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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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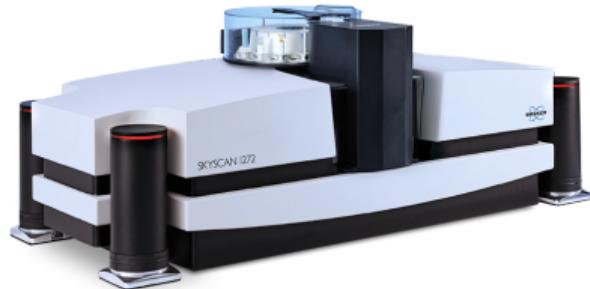
Grüessech from the µCT group



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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

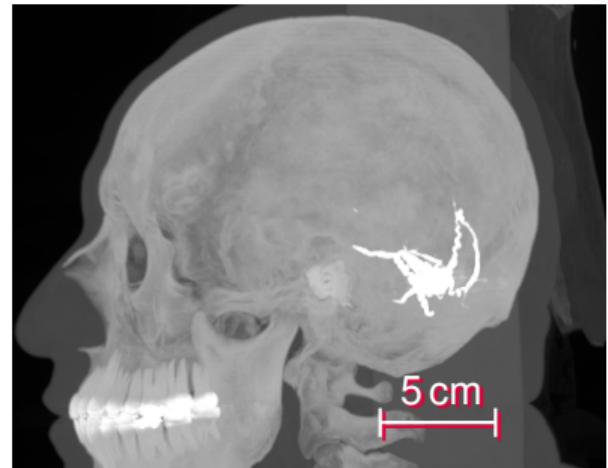
 Materials & Methods

 Results

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micro-Computed tomography

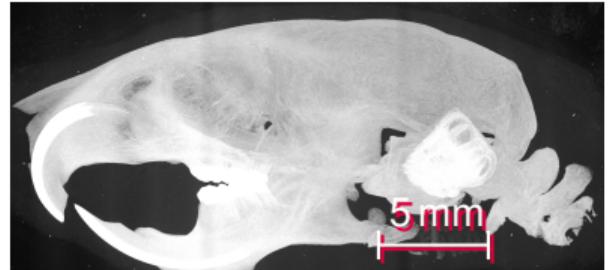
- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a very large range of sample sizes



From [13], Subject C3L-02465

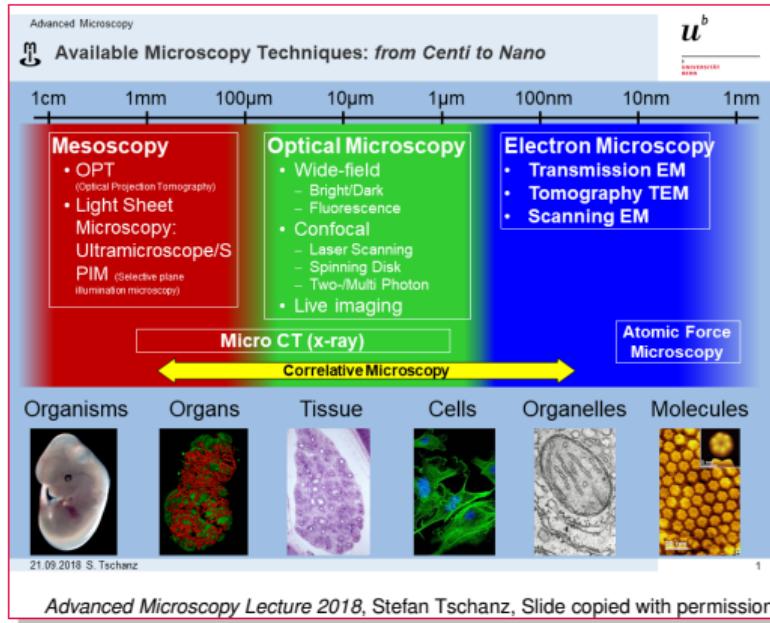
micro-Computed tomography

- 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



- *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

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CT-Scanner



youtu.be/2CWpZKuy-NE

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CT History

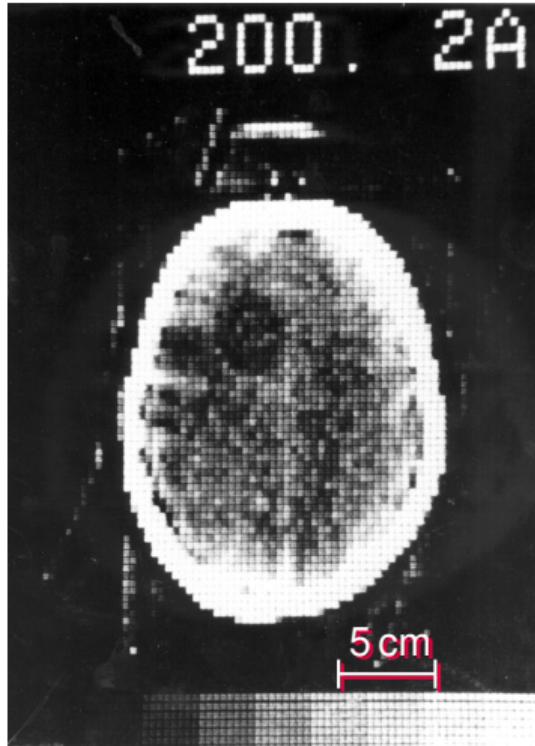
- 1895: Wilhelm Conrad Röntgen discovers X-rays



w.wiki/BHAN ©

CT History

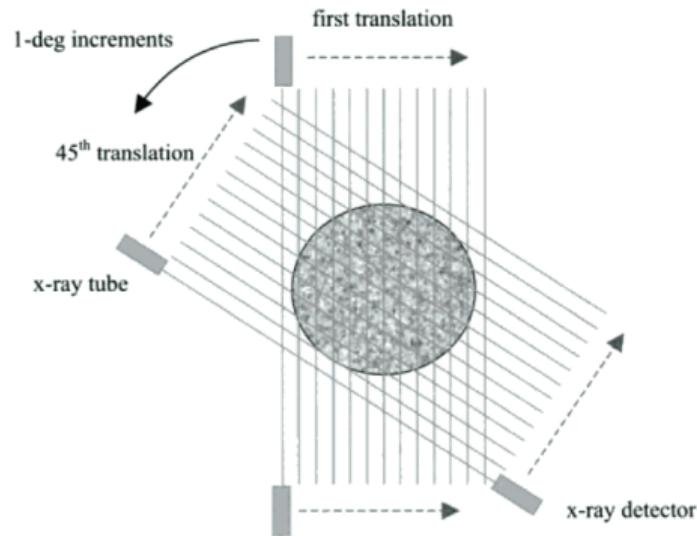
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]



From [16], Figure 5

CT History

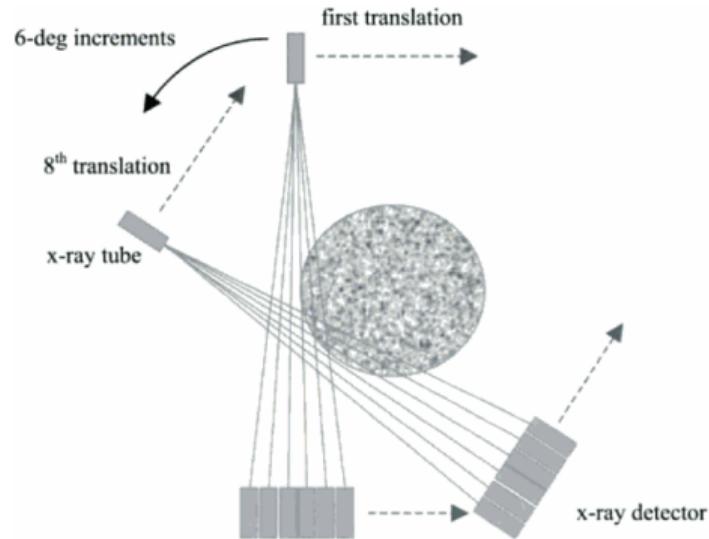
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- 1976: Hounsfield worked on first clinical scanner [15]
- CT scanner generations
 - First generation



From [17], Figure 1.12

CT History

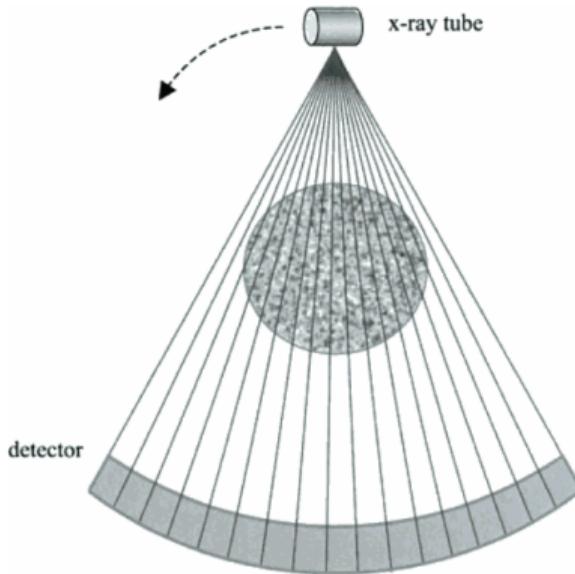
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- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- CT scanner generations
 - First generation
 - Second generation



From [17], Figure 1.13

CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 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

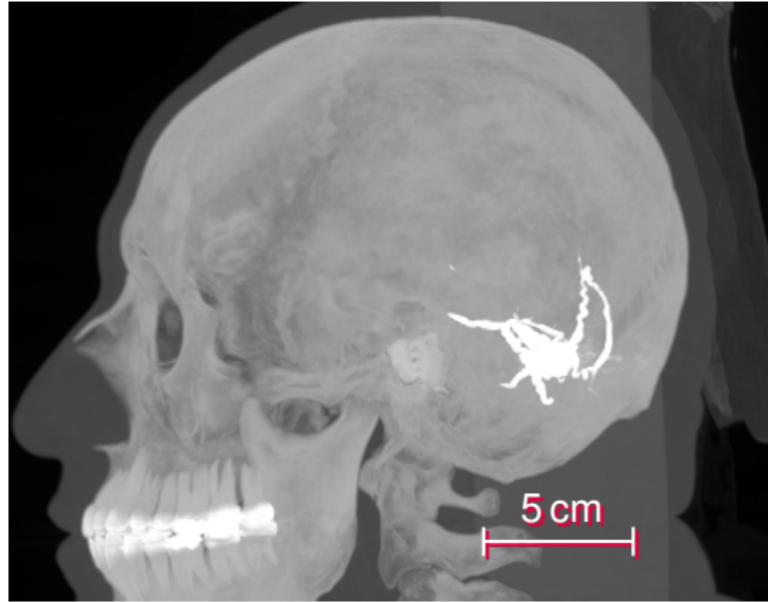
- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [18]
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp [19] developed one of the early laboratory microCT systems by assembling a micro-focus 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
- 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

μ CT History II

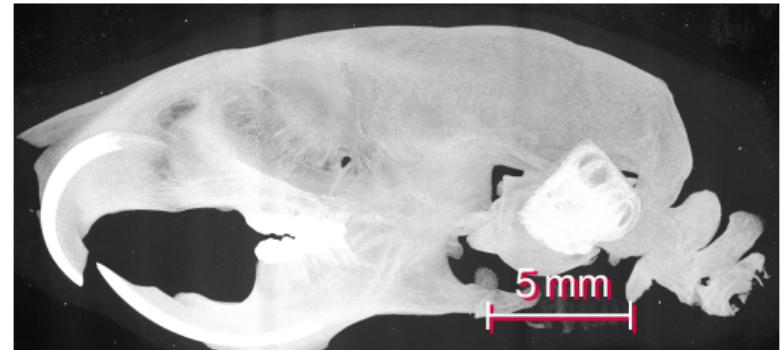
- Mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
- Teeth and their internal details
- Soft tissues and vasculature using radio-opaque contrast agents
- Characterization of anatomical details in high resolution
- \approx 2500 μ CT systems are in use worldwide with over 1000 publications annually

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Why μ CT?



From [13], Subject C3L-02465



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Why μ CT?

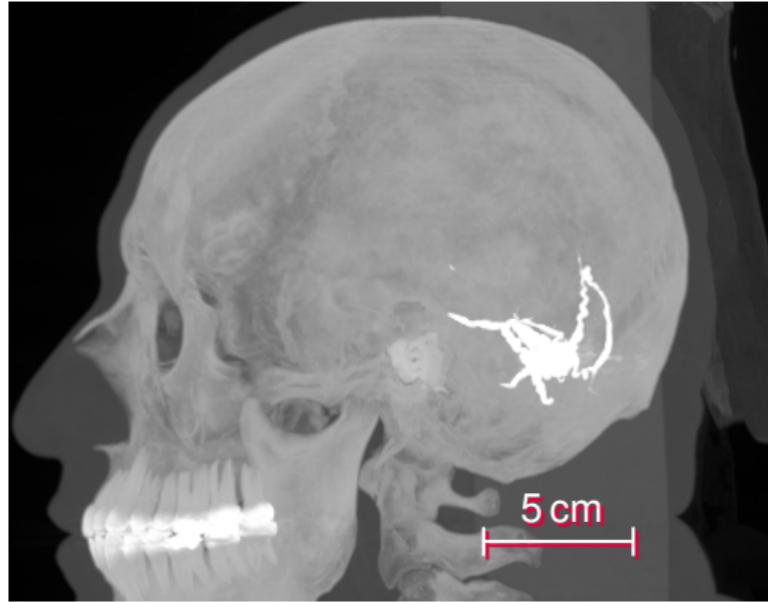


From [13], Subject C3L-02465

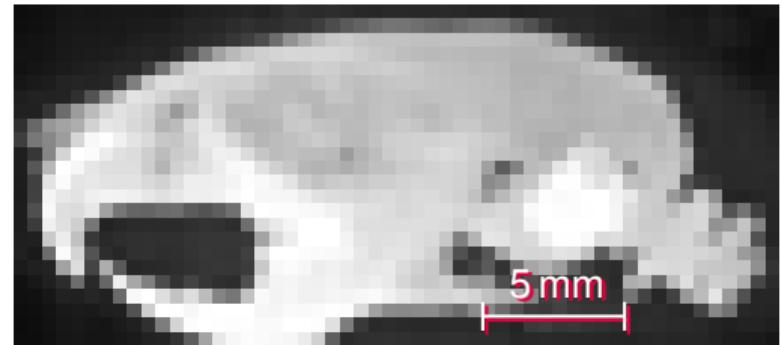


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Why μ CT?

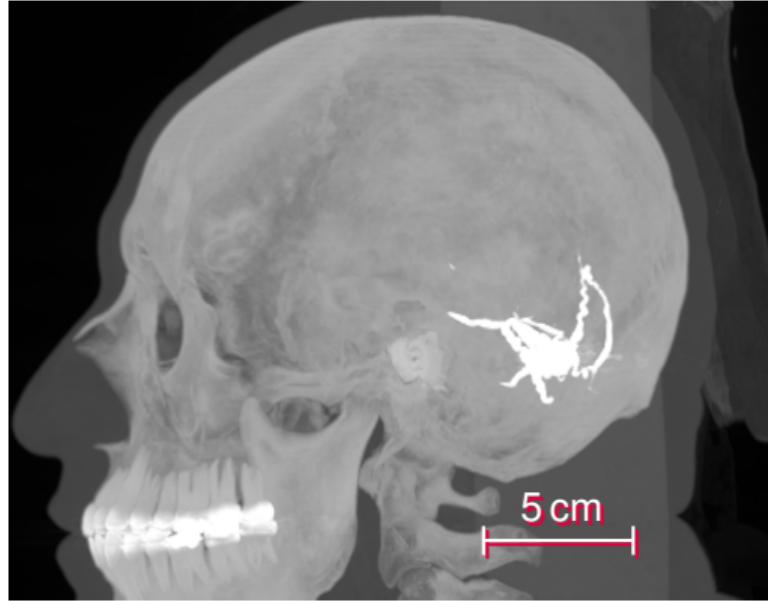


From [13], Subject C3L-02465

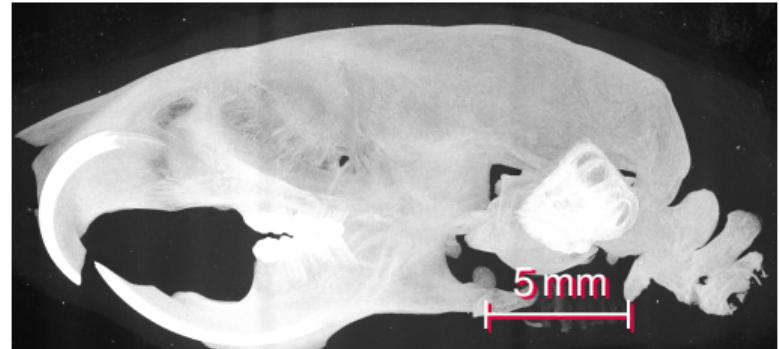


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Why μ CT?

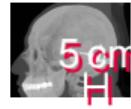


From [13], Subject C3L-02465

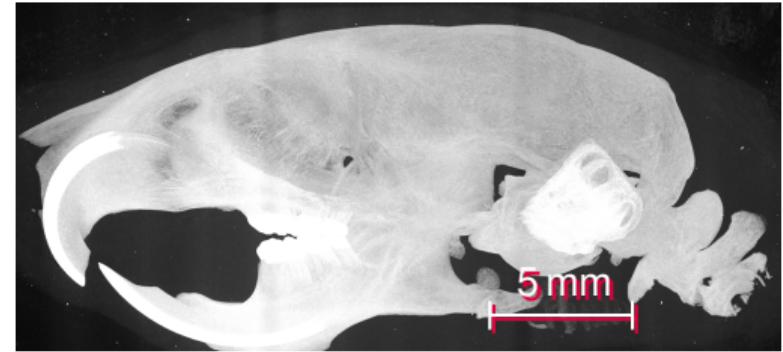


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Why μ CT?



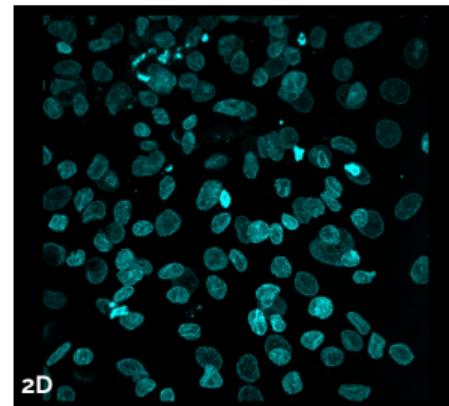
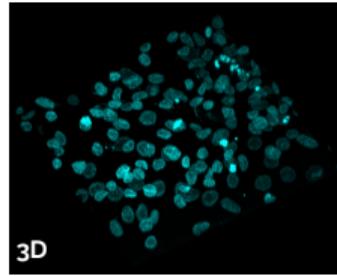
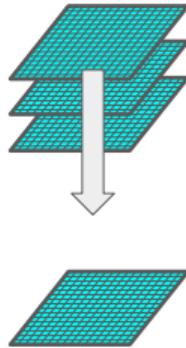
From [13], Subject C3L-02465



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

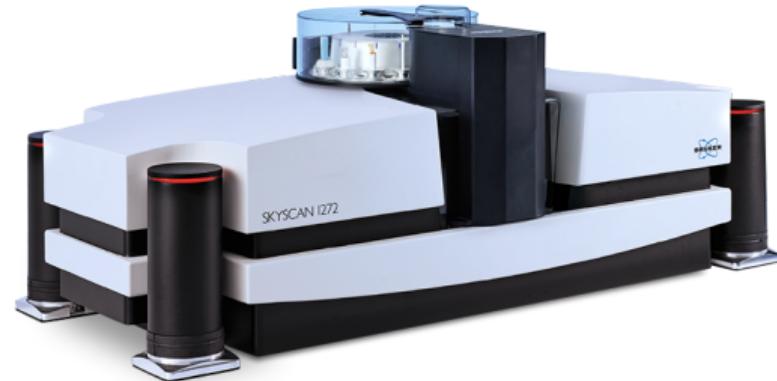
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flic.kr/p/fpTrGu @@

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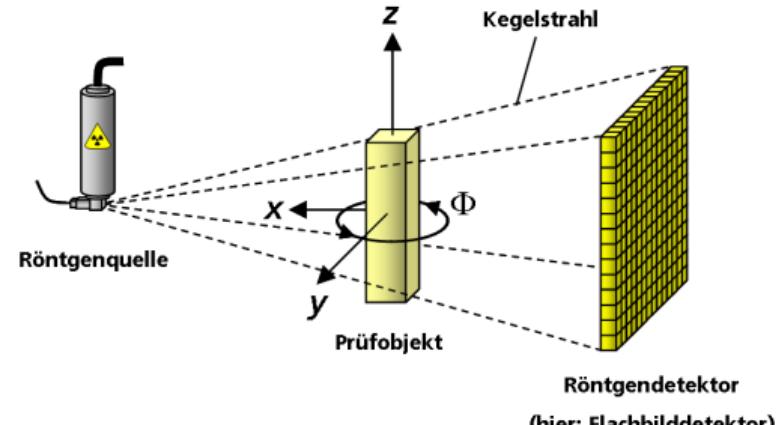
flic.kr/p/7Xhk2Y

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What is happening?

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

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



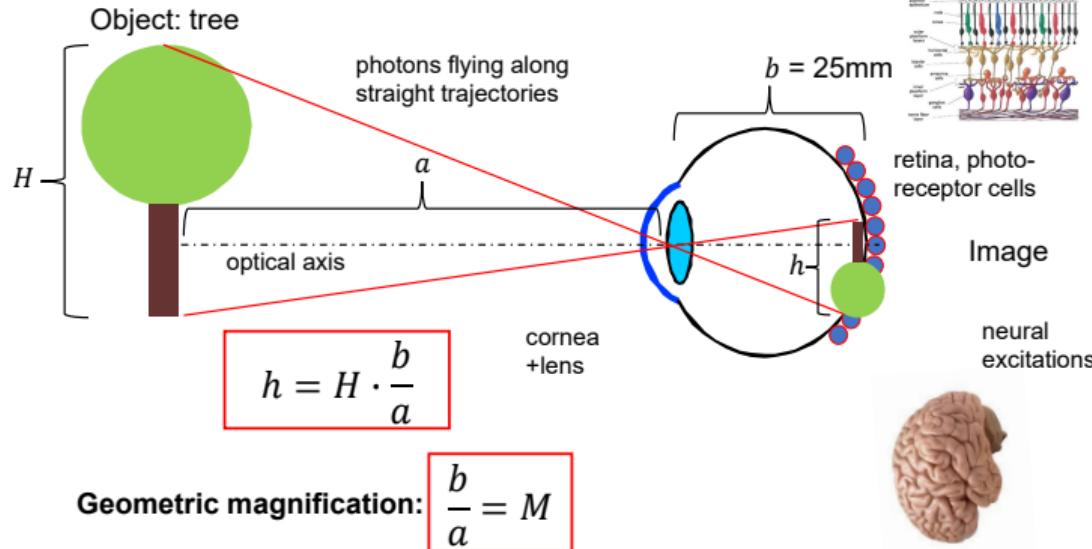
w.wiki/7g3 @①②

Magnification

Introduction – why do we need microscopes?

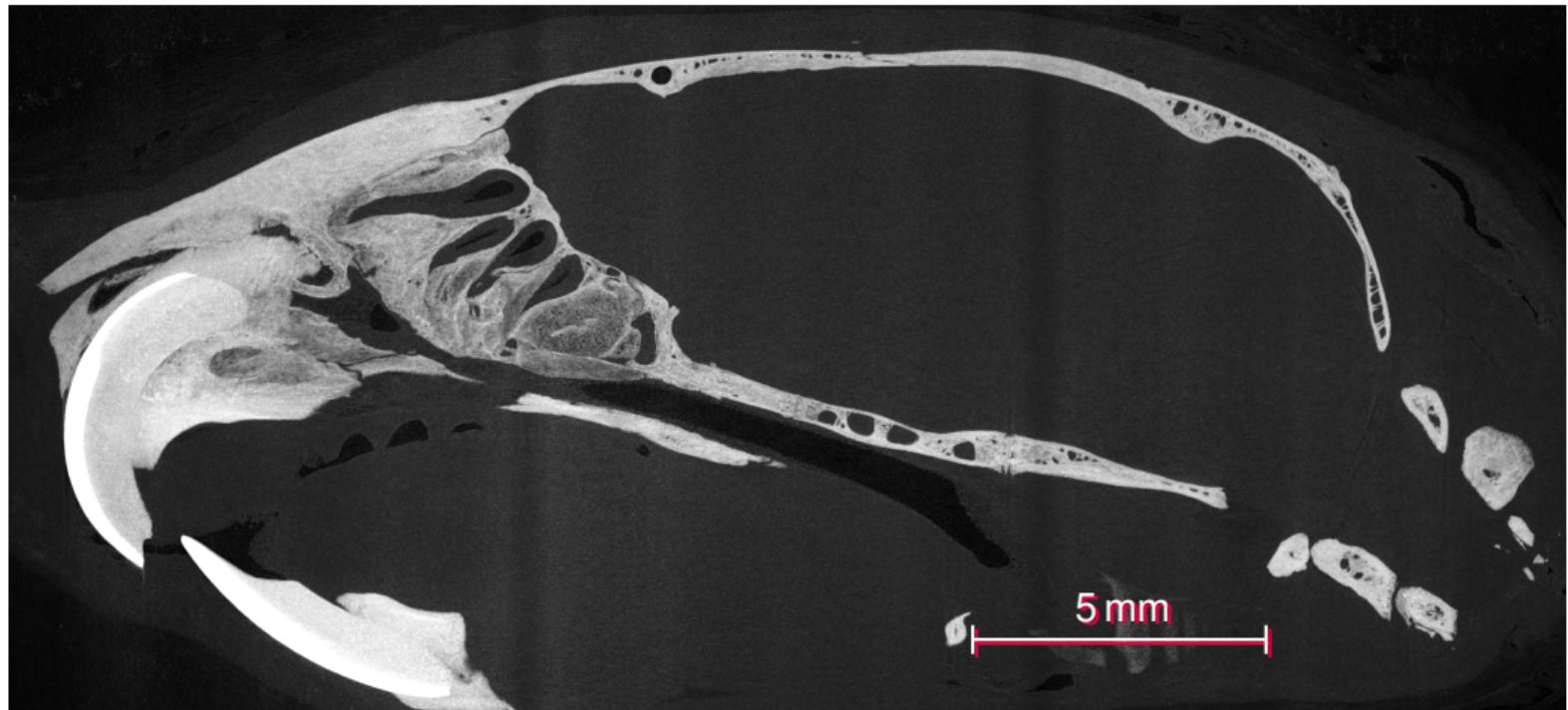
4

- Basic geometric optics: how does the eye form an image?



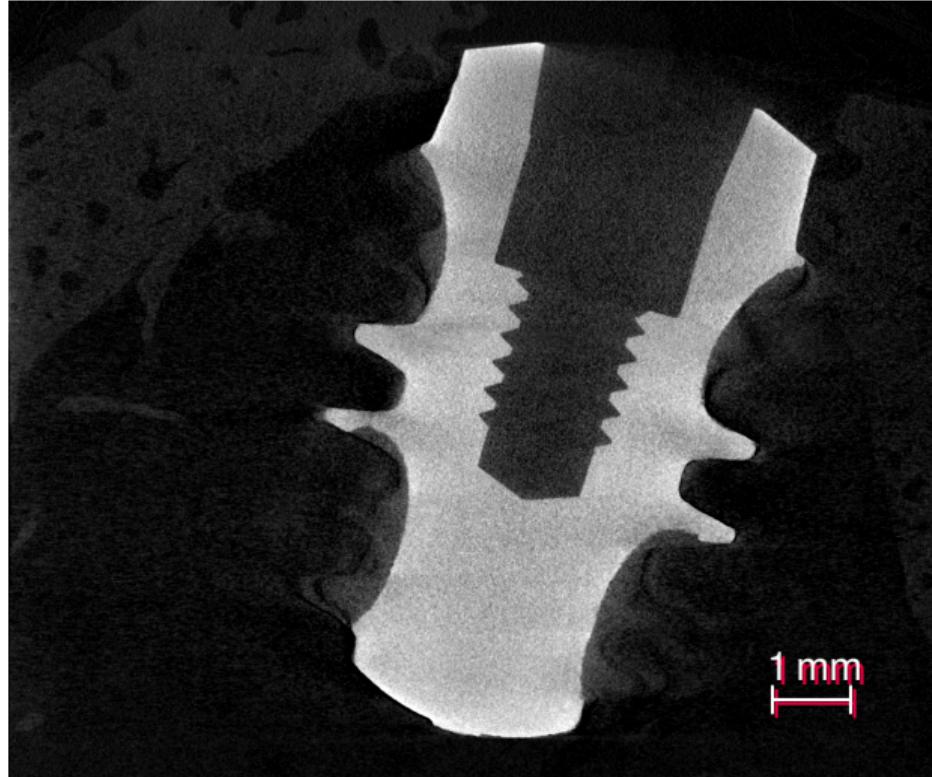
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Examples



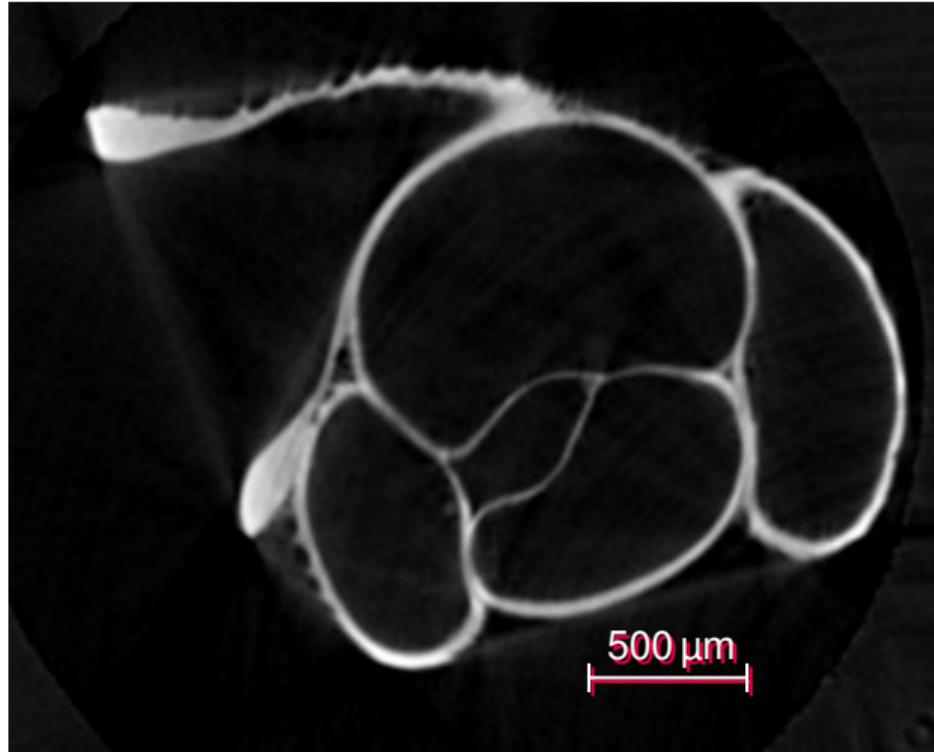
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Examples



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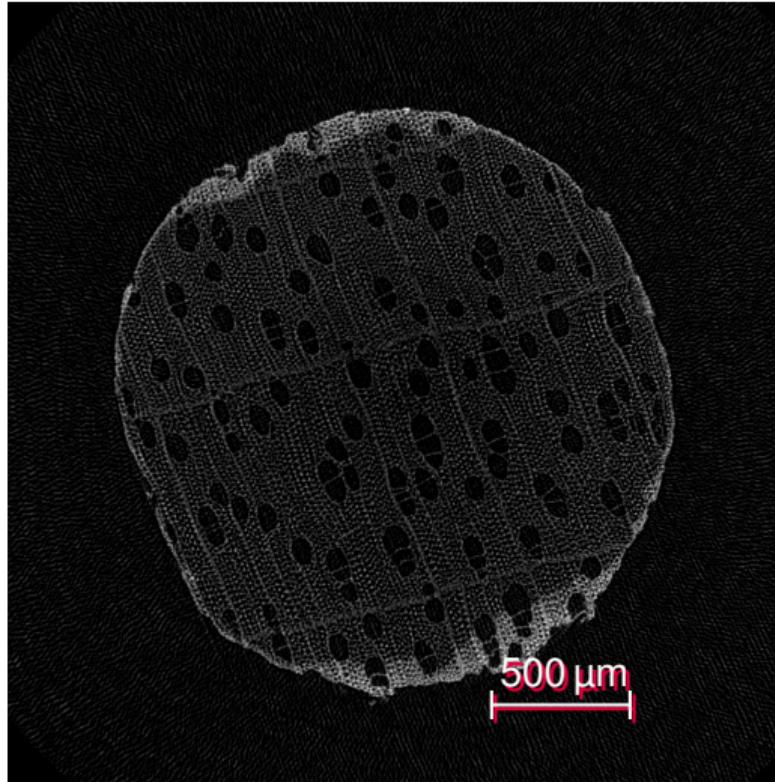
Examples



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

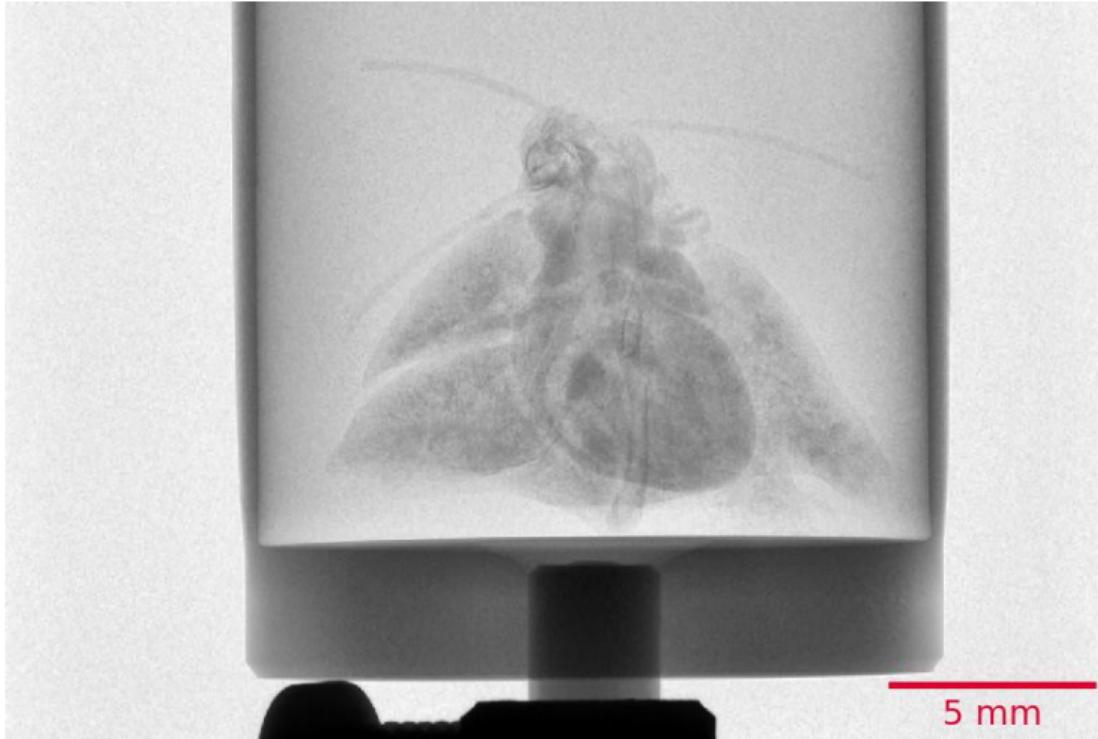
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Preparation

- Study design
- Sample preparation

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Projections

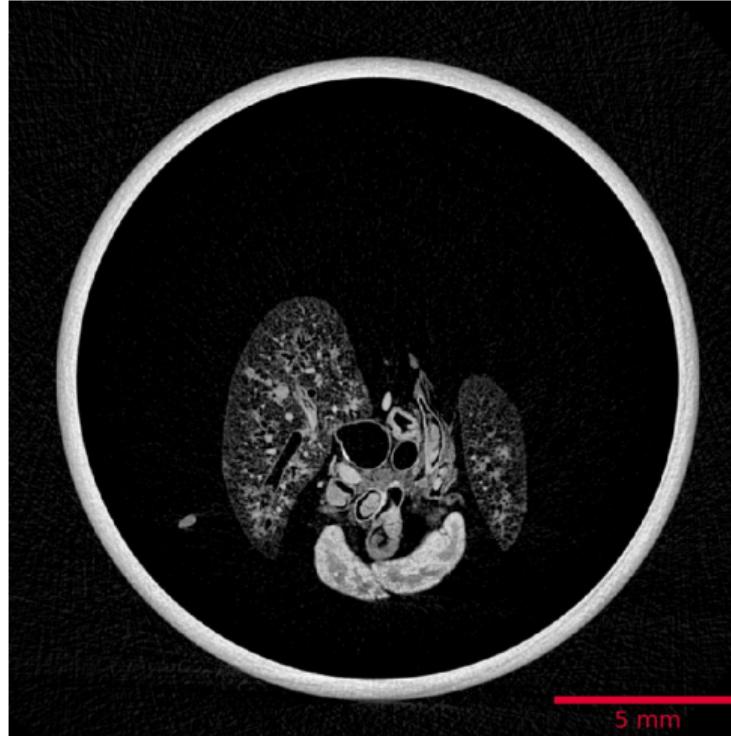


Projections

- 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



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

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Visualization



Visualization

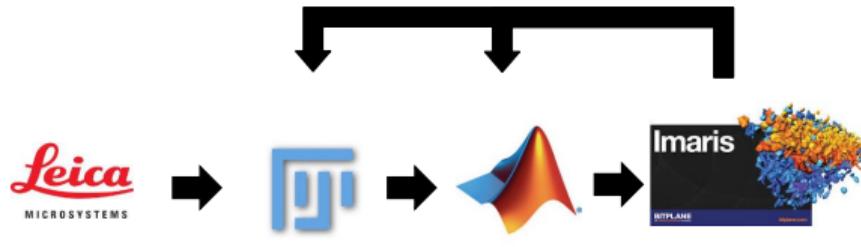
- Based on the calculated reconstructions, a computer synthesizes a (three-dimensional) visualization of the scanned sample

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Imaging

CEM 2024-Light Sheet Microscopy

How to deal with large multidimensional data?



Customized macro
for file conversion
Beatsync (Michael Liebling)
Heart synchronization

CEM 2024-Light Sheet Microscopy

Light Sheet Microscopy, Nadia Mercader, Slide 43

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What to use?

- ImageJ/Fiji [21]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
 -  in Jupyter [22]
 - **git**
 - Script all your things!
 - Data repositories; i. e. sharing is caring!

Quantitative data

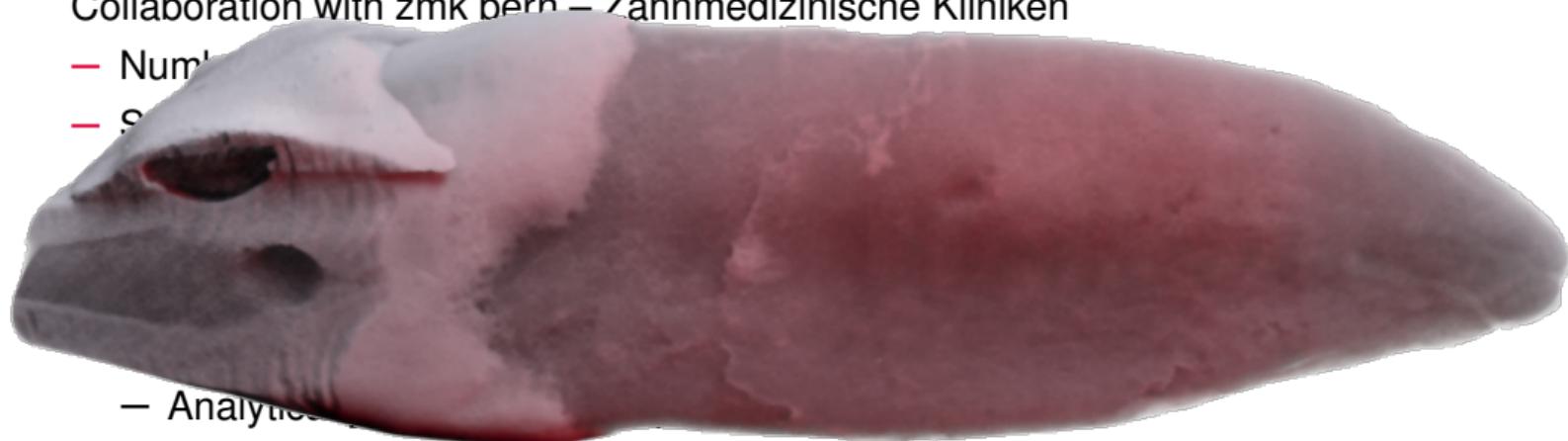
- Pretty images are nice, but we need quantitative numbers
- Segmentation
- Characterization

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Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number of teeth
- Structure of teeth



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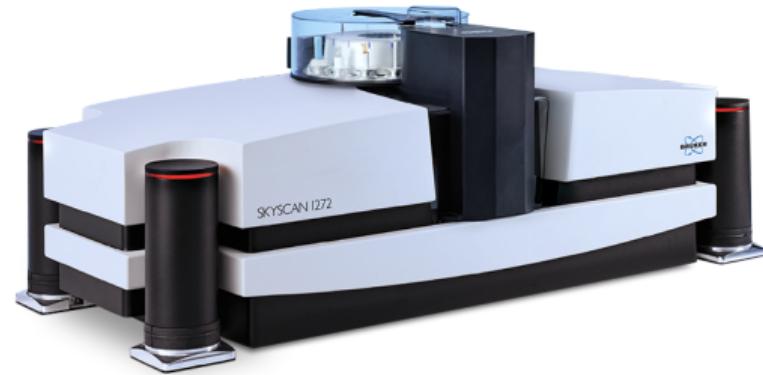
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín *et al.* [24]
- *Reproducible* analysis [25], 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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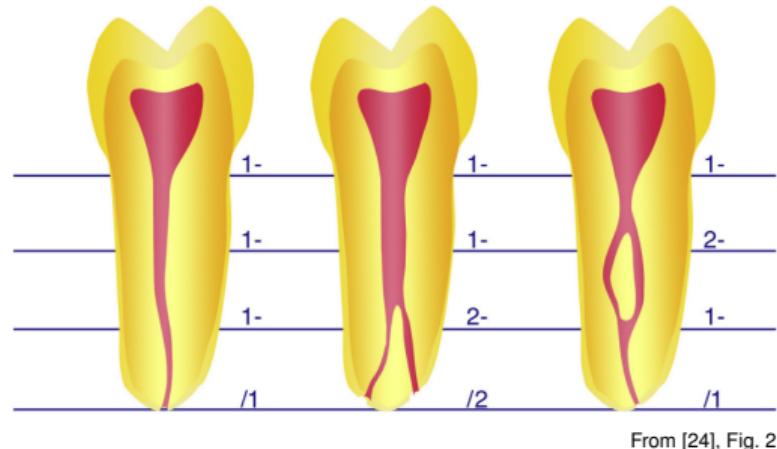
Sample changer on the SkyScan 1272

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

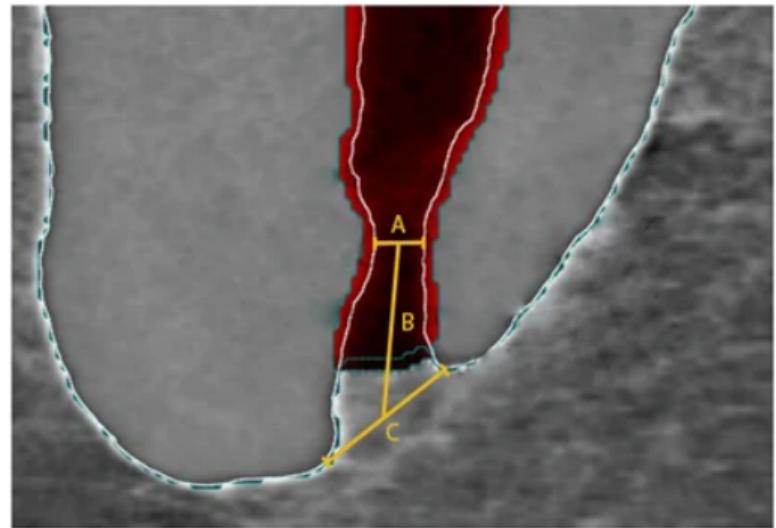
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From [26], Fig. 1

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The screenshot shows a GitHub repository interface. At the top, it displays 'master' branch, 1 branch, 1 tag, and a green 'Code' button. Below is a list of commits from user 'habi':

File	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

Below the commits is the 'README.md' file content:

```
README.md
DOI 10.5281/zenodo.3999402 treebeard.yml failing
launch binder
```

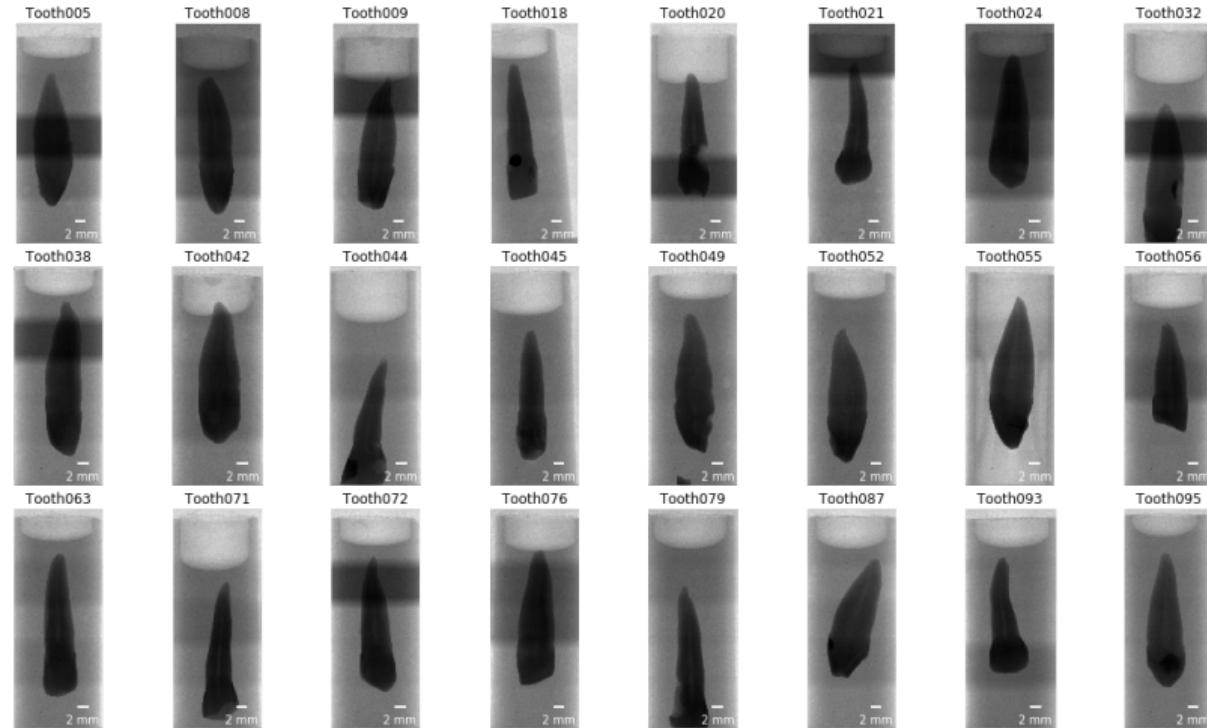
A big tooth cohort

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 b

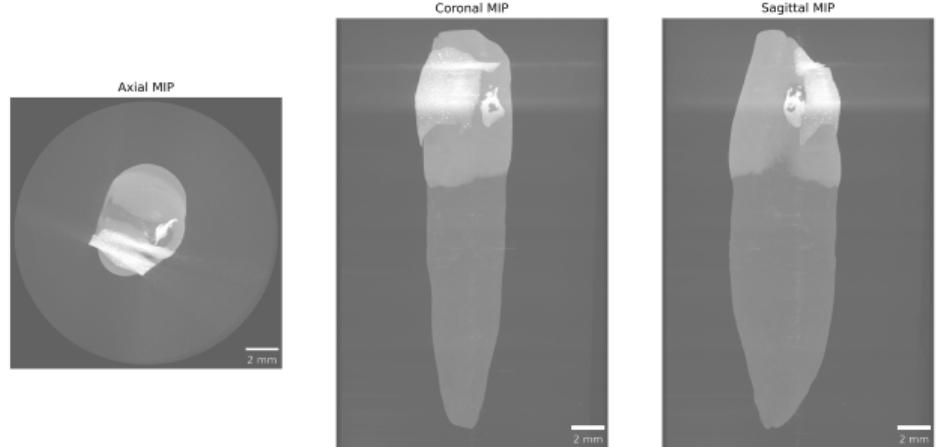
μ CT imaging



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Dataset cropping

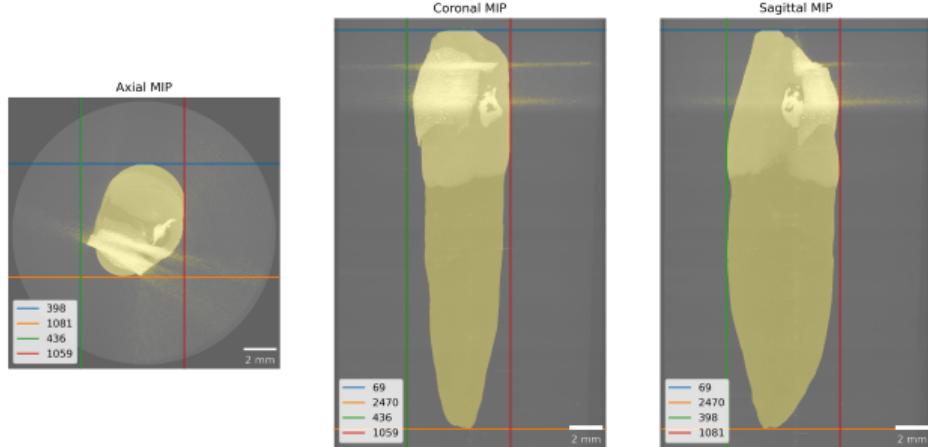
- Full datasets: 326 GB
- Cropped datasets: 115 GB



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Dataset cropping

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



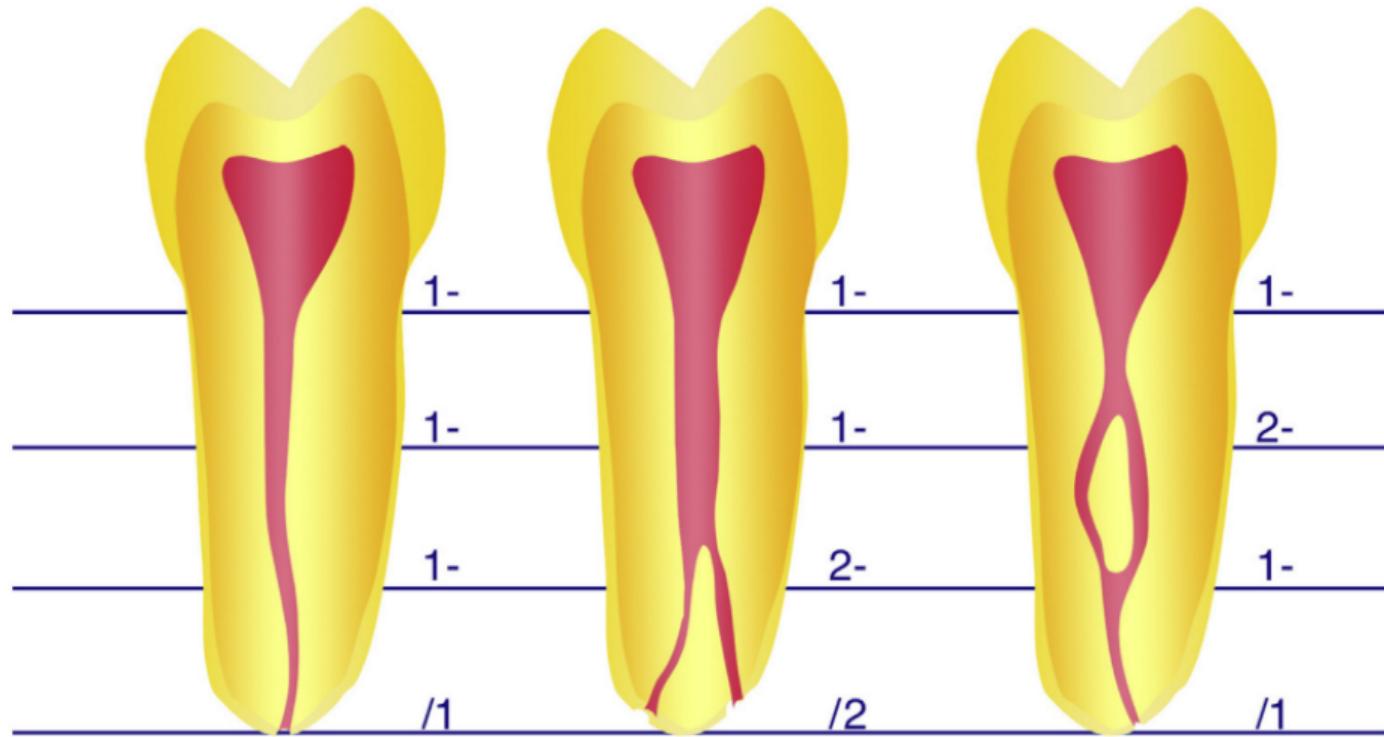
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Tooth morphology



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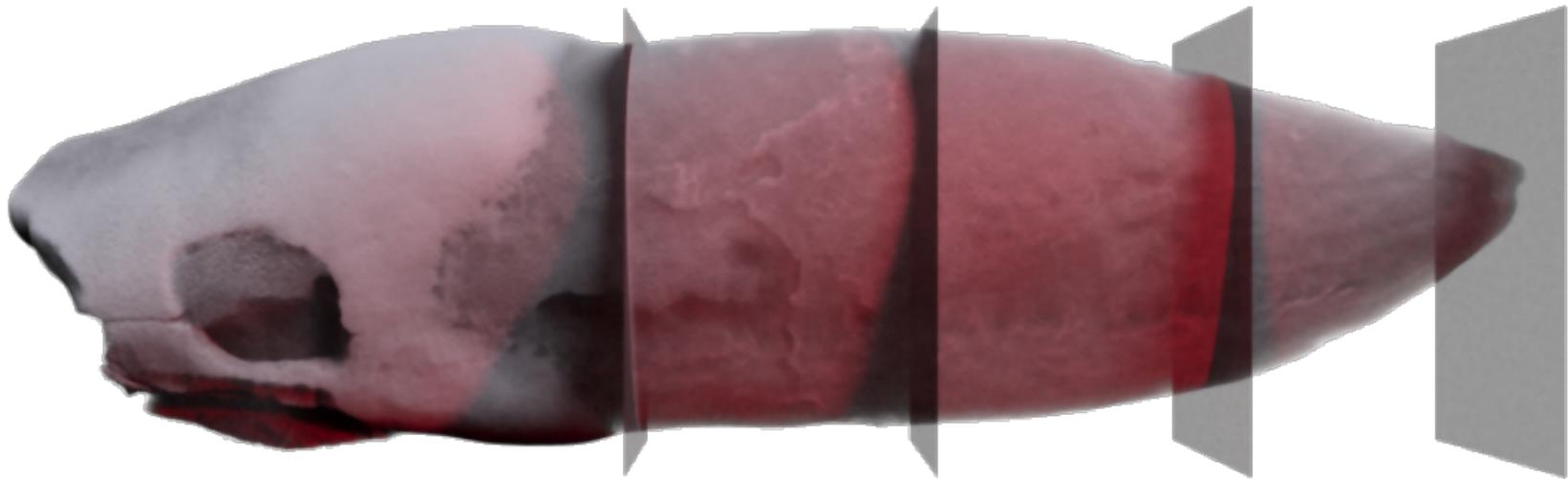
Tooth morphology



From [24], Fig. 2

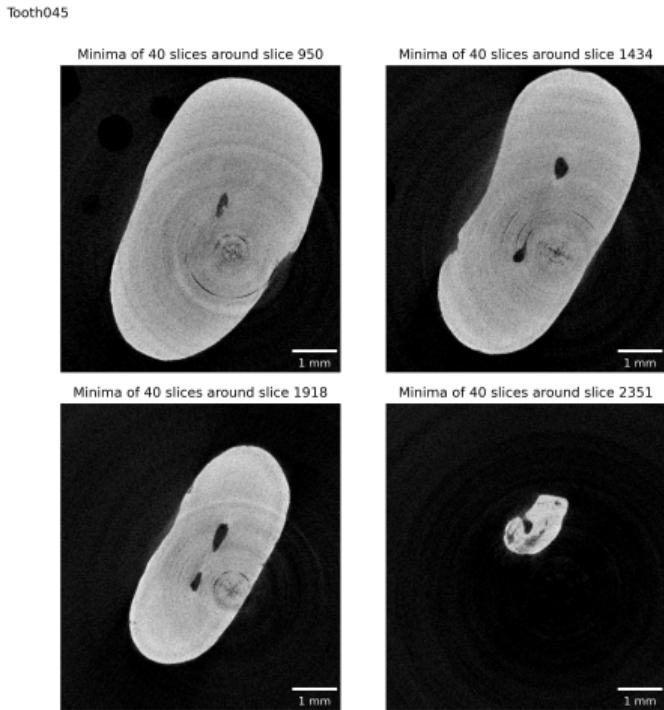
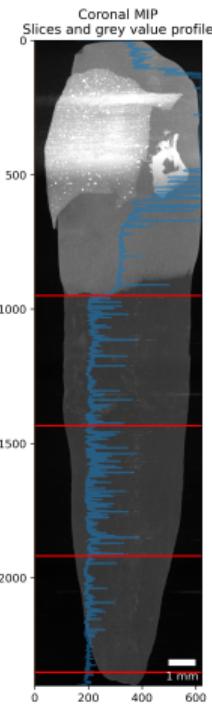
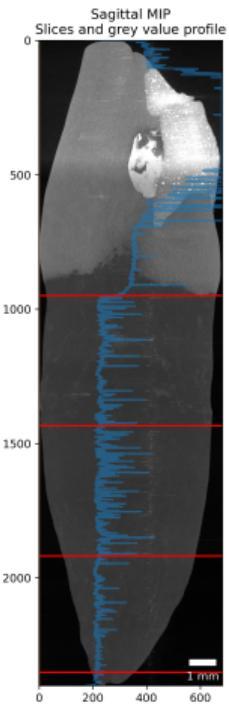
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Tooth morphology



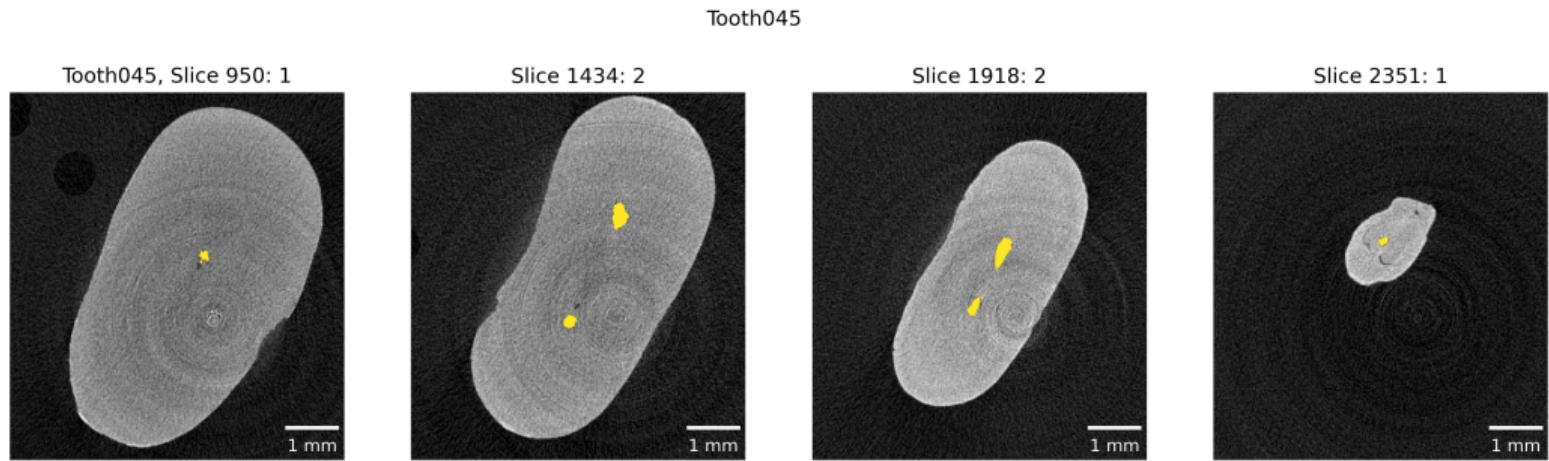
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Detection of enamel-dentin border



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Detection of enamel-dentin border



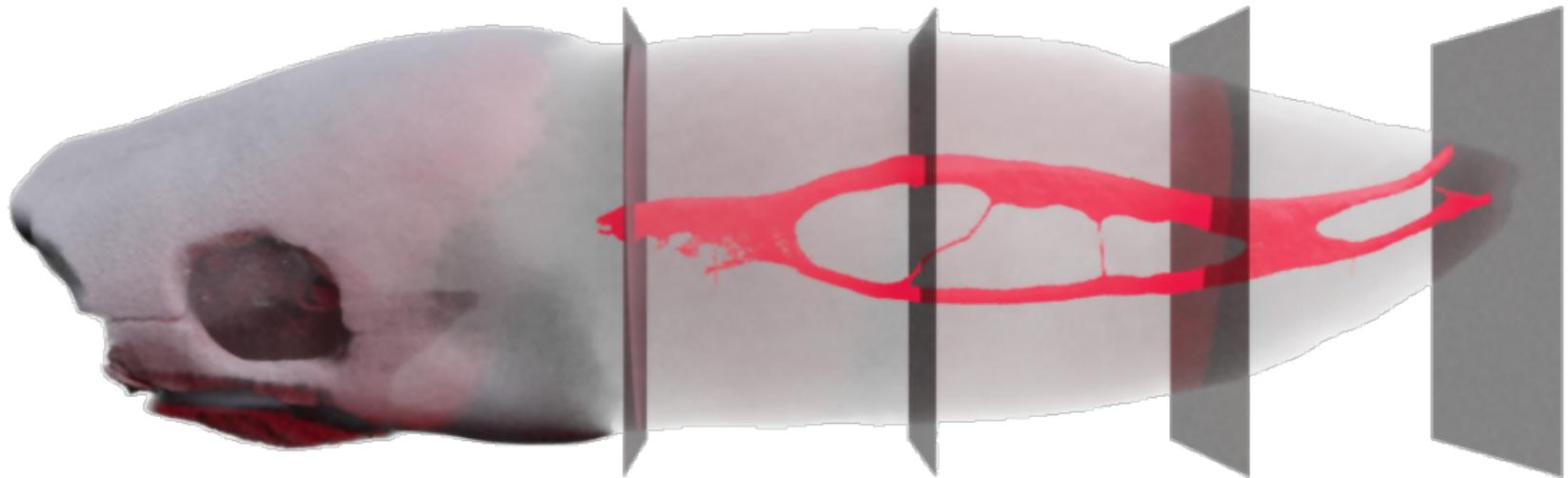
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Outcome root canal configuration classification

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

u^b

Extraction of root canal space



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

u^b

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