

X-ray microtomography

David Haberthür

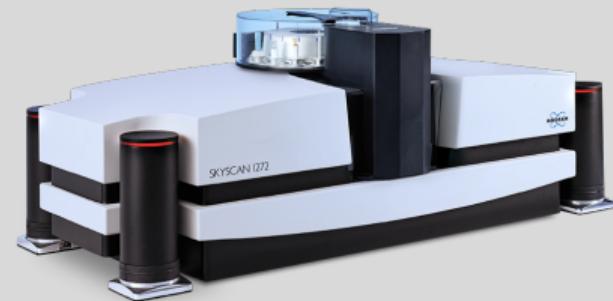
December 23, 2021 | 9256-HS2022-0: Advanced Microscopy

Grüessech!

- David Haberthür
 - Physicist by trade
 - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
 - Post-Doc I: Tomographic imaging at TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland and working on the detector of the GlobalDiagnosiX project
 - Post-Doc II & currently: Tomographic imaging in the μ CT group, Institute of Anatomy, University of Bern, Switzerland

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 our institute [9] to scan a wide range of specimens
- Automate *all* the things! [10]



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Contents

Overview

Imaging

Tomography

History

Interaction of X-rays with matter

Tomography today

A scan, from *getting started* to *getting nice images*

Example of a complete study (about teeth)

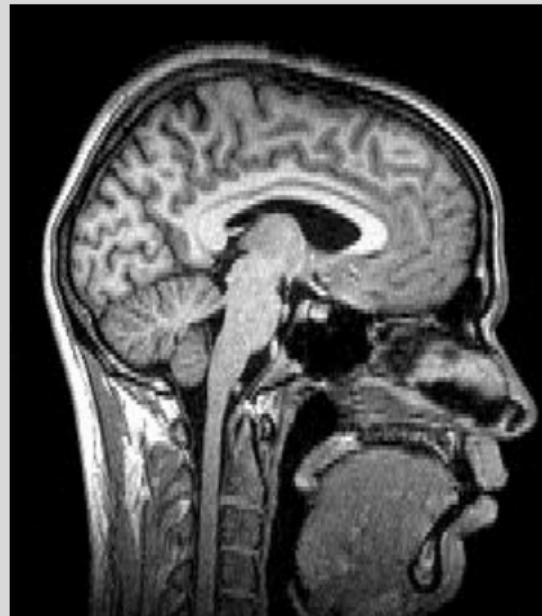
Overview

Materials & Methods

Results

Biomedical imaging

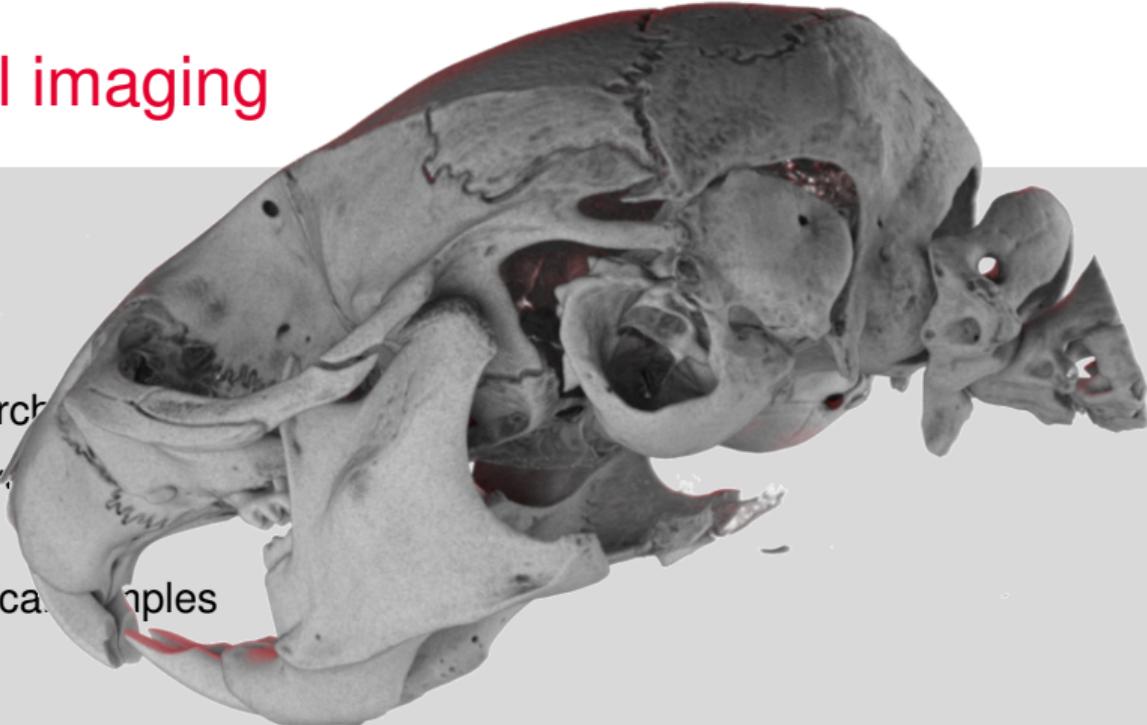
- Medical research
- Non-destructive insights into the samples
- (Small) biological samples



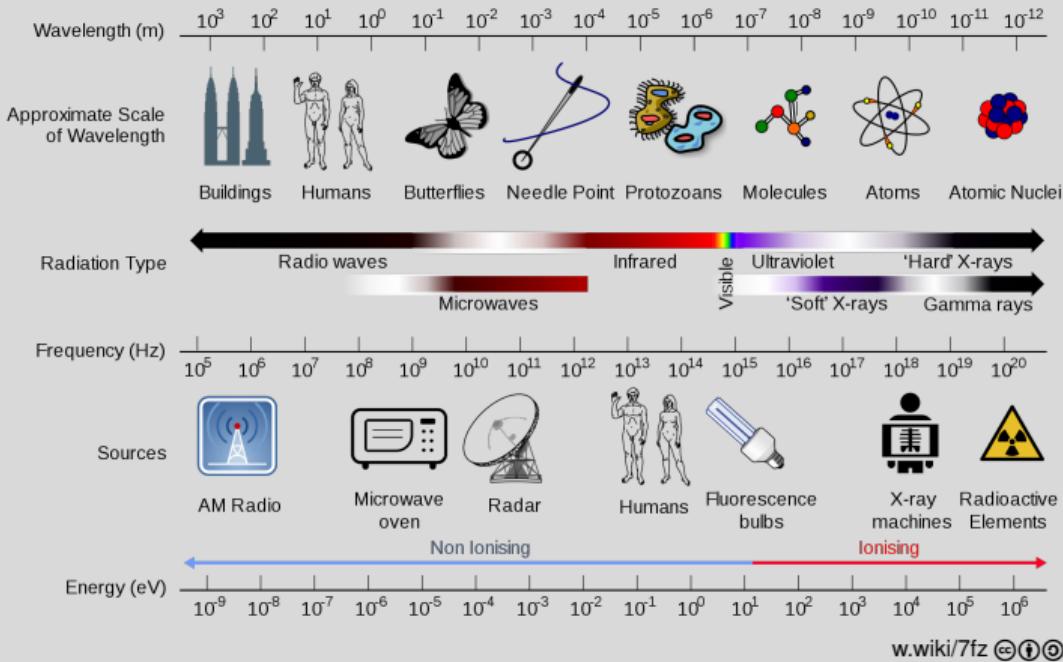
w.wiki/7g4 CC BY NC SA

Biomedical imaging

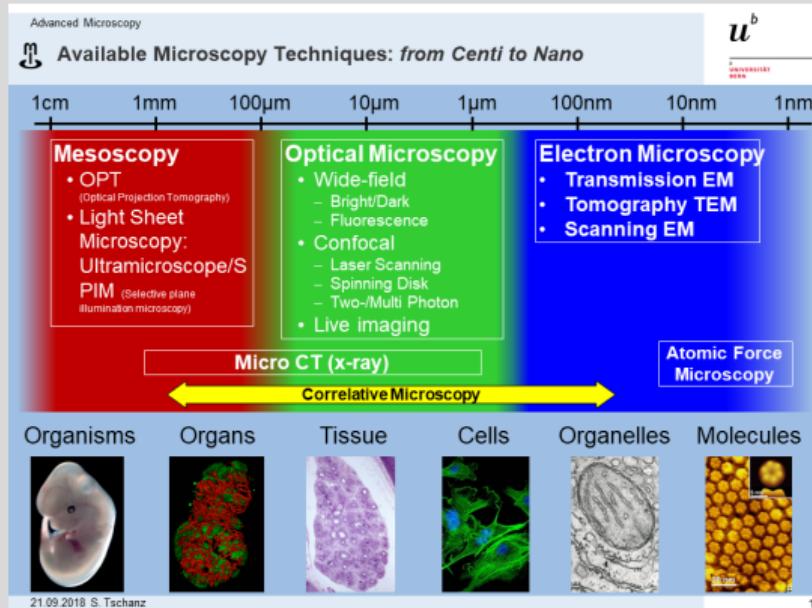
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- Non-destructive analysis of the samples
- (Small) biological samples



Wavelength and scales



Wavelength and scales

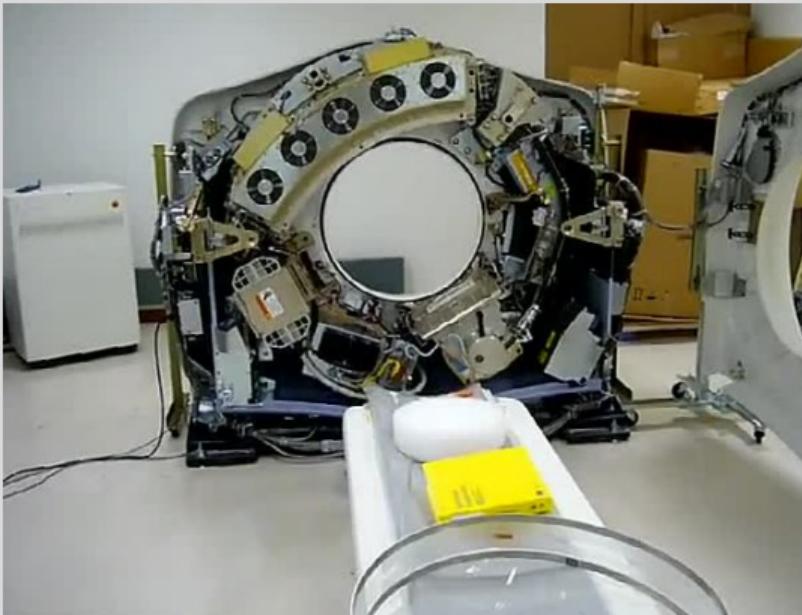


Stefan Tschanz, , with permission

Imaging methods

- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy: see lectures *Transmission Electron Microscopy* by Dimitri Vanhecke, *Scanning Electron Microscopy* by Sabine Kässmeyer & Ivana Jaric and *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

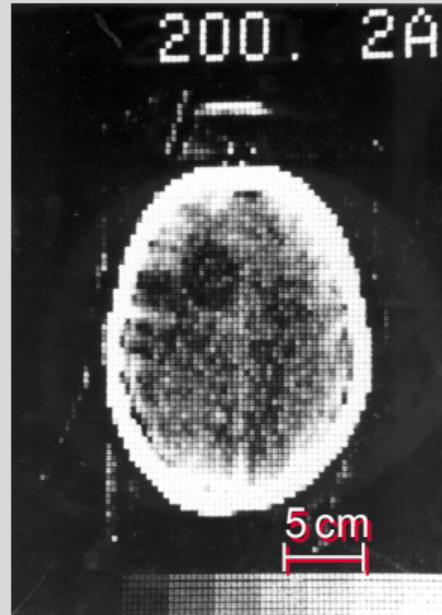
CT-Scanner



youtu.be/2CWpZKuy-NE

History

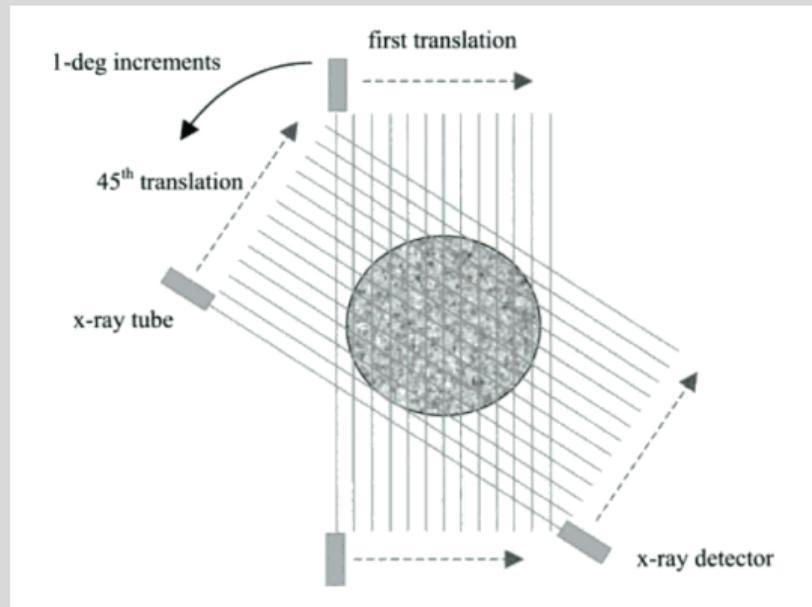
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]



From [14], Figure 5

History

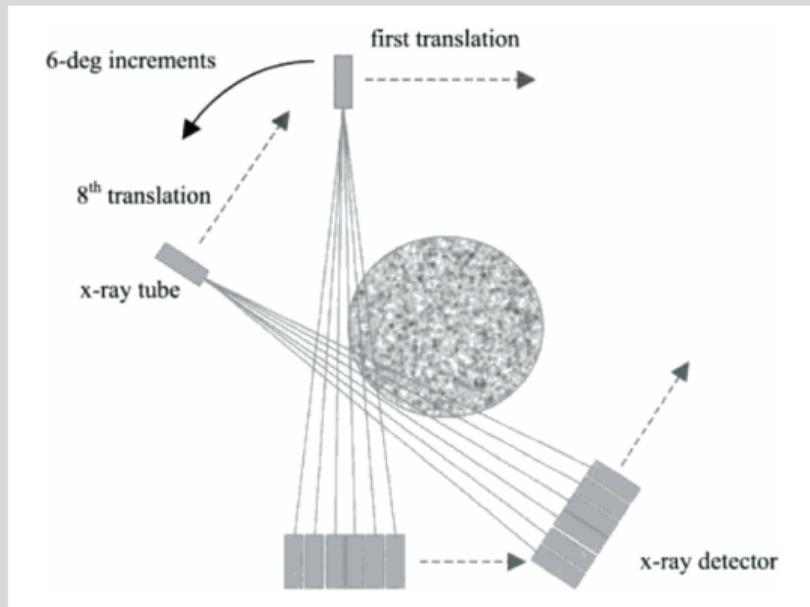
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- CT scanner generations: First



From [13], Figure 1.12

History

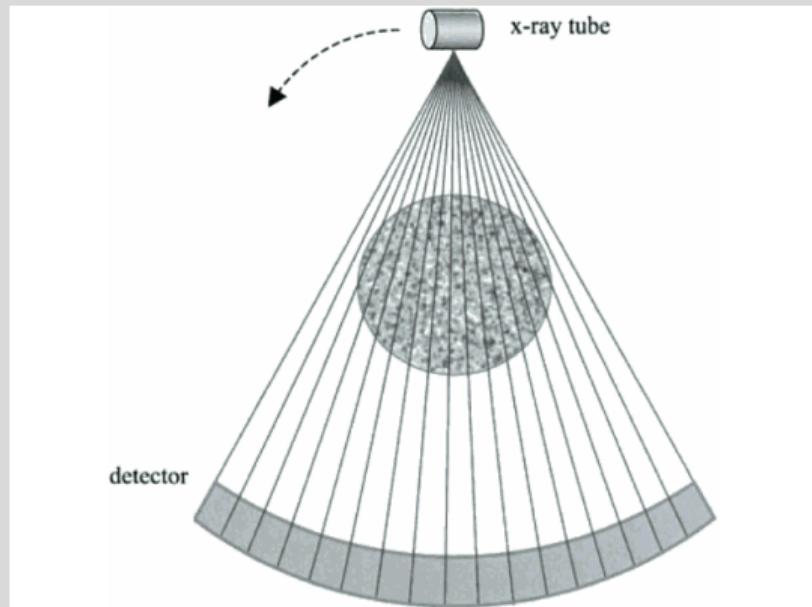
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]
- CT scanner generations: First, second



From [13], Figure 1.13

History

- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]
- CT scanner generations: First, second and third



From [13], Figure 1.14

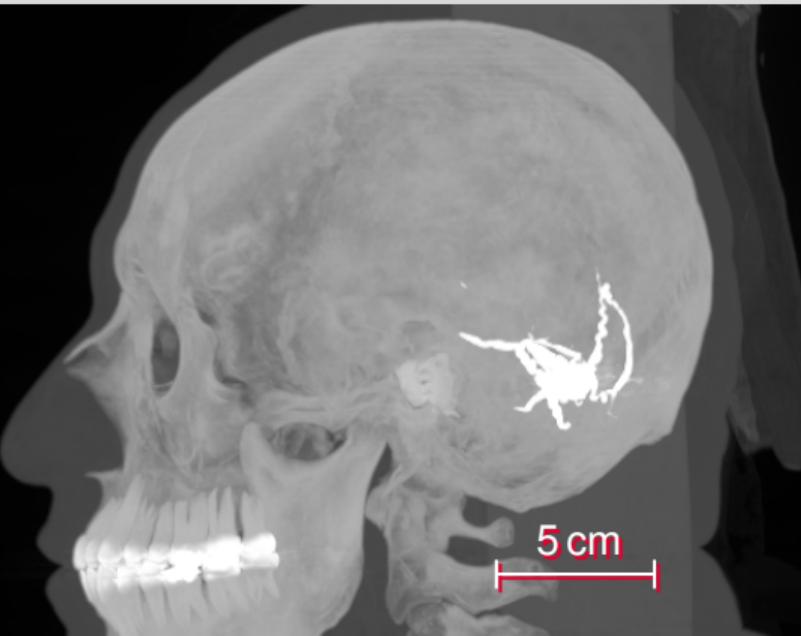
X-ray interaction

- “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 density and physical density of the material. Compton scattering 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] $I(t) = I_0 e^{-\alpha z}$)

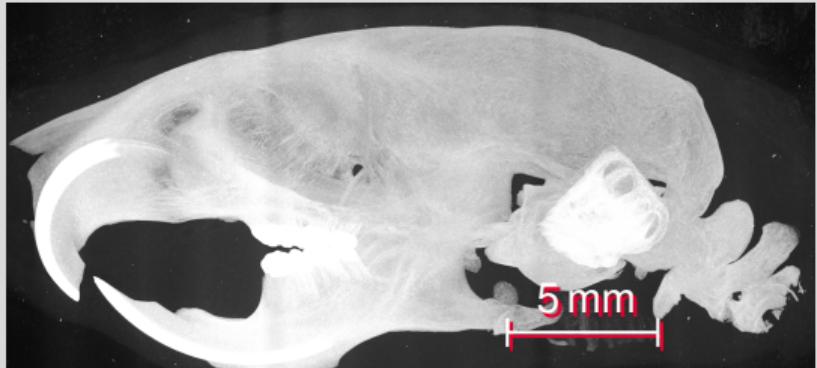
Composition of biological tissues

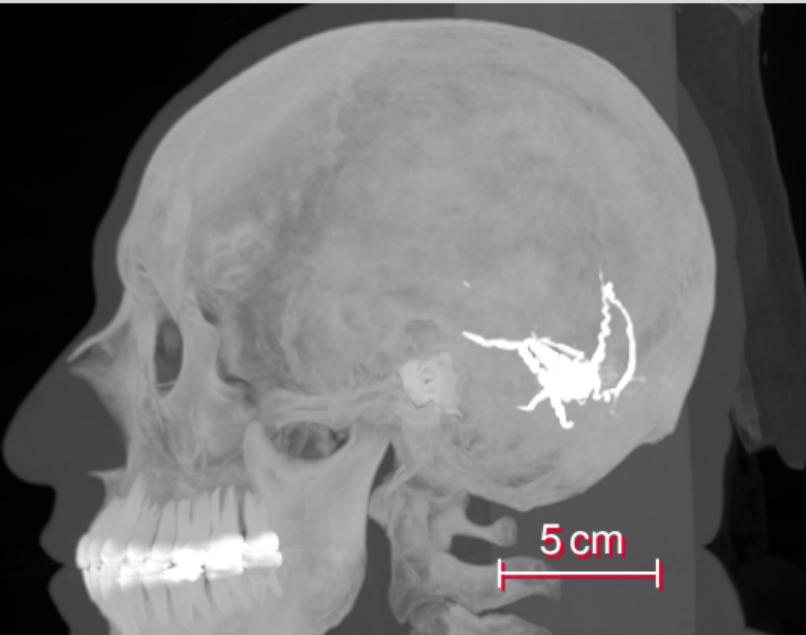
Tissue: content by mass percentage

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



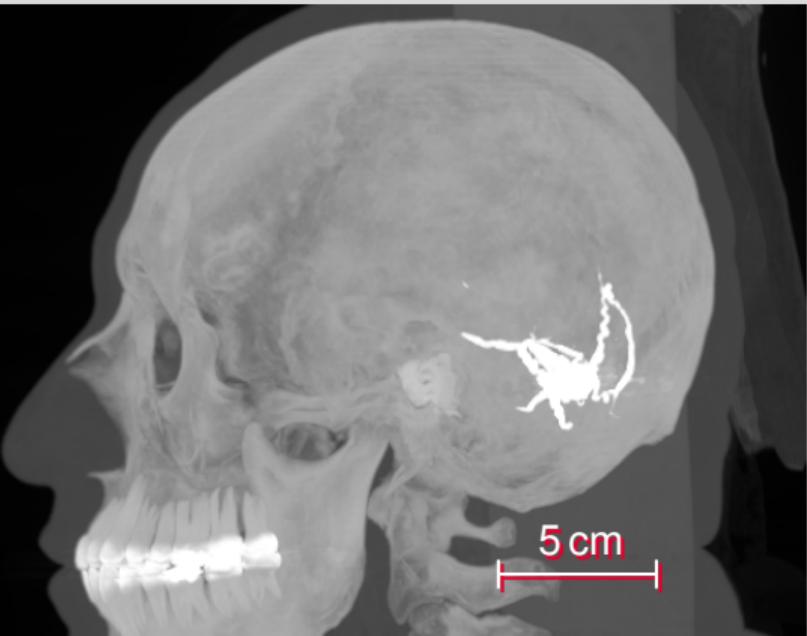
From [17], Subject C3L-02465



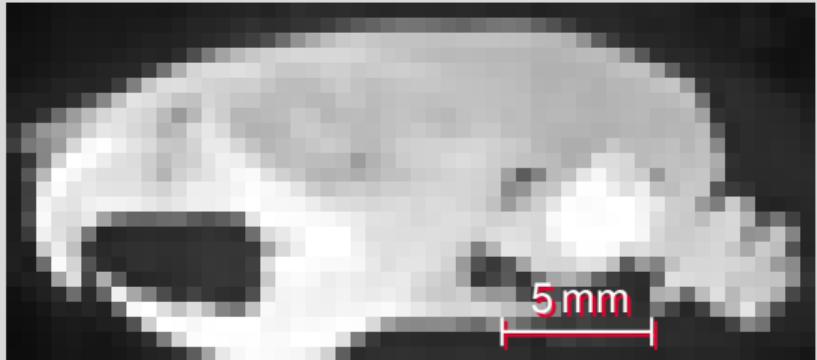


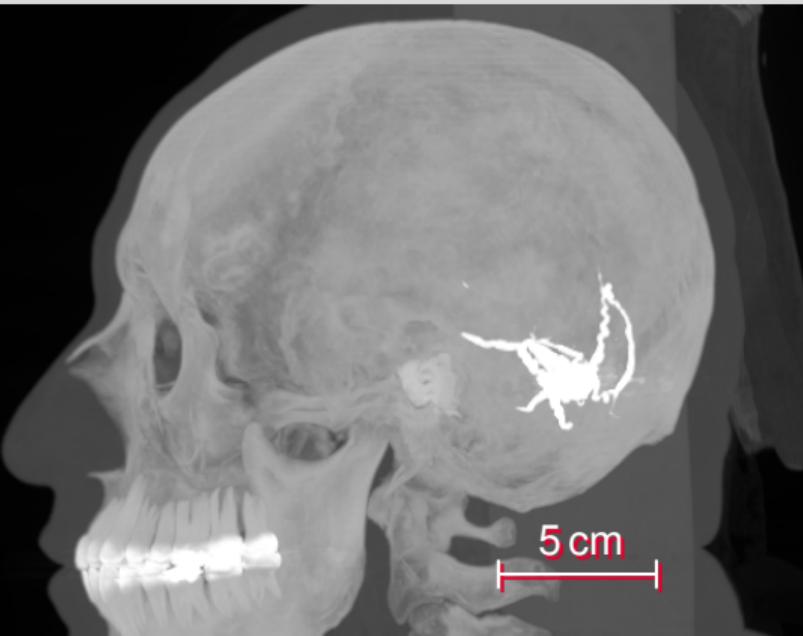
From [17], Subject C3L-02465



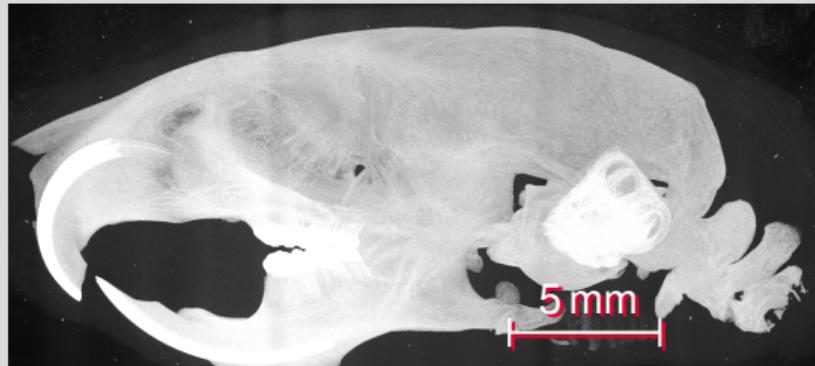


From [17], Subject C3L-02465

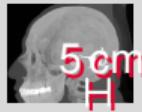




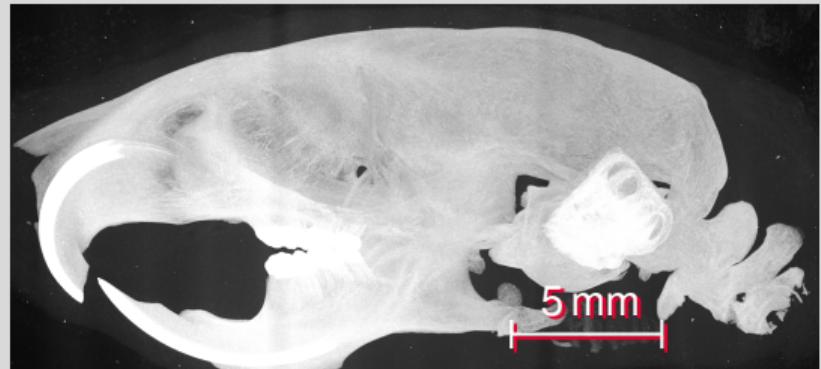
From [17], Subject C3L-02465



Why μ CT?



From [17], Subject C3L-02465

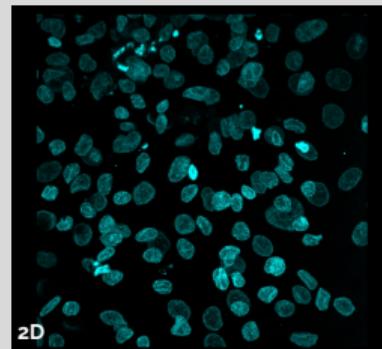
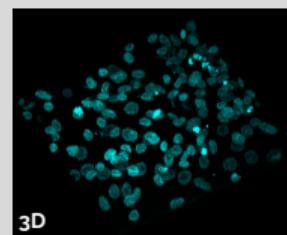
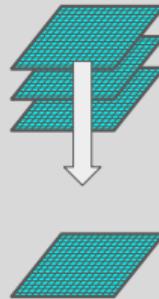


Maximum intensity projection

Projections



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



Fundamentals of Digital Image Processing by Guillaume Witz, Slide 23

Machinery

- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around $7 \mu\text{m}$ (*in vivo*) or $0.5 \mu\text{m}$ (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



flic.kr/p/D4rbom

Machinery

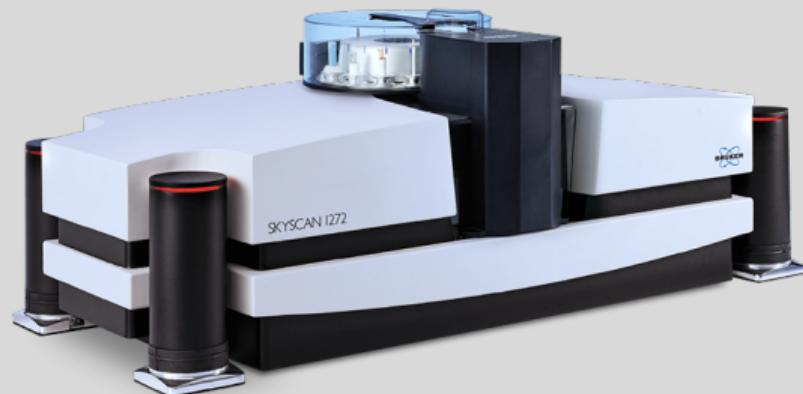
- Hospital CT
 - Voxel size around 0.5 mm
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 - Voxel size around 7 μm (*in vivo*) or 0.5 μm (*ex vivo*)
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flic.kr/p/fpTrGu

Machinery

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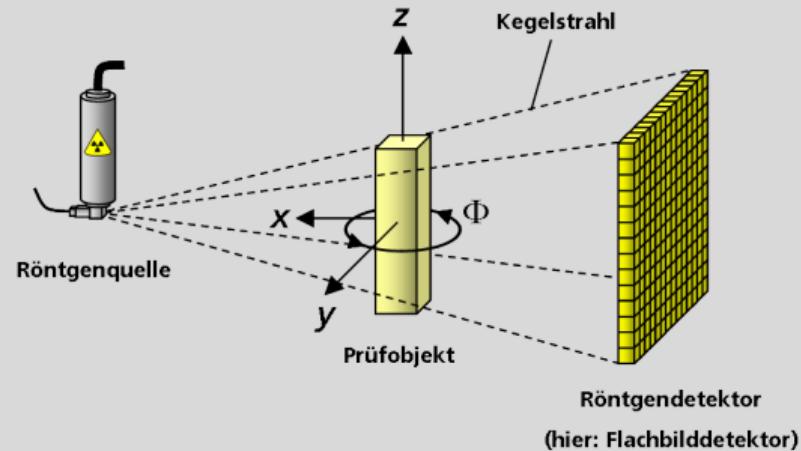


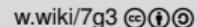
flic.kr/p/7Xhk2Y

What is happening?

The basic components of a computer tomography device are always

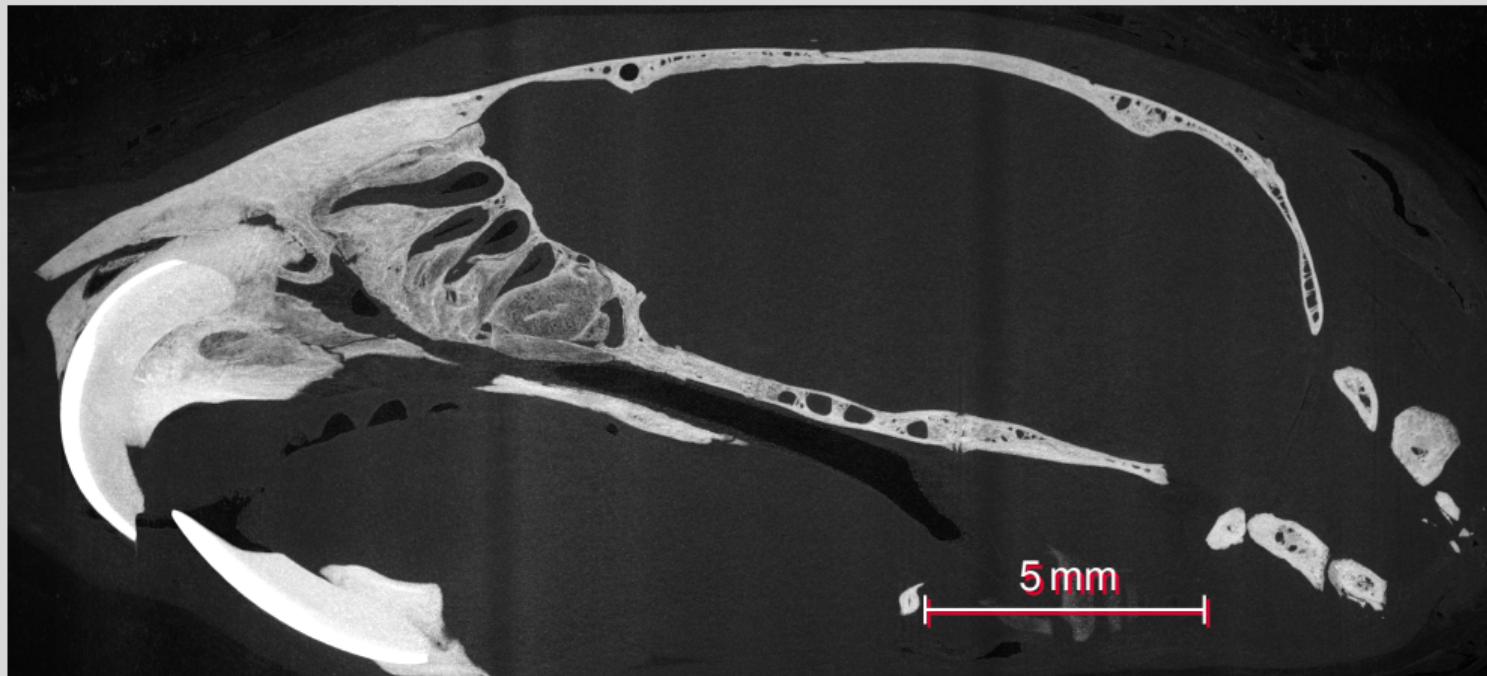
- an X-ray source
- something to image
- a detector



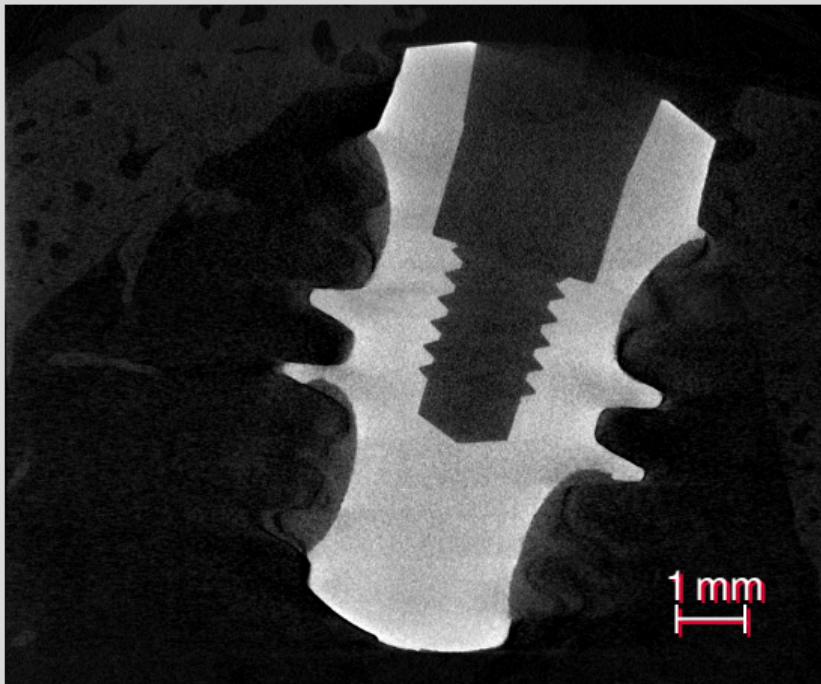
w.wiki/7g3 

Machinery

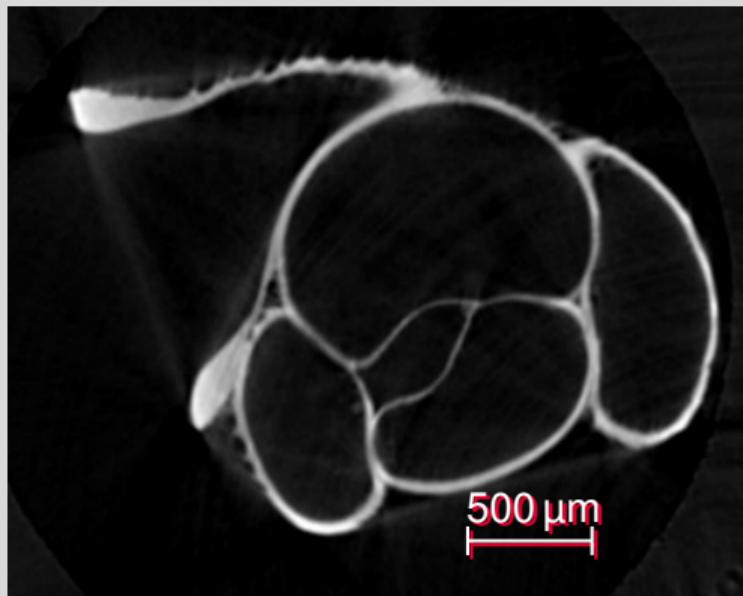
Examples



Examples

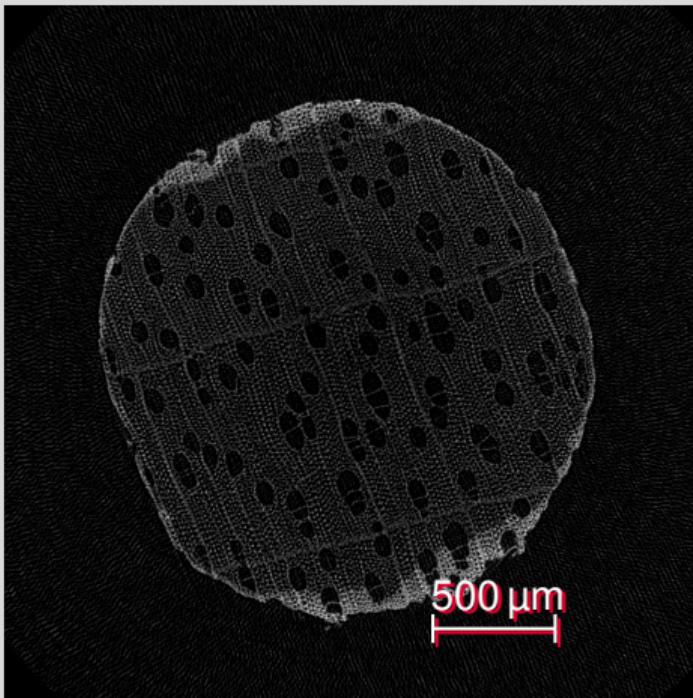


Examples



From [8], *Diancta phoenix*

Examples



Examples



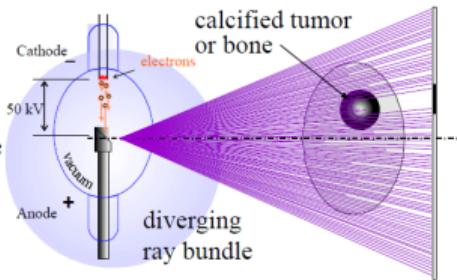
Preparation

- Study design
- Sample preparation

Projections

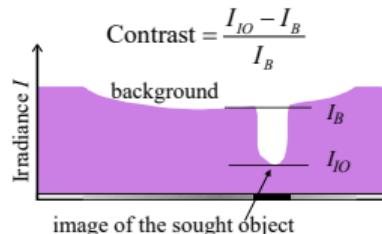
X-ray generation and contrast

X-ray tube:
nearly point like
photon source



Contrast is given by
absorption of intensity I

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



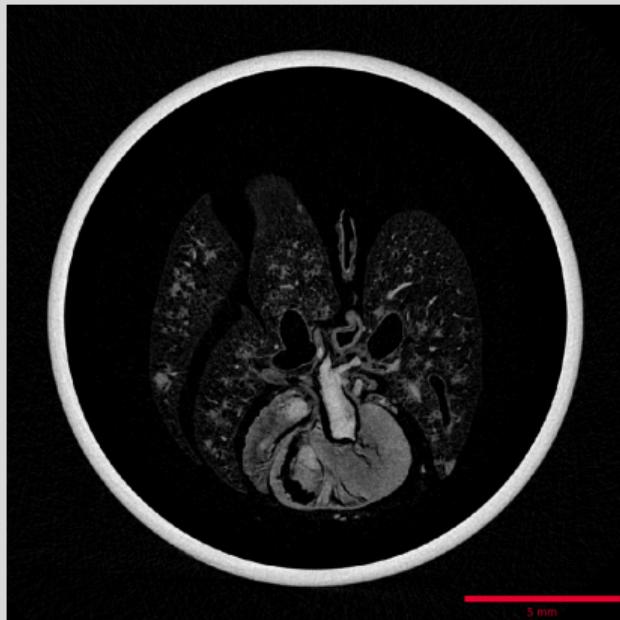
Projections



Projections

- A (micro-focus) X-ray source illuminates the object
- The X-rays penetrate the sample and are attenuated
- A scintillator converts the X-rays to visible light
- A (planar) X-ray detector collects (magnified) projection images.
- The projections are recorded on disk

Reconstructions



Reconstructions

- Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.
- Radon Transformation
- Filtered back projection
- Fan beam reconstruction
- Corrections (beam hardening, etc.)
- Writing to stack

Visualization



Visualization

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

What to use?

- ImageJ/Fiji [18]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
 -  in Jupyter [19]
 - `git`
 - Script all your things!
 - Data repositories; i. e. sharing is caring!

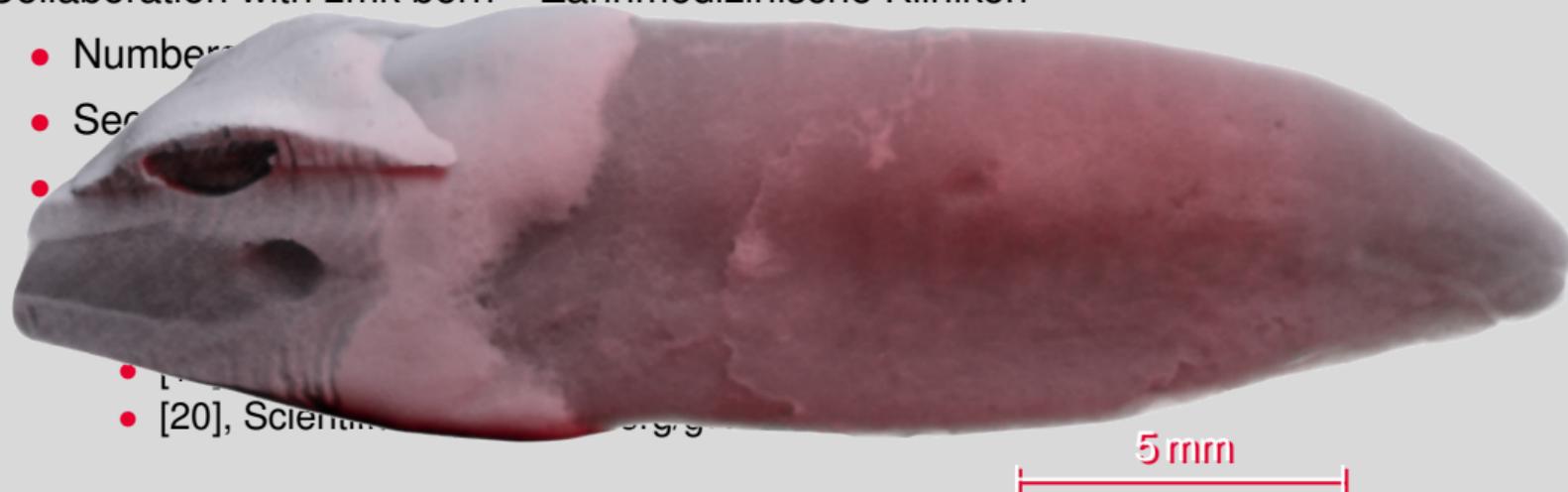
Quantitative data

- Pretty images are nice, but we need quantitative numbers
- Segmentation
- Characterization

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers
 - Sec
 - L [20], Scien



Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers instead of just pretty images
- Segmentation of teeth and root canal
- (Unbiased) Characterization
- Reproducible and automated image analysis ( in Jupyter [19])
- Two publications:
 - [10], BMC Oral Health, doi.org/gjpw2d
 - [20], Scientific Reports, doi.org/g7r8

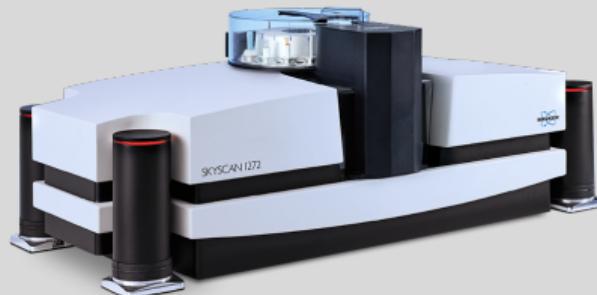
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín et al. [21]
- *Reproducible* analysis [22], e. g. you can click a button to double-check or recalculate the results yourself!



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

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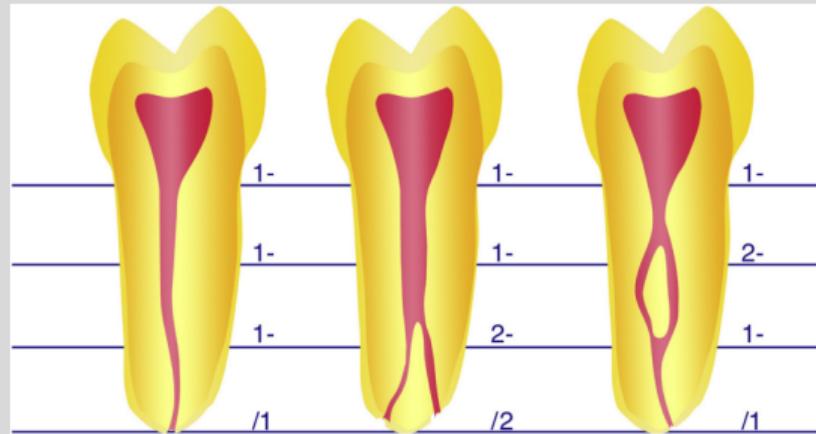
Sample changer on the SkyScan 1272

In total:

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

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From [21], Fig. 2

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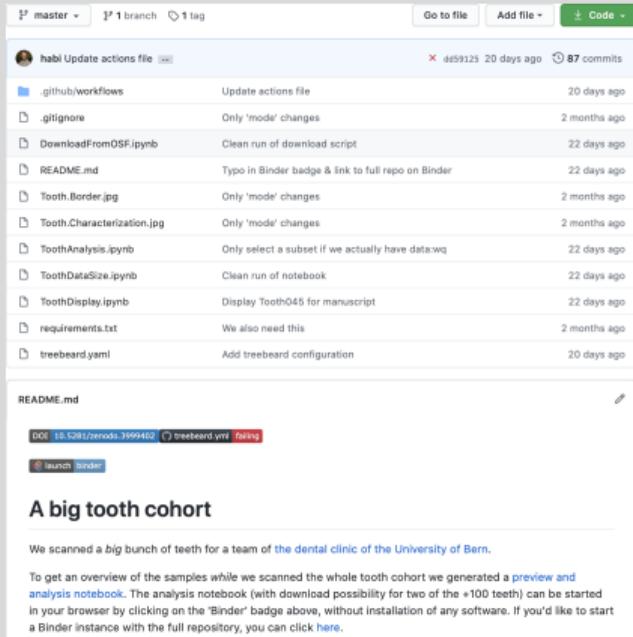
gph.is/2nqkple

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The screenshot shows a GitHub repository page for a project named "habi". The repository has 1 branch and 1 tag. There are 87 commits from user "dab9125" made 20 days ago. The commits are listed as follows:

File / Action	Description	Date
.github/workflows	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

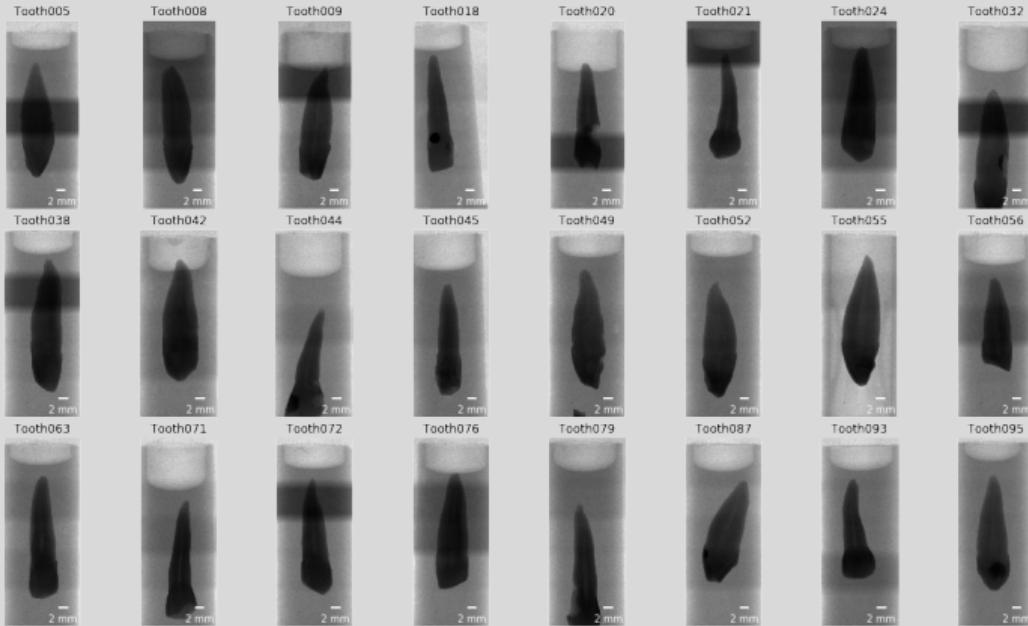
The README.md file contains the following text:

A big tooth cohort

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

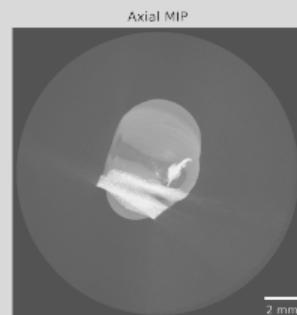
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the "Binder" badge above, without installation of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

μ CT imaging



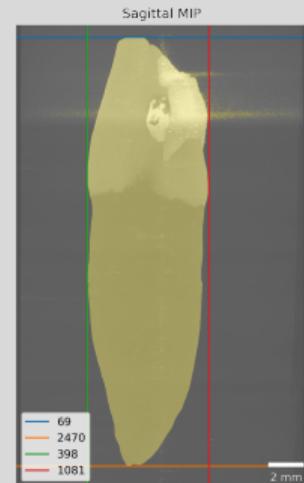
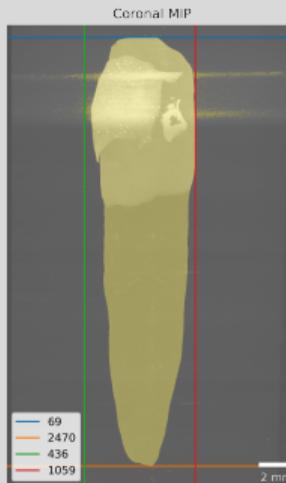
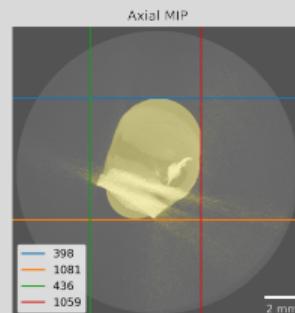
Dataset cropping

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

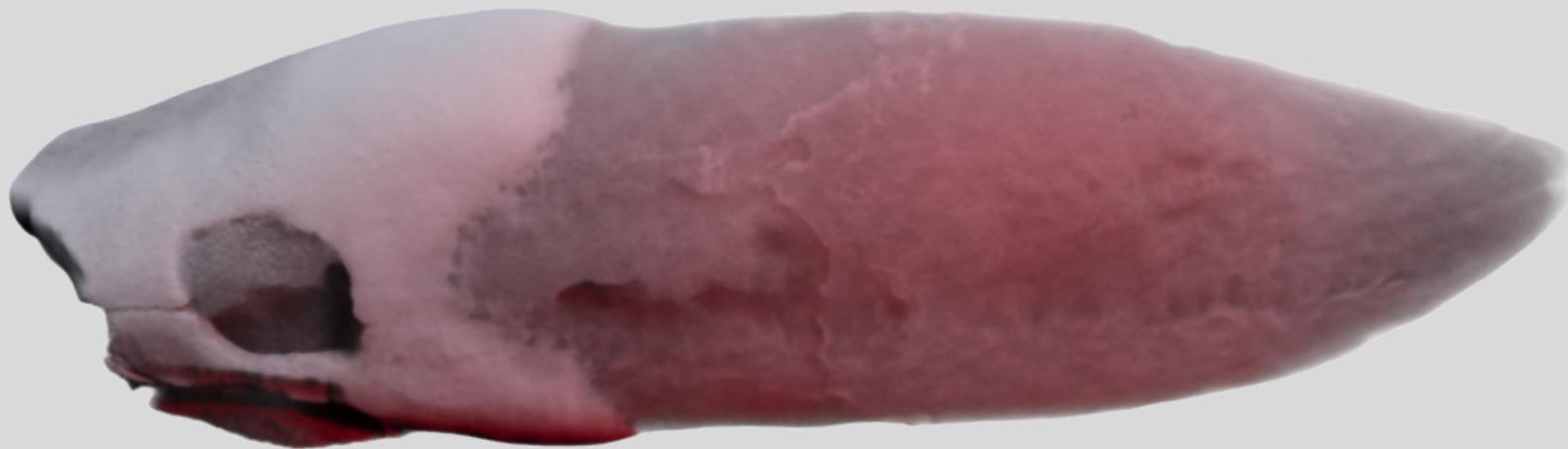


Dataset cropping

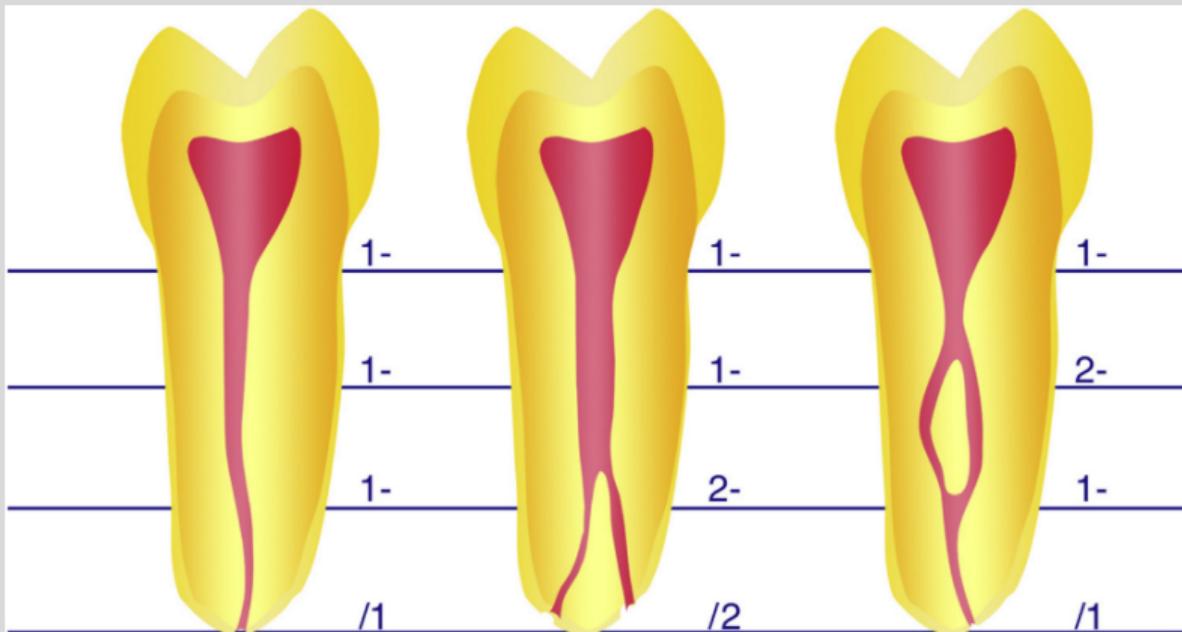
- Full datasets: 326 GB
- Cropped datasets: 115 GB



Tooth morphology

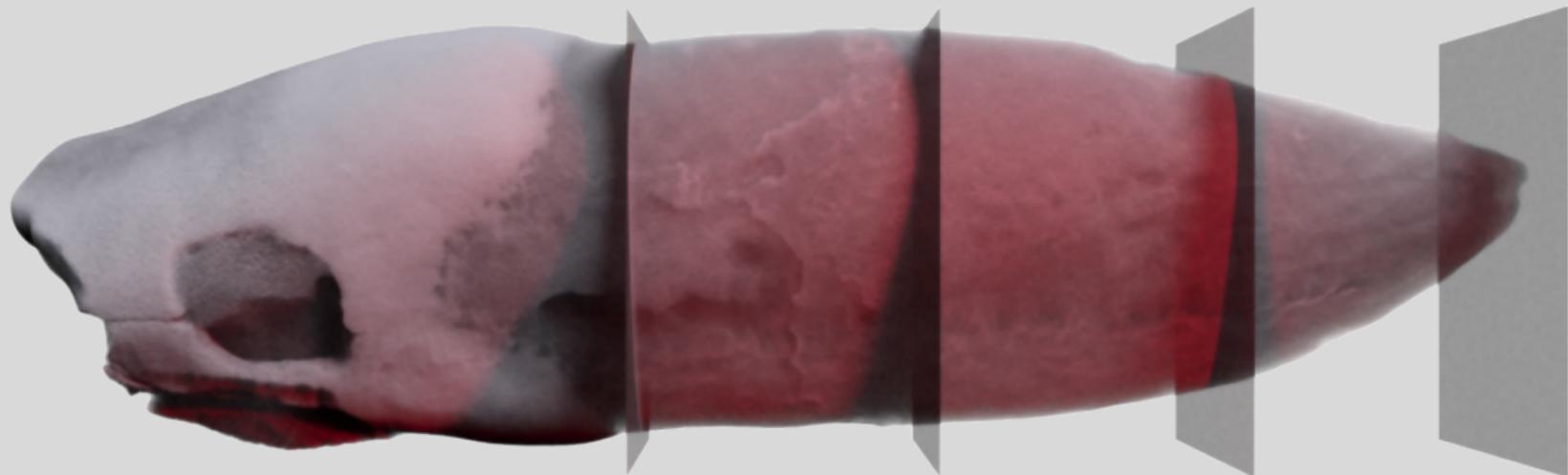


Tooth morphology

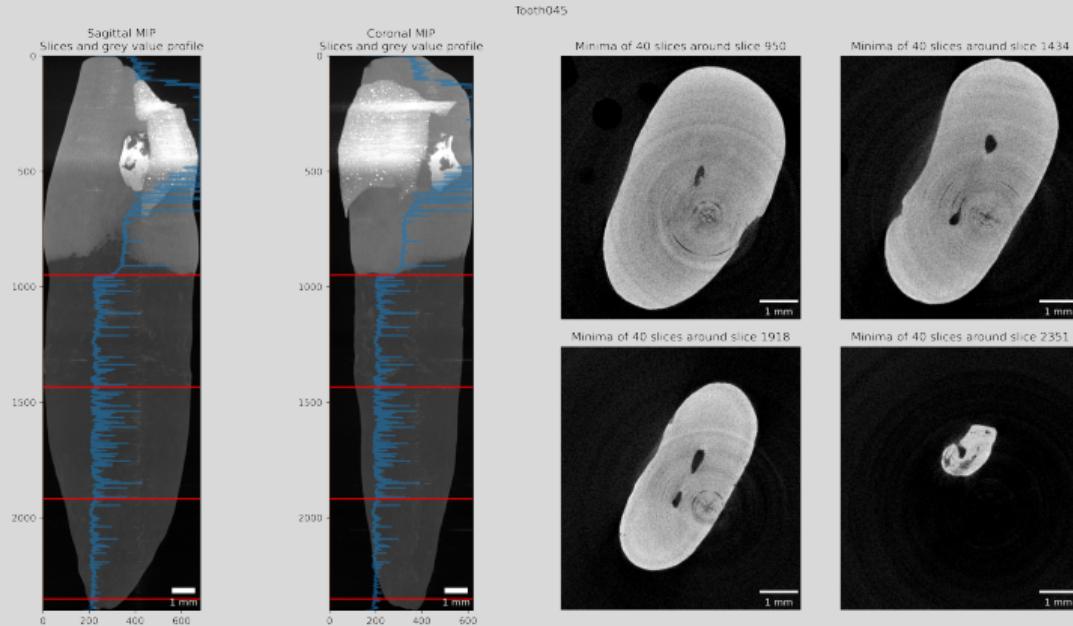


From [21], Fig. 2

Tooth morphology

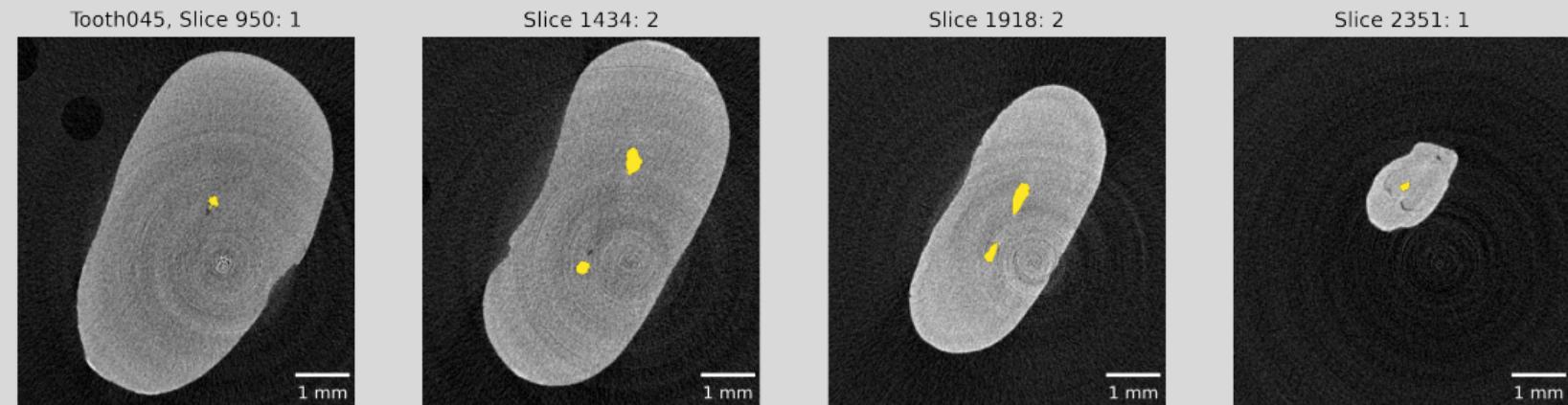


Detection of enamel-dentin border

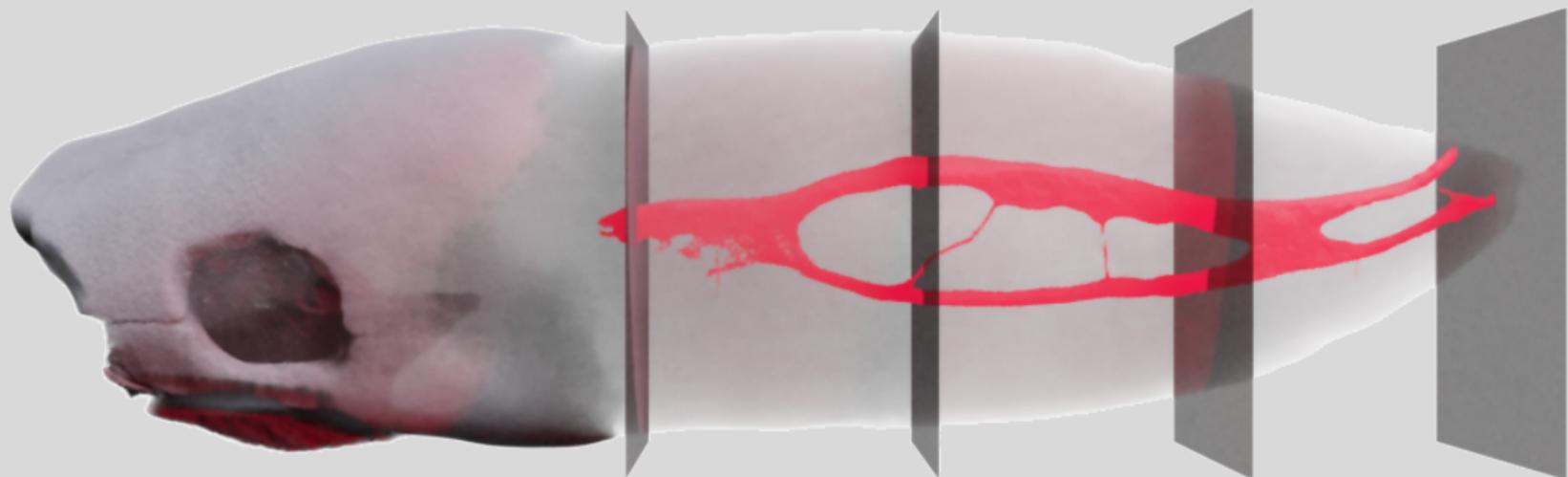


Detection of enamel-dentin border

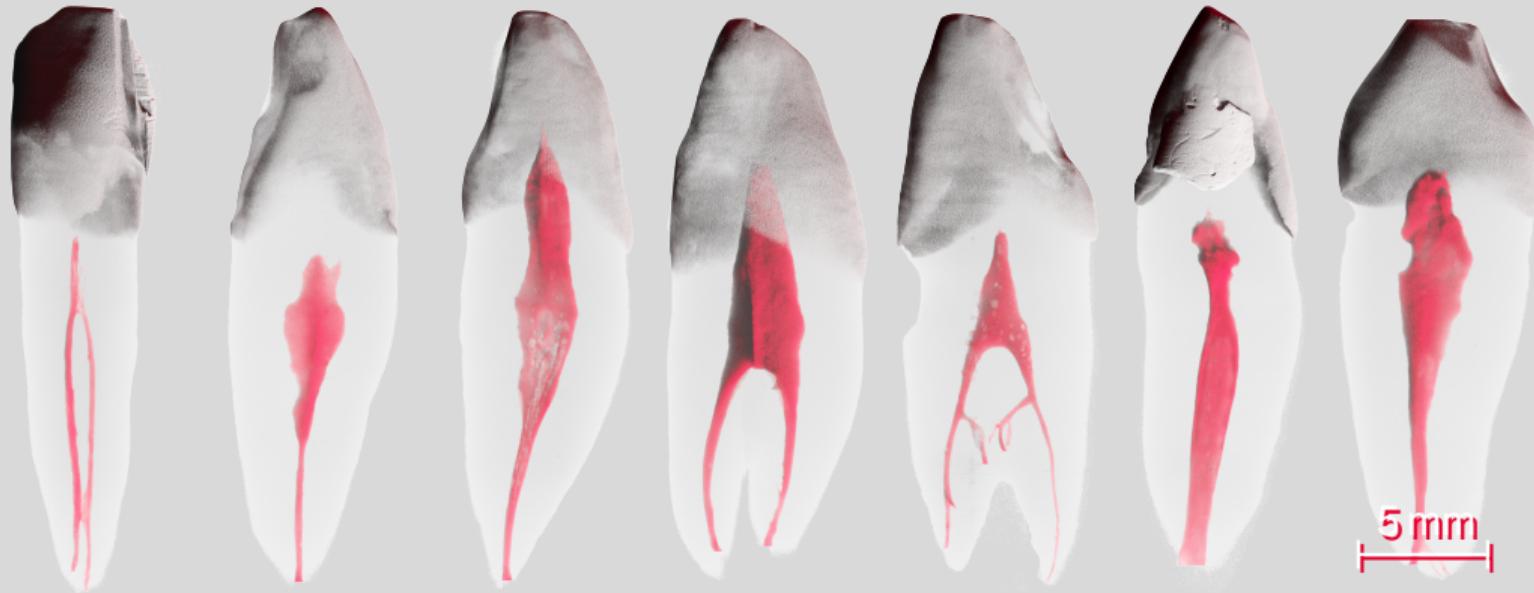
Tooth045



Extraction of root canal space



Results of root canal space extraction



Conclusion

- 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

Thanks!

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

Colophon

- This BEAMER presentation was crafted in L^AT_EX with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
 - Complete source code: git.io/fjpP7
 - The L^AT_EX 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

References I

- [1] Ruslan Hlushchuk et al. "Ex Vivo microangioCT: Advances in Microvascular Imaging". DOI: [10.1016/j.vph.2018.09.003](https://doi.org/10.1016/j.vph.2018.09.003).
- [2] Henry Nording et al. "The C5a/C5a Receptor 1 Axis Controls Tissue Neovascularization through CXCL4 Release from Platelets". DOI: [10.1038/s41467-021-23499-w](https://doi.org/10.1038/s41467-021-23499-w).
- [3] Ruslan Hlushchuk et al. "Innovative High-Resolution microCT Imaging of Animal Brain Vasculature". DOI: [10.1007/s00429-020-02158-8](https://doi.org/10.1007/s00429-020-02158-8).
- [4] Tsering Wüthrich et al. "Development of Vascularized Nerve Scaffold Using Perfusion-Decellularization and Recellularization". DOI: [10.1016/j.msec.2020.111311](https://doi.org/10.1016/j.msec.2020.111311).
- [5] Cédric Zubler et al. "The Anatomical Reliability of the Superficial Circumflex Iliac Artery Perforator (SCIP) Flap". DOI: [10.1016/j.aanat.2020.151624](https://doi.org/10.1016/j.aanat.2020.151624).
- [6] Matthias Messerli et al. "Adaptation Mechanism of the Adult Zebrafish Respiratory Organ to Endurance Training". DOI: [10.1371/journal.pone.0228333](https://doi.org/10.1371/journal.pone.0228333).
- [7] Verdiana Trappetti et al. "Synchrotron Microbeam Radiotherapy for the Treatment of Lung Carcinoma: A Pre-Clinical Study". DOI: [10.1016/j.ijrobp.2021.07.1717](https://doi.org/10.1016/j.ijrobp.2021.07.1717).

References II

- [8] Estée Bochud et al. "A New Diancta Species of the Family Diplommatinidae (Cyclophoroidea) from Vanua Levu Island, Fiji". DOI: 10.3897/zookeys.1073.73241.
- [9] Sebastian Halm et al. "Micro-CT Imaging of Thiel-embalmed and Iodine-Stained Human Temporal Bone for 3D Modeling". 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". DOI: 10.1186/s12903-021-01551-x.
- [11] A. M. Cormack. "Representation of a Function by Its Line Integrals, with Some Radiological Applications". DOI: 10.1063/1.1729798.
- [12] Godfrey Newbold Hounsfield. "Historical Notes on Computerized Axial Tomography.".
- [13] J Hsieh. *Computed Tomography: Principles, Design, Artifacts, and Recent Advances*. Society of Photo Optical.
- [14] E C Beckmann. "CT Scanning the Early Days.". DOI: 10.1259/bjr/29444122.
- [15] Mark Hammer. *X-Ray Physics: X-Ray Interaction with Matter, X-Ray Contrast, and Dose*.
<http://xrayphysics.com/attenuation.html>.

References III

- [16] Wikipedia contributors. *Beer–Lambert Law — Wikipedia, The Free Encyclopedia*.
- [17] Kenneth Clark et al. “The Cancer Imaging Archive (TCIA): Maintaining and Operating a Public Information Repository”. DOI: 10.1007/s10278-013-9622-7.
- [18] Johannes Schindelin et al. “Fiji: An Open-Source Platform for Biological-Image Analysis”. DOI: 10.1038/nmeth.2019.
- [19] Thomas Kluyver et al. “Jupyter Notebooks – a Publishing Format for Reproducible Computational Workflows”. DOI: 10.3233/978-1-61499-649-1-87.
- [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.
- [21] 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”. DOI: 10.1016/j.joen.2015.09.007.
- [22] David Haberthür. “Habi/Zmk-Tooth-Cohort: Used for Manuscript about Method”. DOI: 10.5281/ZENODO.3999402.