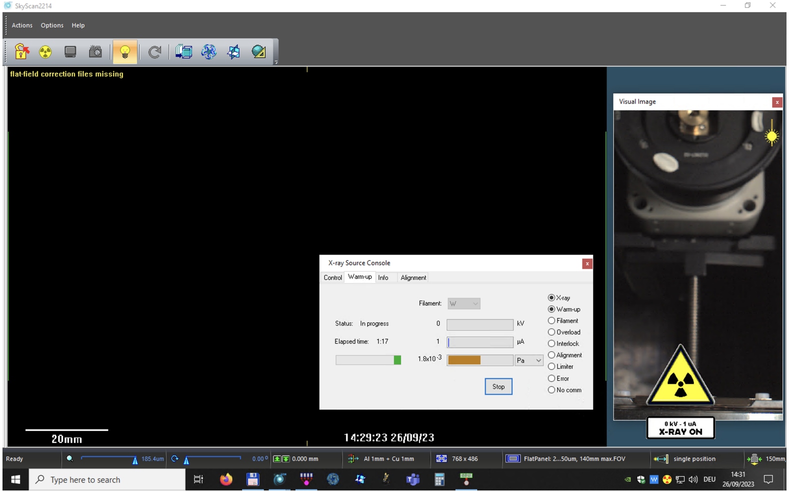
- Study design
- Sample preparation

Projections



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

Projection acquisition



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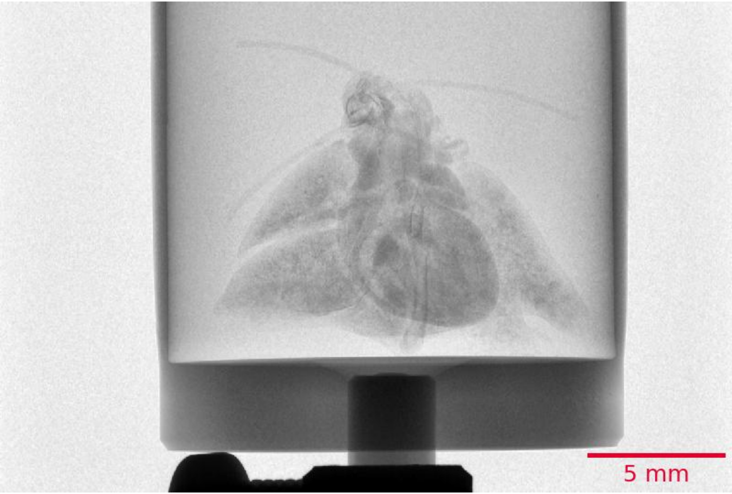
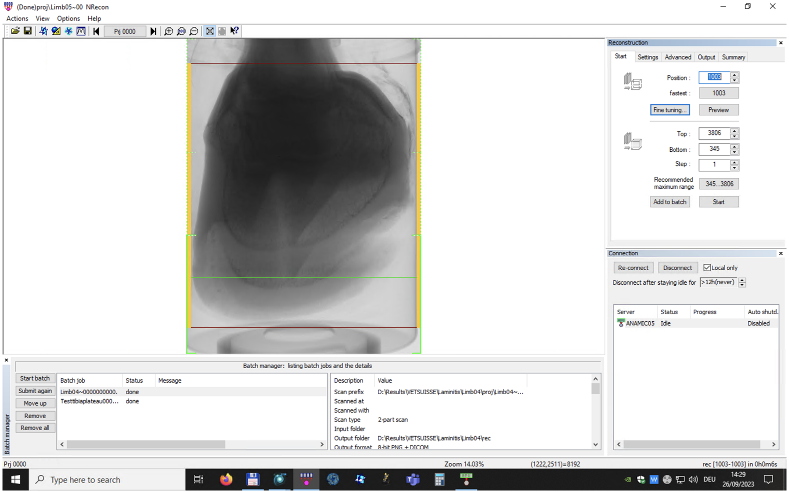


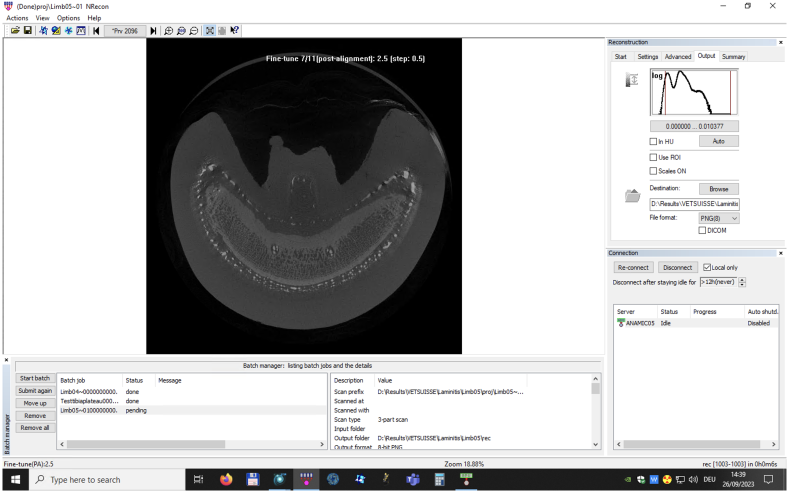
Projections

- 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 on the 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!

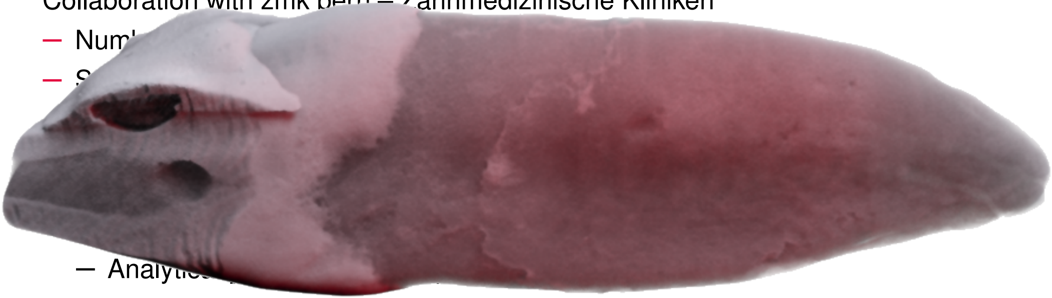
Quantitative data

- Pretty images are nice to have, but science is built on 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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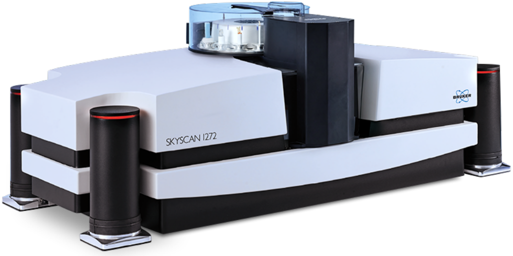

bruker.com/skyscan1272

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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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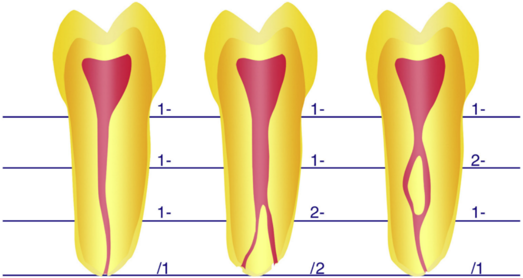

From [25], Fig. 2

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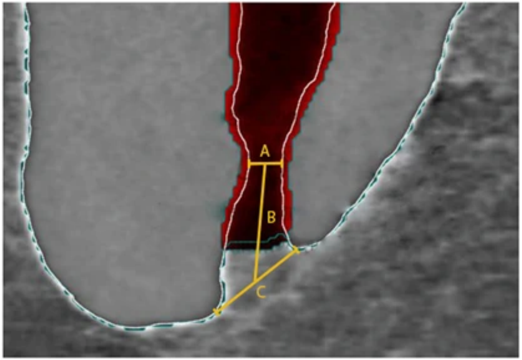

From [27], Fig. 1

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gph.is/2nqkple

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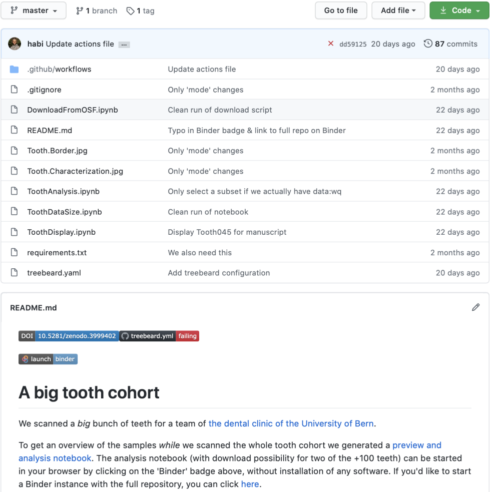
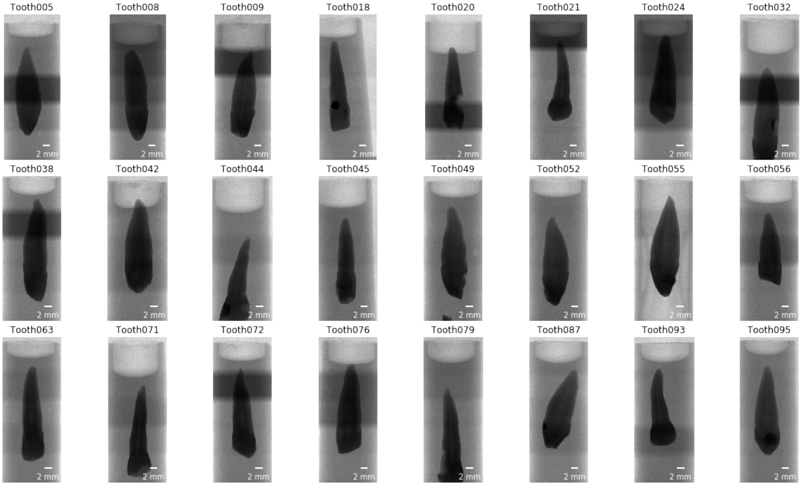


µCT imaging


Tooth005
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Tooth044
Tooth045
Tooth049
Tooth052
Tooth055
Tooth056
Tooth063
Tooth071
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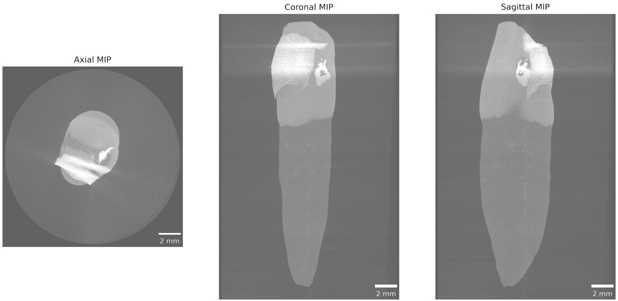
Tooth079
Tooth087
Tooth093
Tooth095
2 mm

Dataset cropping

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



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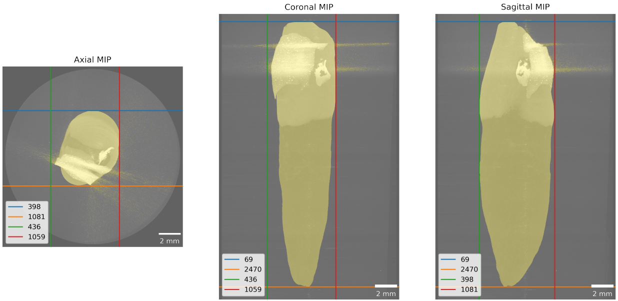
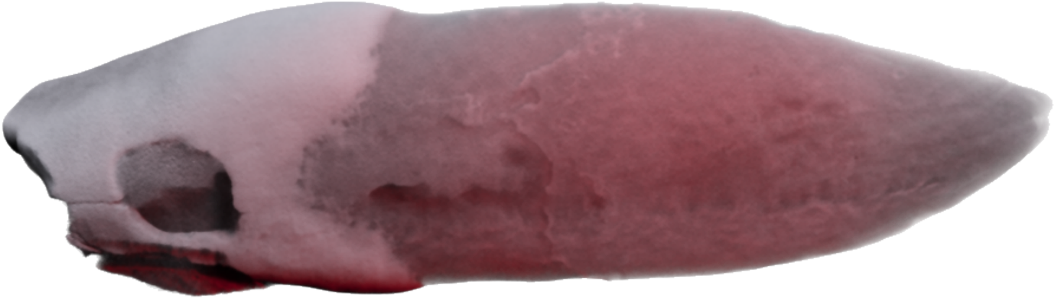
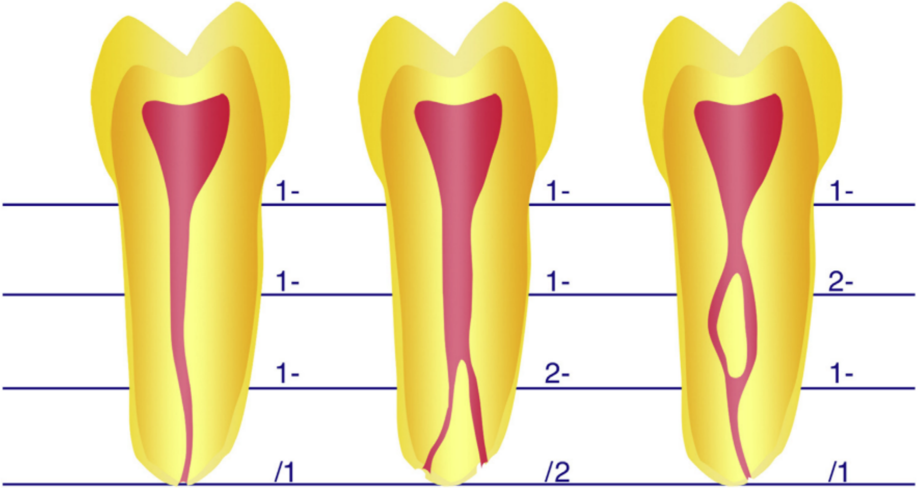


Tooth morphology

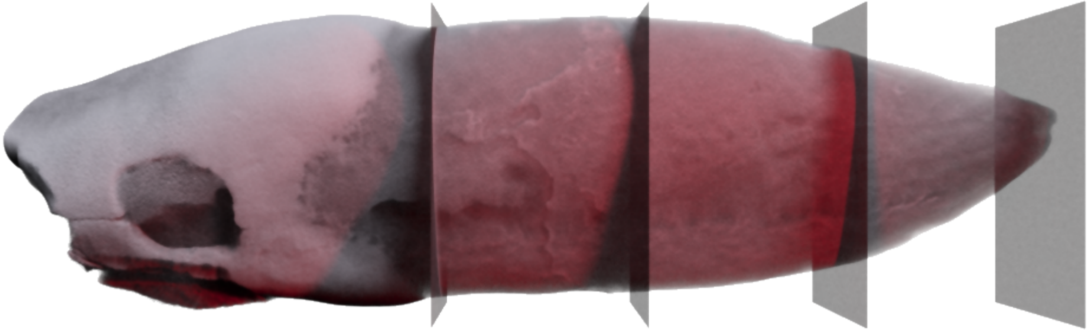


Tooth morphology

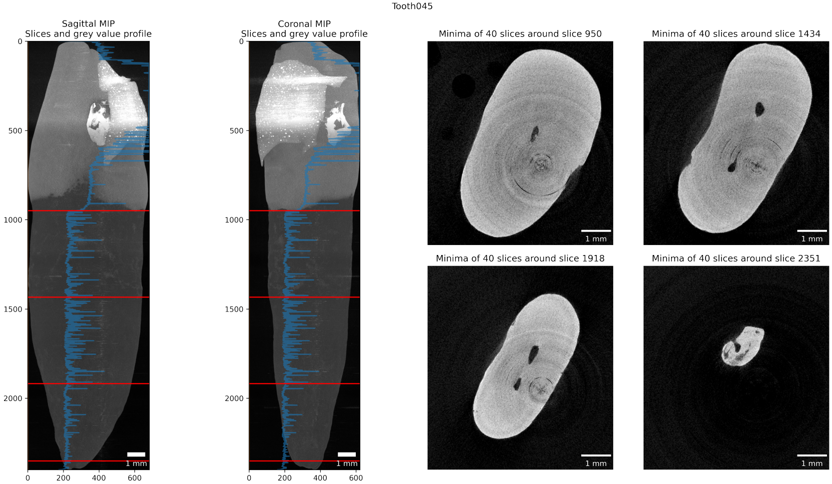


From [25], Fig. 2

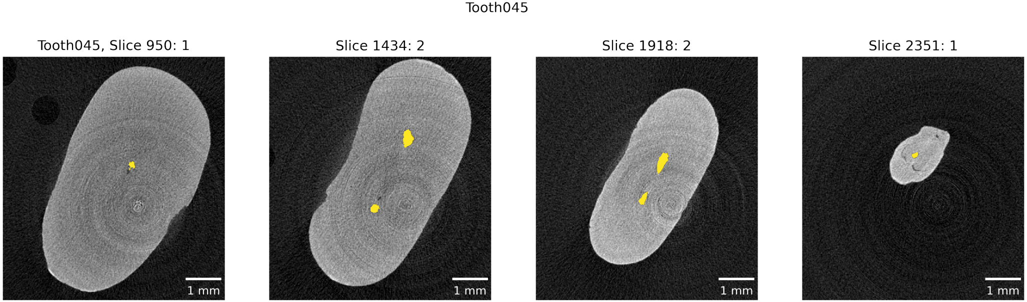
Tooth morphology



Detection of enamel-dentin border



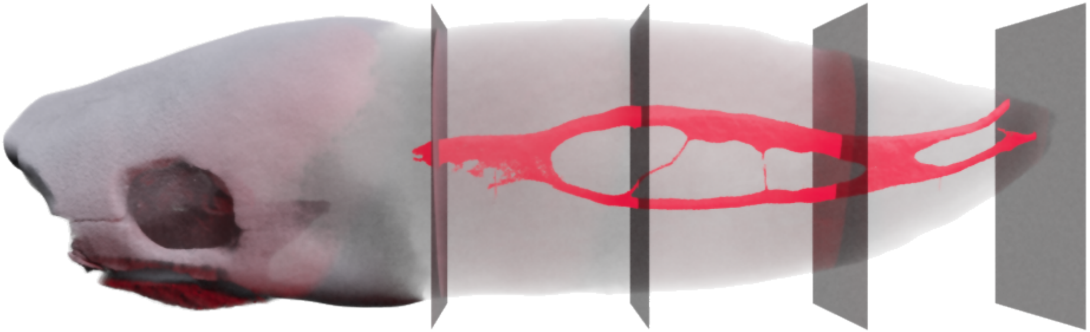
Detection of enamel-dentin border



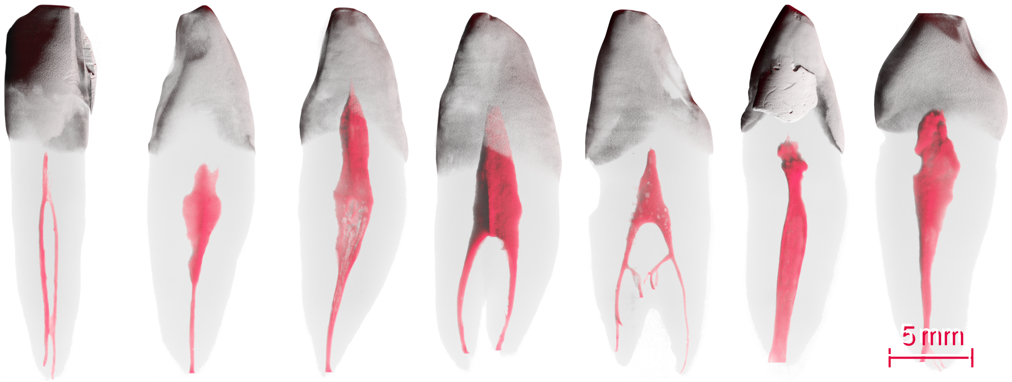
Classification of root canal configurations

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

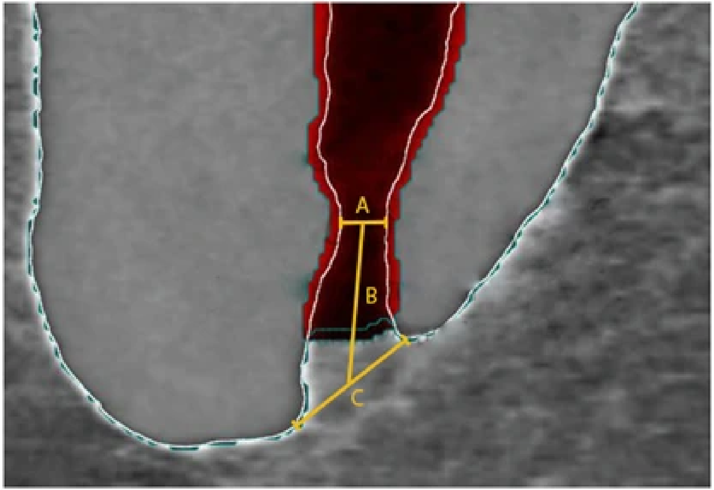
Extraction of root canal space



Results of root canal space extraction

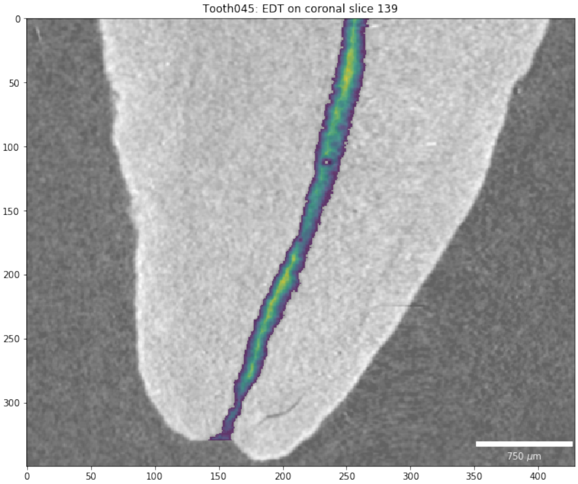


Physiological foramen geometry



From [27], Fig. 1

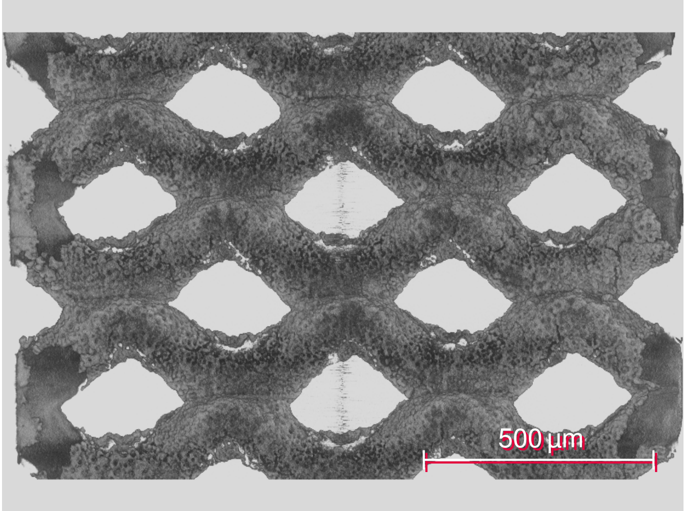
Physiological foramen geometry



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

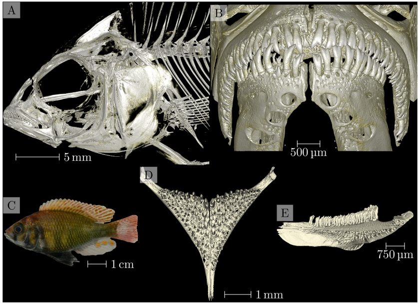


Etienne Berner NanoElectroCatalysis Group

Data wrangling by example: Cichlids

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

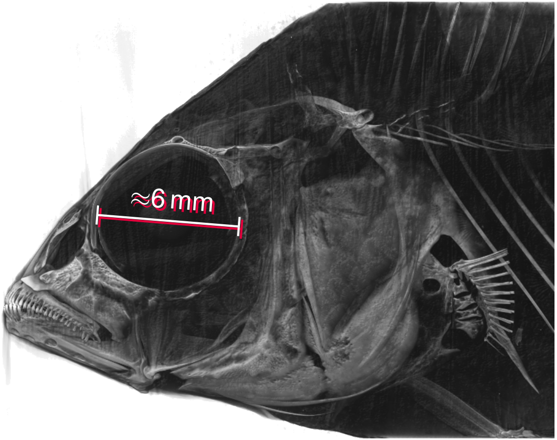
- 133 Cichlids from Lake Victoria, East Africa
  - Functional anatomy of the skulls and jaws
  - 6–18 cm in size
- 375 scans in total
  - Voxelsizes from 3.5–50 µm
  - 46 days of scanning time
  - 9.8 TB of raw data
  - 1.5 TB/+1 000 000 reconstructions



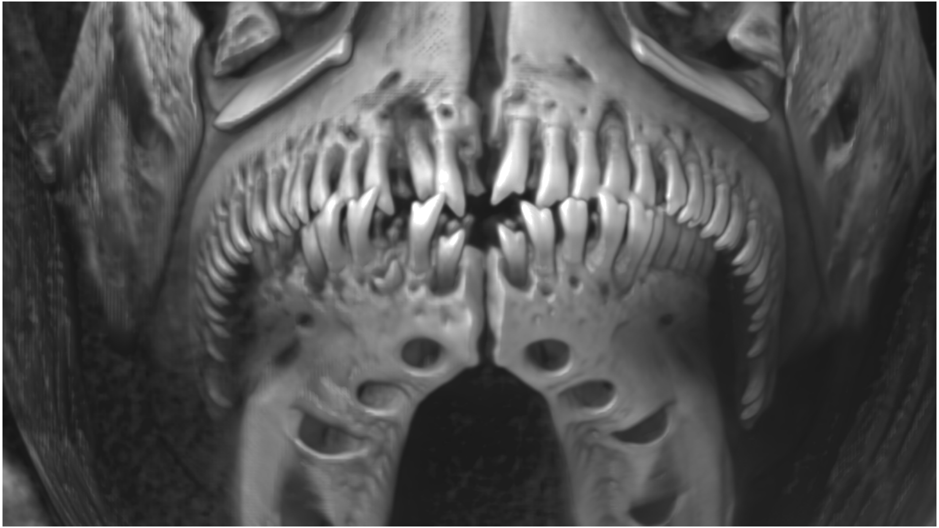
DOI:gsst8t, Fig. 1

[a] 11.

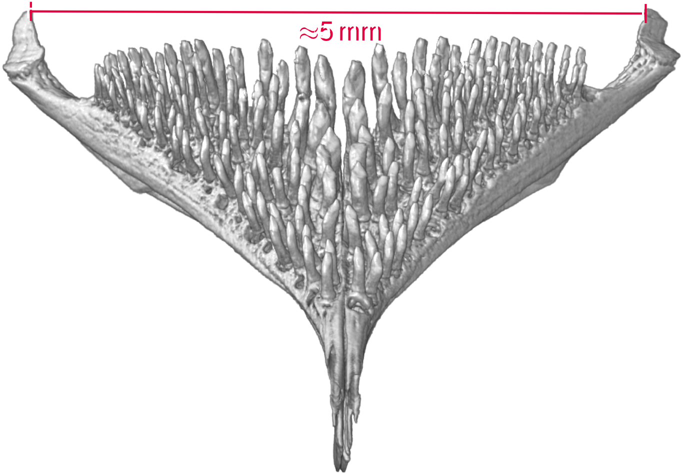
Visualization of cichlid head



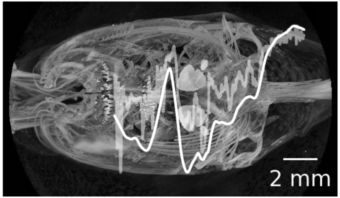
Visualization of cichlid head

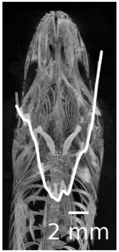


Visualization of segmented pharyngeal jaw

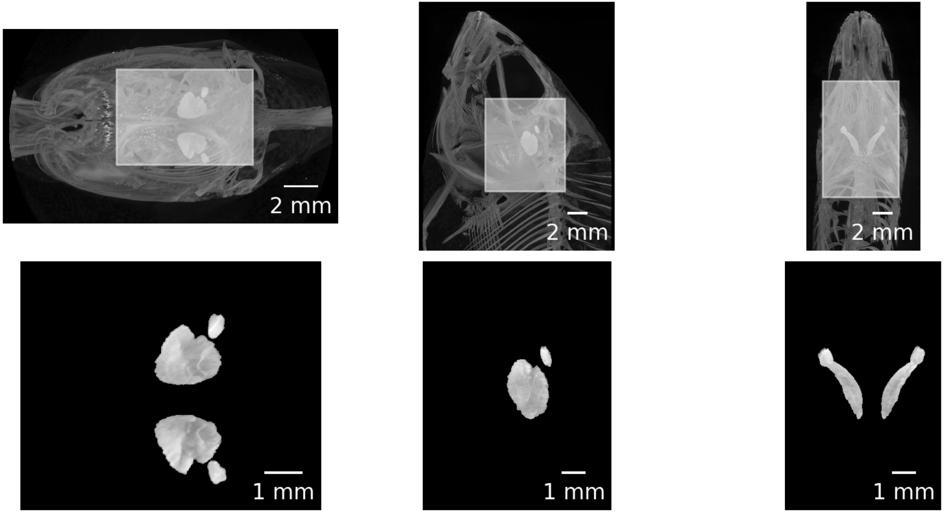


Data wrangling by example: Cichlids





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] Ruslan Hlushchuk et al. “Cutting-Edge Microangio-CT: New Dimensions in Vascular Imaging and Kidney Morphometry”. In: (Mar. 2018). DOI: 10.1152/ajprenal.00099.2017.

[2] Henry Nording et al. “The C5a/C5a Receptor 1 Axis Controls Tissue Neovascularization through CXCL4 Release from Platelets”. In: (Dec. 2021). DOI: 10.1038/s41467-021-23499-w.

[3] Ruslan Hlushchuk et al. “Innovative High-Resolution microCT Imaging of Animal Brain Vasculature”. In: (Oct. 2020). DOI: 10.1007/s00429-020-02158-8.

[4] Tsering Wüthrich et al. “Development of Vascularized Nerve Scaffold Using Perfusion-Decellularization and Recellularization”. In: (Aug. 2020). DOI: 10.1016/j.msec.2020.111311.

[5] Cédric Zubler et al. “The Anatomical Reliability of the Superficial Circumflex Iliac Artery Perforator (SCIP) Flap”. In: (Mar. 2021). DOI: 10.1016/j.aanat.2020.151624.

[6] Matthias Messerli et al. “Adaptation Mechanism of the Adult Zebrafish Respiratory Organ to Endurance Training”. In: (Feb. 2020). DOI: 10.1371/journal.pone.0228333.

[7] Verdiana Trappetti et al. “Synchrotron Microbeam Radiotherapy for the Treatment of Lung Carcinoma: A Pre-Clinical Study”. In: (Aug. 2021). DOI: 10.1016/j.ijrobp.2021.07.1717.

[8] Estée Bochud et al. “A New Diancta Species of the Family Diplommatinidae (Cyclophoroidea) from Vanua Levu Island, Fiji”. In: (Nov. 2021). DOI: 10.3897/zookeys.1073.73241.

References II

[9] Sebastian Halm et al. “Micro-CT Imaging of Thiel-embalmed and Iodine-Stained Human Temporal Bone for 3D Modeling”. In: (2021). DOI: 10.1186/s40463-021-00522-0.

[10] David Haberthür et al. “Automated Segmentation and Description of the Internal Morphology of Human Permanent Teeth by Means of Micro-CT”. In: (Apr. 2021). DOI: gjpw2d.

[11] David Haberthür et al. “Microtomographic Investigation of a Large Corpus of Cichlids”. In: (Sept. 2023). DOI: gsst8t.

[12] A. M. Cormack. “Representation of a Function by Its Line Integrals, with Some Radiological Applications”. In: (Sept. 1963). DOI: 10.1063/1.1729798.

[13] Godfrey Newbold Hounsfield. “Historical Notes on Computerized Axial Tomography.”. In: (1976).

[14] E C Beckmann. “CT Scanning the Early Days.”. In: (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”. In: (June 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”. In: (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”. In: (Nov. 1988). DOI: 10.1002/art.1780311109.

References III

[19] Mark 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] Kenneth Clark et al. “The Cancer Imaging Archive (TCIA): Maintaining and Operating a Public Information Repository”. In: (Dec. 2013). DOI: 10.1007/s10278-013-9622-7.

[22] Johannes Schindelin et al. “Fiji: An Open-Source Platform for Biological-Image Analysis”. In: (July 2012). DOI: 10.1038/nmeth.2019.

[23] Thomas Kluyver et al. “Jupyter Notebooks – a Publishing Format for Reproducible Computational Workflows”. In: (2016). DOI: 10.3233/978-1-61499-649-1-87.

[24] 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”. In: (2021). DOI: g7r8.

[25] Benjamín Briseño-Marroquín et al. “Root Canal Morphology and Configuration of 179 Maxillary First Molars by Means of Micro–Computed Tomography: An Ex Vivo Study”. In: (Dec. 2015). DOI: 10.1016/j.joen.2015.09.007.

[26] David Haberthür. “Habi/Zmk-Tooth-Cohort: Used for Manuscript about Method”. In: (Aug. 2020). DOI: 10.5281/ZENODO.3999402.

[27] Thomas Gerhard Wolf et al. “Three-Dimensional Analysis of the Physiological Foramen Geometry of Maxillary and Mandibular Molars by Means of Micro-CT”. In: (Sept. 2017). DOI: 10.1038/ijos.2017.29.