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**UNIVERSITÄT
BERN**

X-ray microtomography

David Haberthür

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

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: μ CT group, Institute of Anatomy, University of Bern, Switzerland.

Grüessech from the μ CT-group



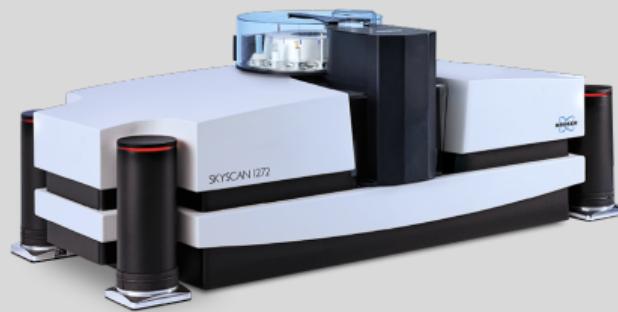
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Oleksiy.Khoma@unibe.ch

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

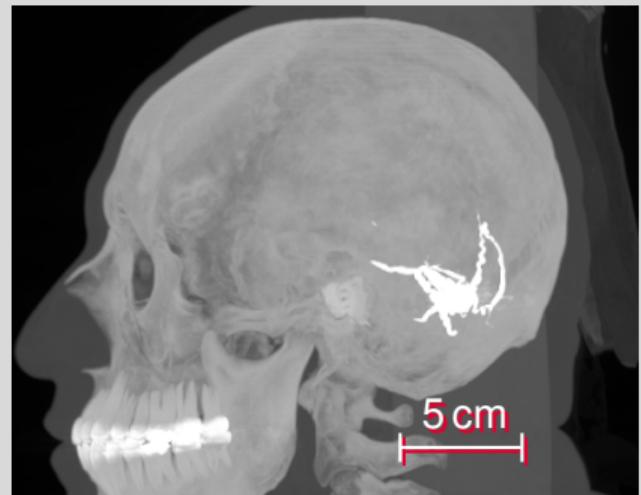
Overview

Materials & Methods

Results

micro-Computed tomography

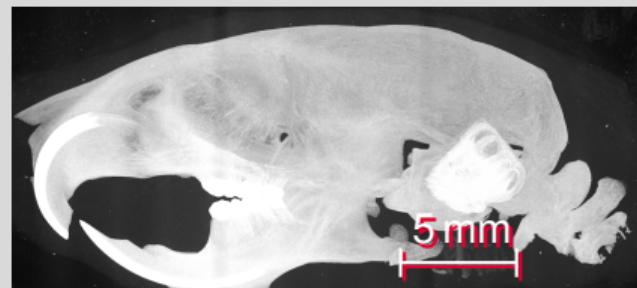
- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a very large range of sample sizes



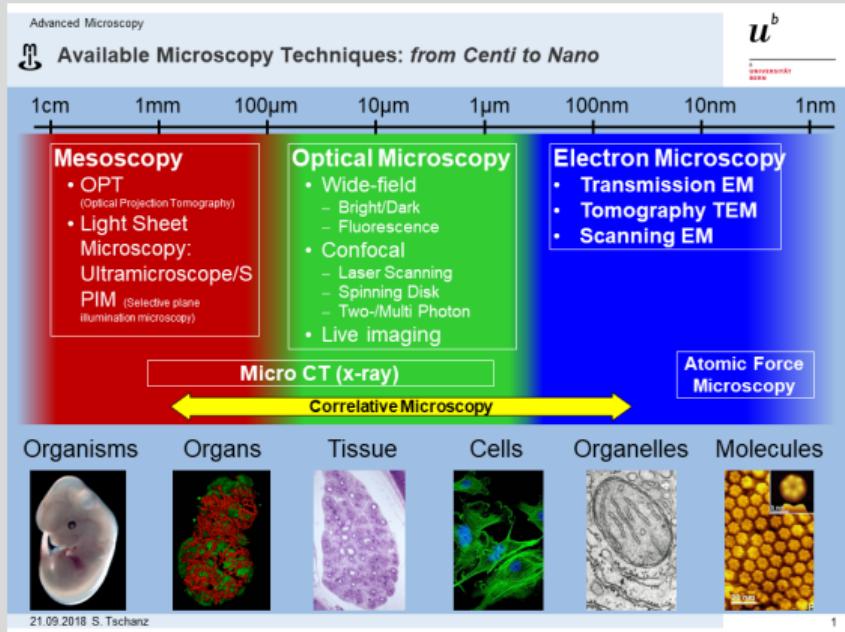
From [13], Subject C3L-02465

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



Imaging methods



- *Light Sheet Microscopy* by Nadia Mercader Huber
- X-ray imaging
- Electron microscopy
 - *Transmission Electron Microscopy* by Dimitri
 - *Scanning Electron Microscopy* by Sabine Kässmeyer and Ivana Jaric
 - *Cryoelectron Microscopy & Serial Block Face SEM* by Iordan

Stefan Tschanz, with permission

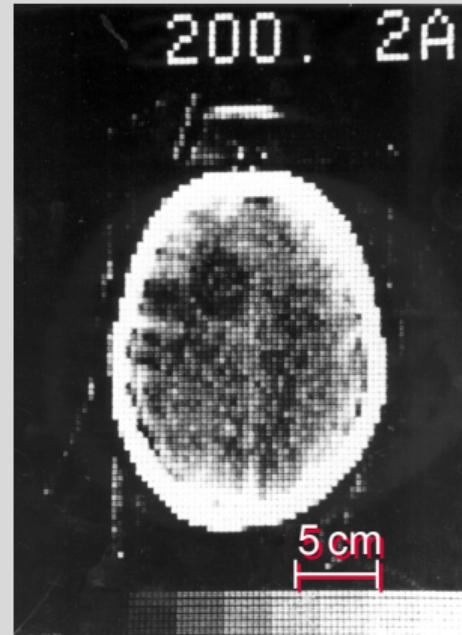
CT-Scanner



youtu.be/2CWpZKuy-NE

CT History

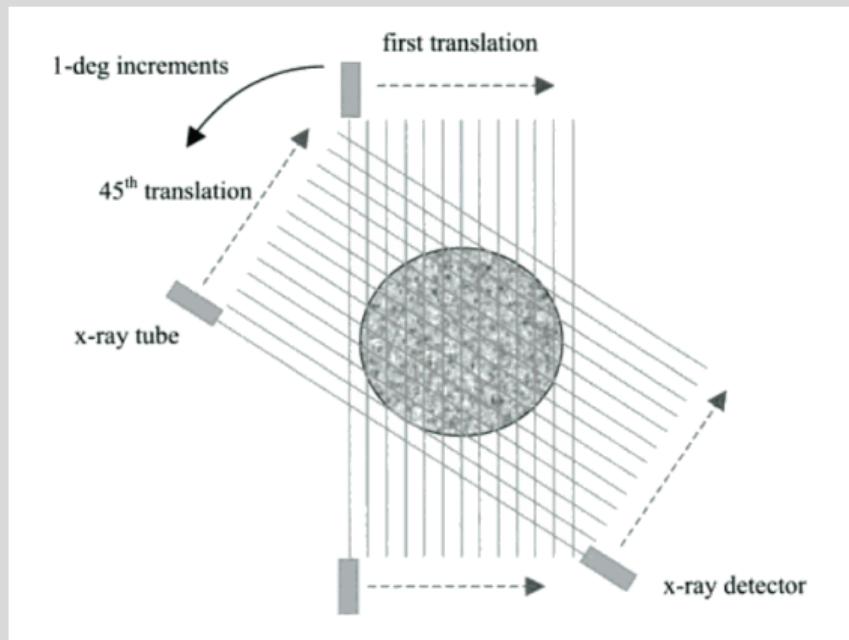
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]



From [16], Figure 5

CT History

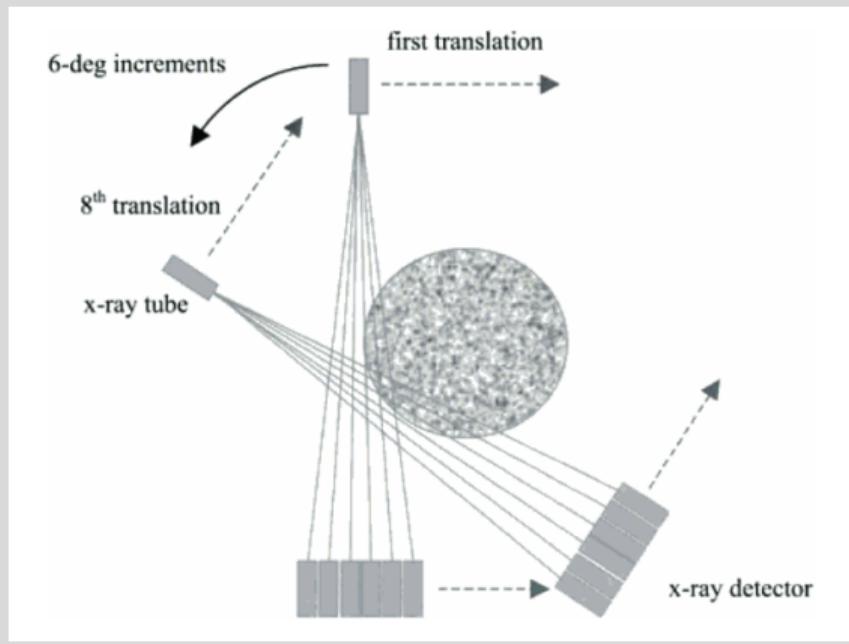
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- CT scanner generations
 - First generation



From [17], Figure 1.12

CT History

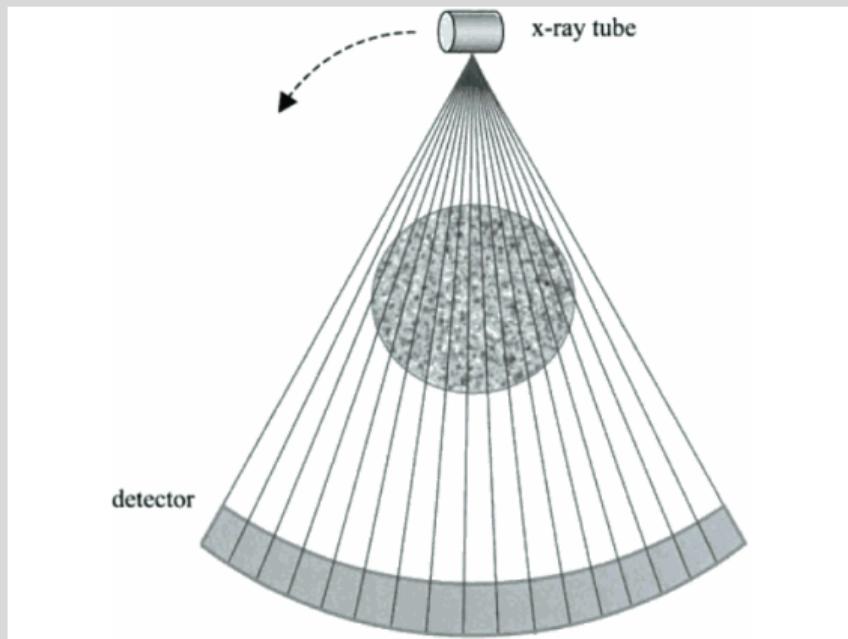
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- CT scanner generations
 - First generation
 - Second generation



From [17], Figure 1.13

CT History

- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- CT scanner generations
 - First generation
 - Second generation
 - Third generation



From [17], Figure 1.14

μ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [18]
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp [19] 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

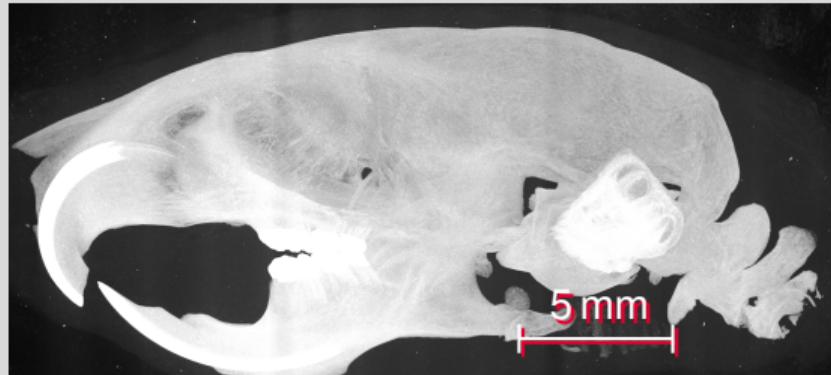
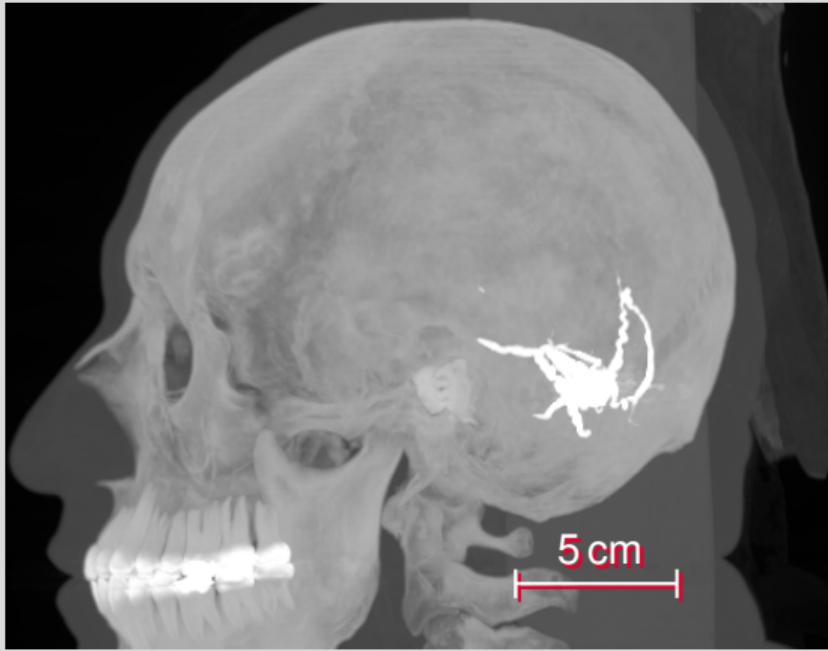
μ CT History II

- 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 [20]
- 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

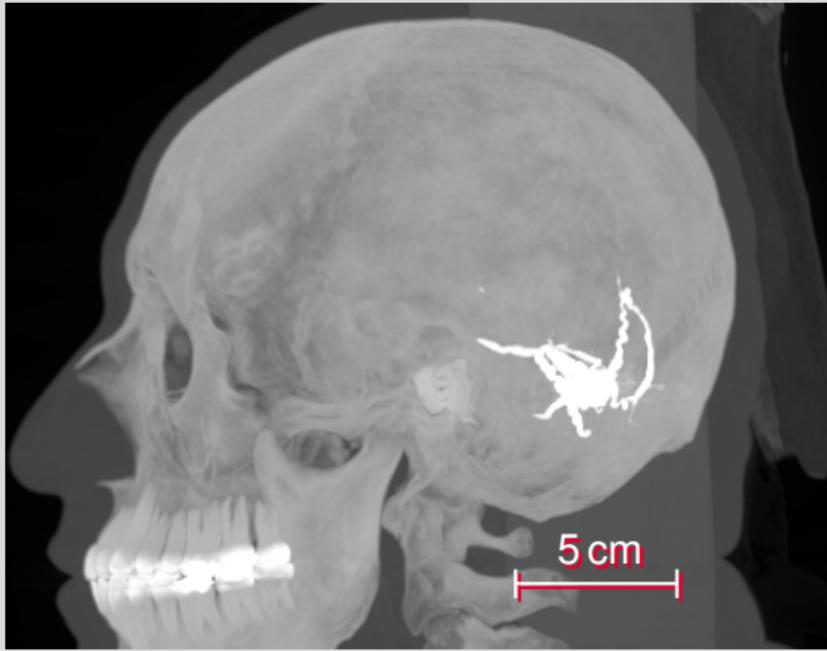
µCT History III

- ≈2500 µCT systems are in use worldwide with over 1000 publications annually

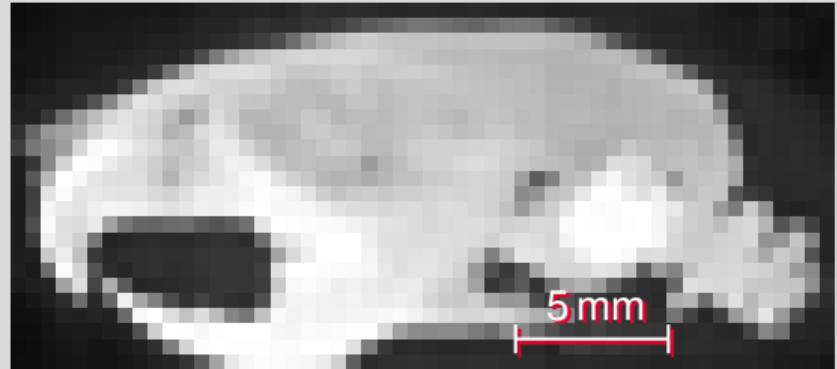
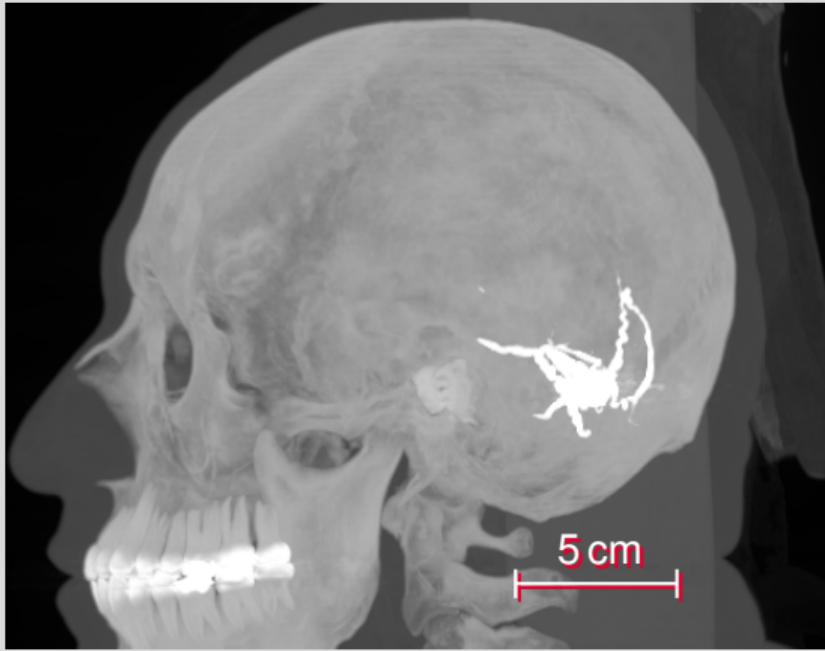
Why μ CT?



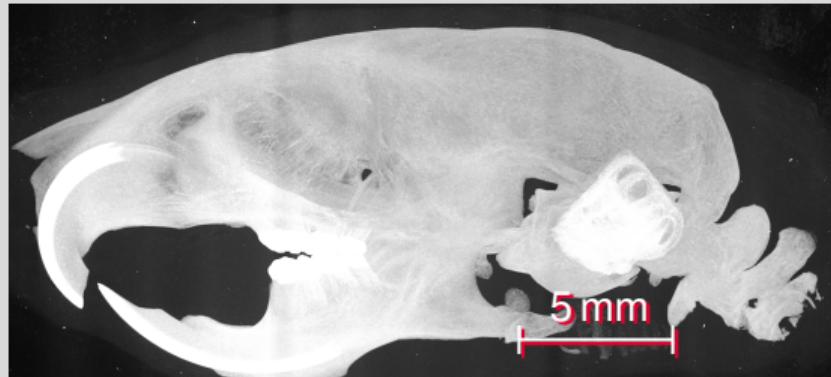
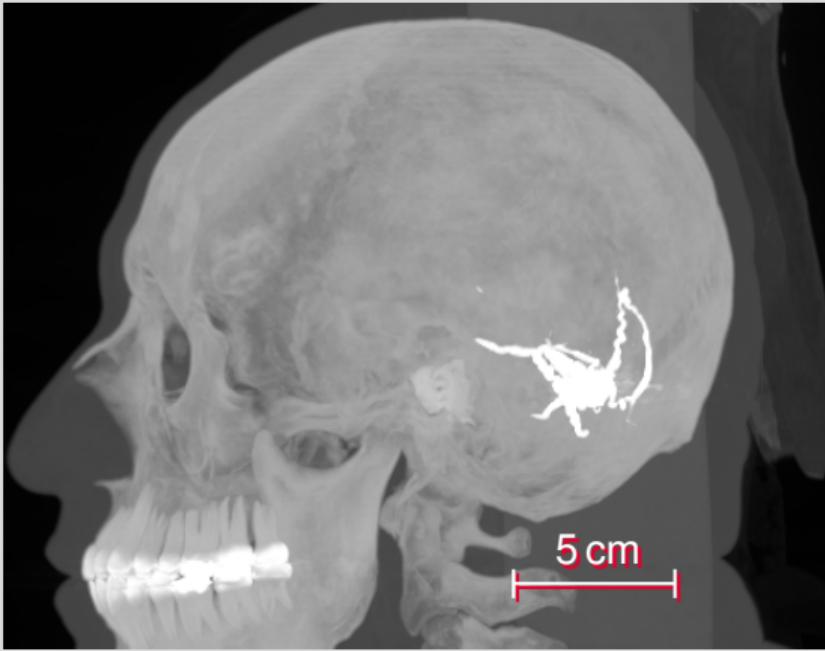
Why μ CT?



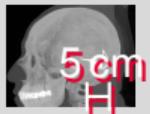
Why μ CT?



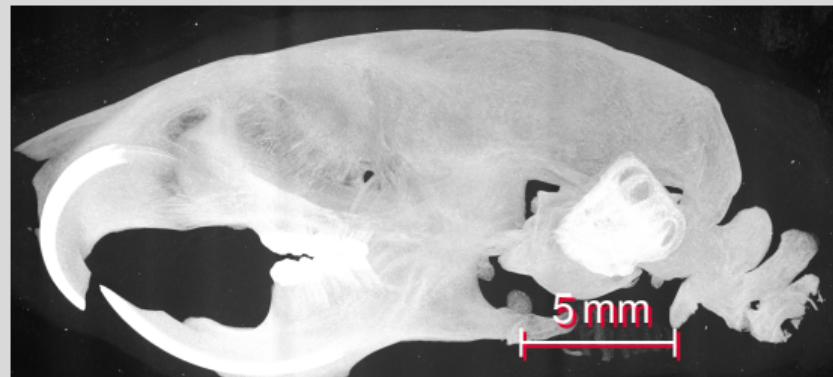
Why μ CT?



Why μ CT?



From [13], Subject C3L-02465

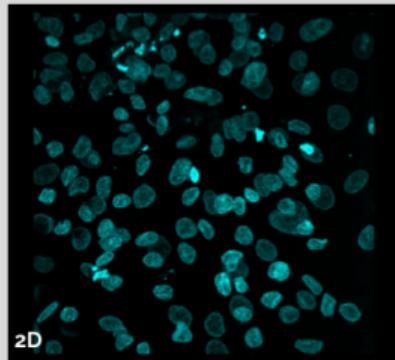
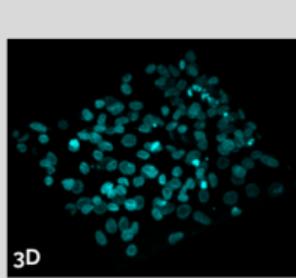
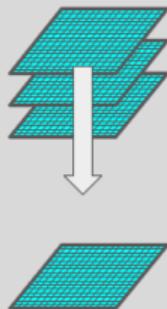


Maximum intensity projection

Projections

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



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

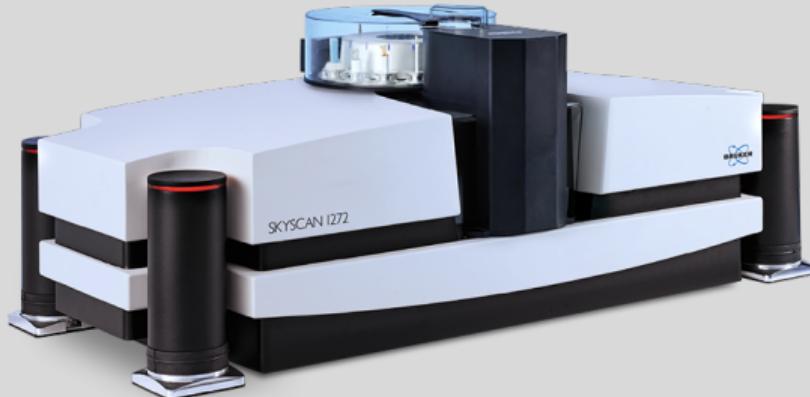
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flic.kr/p/fpTrGu

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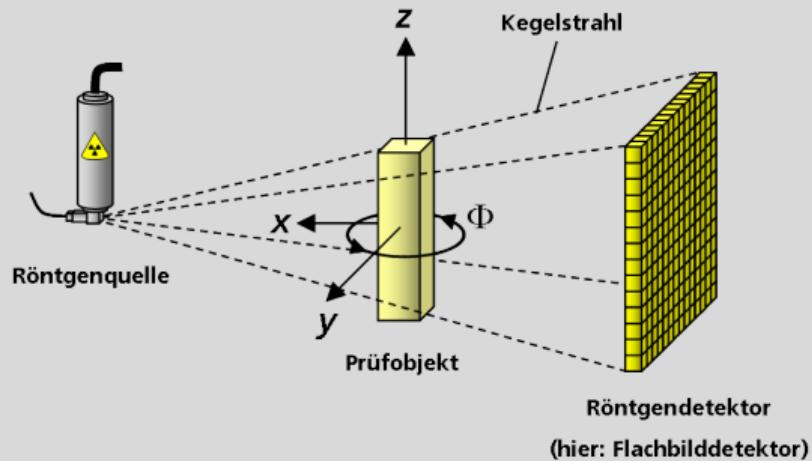


flic.kr/p/7Xhk2Y

What is happening?

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

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

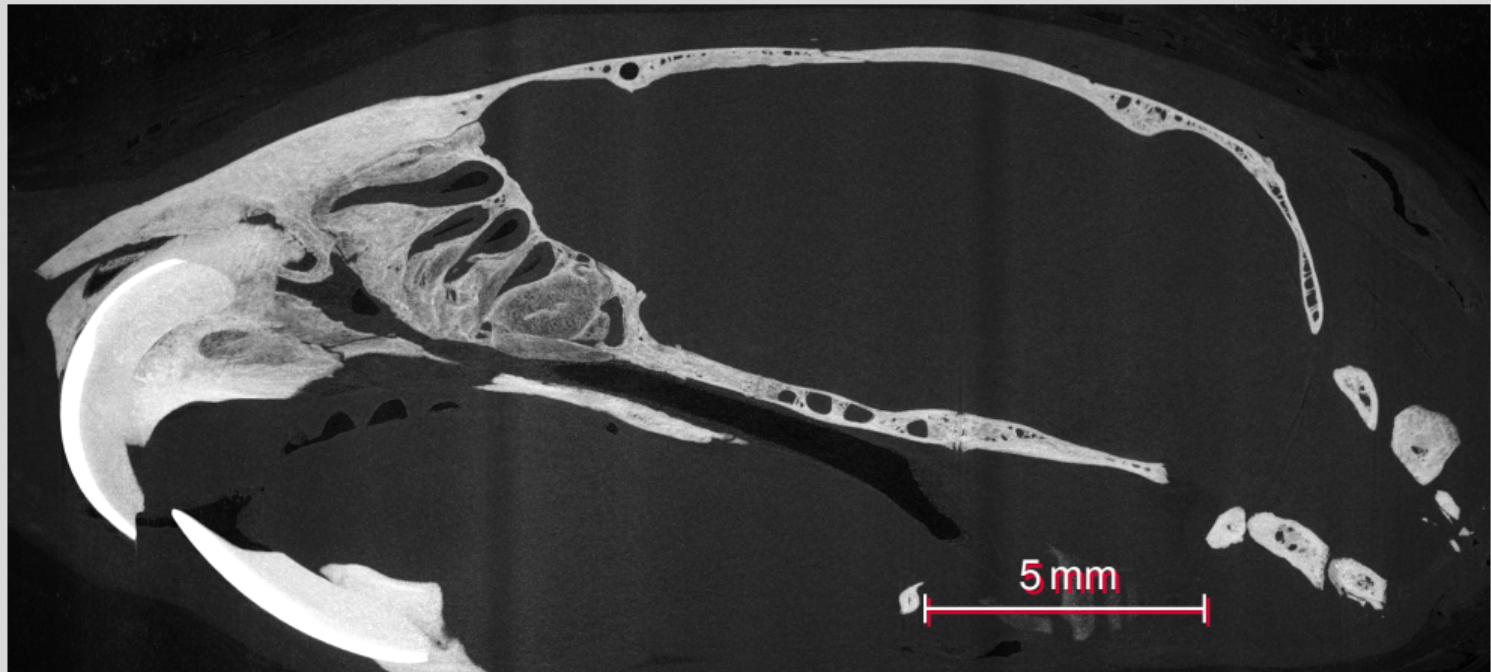


(hier: Flachbilddetektor)

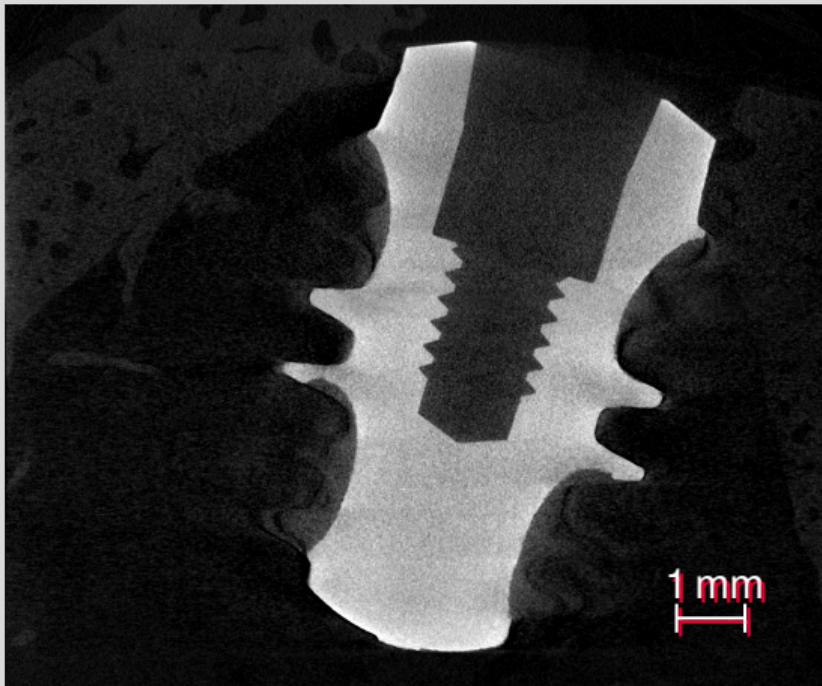
w.wiki/7g3

Machinery

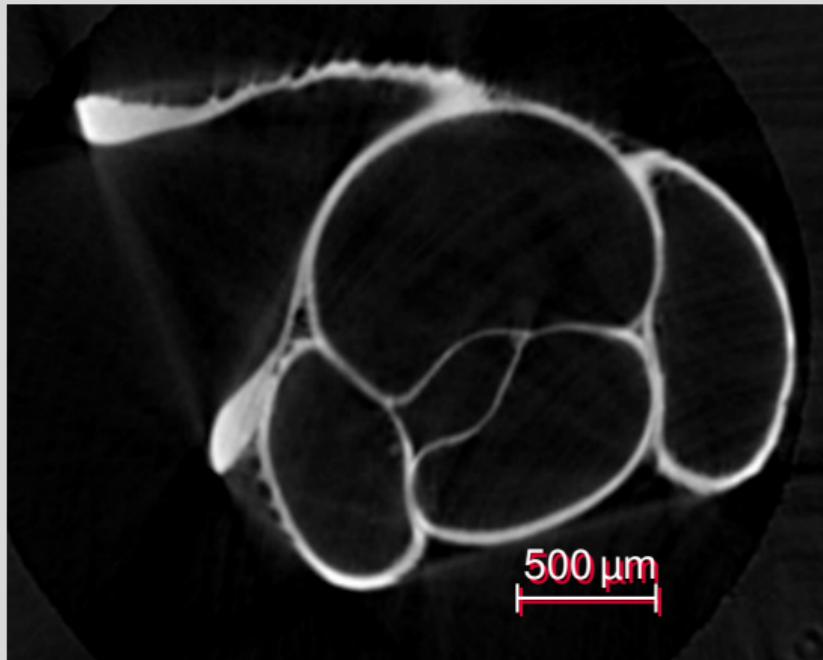
Examples



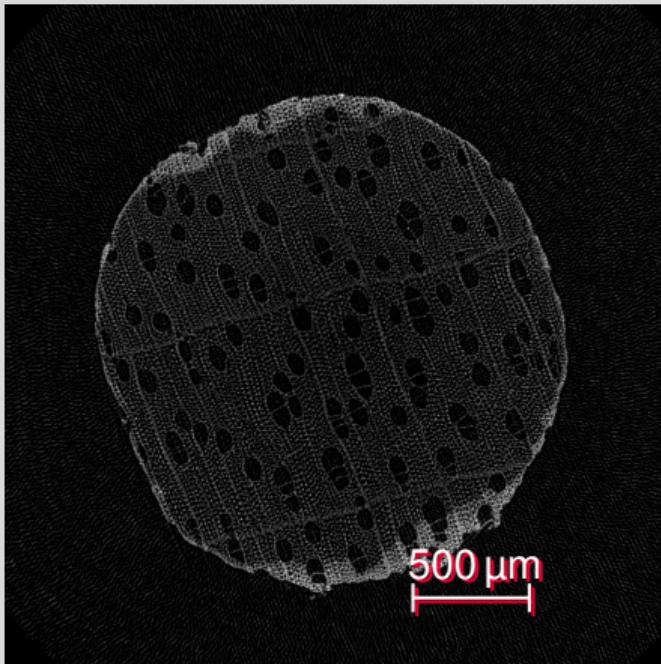
Examples



Examples



Examples



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.” ([21])
 - 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 [22, 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

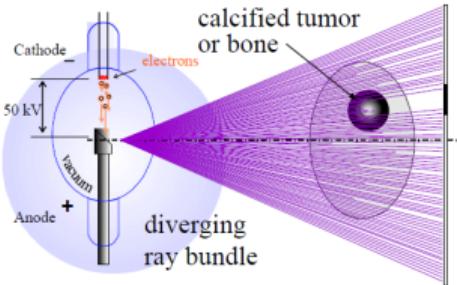
Preparation

- Study design
- Sample preparation

Projections

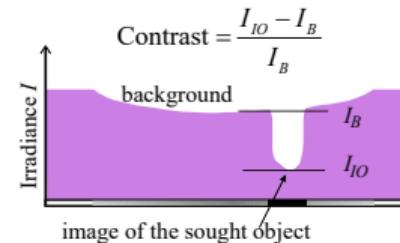
X-ray generation and contrast

X-ray tube:
nearly point like
photon source

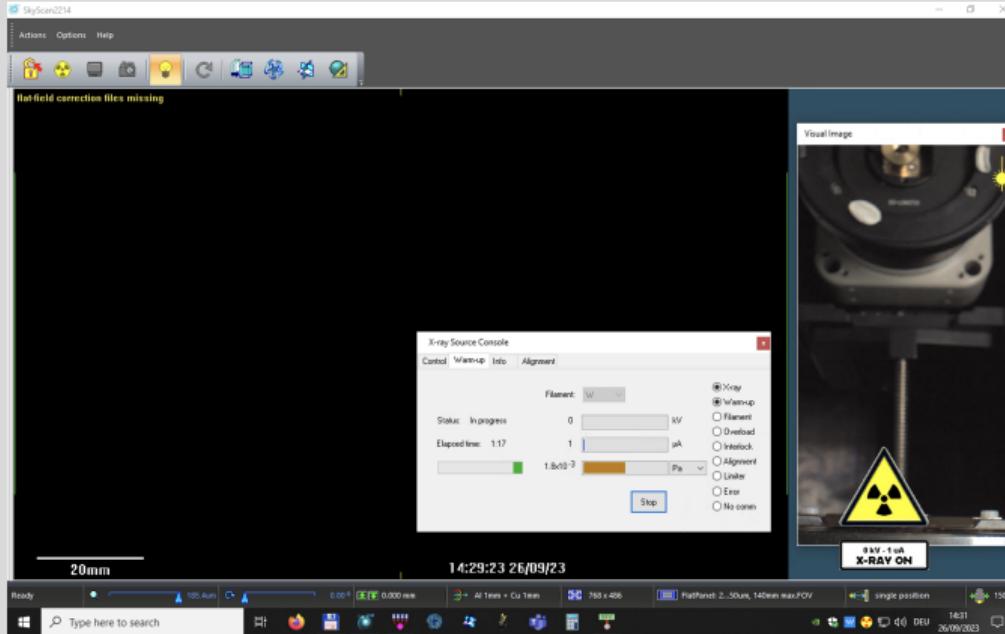


Contrast is given by
absorption of intensity I

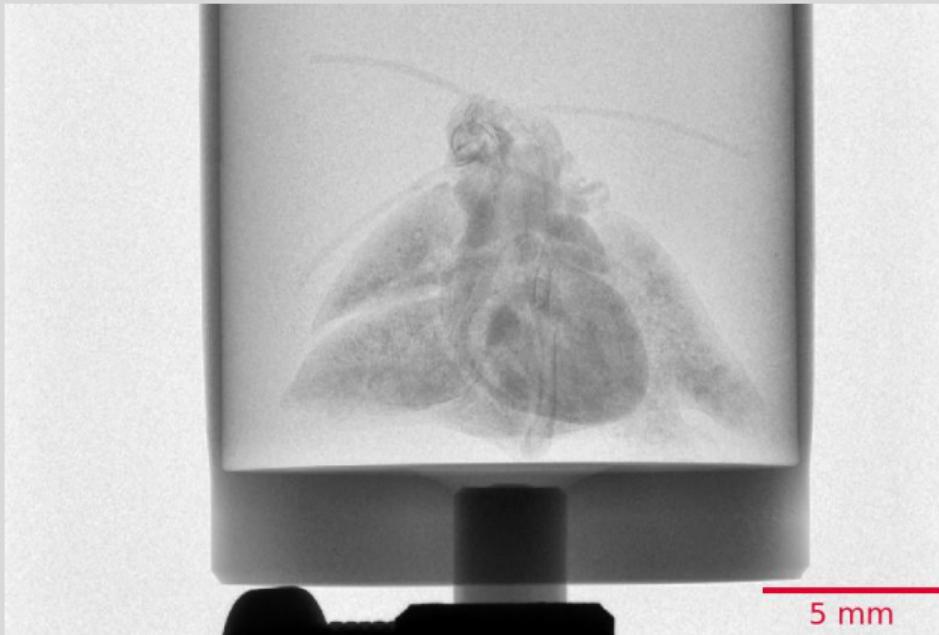
Note that contrast is negative
X-ray shadowgraphy
is a bright field technique



Projection acquisition



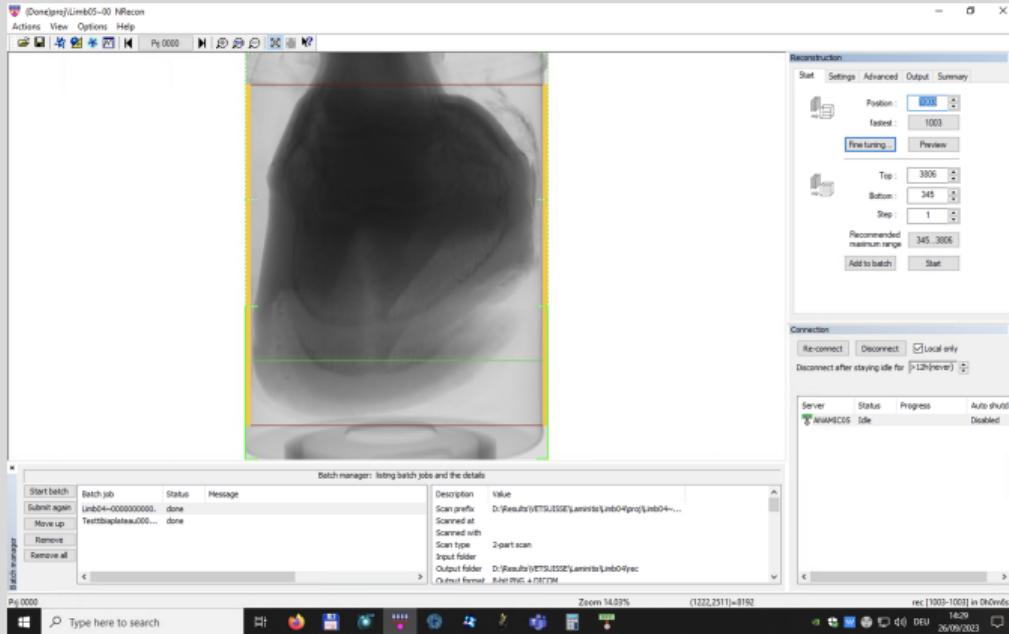
Projections



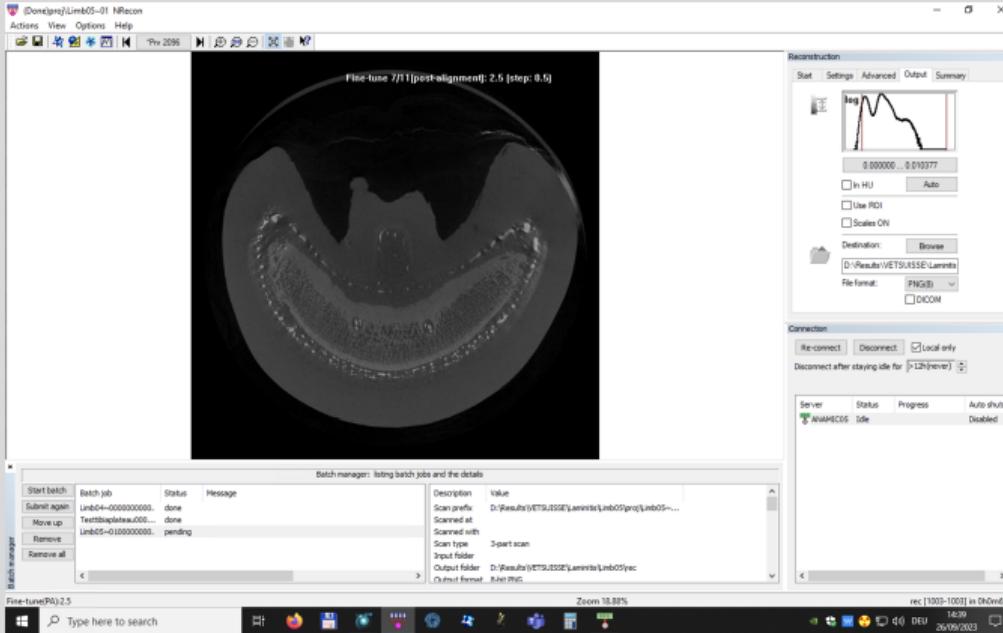
Projections

- 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



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 [19]
- Corrections (beam hardening, etc.)
- Writing to stack

Visualization



Visualization

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

What to use?

- ImageJ/Fiji [23]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
 -  in Jupyter [24]
 - `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 instead of just pretty images
- Segmentation of teeth and root canal
- (Unbiased) Characterization
- Reproducible and automated image analysis ( in Jupyter [24])
- Two publications:
 - [11]: doi.org/gjpw2d
 - [25]: doi.org/g7r8

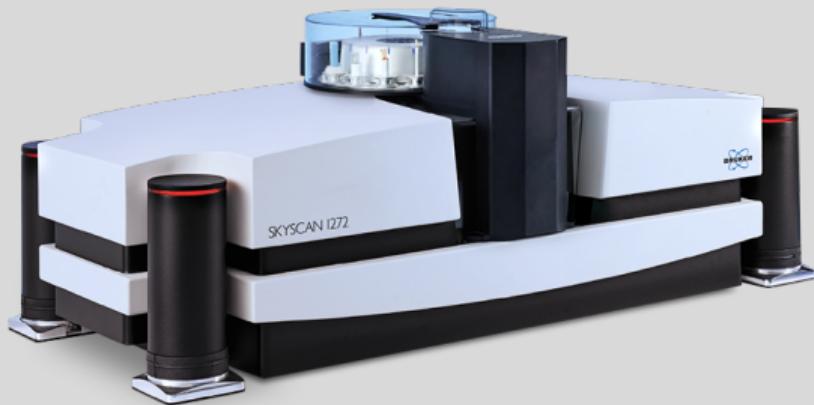
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín et al. [26]
- *Reproducible* analysis [27], 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

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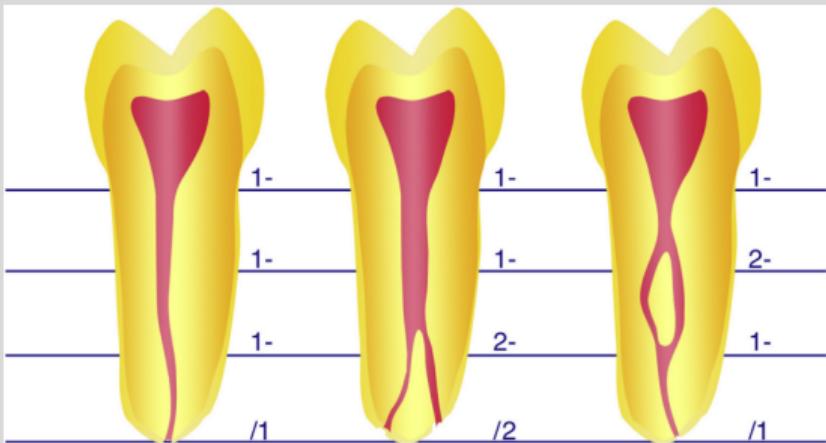
Sample changer on the SkyScan 1272

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

How?

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From [26], Fig. 2

How?

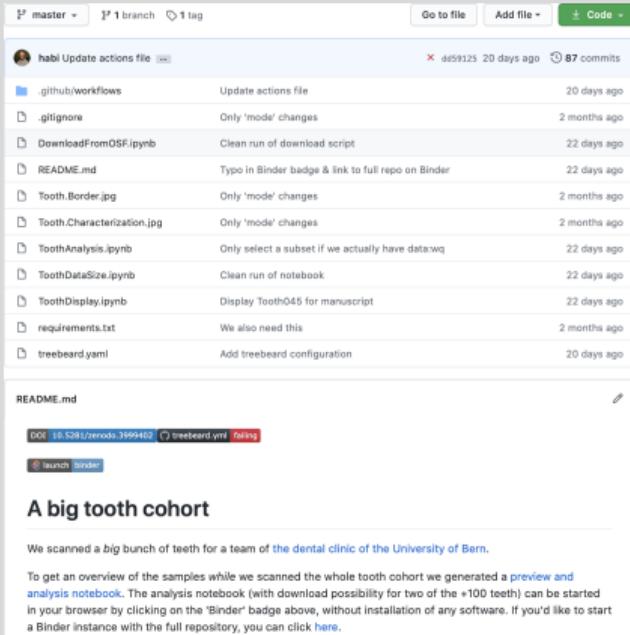
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gph.is/2nqkple

How?

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The screenshot shows a GitHub repository interface. At the top, there are buttons for 'master' (with a dropdown arrow), '1 branch', '1 tag', 'Go to file', 'Add file', and 'Code'. Below this is a list of files and their details:

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
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
treeboard.yaml	Add treeboard configuration	20 days ago

Below the file list is the 'README.md' page, which contains the following content:

```
README.md

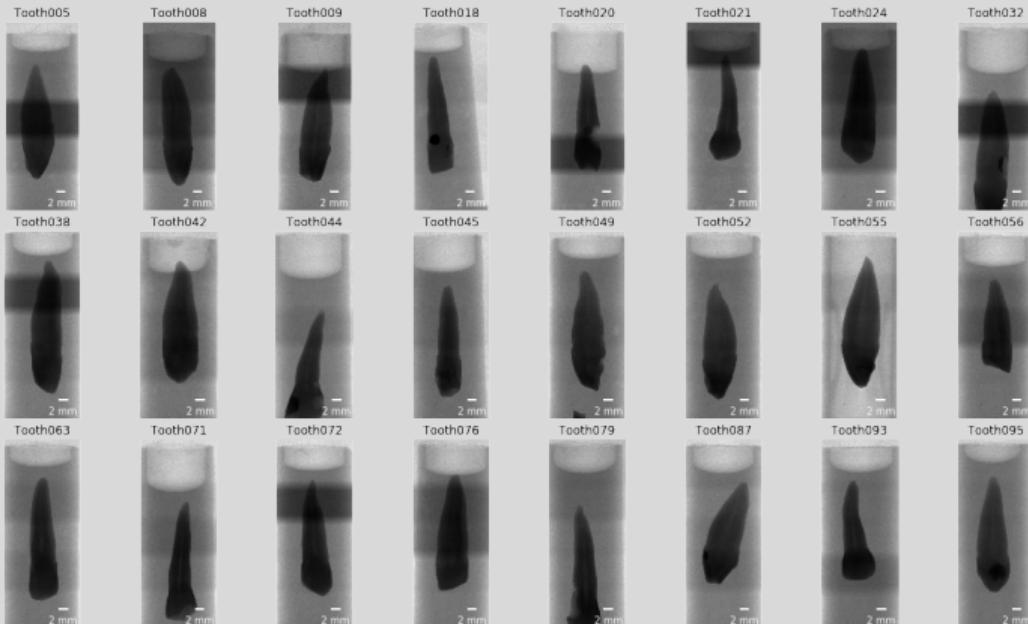
DOI: 10.5281/zenodo.3999462 treeboard.yaml failing
[Launch Binder]

A big tooth cohort

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

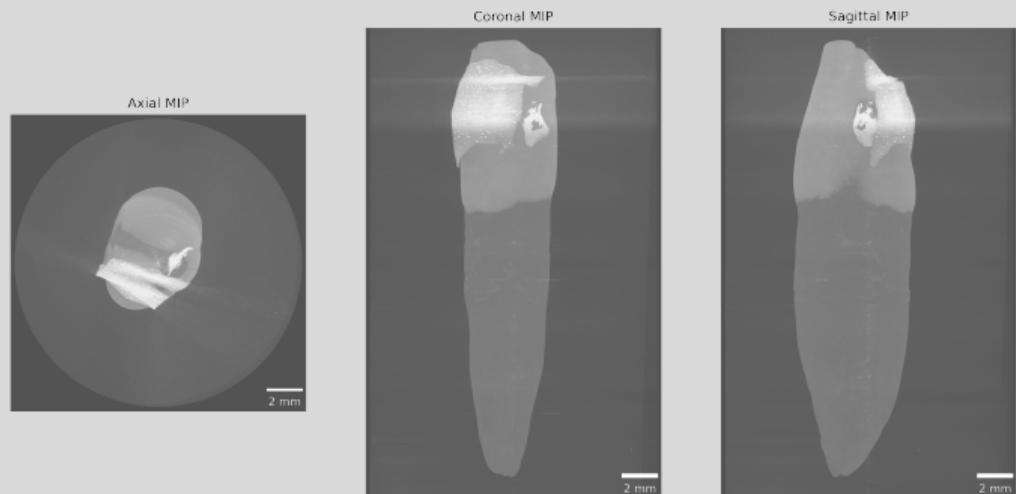
To get an overview of the samples while we scanned the whole tooth cohort we generated a preview and analysis notebook. The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of any software.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

μ CT imaging



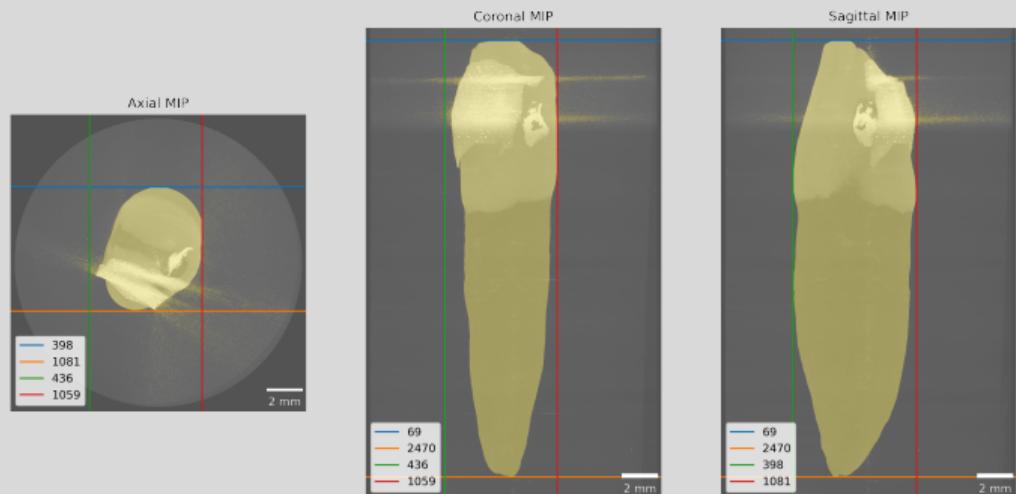
Dataset cropping

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



Dataset cropping

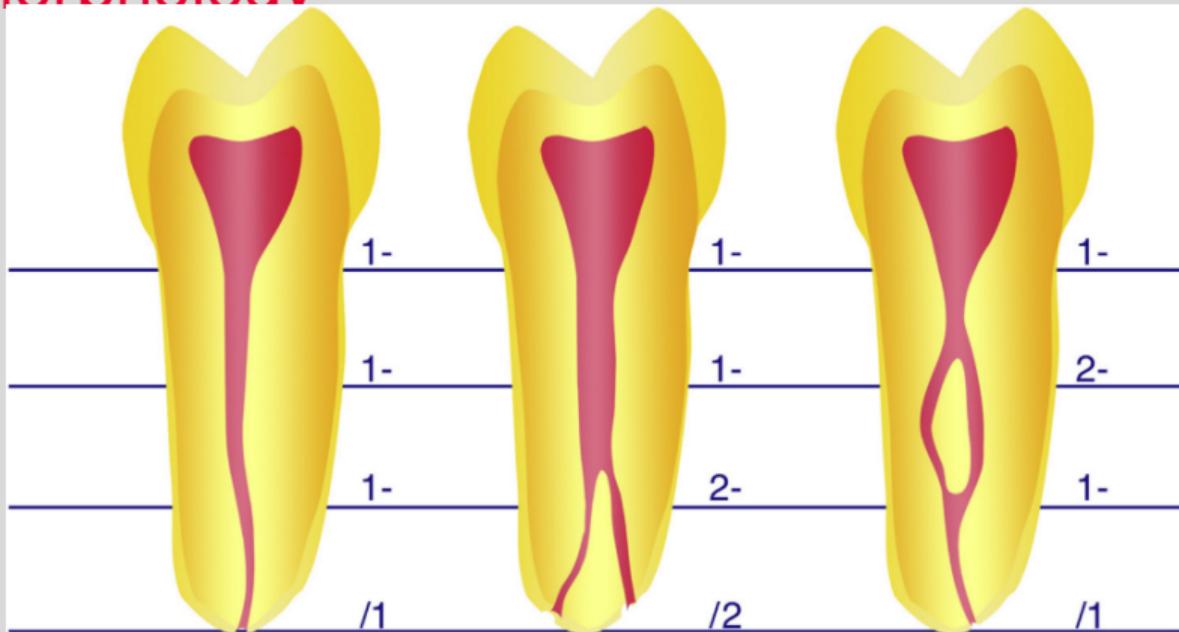
- Full datasets: 326 GB
- Cropped datasets: 115 GB



Tooth morphology

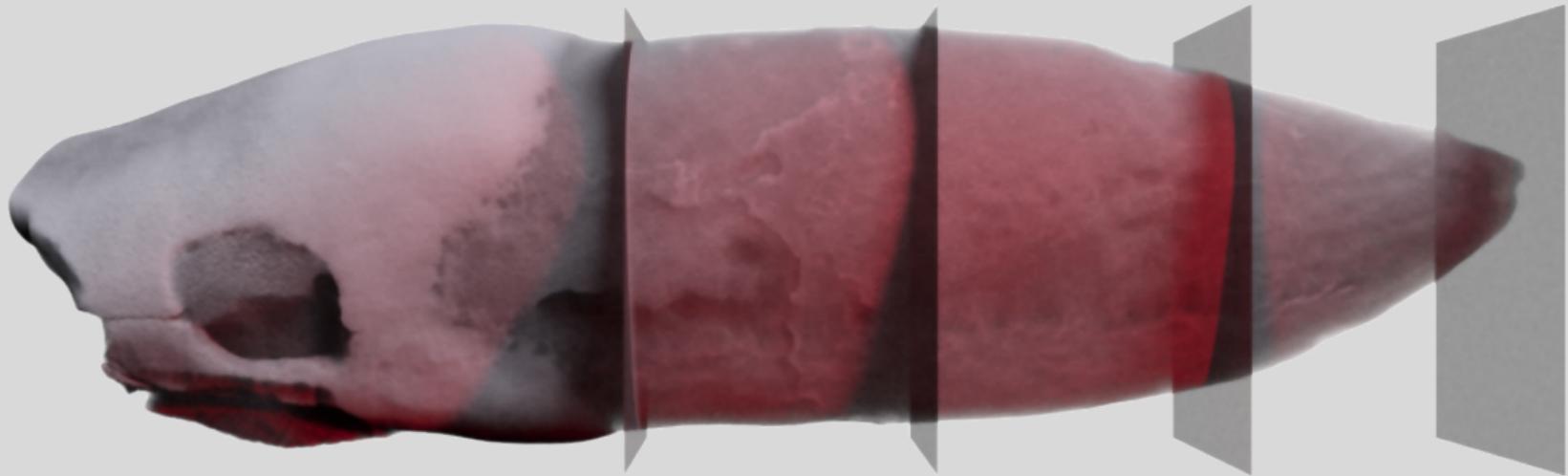


Tooth morphology

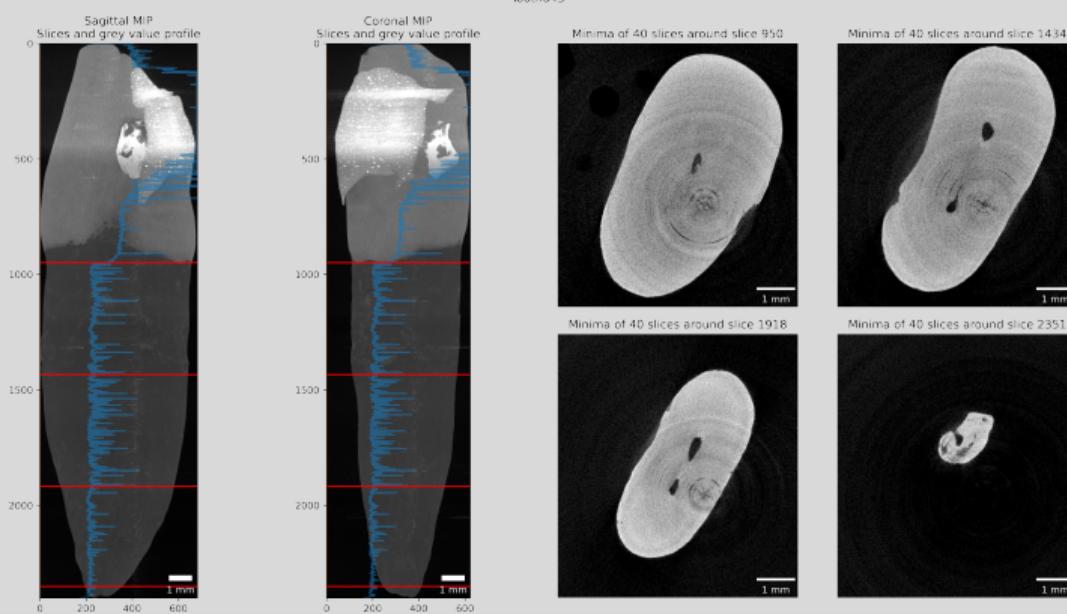


From [26], Fig. 2

Tooth morphology

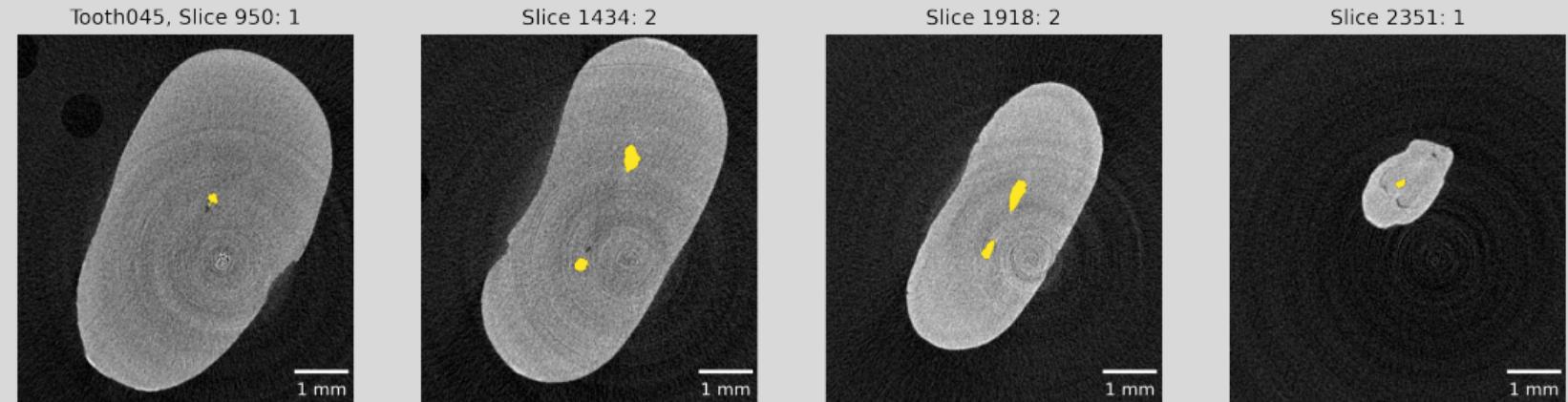


Detection of enamel-dentin border



Detection of enamel-dentin border

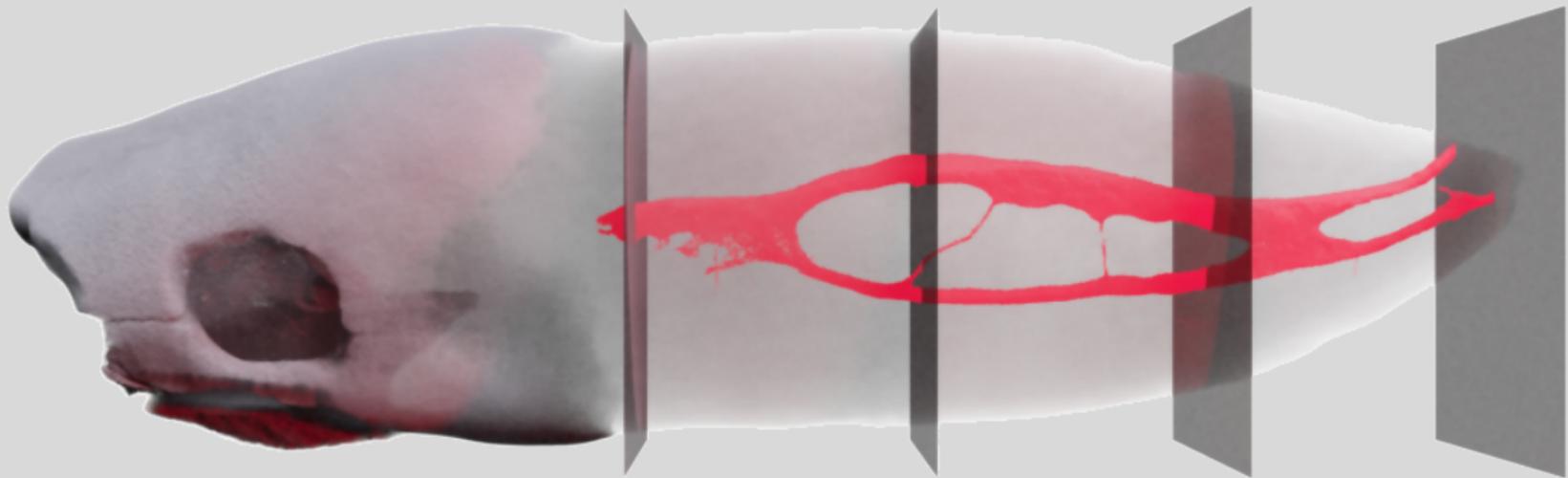
Tooth045



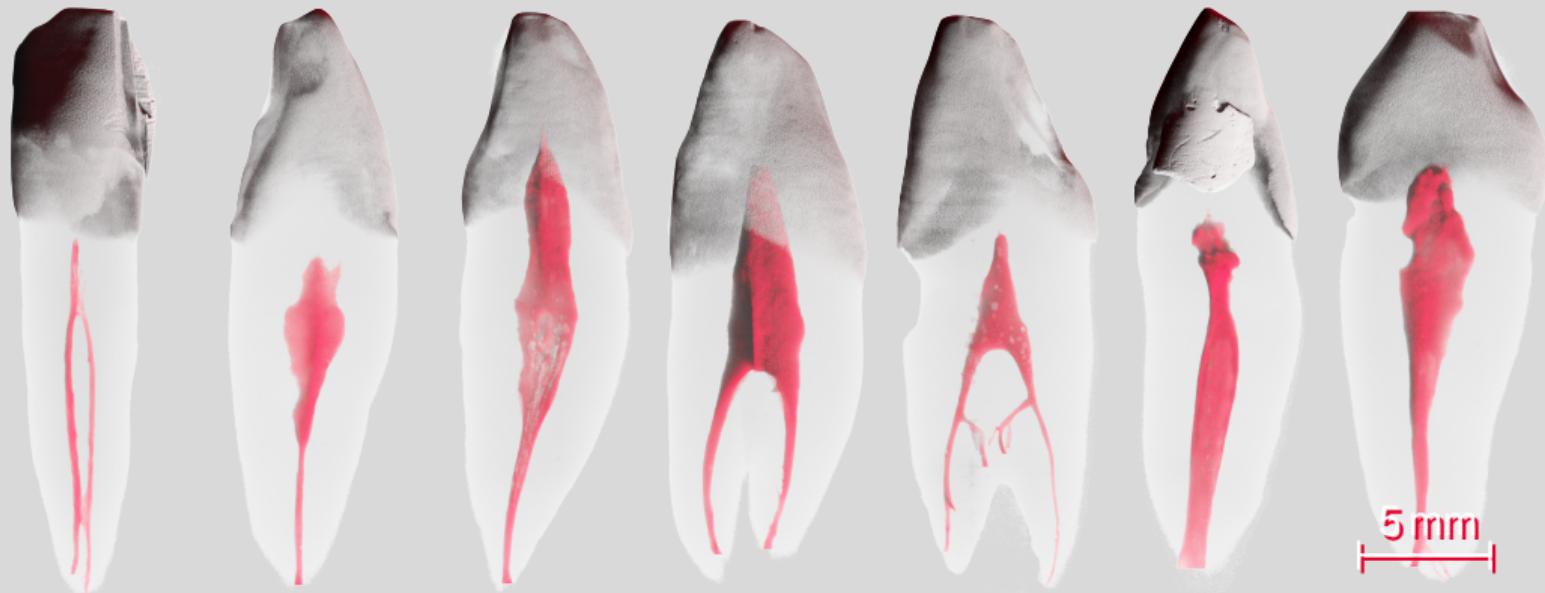
Outcome root canal configuration classification

Roots	RCC	#	%	
Single (N=98)	1–1–1/1	73	74.5	
	1–1–1/2	14	14.3	
	1–1–1/3	1	1.0	
	1–1–1/4	2	2.1	
	1–1–2/1	1	1.0	
	1–2–1/1	4	4.1	
	1–2–1/2	1	1.0	
	1–2–2/2	1	1.0	
	2–3–1/1	1	1.0	
Double (N=3)	Buccal	1–1–1/1	2	66.6
		1–2–1/1	1	33.3
	Lingual	1–1–1/1	2	66.6
		1–1–1/2	1	33.3

Extraction of root canal space



Results of root canal space extraction



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

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 the (handout) PDF linked on ILIAS (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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