

X-ray microtomography

David Haberthür

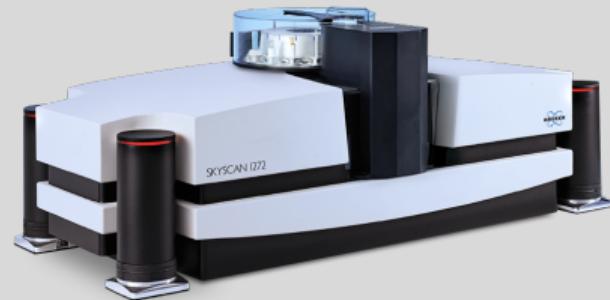
December 22, 2023 | 9256-HS2023-0: Advanced Microscopy

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



bruker.com/skyscan1272

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Overview

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History

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Tomography today

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

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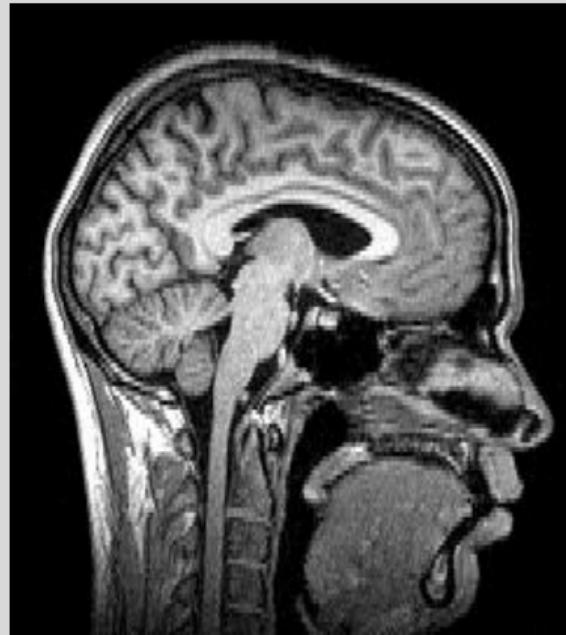
Overview

Materials & Methods

Results

Biomedical imaging

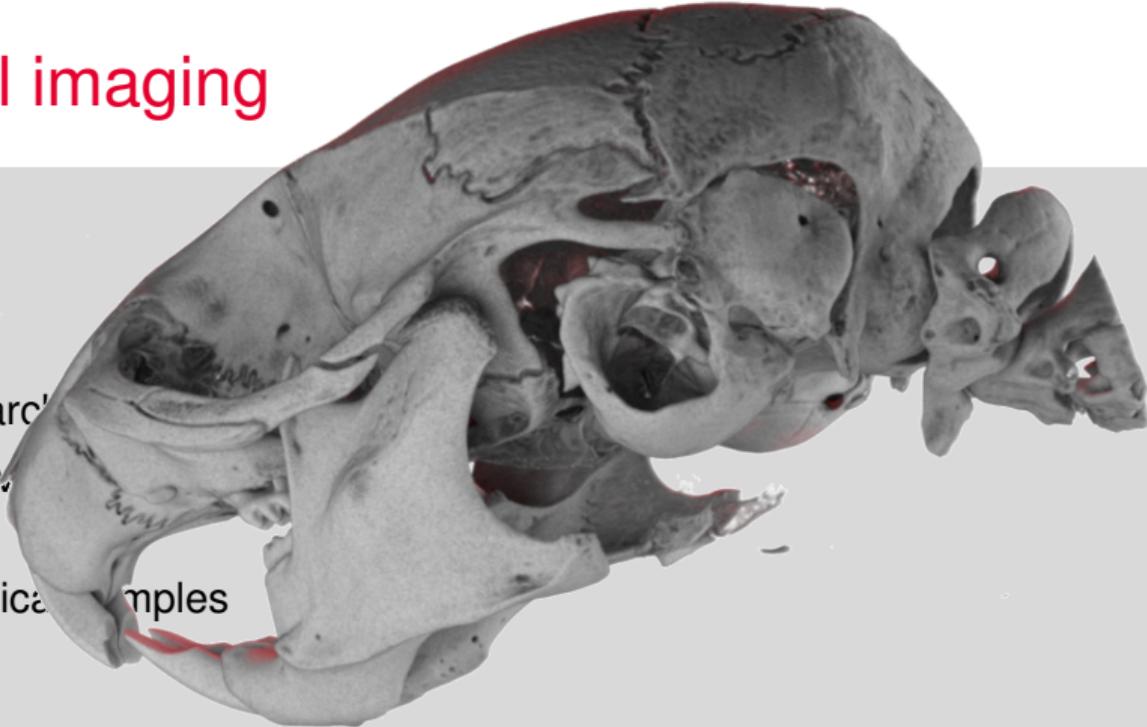
- Medical research
- Non-destructive insights into the samples
- (Small) biological samples



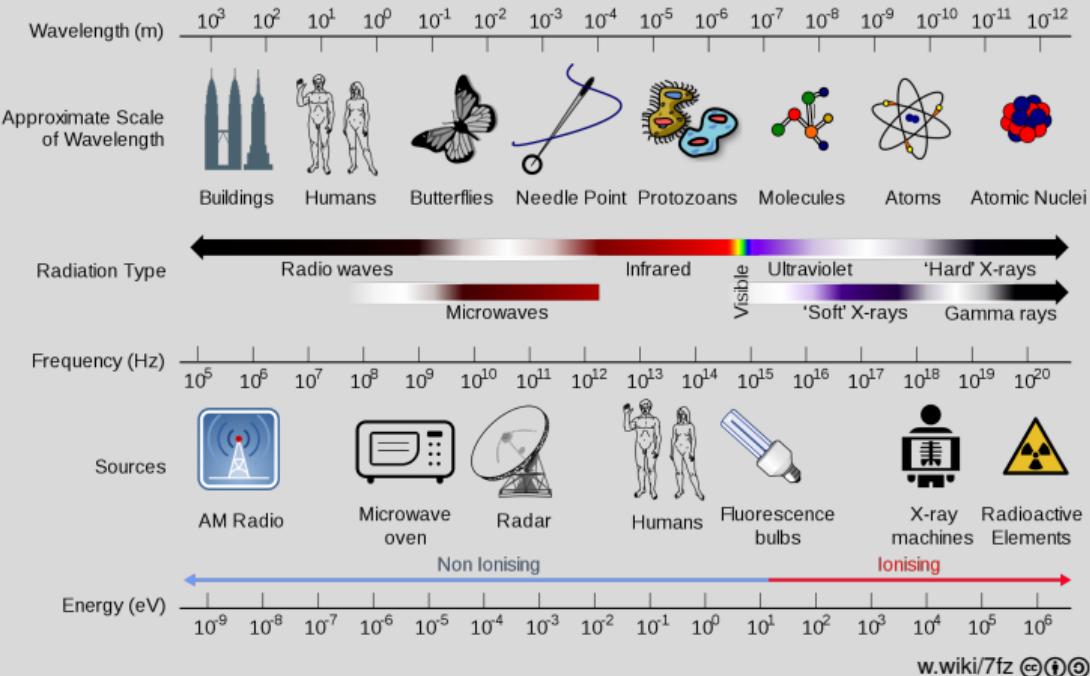
w.wiki/7g4 

Biomedical imaging

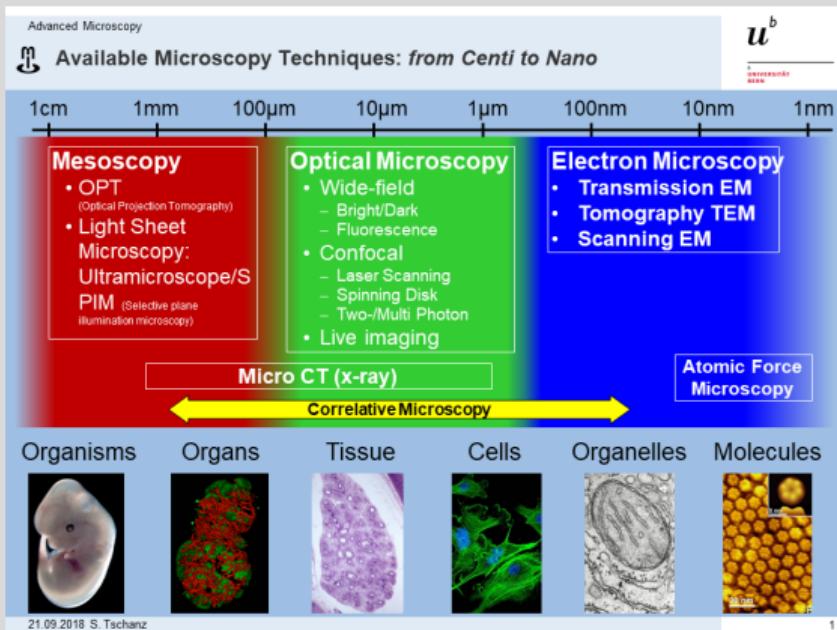
- Medical research
- Non-destructive analysis of the samples
- (Small) biological samples



Wavelength and scales



Wavelength and scales



Stefan Tschanz, with permission

Imaging methods

- Light (sheet) microscopy: see lecture of Myra Chavez
- 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.

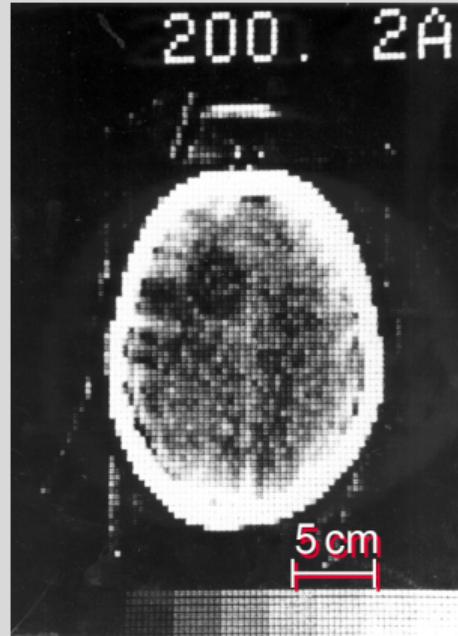
CT-Scanner



youtu.be/2CWpZKuy-NE

History

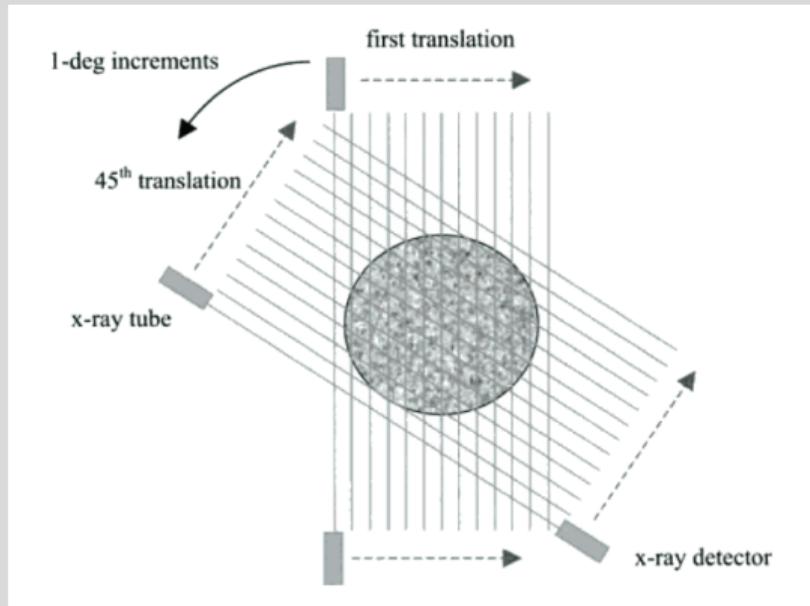
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]



From [14], Figure 5

History

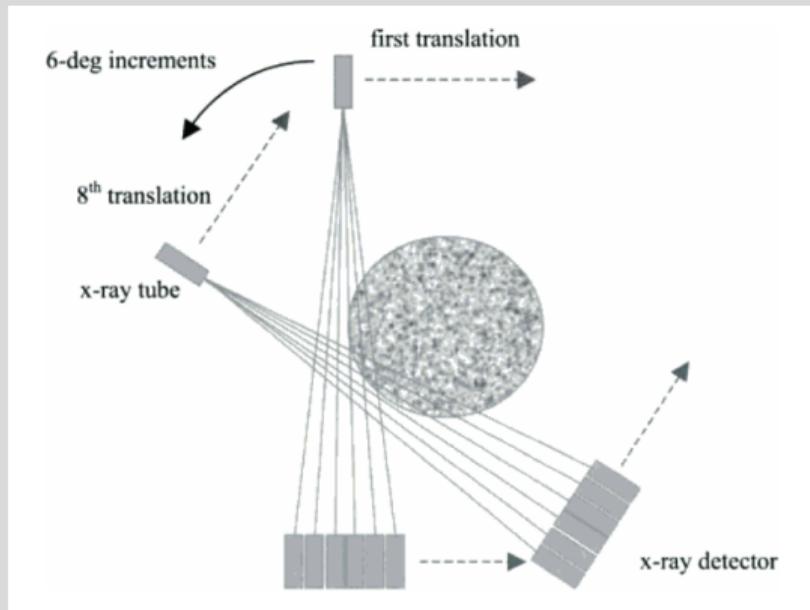
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
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- CT scanner generations: First



From [13], Figure 1.12

History

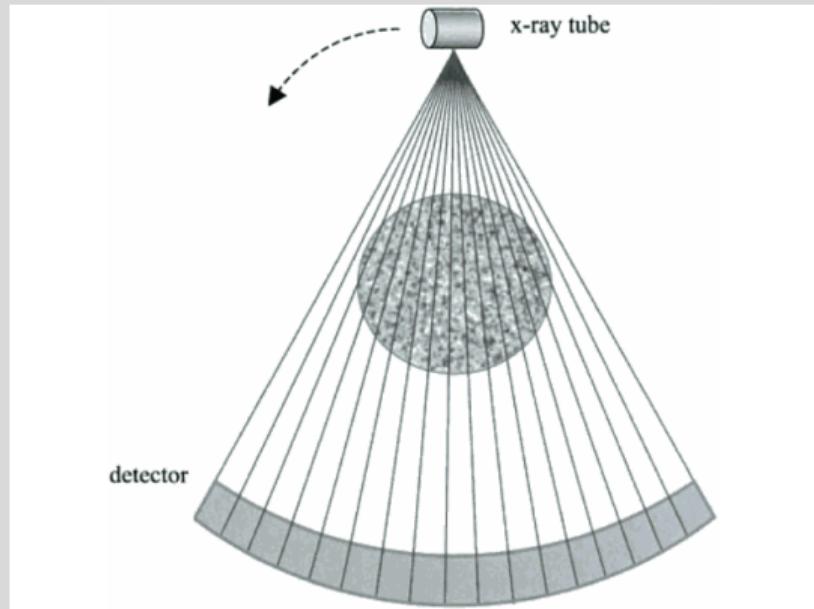
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]
- CT scanner generations: First, second



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

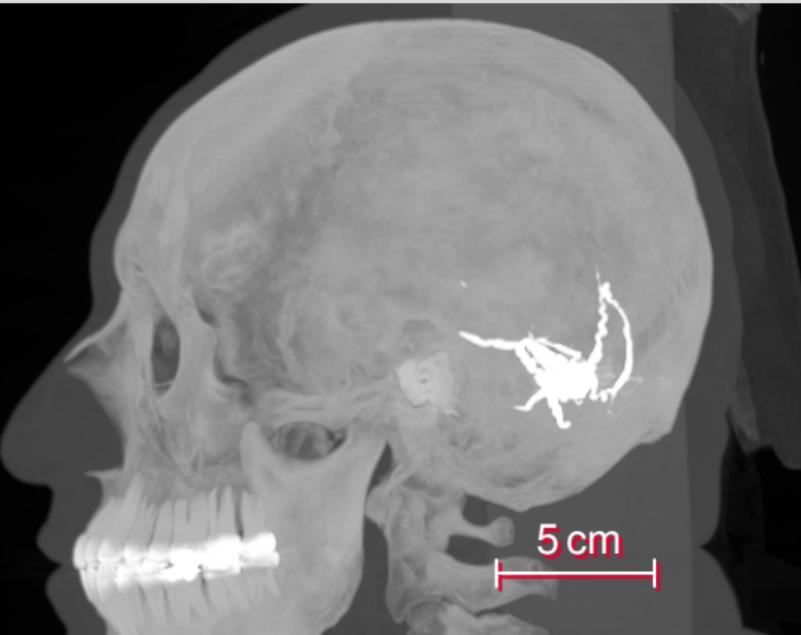
X-ray interaction

- “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] $I(t) = I_0 e^{-\alpha z}$)

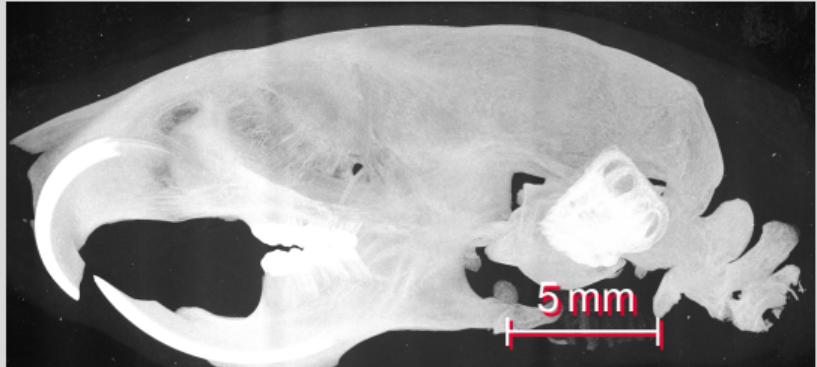
Composition of biological tissues

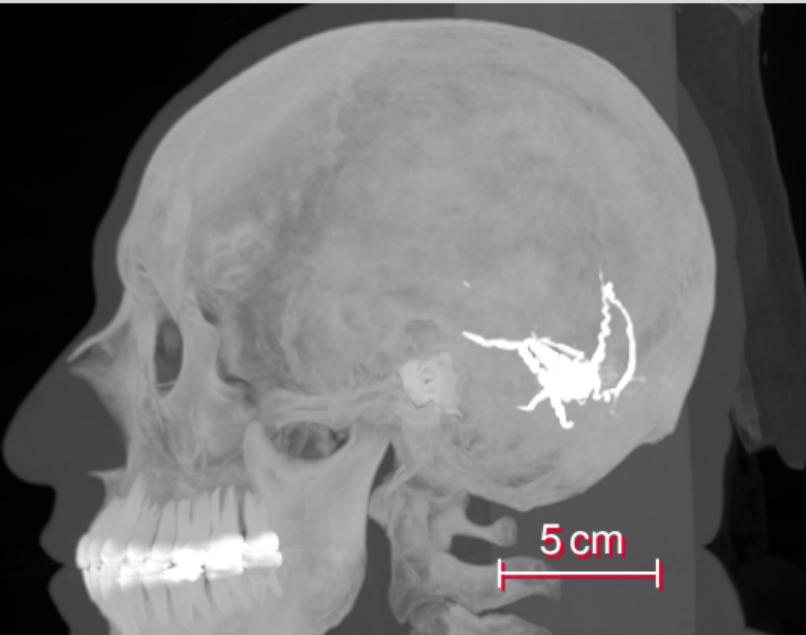
Tissue: content by mass percentage

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



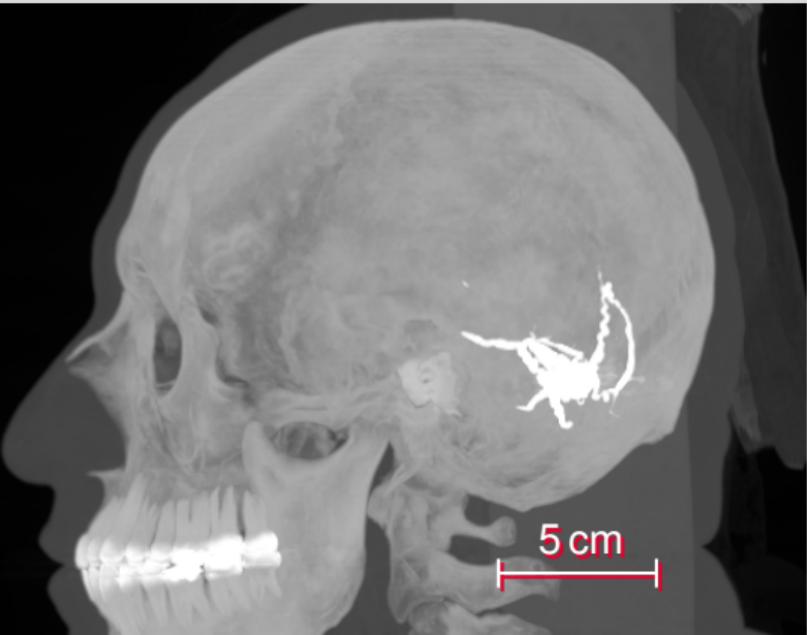
From [17], Subject C3L-02465



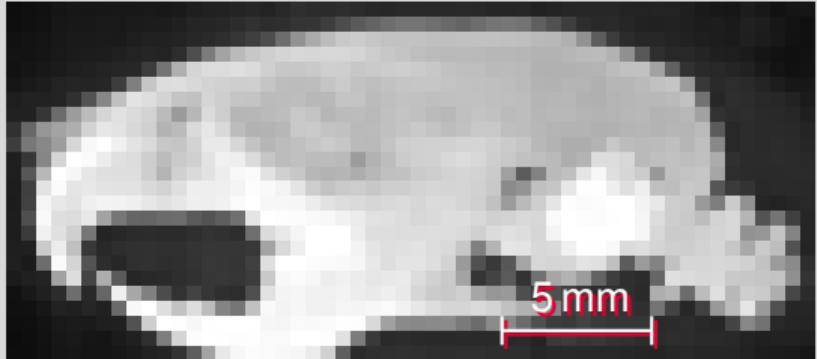


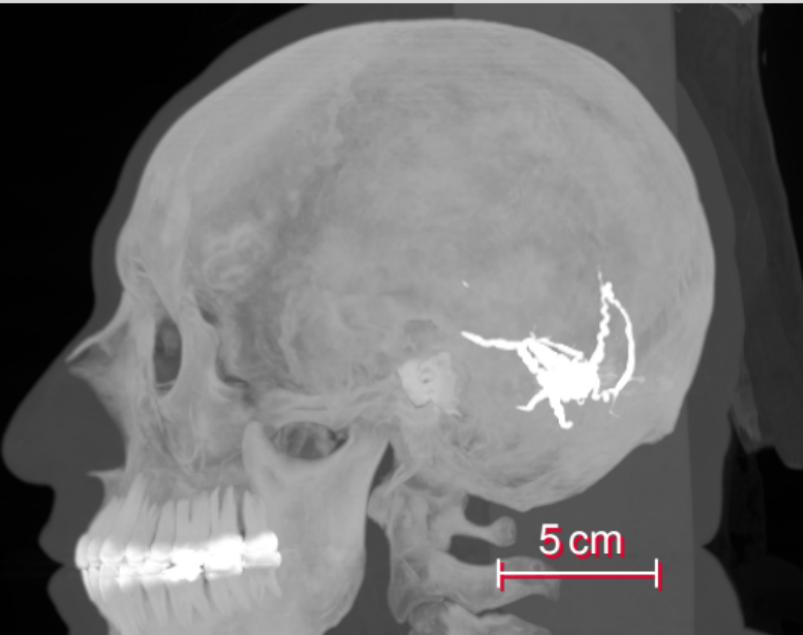
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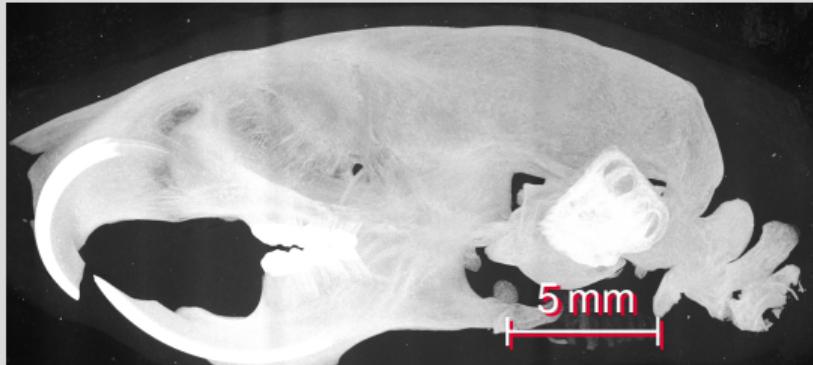


From [17], Subject C3L-02465

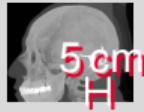




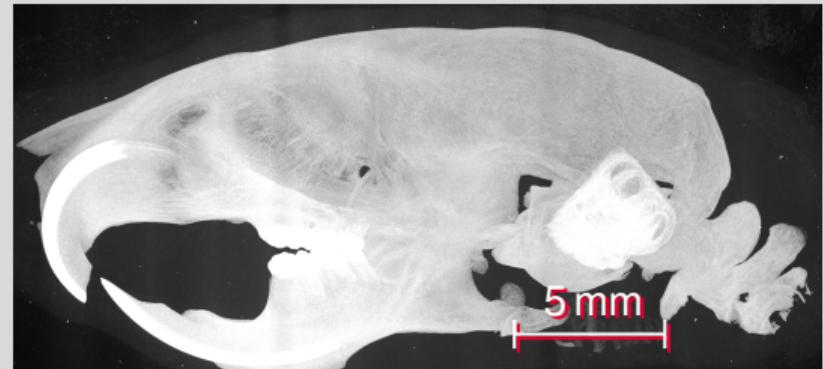
From [17], Subject C3L-02465



Why μ CT?



From [17], Subject C3L-02465

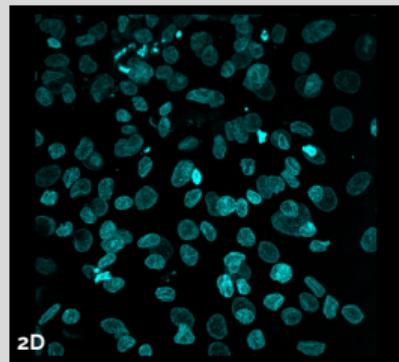
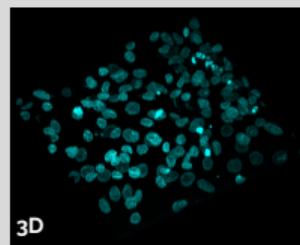
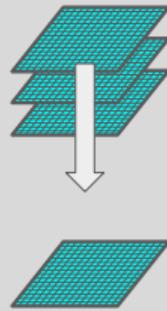


Maximum intensity projection

Projections

 u^b UNIVERSITÄT
BERN

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



flic.kr/p/D4rbom

Machinery

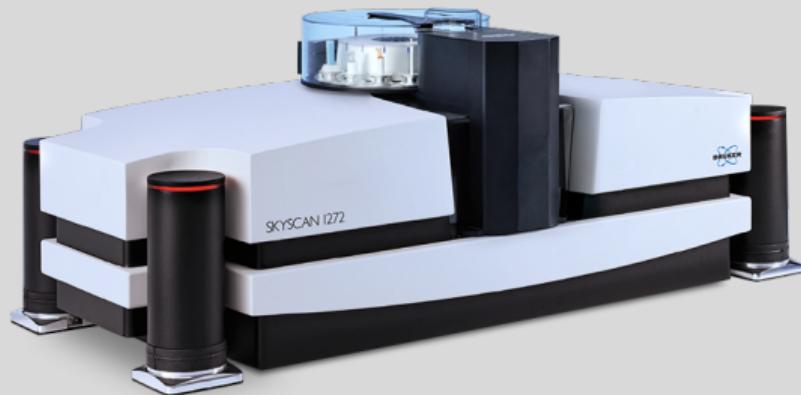
- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around 7 μm (*in vivo*) or 0.5 μm (*ex vivo*)
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flic.kr/p/fpTrGu

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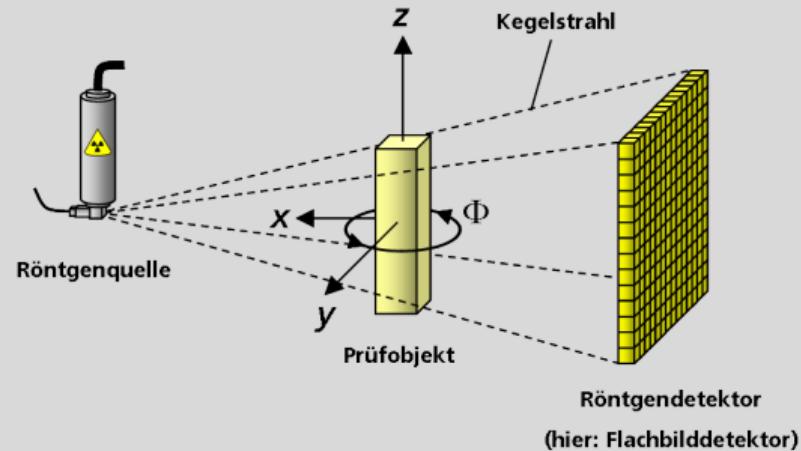


flic.kr/p/7Xhk2Y

What is happening?

The basic components of a computer tomography device are always

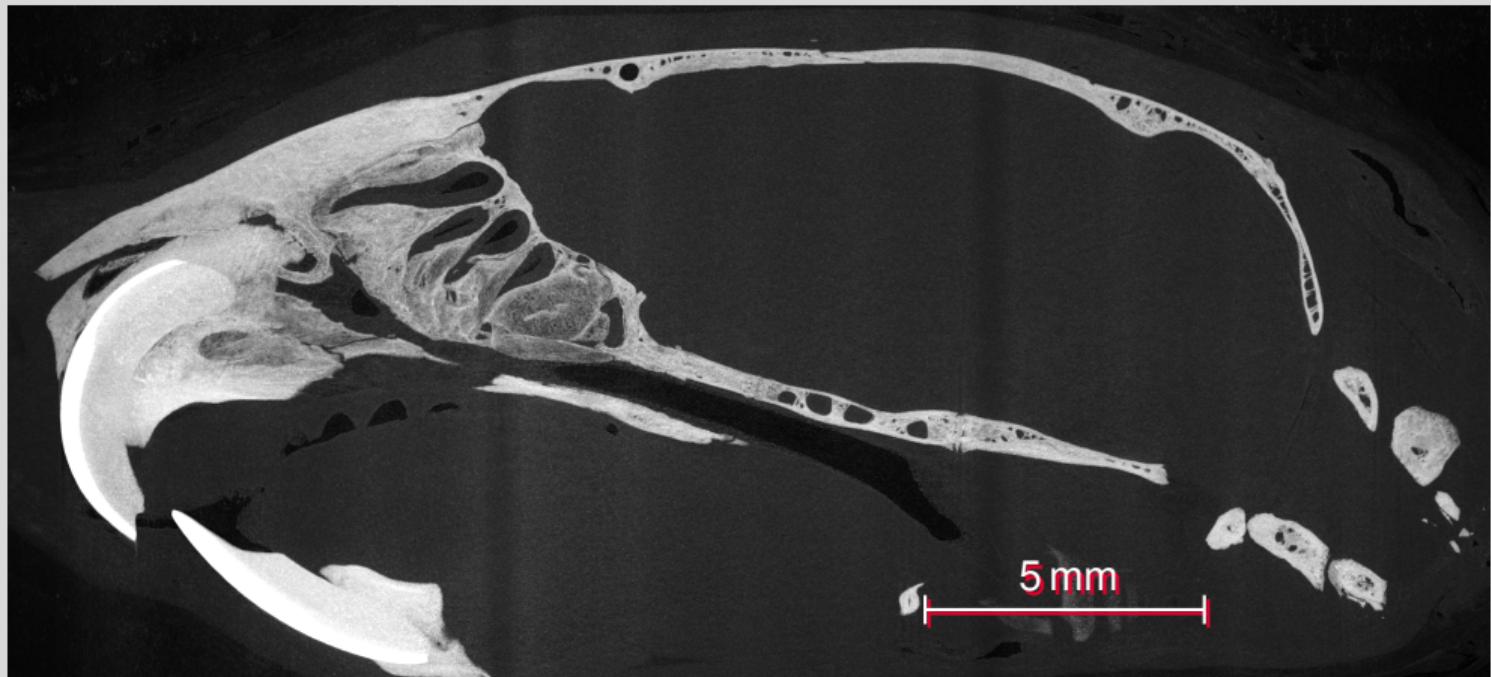
- an X-ray source
- something to image
- a detector



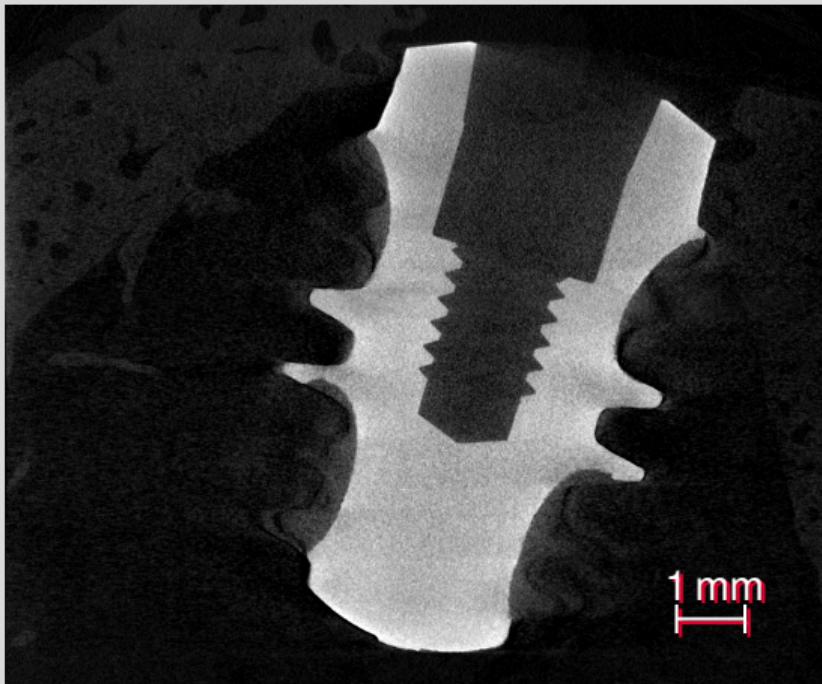
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Machinery

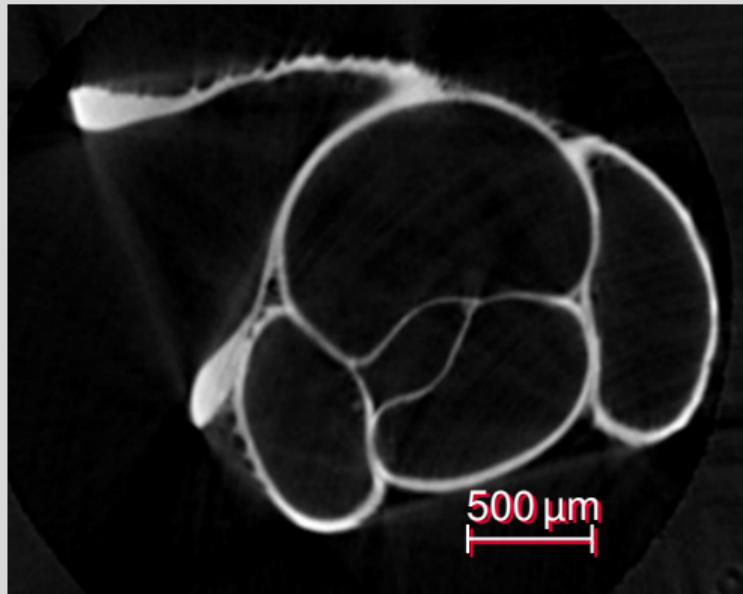
Examples



Examples

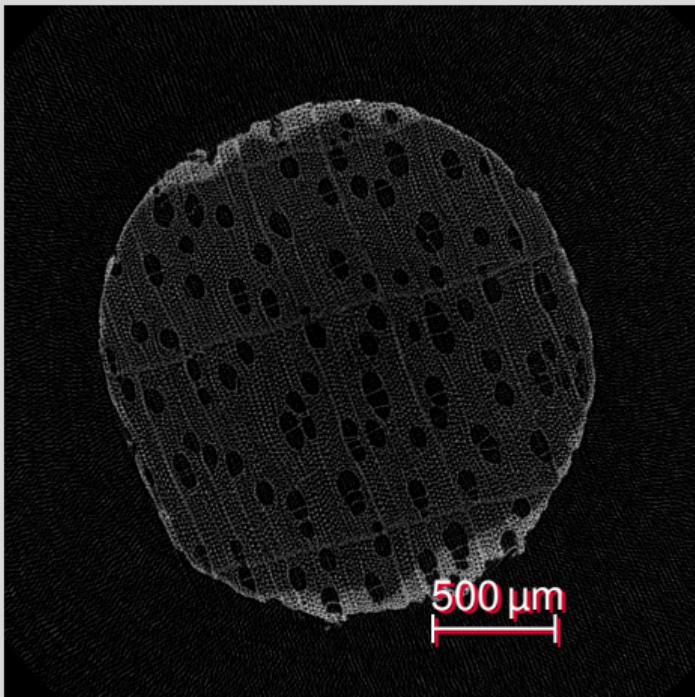


Examples



From [8], *Diancta phoenix*

Examples



Examples

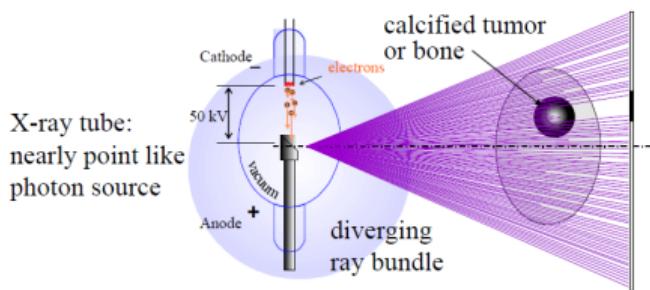


Preparation

- Study design
- Sample preparation

Projections

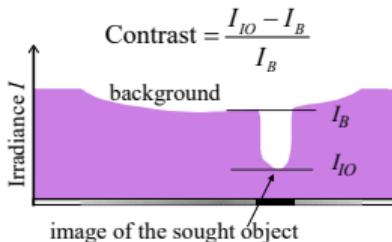
X-ray generation and contrast



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



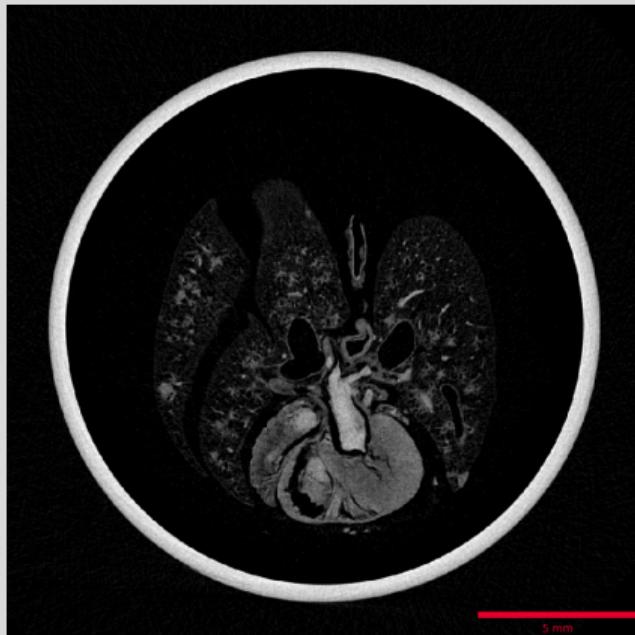
Projections



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

- Number
- Section
- [19], Scientific
- [20], Scientific



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

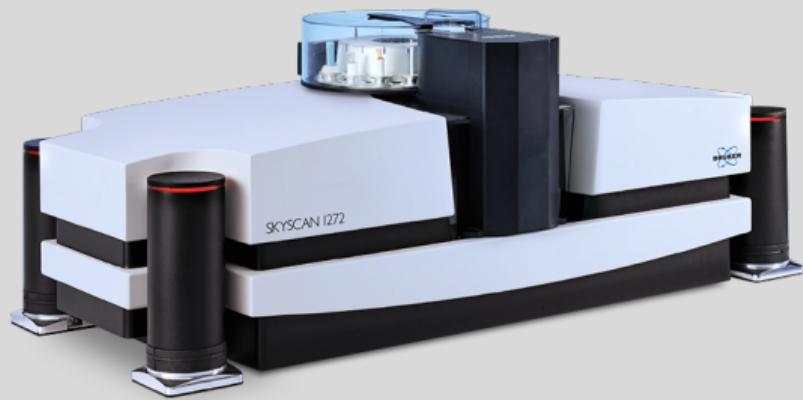
How?

- 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!



How?

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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
```

How?

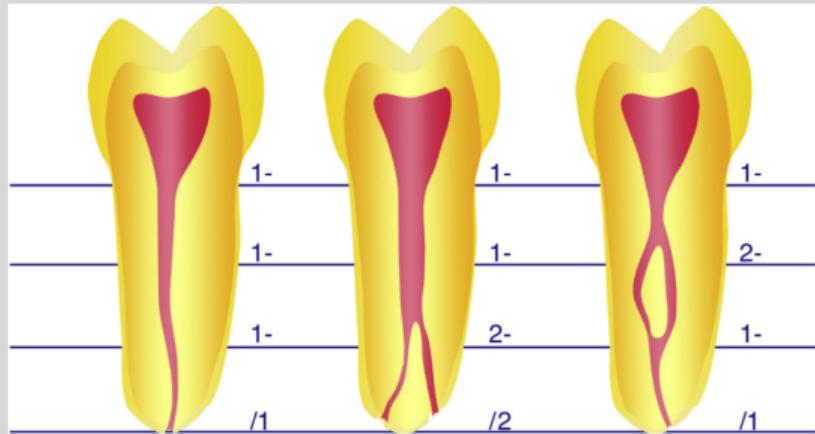
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Sample changer on the SkyScan 1272
In total:

- 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

How?

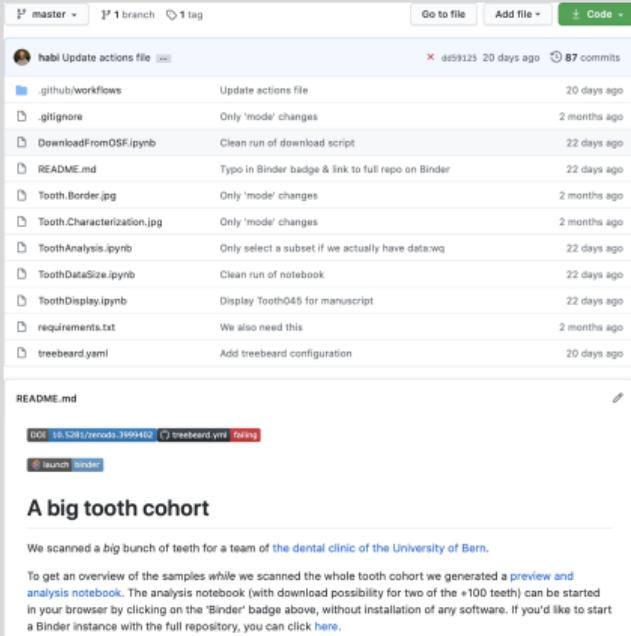
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gph.is/2nqkple

How?

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The screenshot shows a GitHub repository interface. At the top, there are buttons for 'master', '1 branch', '1 tag', 'Go to file', 'Add file', and 'Code'. Below this is a list of commits from a user named 'habi' with the commit message 'Update actions file'. The commits are dated from 20 days ago to 2 months ago. The repository contains files like '.github/workflows', '.gitignore', 'DownloadFromOSF.ipynb', 'README.md', 'Tooth.Border.jpg', 'Tooth.Characterization.jpg', 'ToothAnalysis.ipynb', 'ToothAxisSize.ipynb', 'ToothDisplay.ipynb', 'requirements.txt', and 'treebeard.yaml'. A 'README.md' file is shown below the commits, featuring a DOI link, a 'treebeard.yaml' file link, and a 'launch Binder' button. The text 'A big tooth cohort' is displayed, followed by a note about scanning 100 teeth and generating a preview and analysis notebook.

master | 1 branch | 1 tag | Go to file | Add file | Code

habi Update actions file · 87 commits

.github/workflows · 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

README.md

DOI: 10.5281/zenodo.3969402 · treebeard.yaml · failing

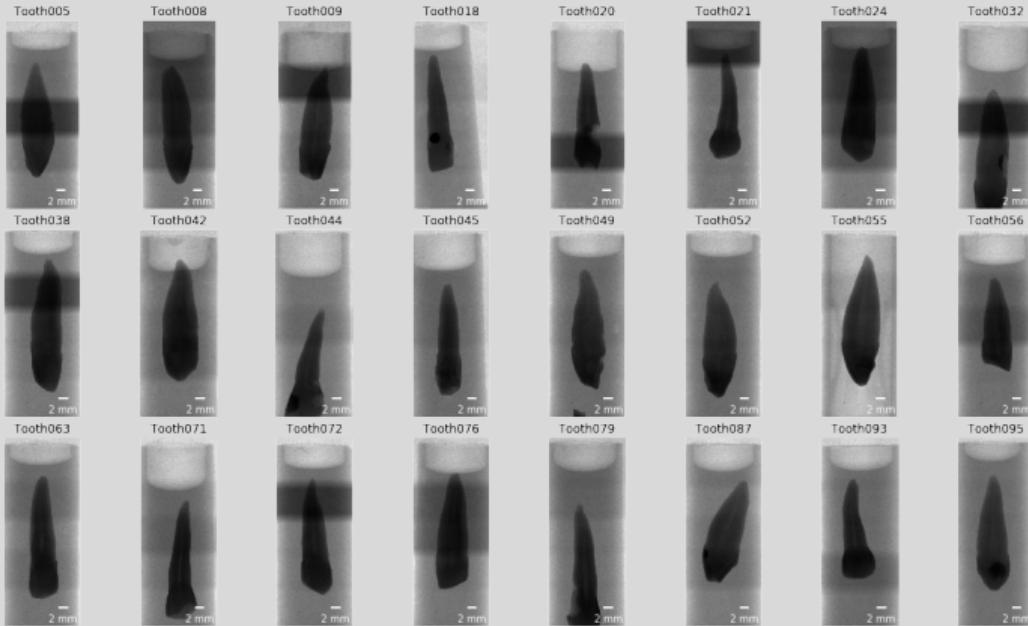
launch Binder

A big tooth cohort

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

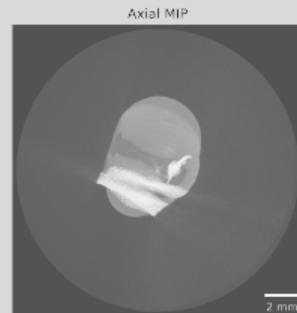
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the "Binder" badge above, without installation of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

μ CT imaging



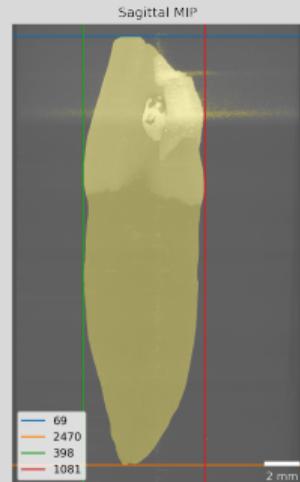
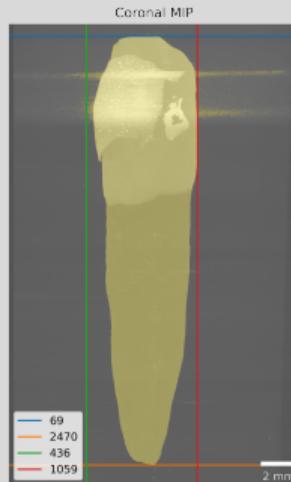
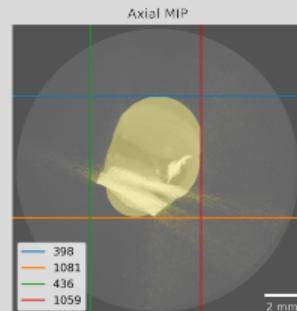
Dataset cropping

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



Dataset cropping

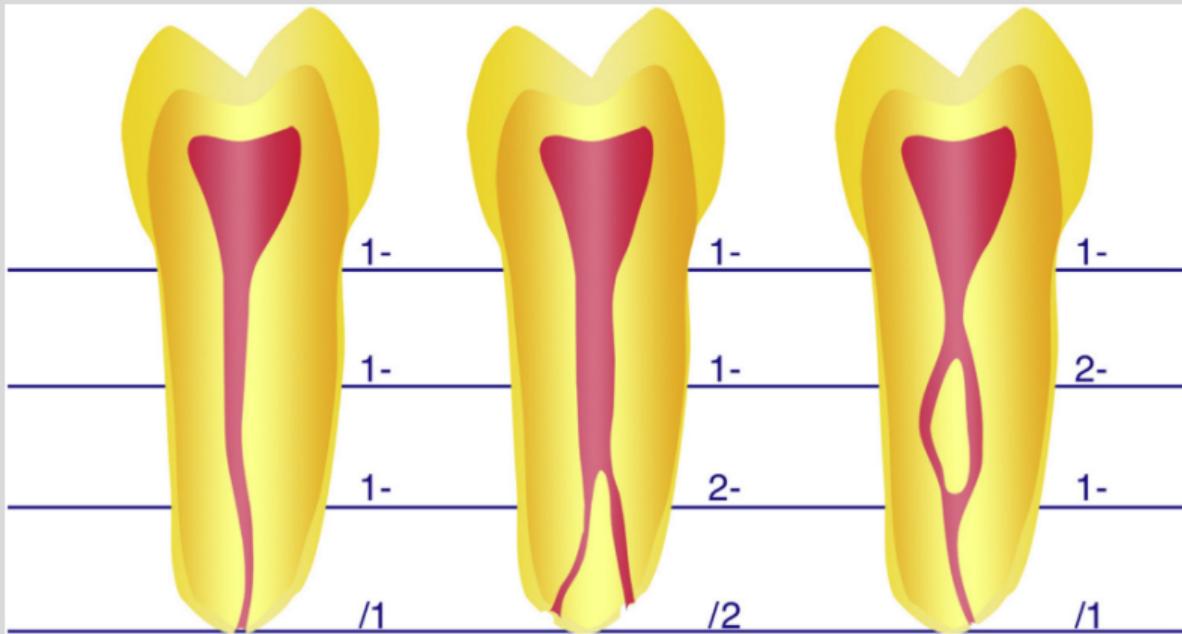
- Full datasets: 326 GB
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Tooth morphology

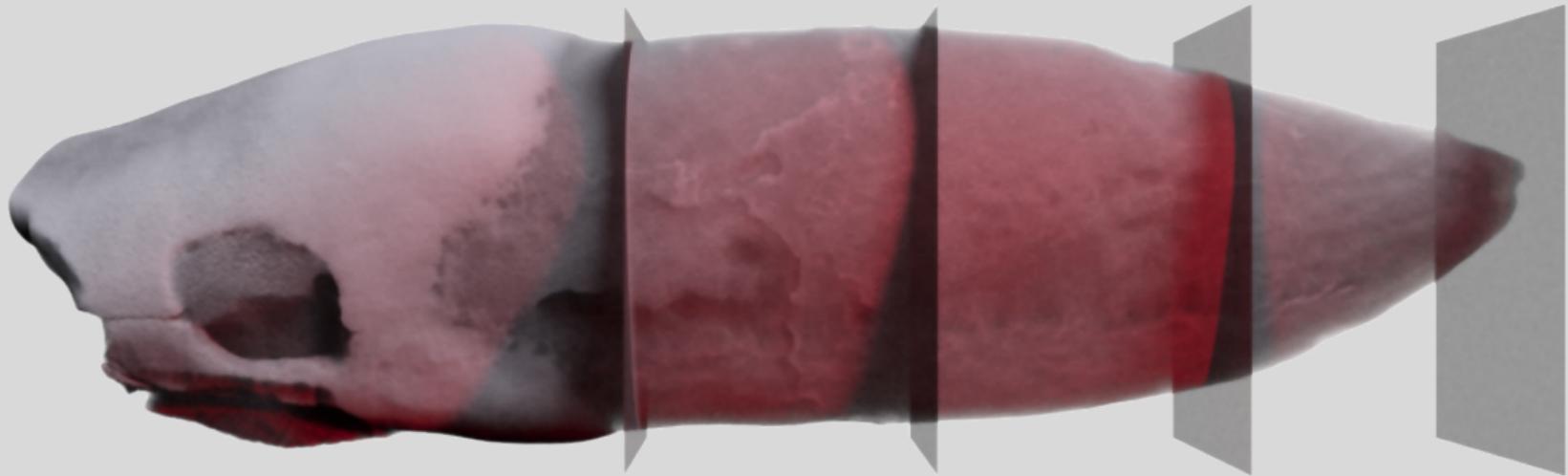


Tooth morphology

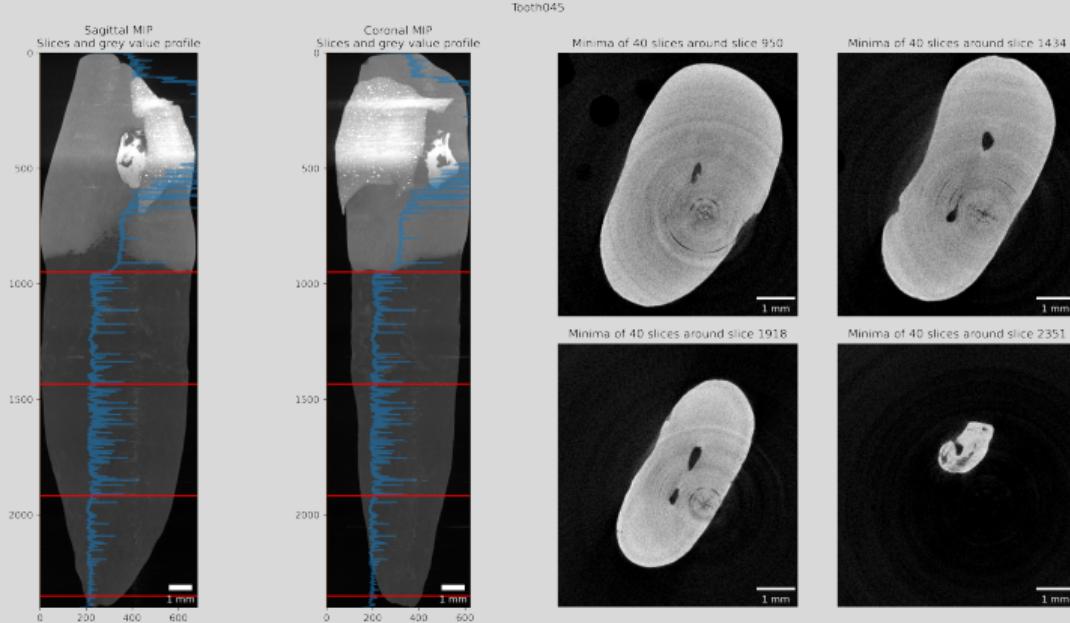


From [21], Fig. 2

Tooth morphology



Detection of enamel-dentin border

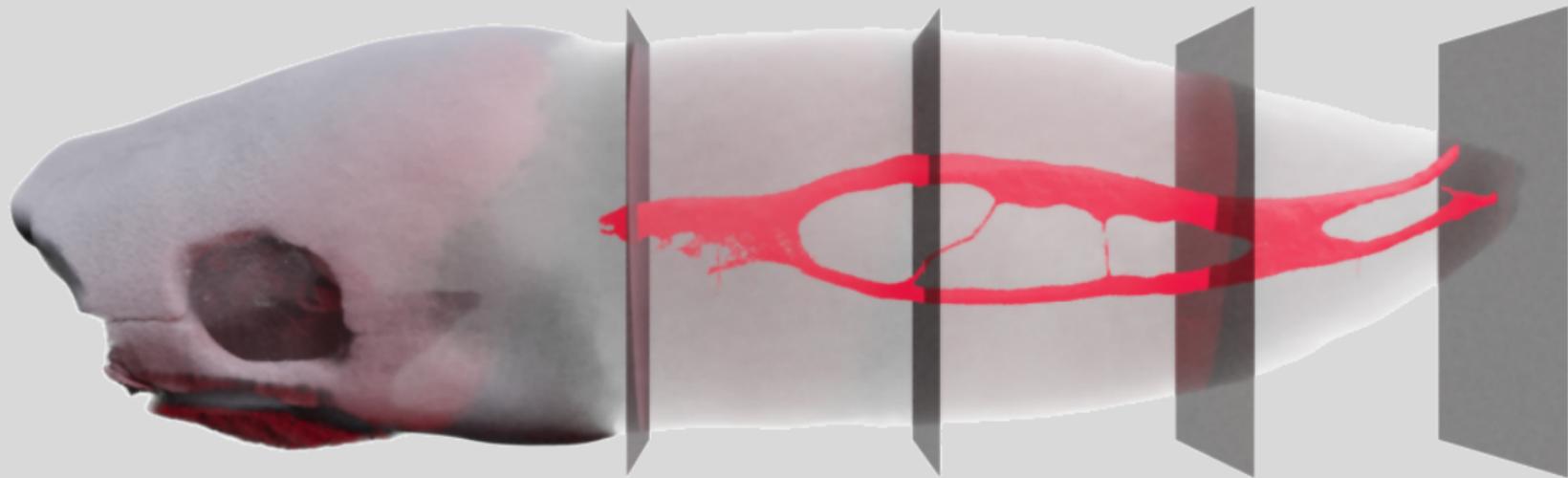


Detection of enamel-dentin border

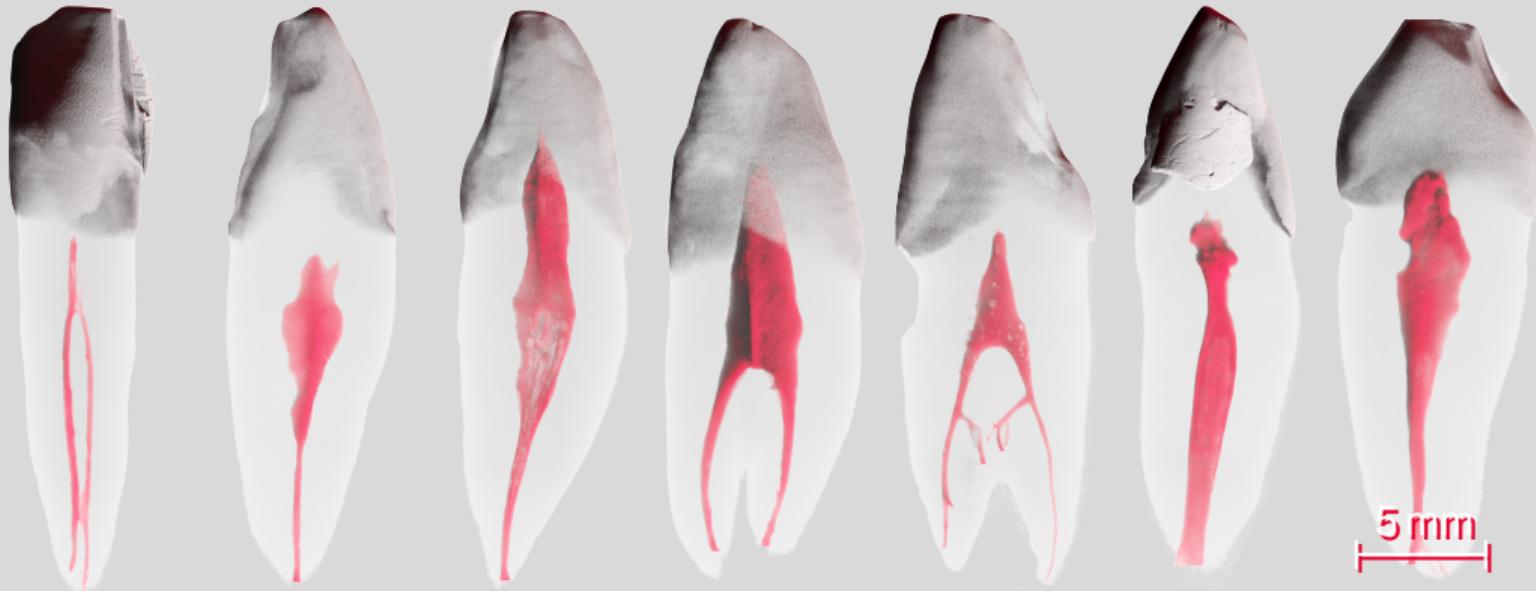
Tooth045



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?

References I

- [1] Ruslan Hlushchuk et al. "Ex Vivo microangioCT: Advances in Microvascular Imaging". DOI: 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.
- [3] Ruslan Hlushchuk et al. "Innovative High-Resolution microCT Imaging of Animal Brain Vasculature". DOI: 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.
- [5] Cédric Zubler et al. "The Anatomical Reliability of the Superficial Circumflex Iliac Artery Perforator (SCIP) Flap". DOI: 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.
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References II

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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.
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[1] Details on how this works are specified in a small test repository here: git.io/JeOOj