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**UNIVERSITY
OF BERN**

u^b X-ray microtomography

9256-HS2024-0: Advanced Microscopy

David Haberthür

Institute of Anatomy, December 20, 2024

u^b Grüessech mitenang!

- David Haberthür
 - Physicist by trade
 - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
 - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
 - Post-Doc II: µCT group, Institute of Anatomy, University of Bern, Switzerland

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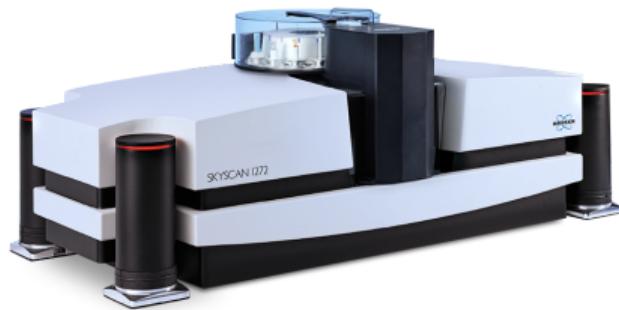
Grüessech from the μ CT group



David.Haberthuer@unibe.ch Ruslan.Hlushchuk@unibe.ch Oleksiy.Khoma@unibe.ch

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], [10] to scan a wide range of specimens, from human hearing bones to meteorites
- Automate all the things! [11], [12]



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Contents

Overview

 Imaging methods

Tomography

 History

 Tomography today

 Interaction of x-rays with matter

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

Example: A study about teeth

 Overview

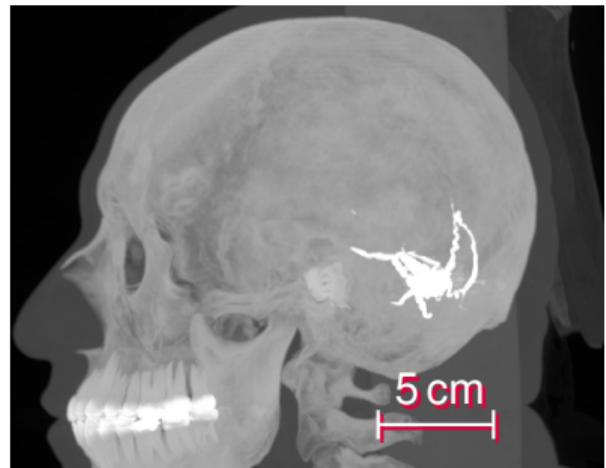
 Materials & Methods

 Results

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micro-Computed tomography

- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a very large range of sample sizes

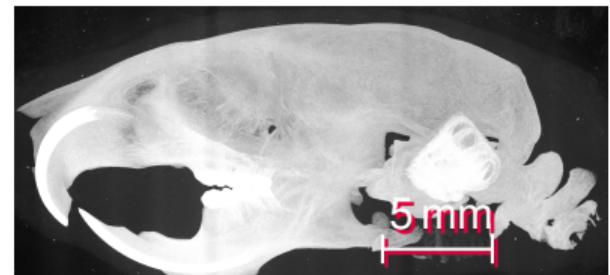


From [13], Subject C3L-02465

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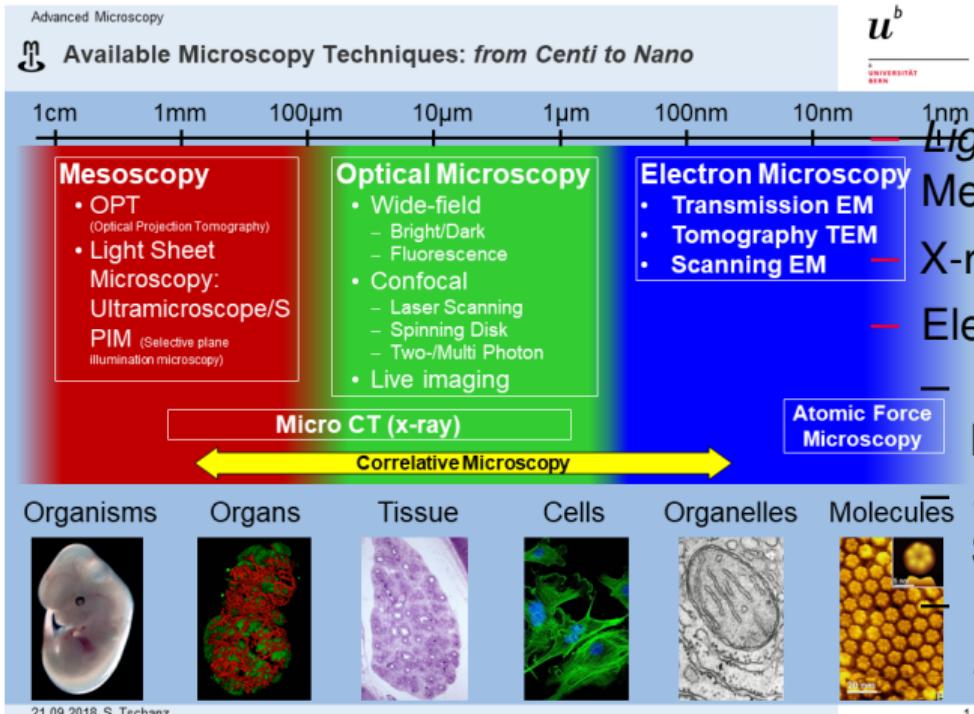
micro-Computed tomography

- Allows for imaging dense and non-transparent samples
- Non-destructive imaging, thus compatible with routine sample preparation
- Results in three-dimensional images with μm resolution
- Covers a very large range of sample sizes
- (Small) biological samples
- Enables correlative imaging pipelines, scanning of precious biological samples, as well as museum & collection material



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Imaging methods



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Light Sheet Microscopy by Nadia Mercader Huber

X-ray imaging

– Electron microscopy

– *Transmission Electron Microscopy* by Dimitri Vanhecke

Scanning Electron Microscopy by Sabine Kässmeyer and Ivana Jaric

Cryoelectron Microscopy & Serial Block Face SEM by Ioan Iacovache

1

Stefan Tschanz, with permission

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CT-Scanner



youtu.be/2CWpZKuy-NE

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CT History

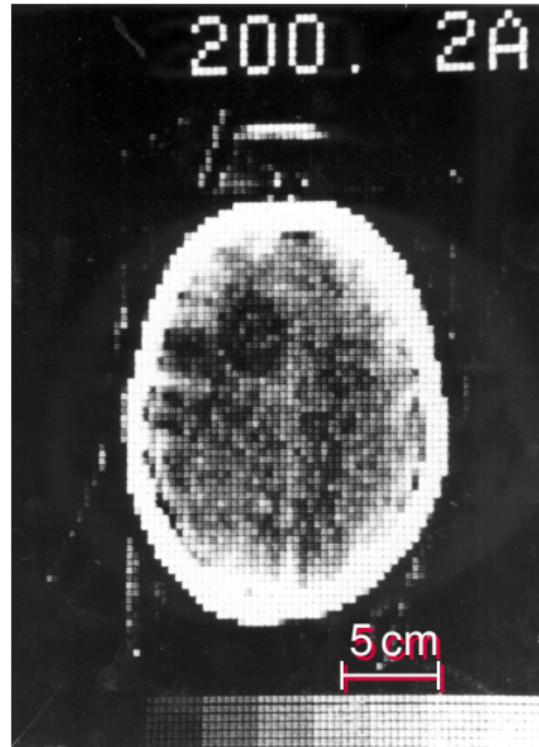
- 1895: Wilhelm Conrad Röntgen discovers X-rays



w.wiki/BHAN

CT History

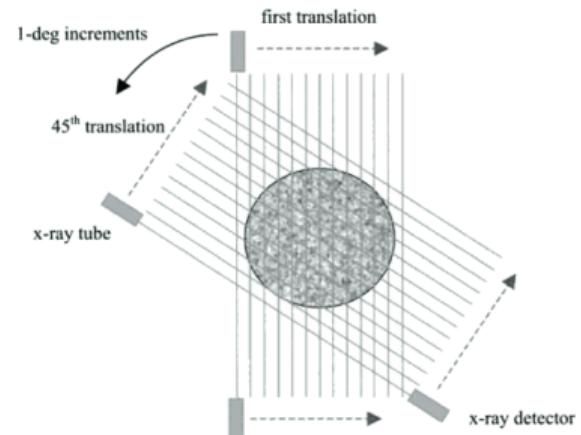
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- **Cormack1963**: Cormack1963 used a collimated ^{60}Co source and a Geiger counter as a detector **Cormack1963**
- **Hounsfield1976a**: Hounsfield1976a worked on first clinical scanner **Hounsfield1976a**



From Beckmann2006, Figure 5

CT History

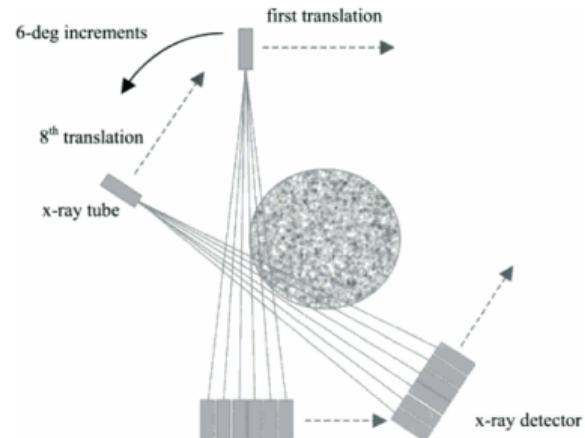
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- Cormack1963: Cormack1963 used a collimated ^{60}Co source and a Geiger counter as a detector Cormack1963
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- CT scanner generations
 - First generation



From Hsieh2003, Figure 1.12

CT History

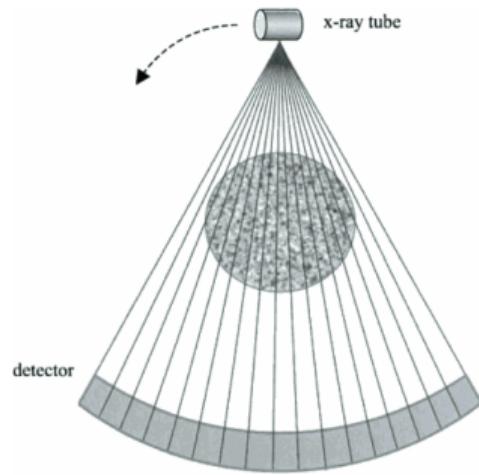
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- CT scanner generations
 - First generation
 - Second generation



From **Hsieh2003**, Figure 1.13

CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays
- Cormack1963: Cormack1963 used a collimated ^{60}Co source and a Geiger counter as a detector Cormack1963
- Hounsfield1976a: Hounsfield1976a worked on first clinical scanner Hounsfield1976a
- CT scanner generations
 - First generation
 - Second generation
 - Third generation



From Hsieh2003, Figure 1.14

μ CT 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 **Feldkamp1984** developed one of the early laboratory microCT systems by assembling a micro-focus cone beam x-ray source, specimen holder and stages, 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**

μ CT History II

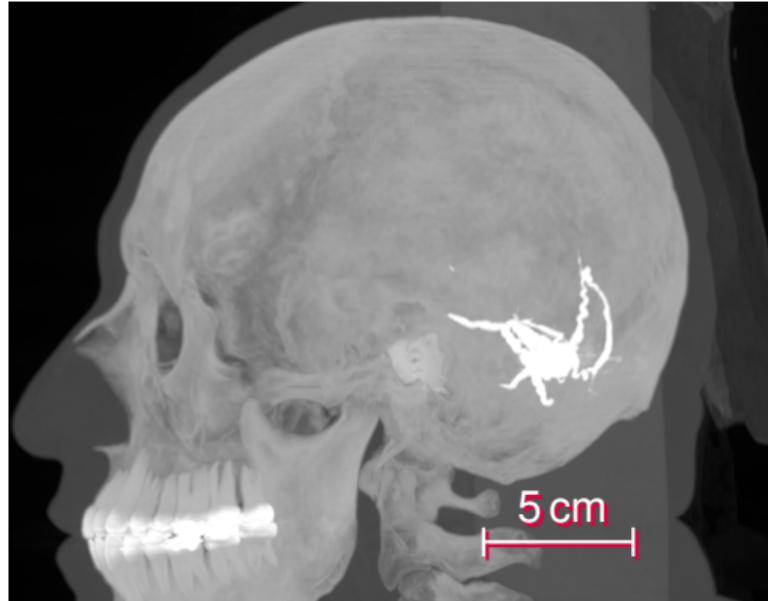
- μ CT was first reported in the 1980s, for scanning gemstones to cut out the largest possible one
- Today: Nondestructive imaging for quantifying the (micro)structure of organic materials
 - Mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
 - Teeth and their internal details
 - Soft tissues and vasculature using radio-opaque contrast agents
 - Characterization of anatomical details in high resolution
- \approx 2500 μ CT systems are in use worldwide with over 1000 publications annually

μ CT History

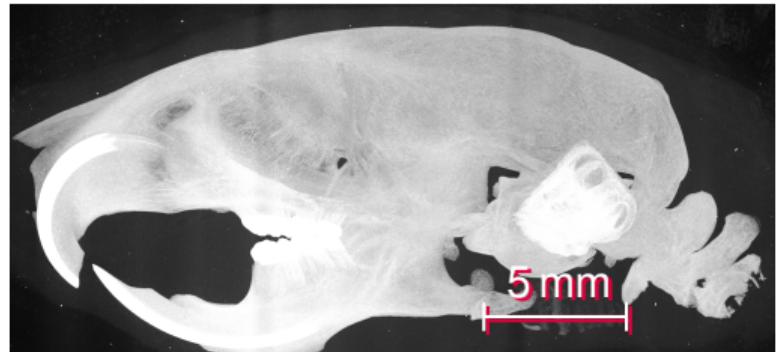
- 1984: Lee Feldkamp **Feldkamp1984** developed an early laboratory microCT system at Ford Motor Company's Scientific Research Laboratory (to nondestructively detect damage in ceramic automobile parts). Then collaborated with scientists at Henry Ford Hospital and University of Michigan interested in understanding bone microstructure **Feldkamp1989** and osteoarthritis **Layton1988**.
- 1987: The first commercially available μ CT scanner was developed by SkyScan (now Bruker)
- Today: μ CT 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, μ CT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
 - Paleontology and Archaeology: study of fossils and archaeological artifacts

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Why μ CT?

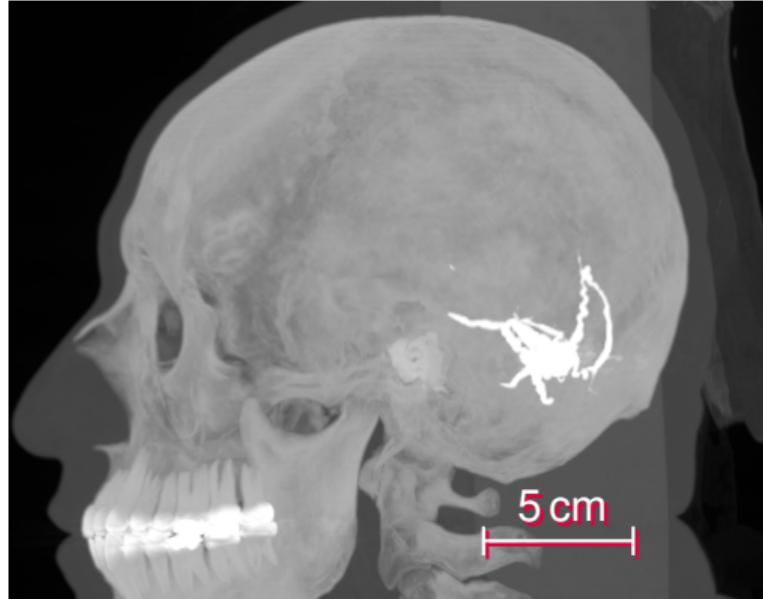


From [13], Subject C3L-02465



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Why μ CT?

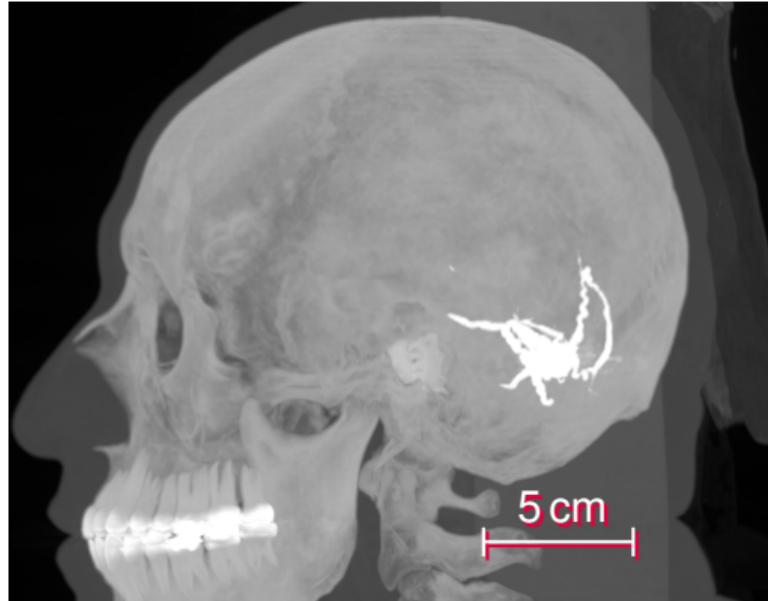


From [13], Subject C3L-02465

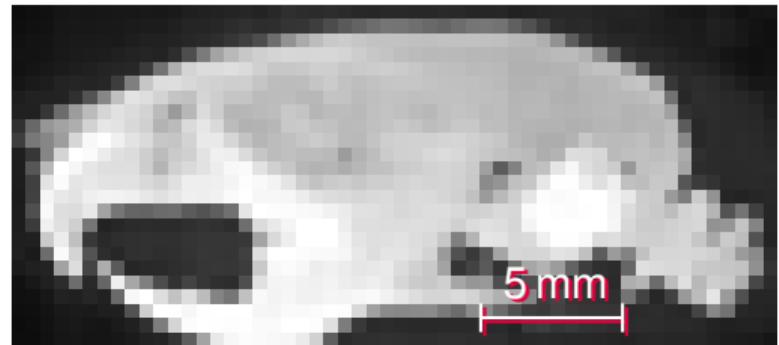


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Why μ CT?

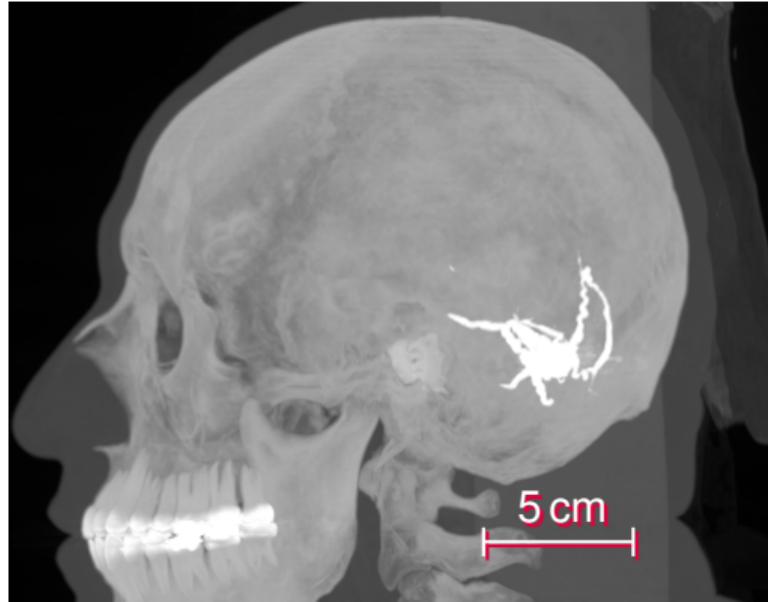


From [13], Subject C3L-02465

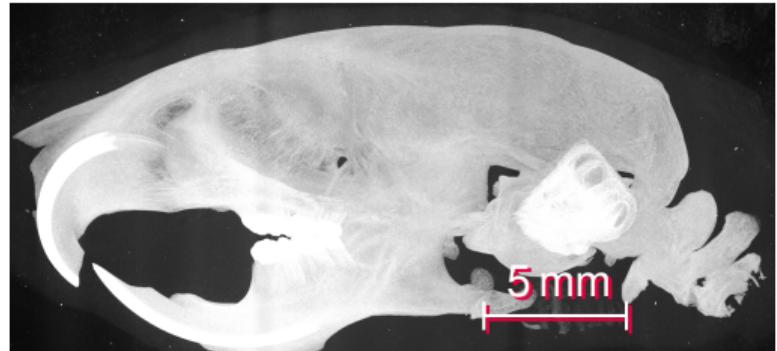


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Why μ CT?

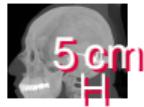


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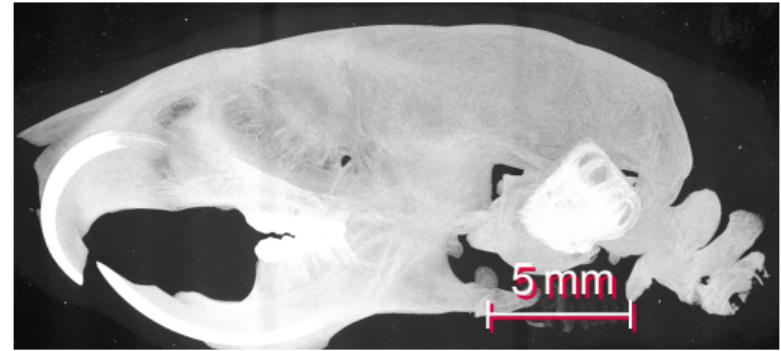


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Why μ CT?



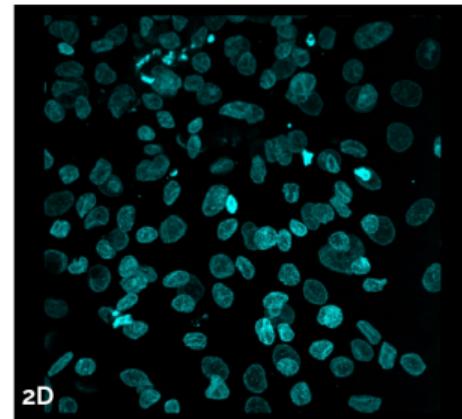
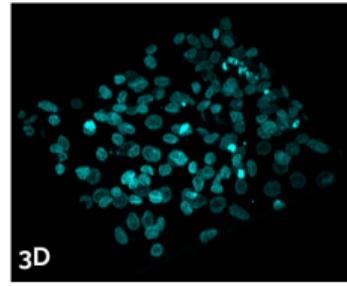
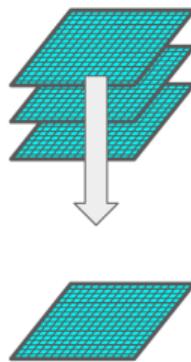
From [13], Subject C3L-02465



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.



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

Machinery

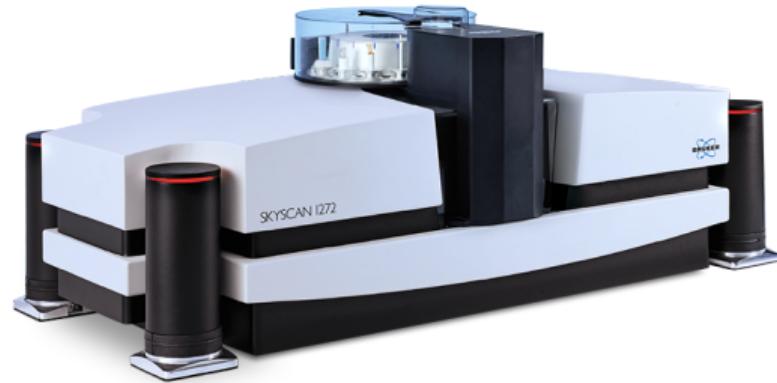
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flic.kr/p/fpTrGu

Machinery

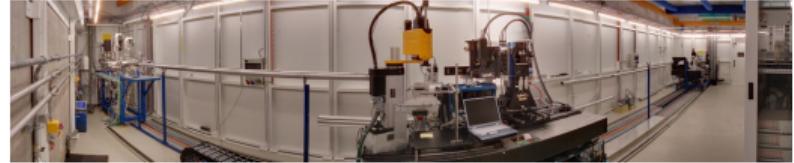
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- Hospital CT
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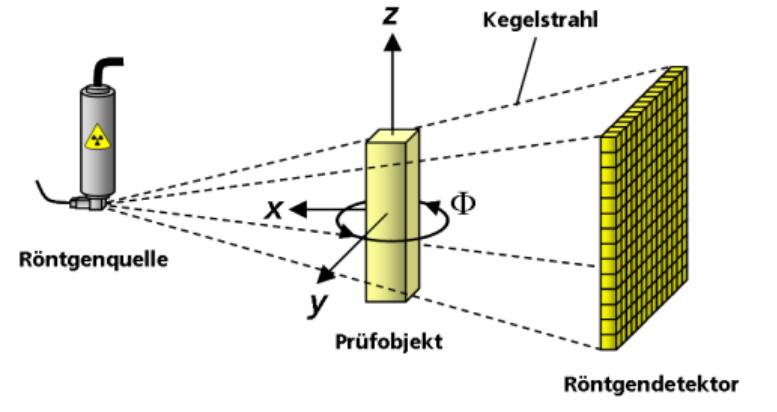


flic.kr/p/7Xhk2Y © ⓘ ⓘ

What is happening?

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

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



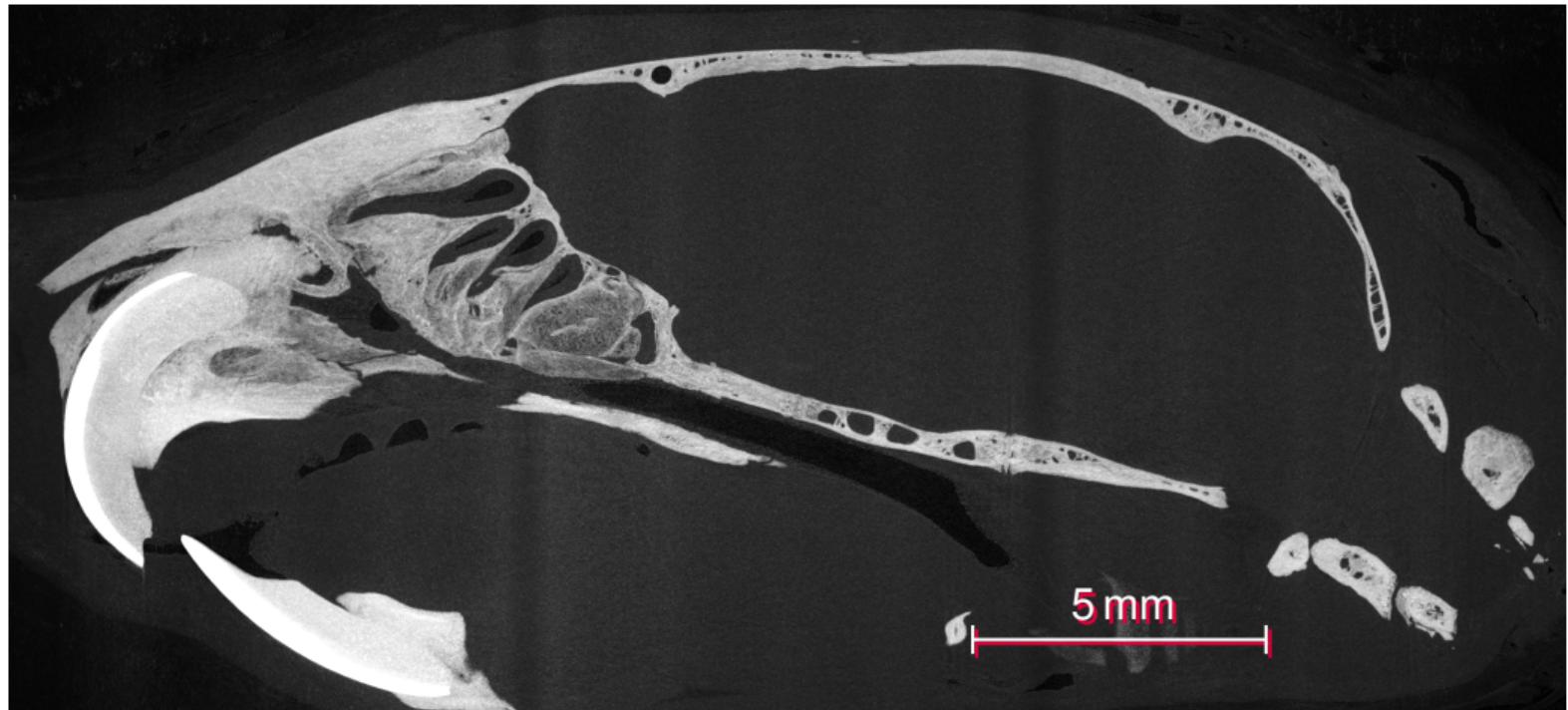
w.wiki/7g3 @①②

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Machinery

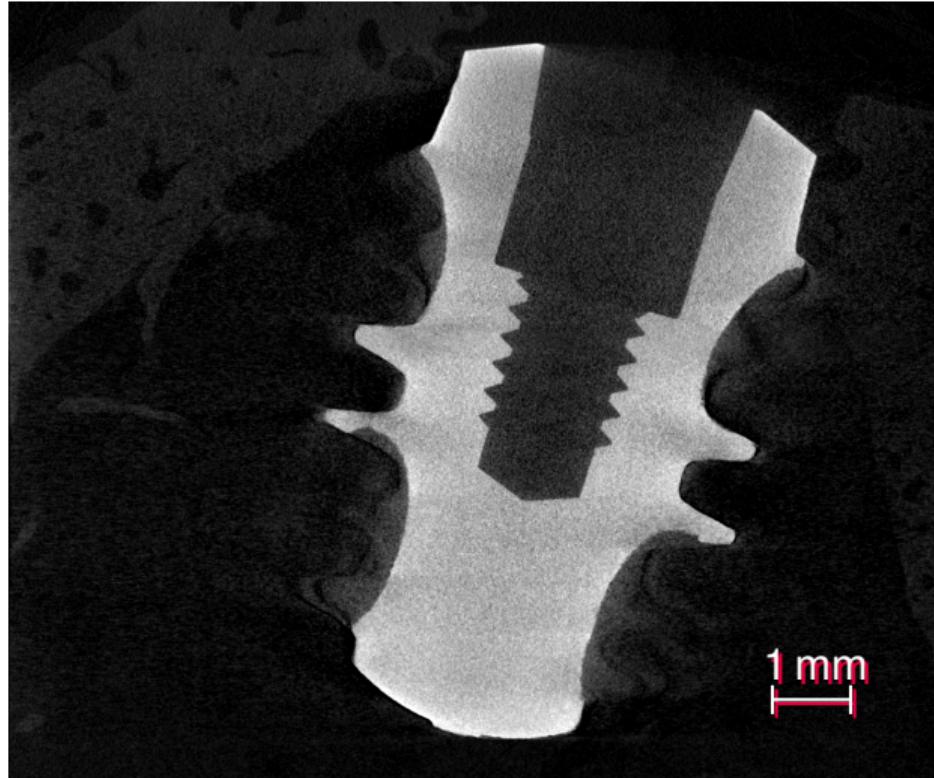
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Examples



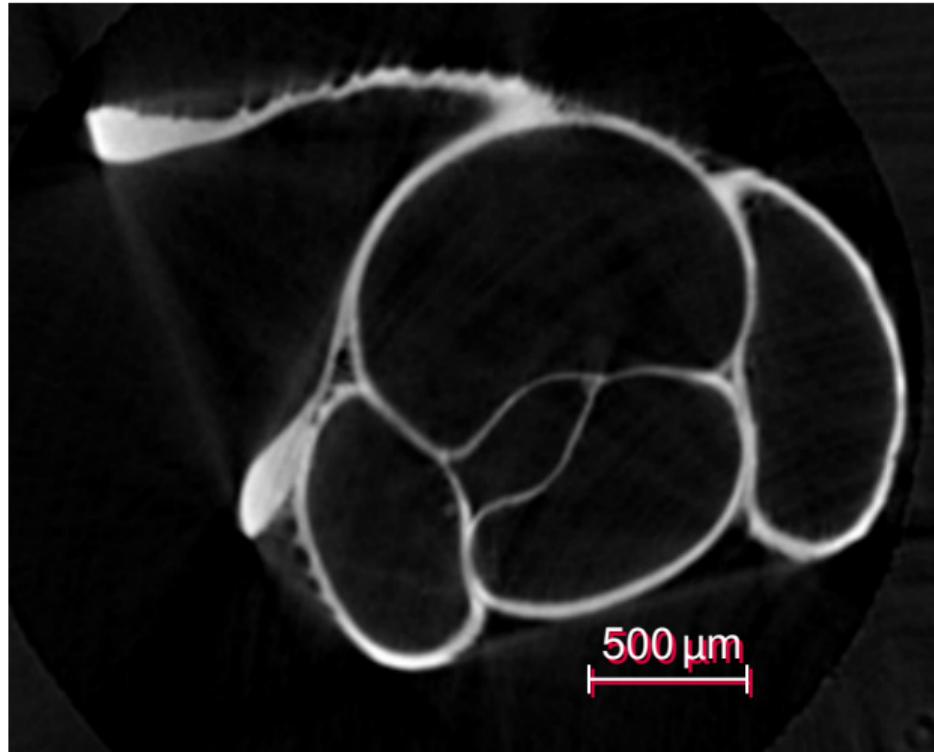
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Examples



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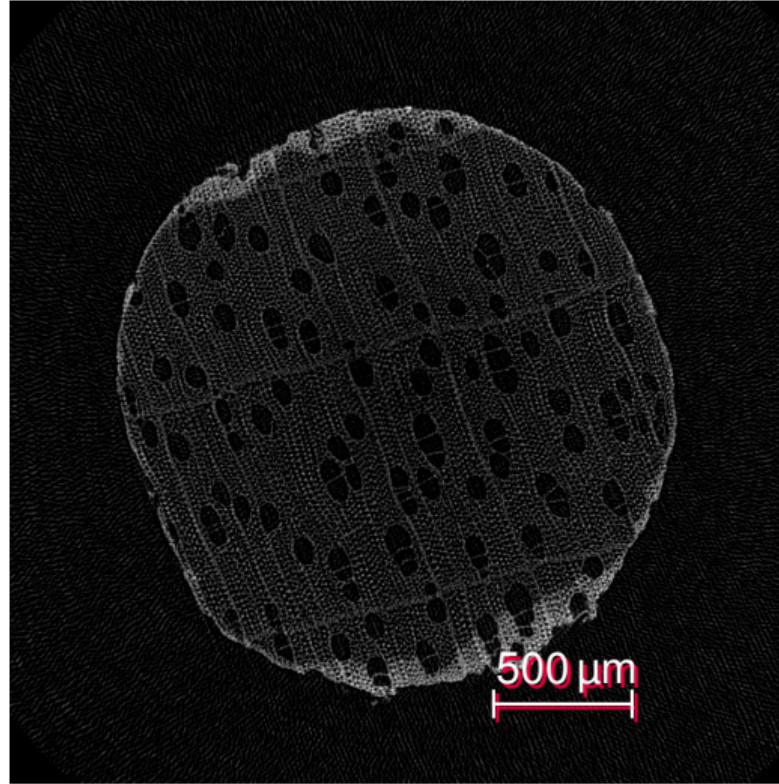
Examples



From [8], *Diancta phoenix*

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Examples



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Examples



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.”
(xrayphysics)
 - 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 **wiki:beer-lambert**: $I(t) = I_0 e^{-\alpha z}$

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

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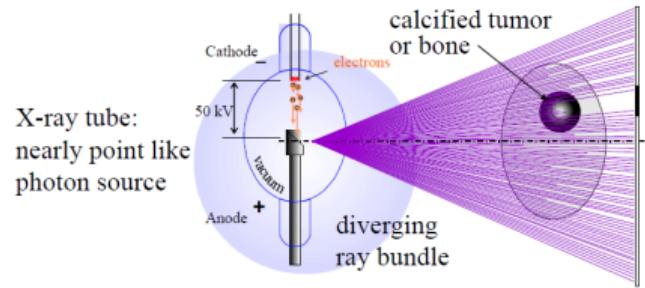
Preparation

- Study design
- Sample preparation

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Projections

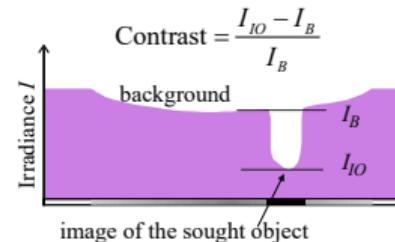
X-ray generation and contrast



X-ray tube:
nearly point like
photon source

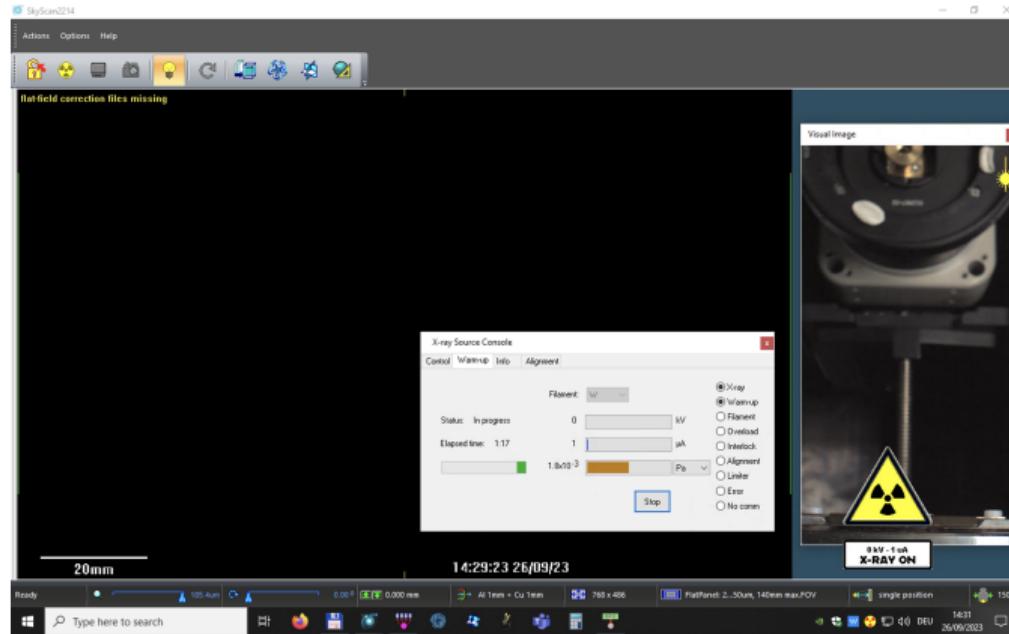
Contrast is given by
absorption of intensity I

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



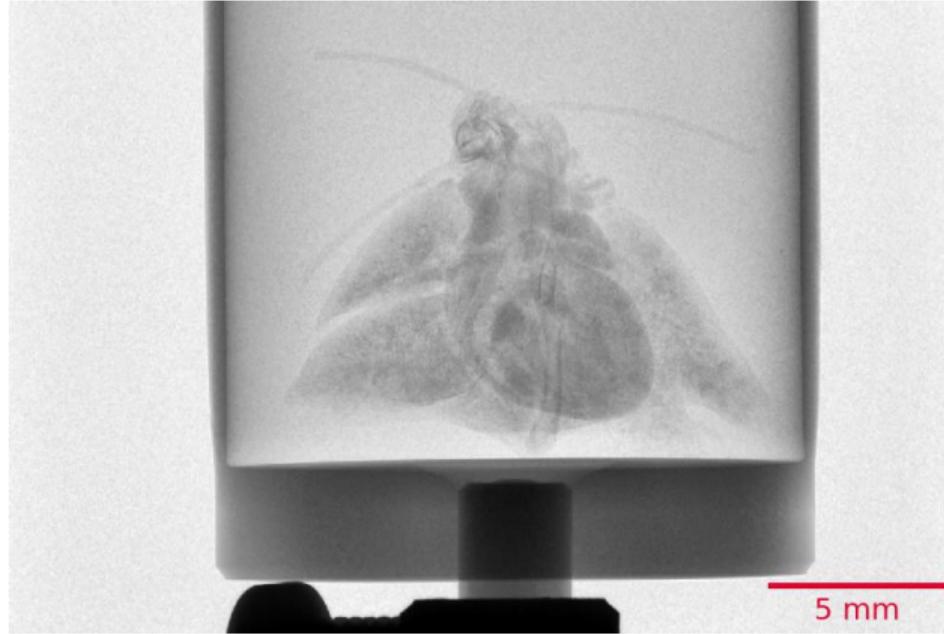
Contrast, Magnification and Resolution—Laws of Physics for Microscopists (1, 2023) by Martin Frenz, Slide 21

u^b Projection acquisition



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Projections

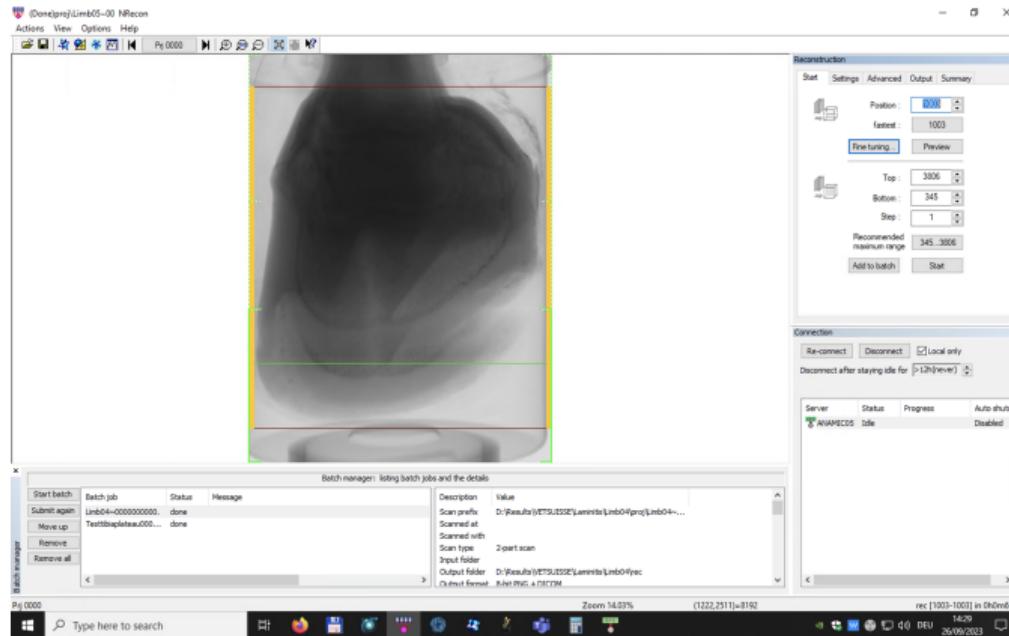


Projections

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

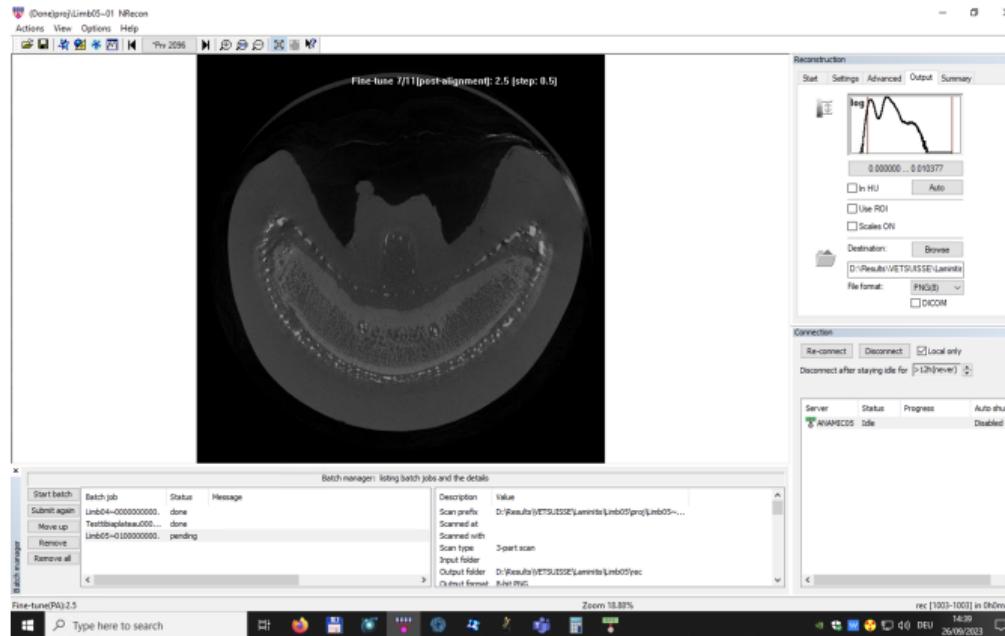
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Reconstructions



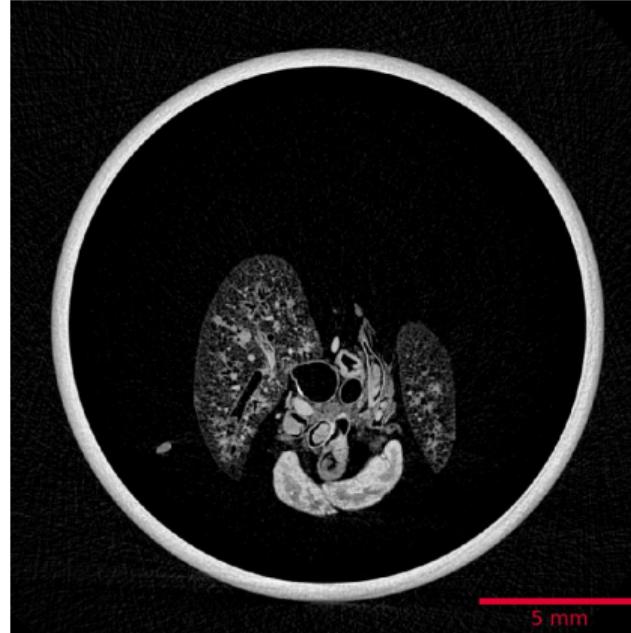
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Reconstructions



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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 **Feldkamp1984**
- Corrections (beam hardening, etc.)
- Writing to stack

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Visualization



Visualization

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

What to use?

- ImageJ/Fiji **Schindelin2012**
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
 -  in Jupyter **Kluyver2016**
 - **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 of teeth
- Structure of teeth



- Morphology of teeth
- Analytical workflow, doi.org/g/r8

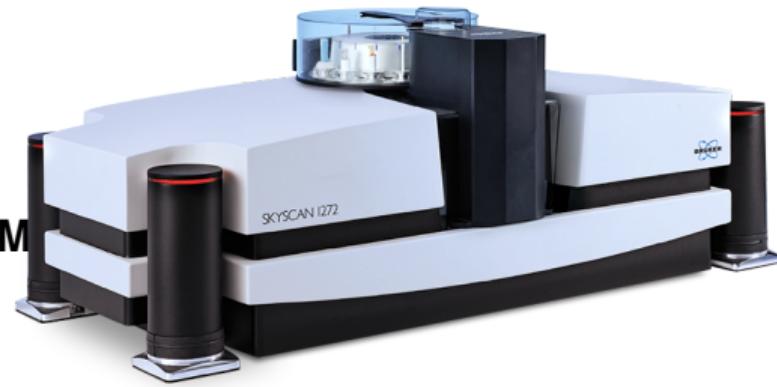
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to **Briseno-Marroquin2015** Briseno-I
- *Reproducible* analysis **Haberthuer2020a**, e.g. you can click a button to double-check or recalculate the results yourself!



How?

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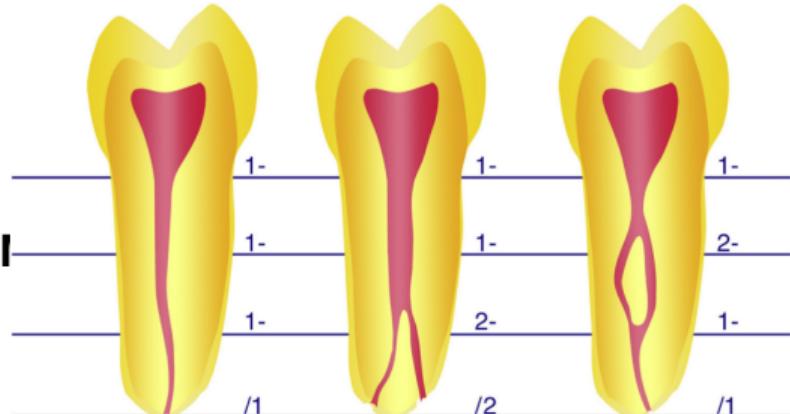
```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files= 482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)= 80
Rotation Speed (deg/s)= 125
Image Pixel Size (µm)=9.999986
Exposure (ms)=950
Rotation Step (deg)=0.400
Frame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

How?

- 104 extracted human permanent mandibular canines
 - μ CT imaging
 - Root canal configuration, according to **Briseno-Marroquin2015** ~~Briseno-Marroquin2015~~
 - *Reproducible* analysis **Haberthuer2020a**, e.g. you can click a button to double-check or recalculate the results yourself!
- 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

How?

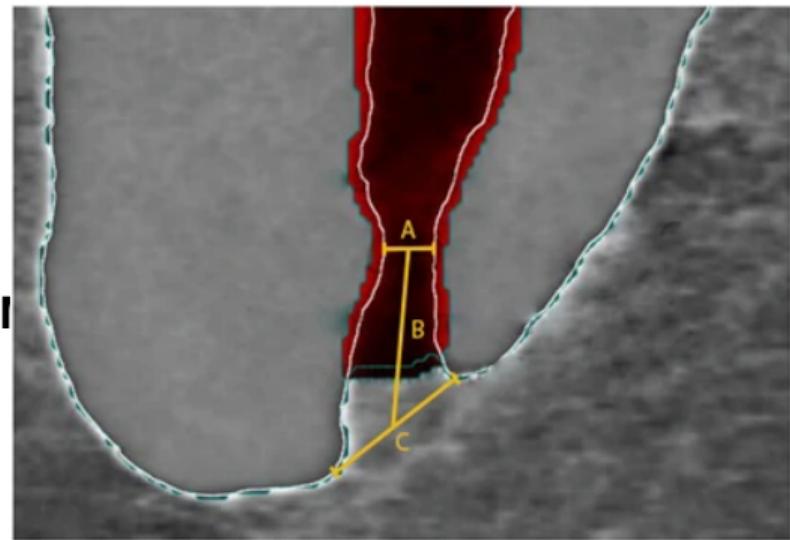
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From **Briseno-Marroquin2015**, Fig. 2

How?

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From **Wolf2017**, Fig. 1

How?

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gph.is/2nqkple

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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 table of file commits:

File	Description	Last Commit
.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
ToothDataSize.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

Below the table is the 'README.md' page content:

README.md

DOI: <https://zenodo.3999402> treebeard.yml failing

[Launch Binder](#)

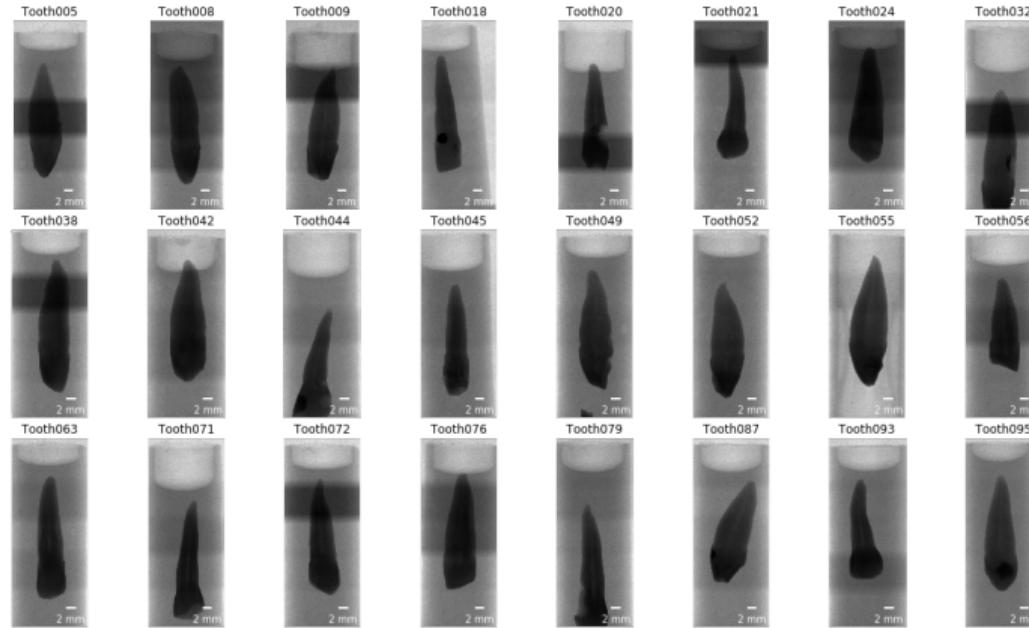
A big tooth cohort

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

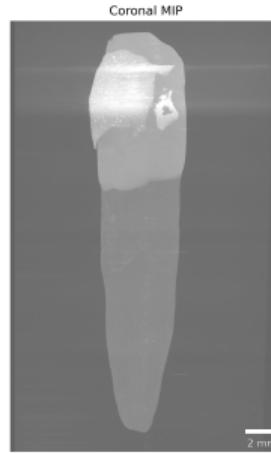
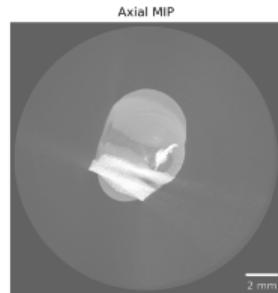
μ CT imaging



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Dataset cropping

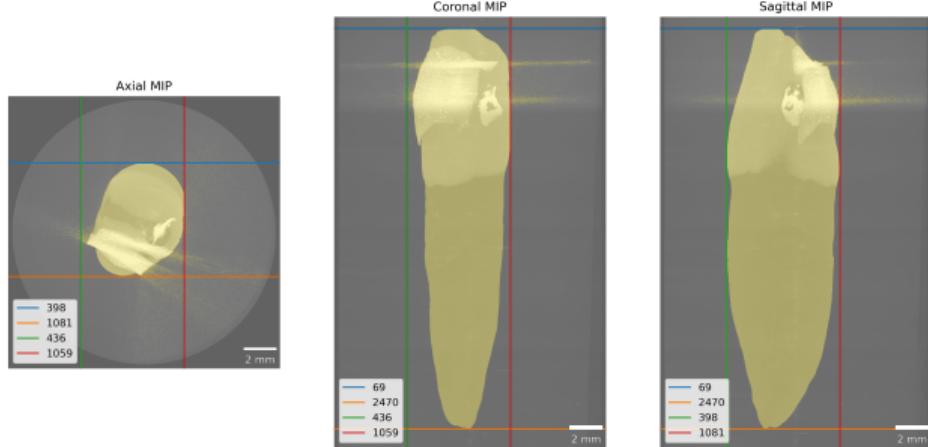
- Full datasets: 326 GB
- Cropped datasets: 115 GB



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Dataset cropping

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



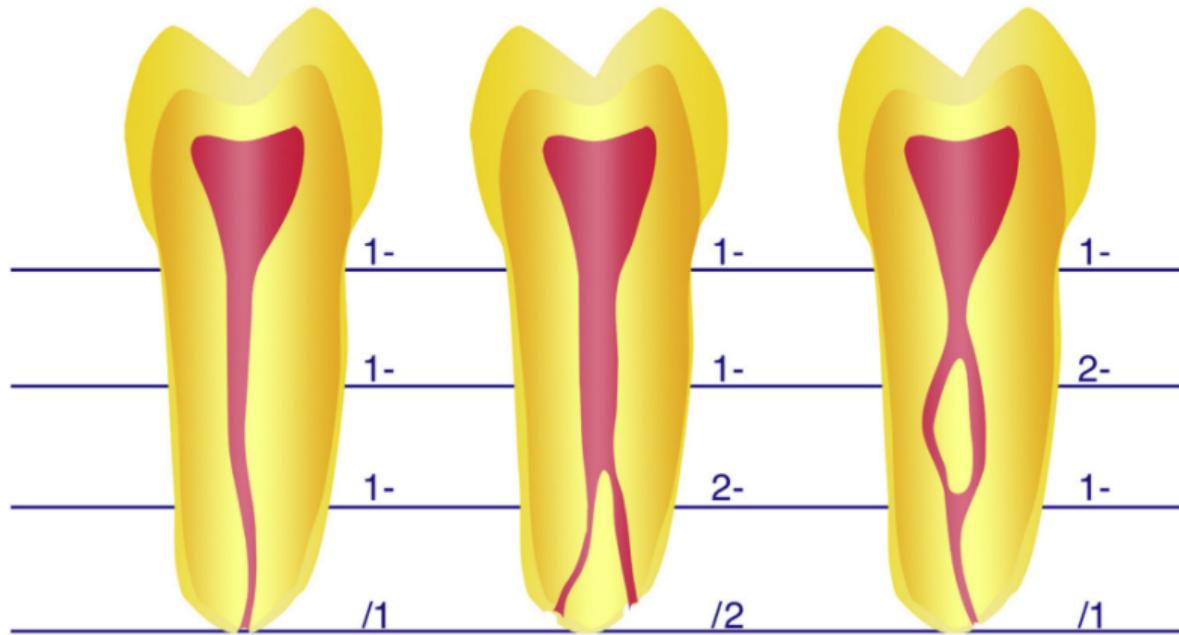
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Tooth morphology



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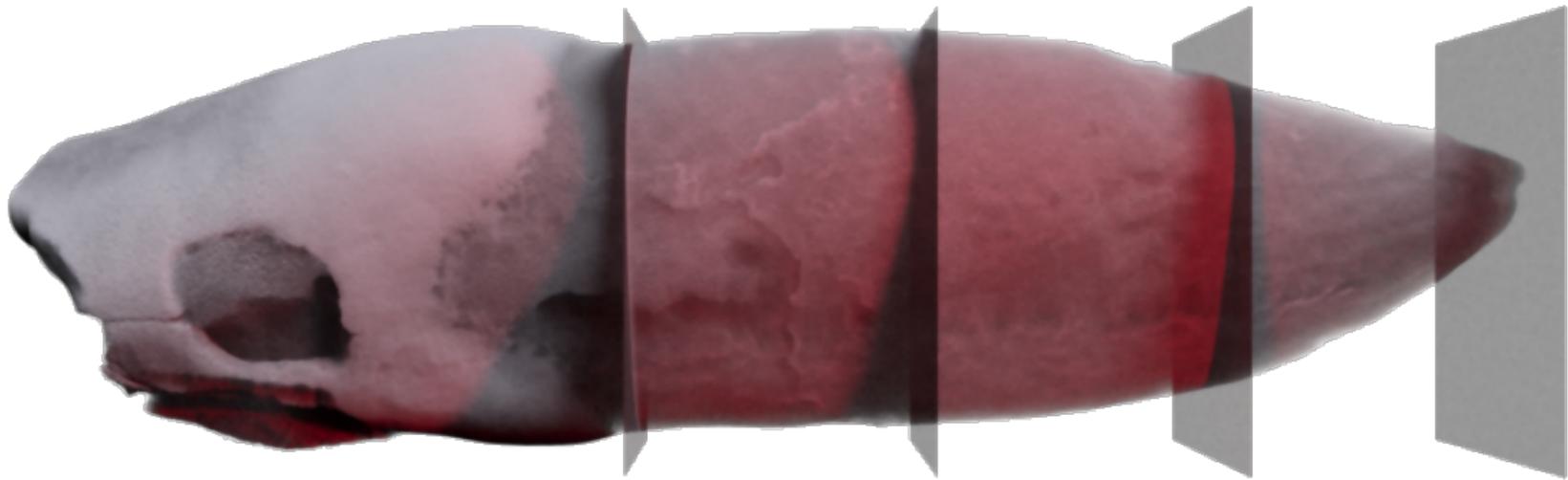
Tooth morphology



From Briseno-Marroquin2015, Fig. 2

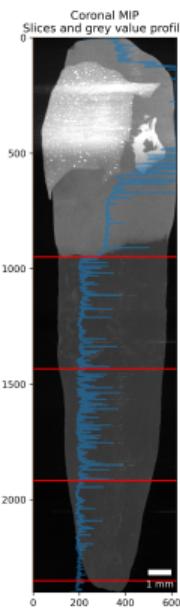
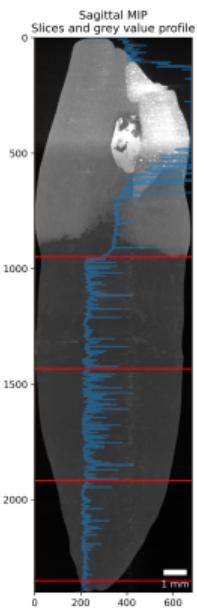
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Tooth morphology

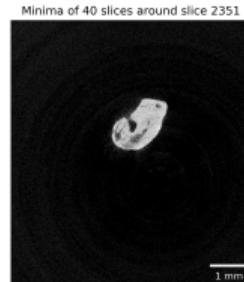
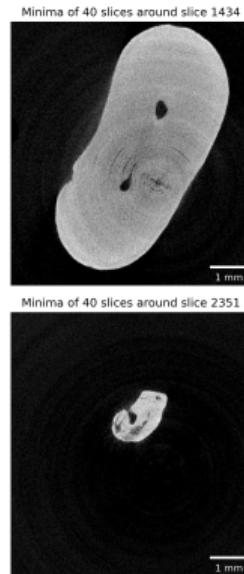
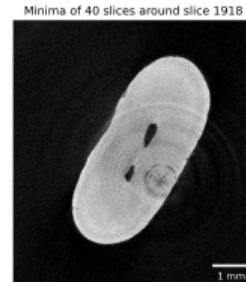
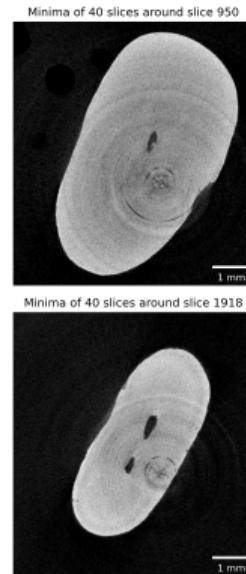


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Detection of enamel-dentin border



Tooth045

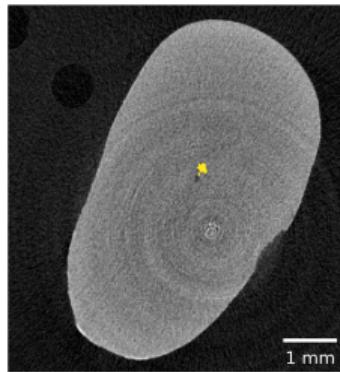


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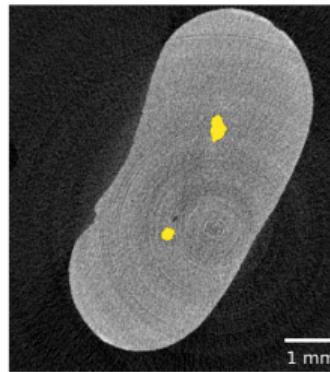
Detection of enamel-dentin border

Tooth045

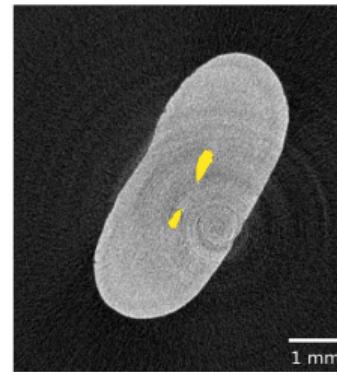
Tooth045, Slice 950: 1



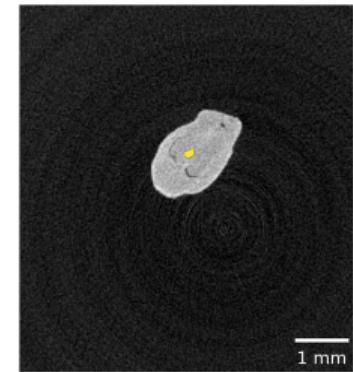
Slice 1434: 2



Slice 1918: 2



Slice 2351: 1



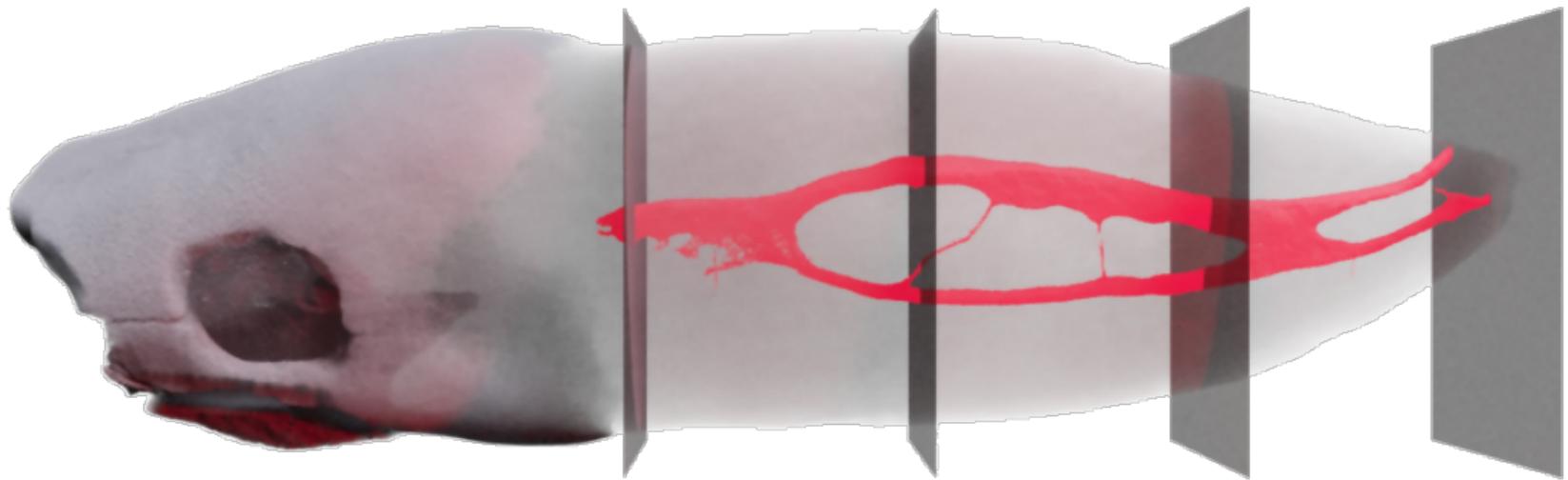
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Outcome root canal configuration classification

Roots	RCC	#	%
Single (N=98)	1-1-1/1	73	74.5
	1-1-1/2	14	14.3
	1-1-1/3	1	1.0
	1-1-1/4	2	2.1
	1-1-2/1	1	1.0
	1-2-1/1	4	4.1
	1-2-1/2	1	1.0
	1-2-2/2	1	1.0
	2-3-1/1	1	1.0
Double (N=3)	Buccal	1-1-1/1	2
		1-2-1/1	1
	Lingual	1-1-1/1	2
		1-1-1/2	1

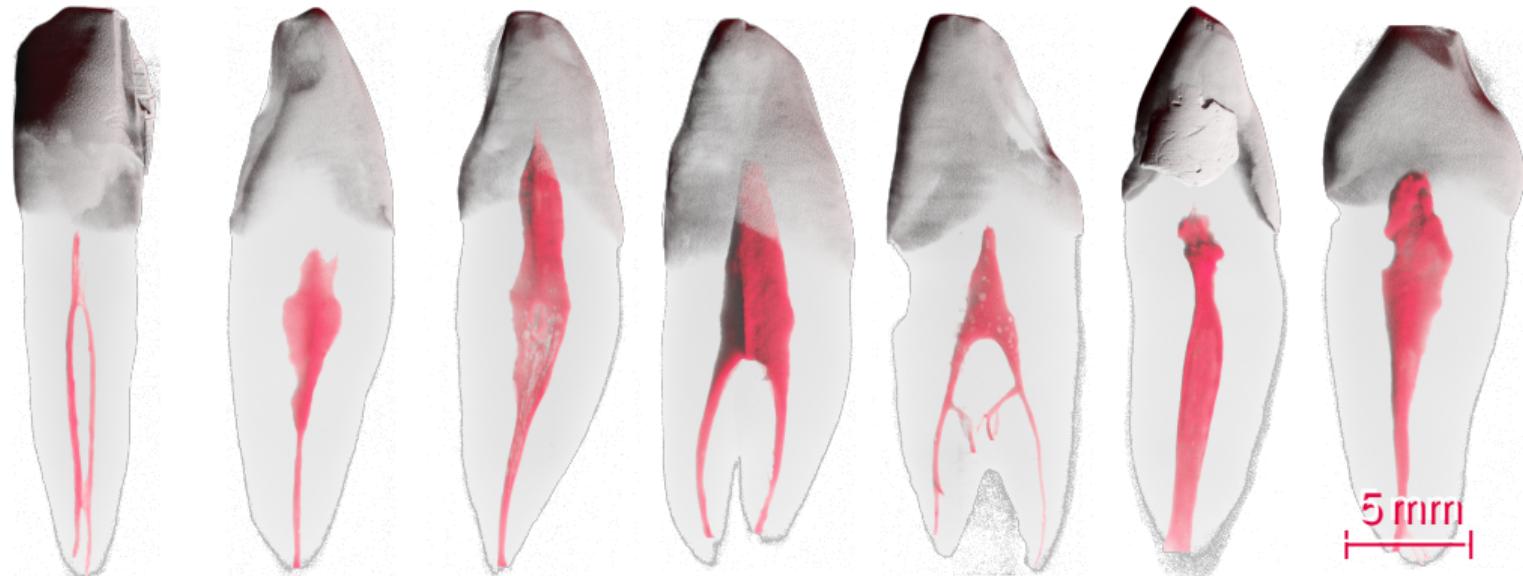
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Extraction of root canal space



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Results of root canal space extraction



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

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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

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