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**UNIVERSITY  
OF BERN**

# *u<sup>b</sup>* X-ray microtomography

## 9256-HS2024-0: Advanced Microscopy

**David Haberthür**

Institute of Anatomy, December 20, 2024

*u<sup>b</sup>*

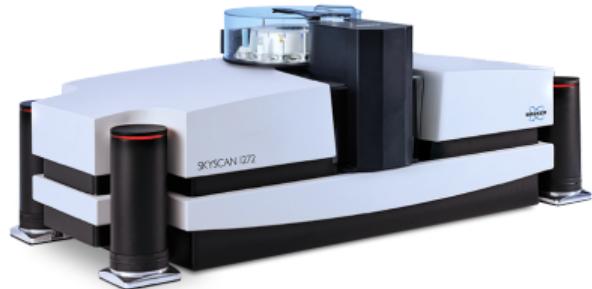
# Grüessech mitenang!



[David.Haberthuer@unibe.ch](mailto:David.Haberthuer@unibe.ch) [Ruslan.Hlushchuk@unibe.ch](mailto:Ruslan.Hlushchuk@unibe.ch) [Oleksiy.Khoma@unibe.ch](mailto:Oleksiy.Khoma@unibe.ch)

# $\mu$ 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]



[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

# 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

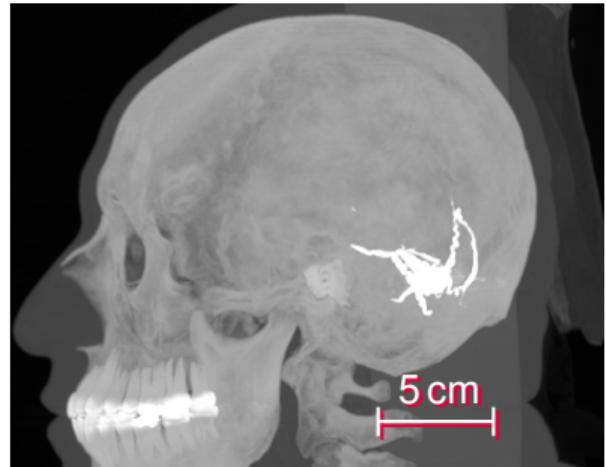
  Overview

  Materials & Methods

  Results

# *u<sup>b</sup>* Computed tomography

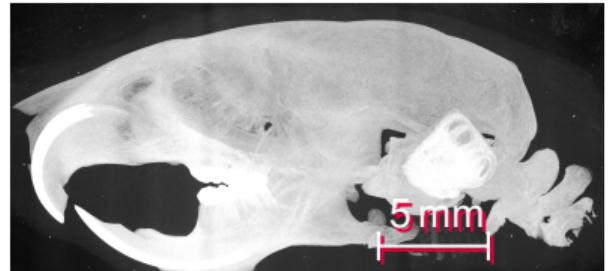
- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a large range of sample sizes



From [13], Subject C3L-02465

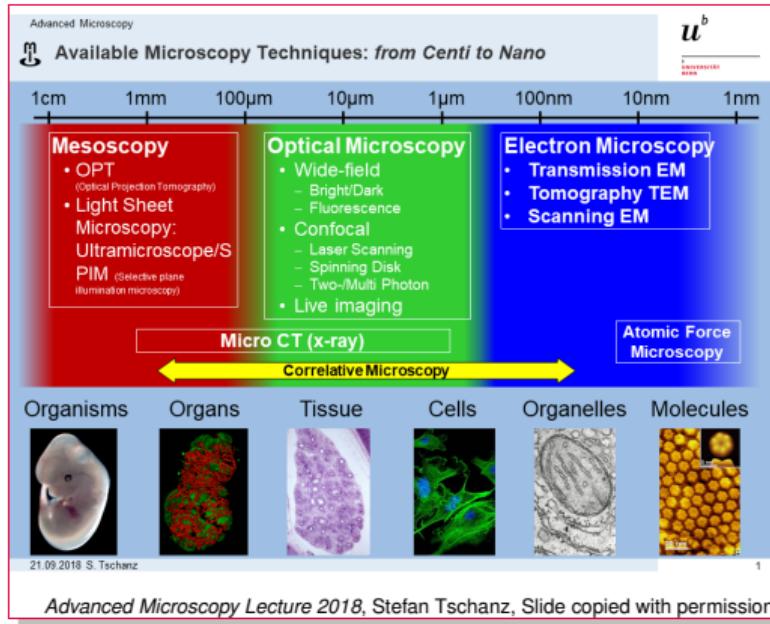
# micro-Computed tomography

- Allows for imaging dense and non-transparent samples
- Non-destructive imaging, thus compatible with routine sample preparation
- Results in three-dimensional images with  $\mu\text{m}$  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



*u*<sup>b</sup>

# 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](https://youtu.be/2CWpZKuy-NE)

*u<sup>b</sup>*

# CT History

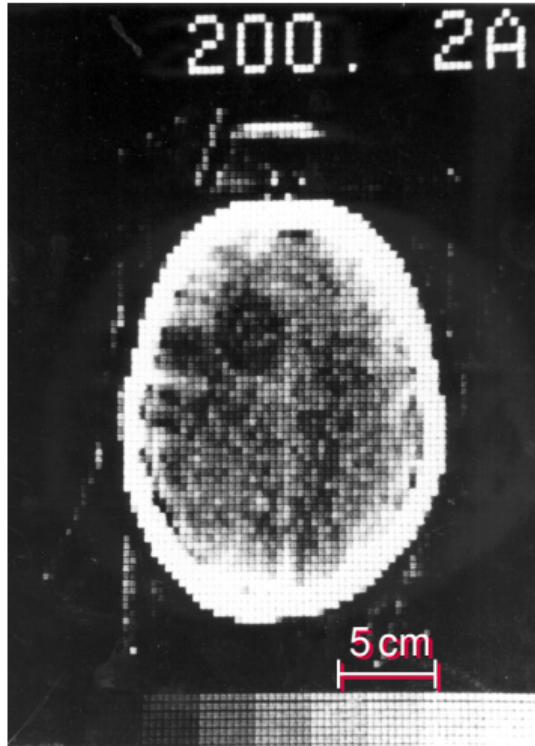
- 1895: Wilhelm Conrad Röntgen discovers X-rays



w.wiki/BHAN ©

# CT History

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

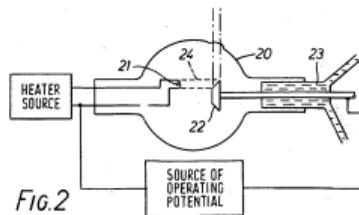
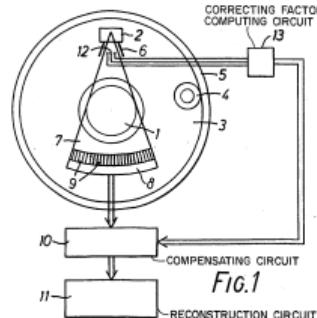


From [16], Figure 5

# CT History

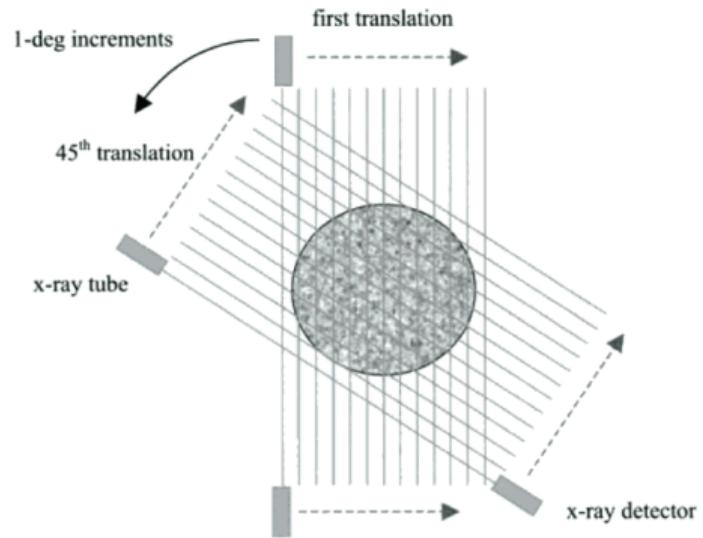
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- Nobel Prize in 1979, jointly for Allan Cormack and Godfrey Hounsfield

U.S. Patent Feb. 24, 1976 Sheet 1 of 2 3,940,625



# CT History

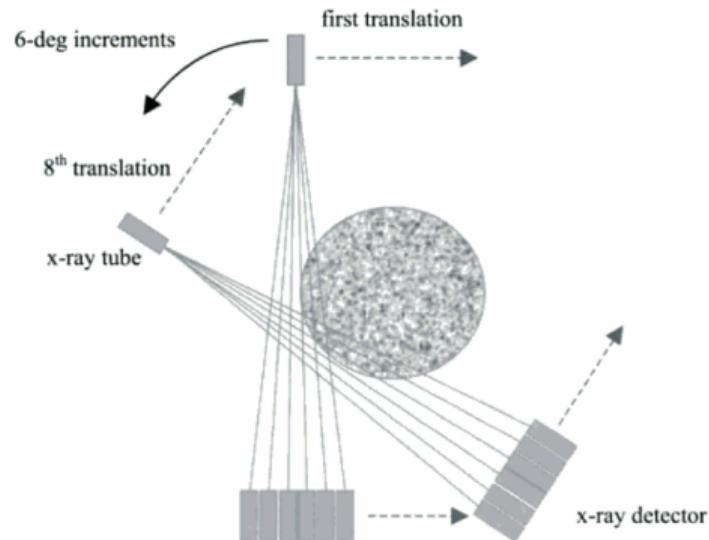
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- CT scanner generations
  - First generation



From [18], Figure 1.12

# CT History

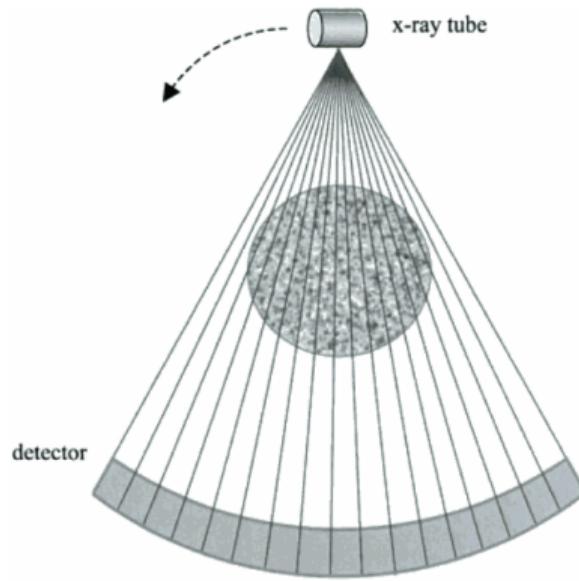
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  - First generation
  - Second generation



From [18], Figure 1.13

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  - First generation
  - Second generation
  - Third generation



From [18], Figure 1.14

# $\mu$ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [19]
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp [20] developed an early laboratory microCT system 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 [21]

# $\mu$ CT History II

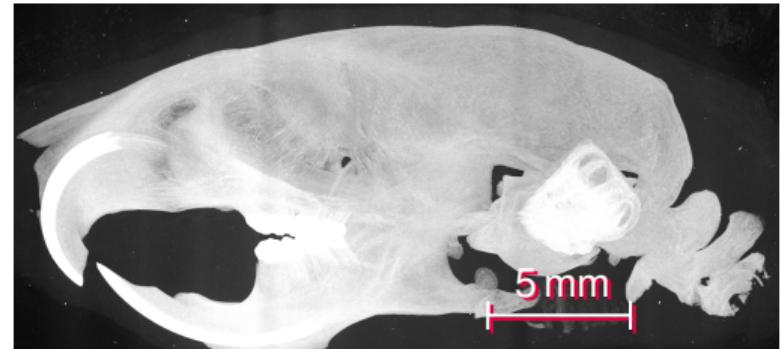
- $\mu$ CT was first reported in the 1980s, for scanning gemstones
- Early 1990s: Manufacturers like SkyScan and Scanco Medical made  $\mu$ CT systems commercially available
- Today: Nondestructive imaging for quantifying the (micro)structure of (organic) materials
  - Mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
  - Teeth and their internal details
  - Tissues, small animals, and medical devices like stents and implants
  - Soft tissues and vasculature using radio-opaque contrast agents
  - Characterization of anatomical details in high resolution
- $\approx$ 2500  $\mu$ CT systems are in use worldwide with over 1000 publications annually

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# Why $\mu$ CT?

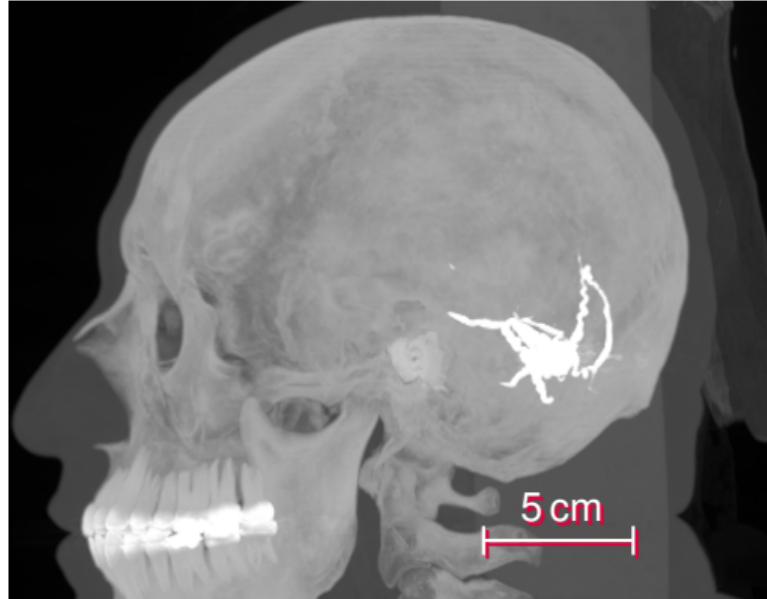


From [13], Subject C3L-02465



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# Why $\mu$ CT?

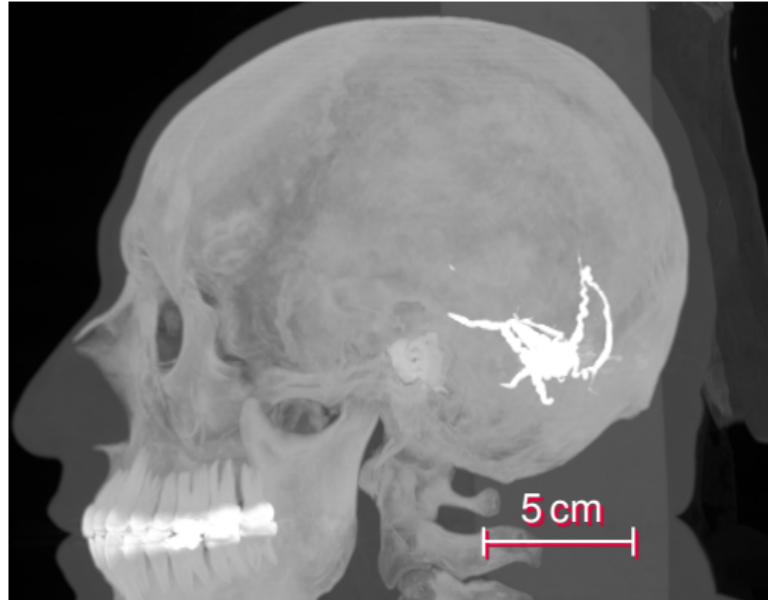


From [13], Subject C3L-02465

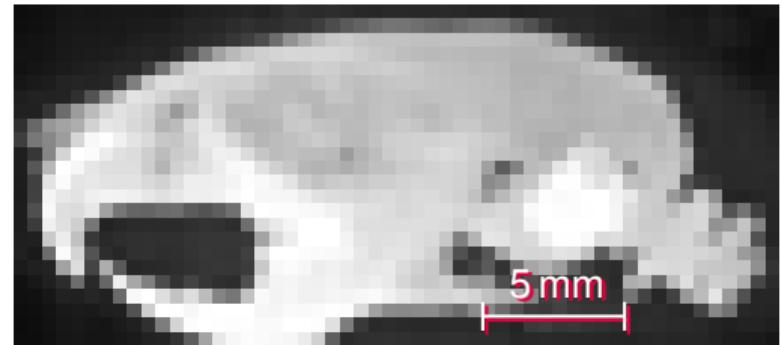


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# Why $\mu$ CT?

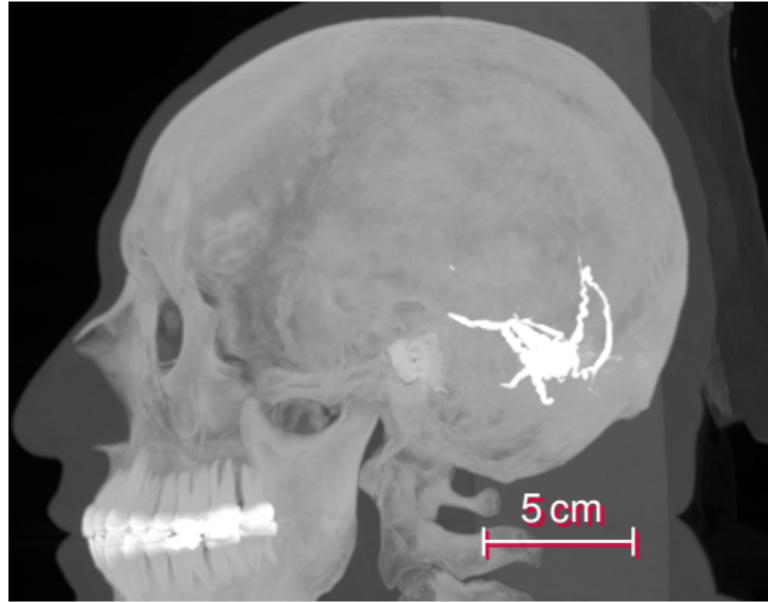


From [13], Subject C3L-02465

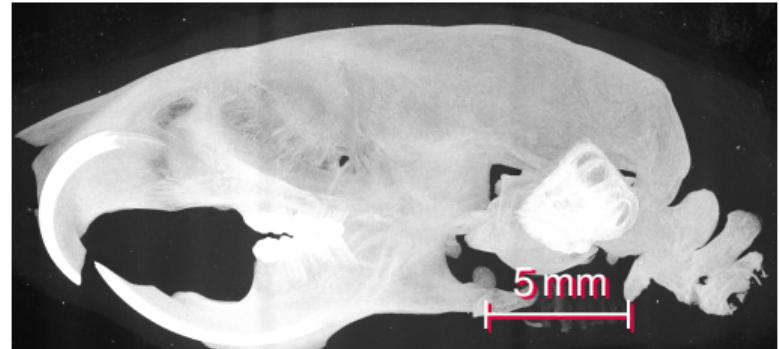


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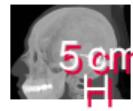


From [13], Subject C3L-02465

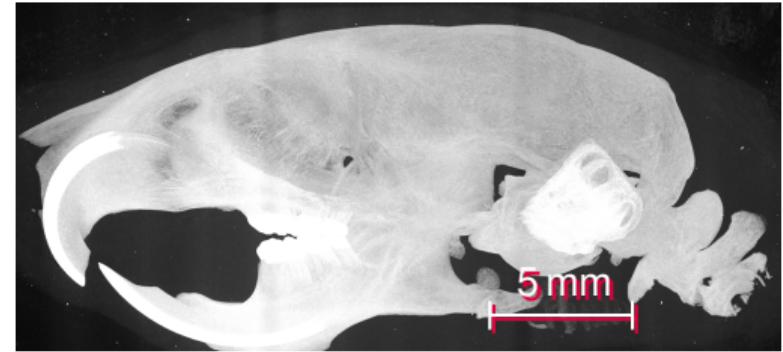


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# Why $\mu$ CT?



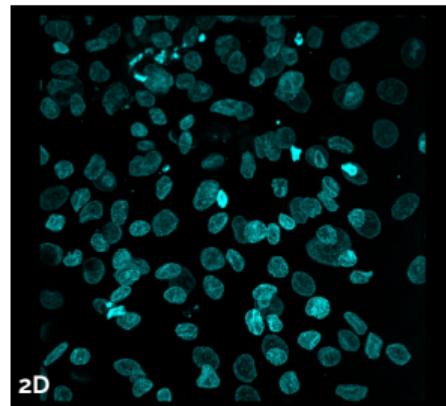
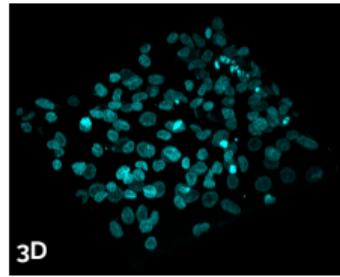
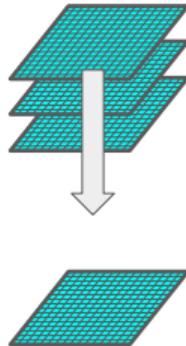
From [13], Subject C3L-02465



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



flic.kr/p/D4rbom

# Machinery

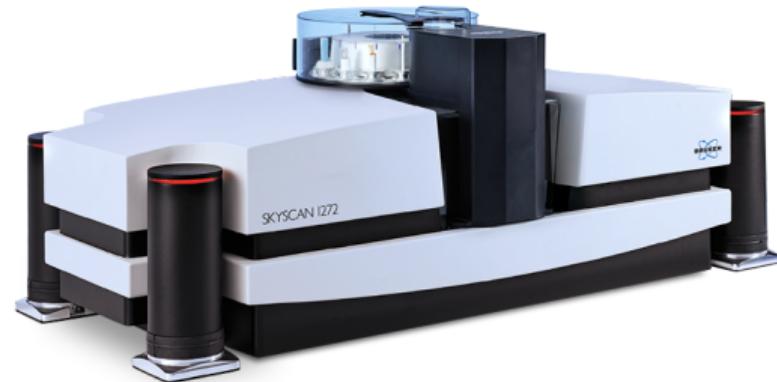
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[flic.kr/p/fpTrGu](https://flic.kr/p/fpTrGu) @@

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[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

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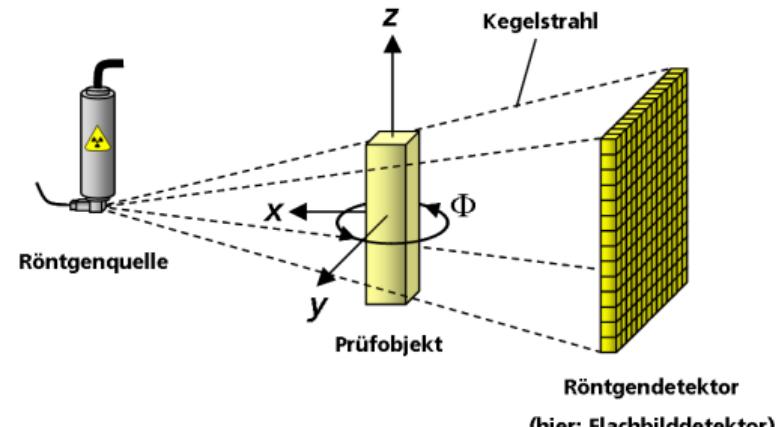


flic.kr/p/7Xhk2Y

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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

*u<sup>b</sup>*

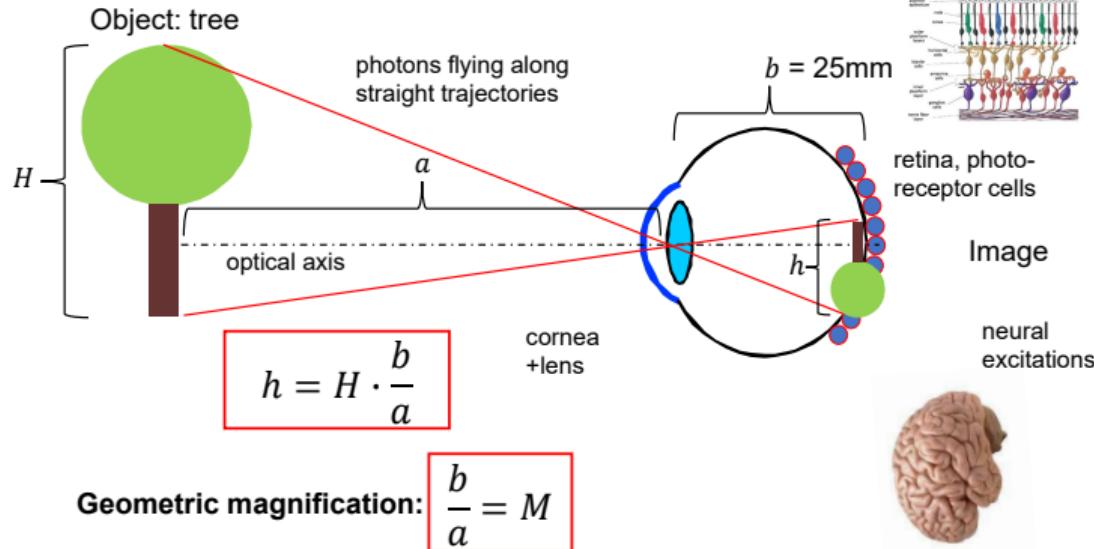
# Machinery

# Magnification

## Introduction – why do we need microscopes?

4

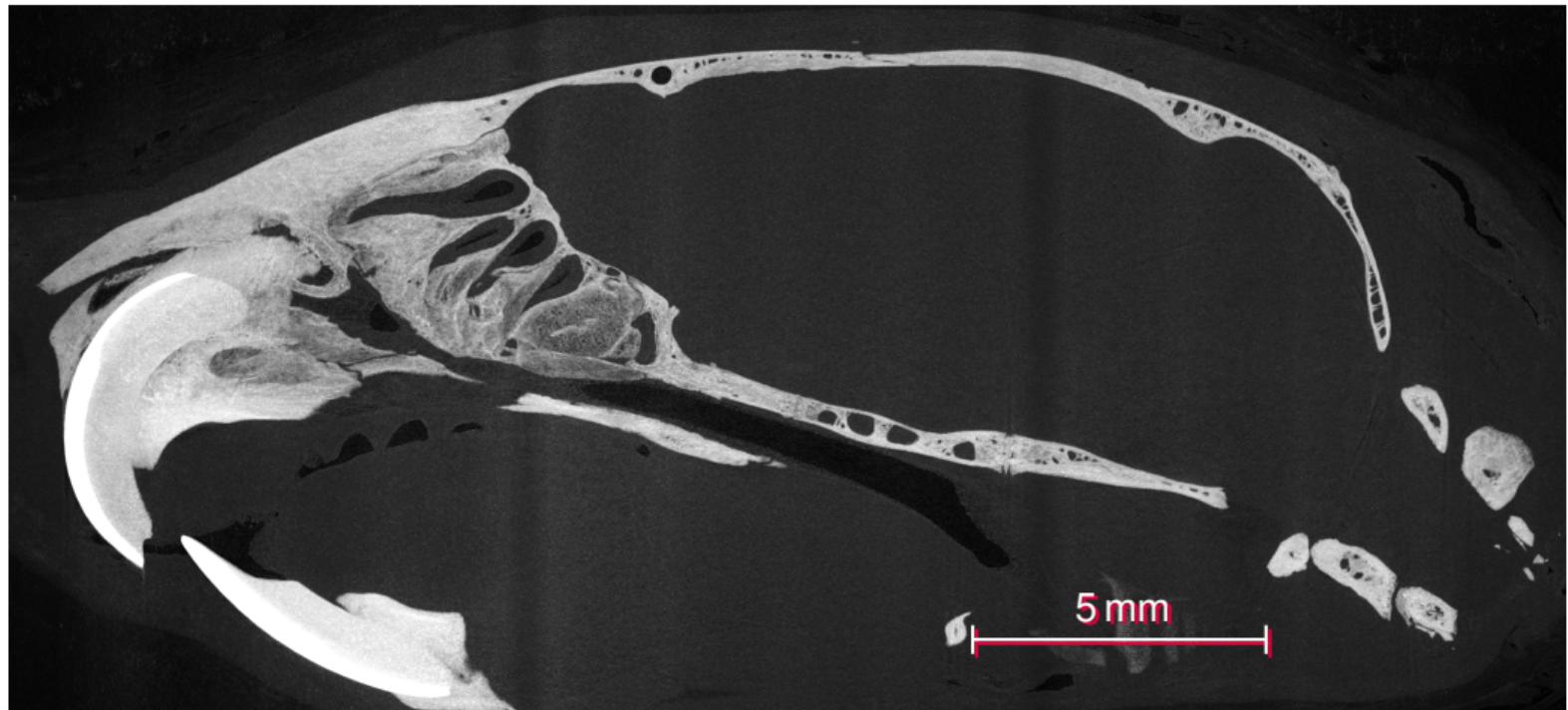
- Basic geometric optics: how does the eye form an image?



Contrast, Magnification and Resolution, Michael Jaeger, Slide 4

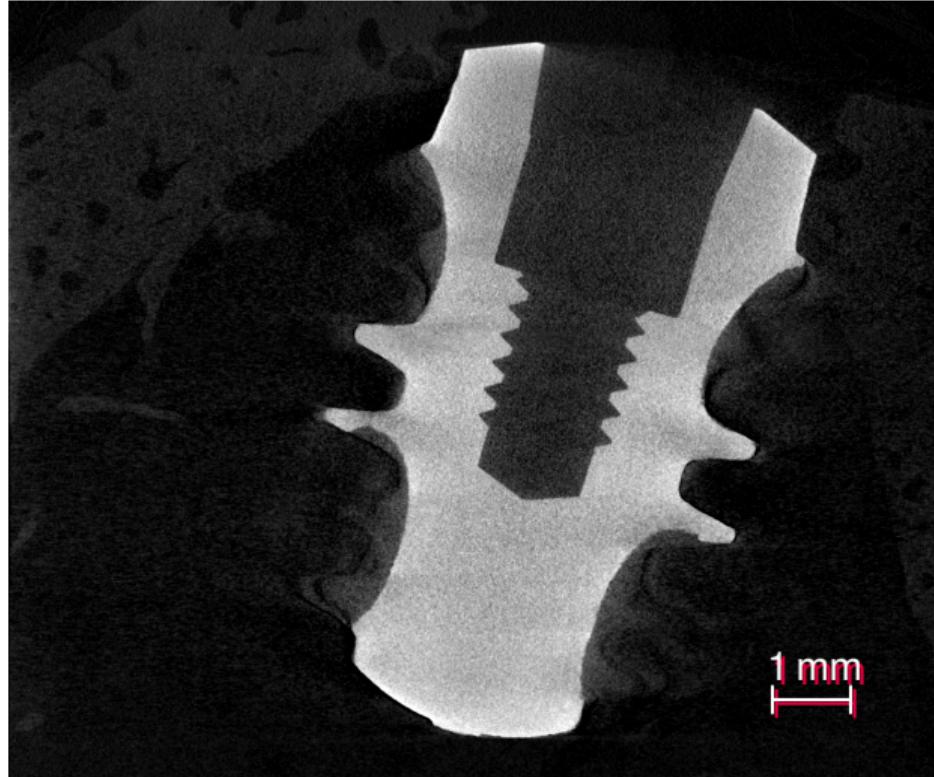
*u*<sup>b</sup>

# Examples



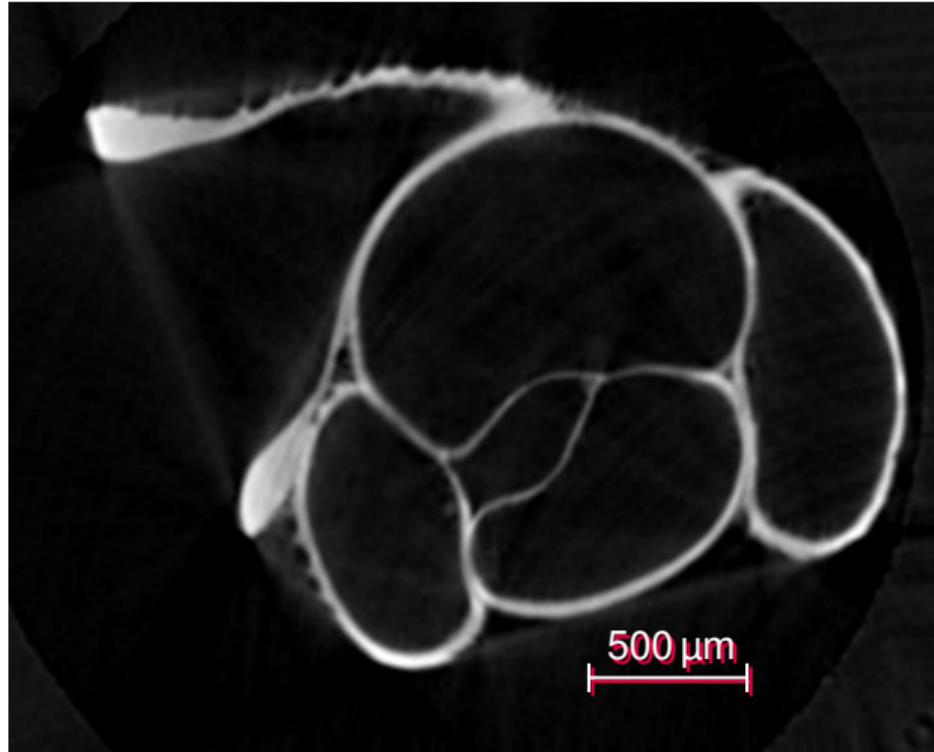
*u*<sup>b</sup>

# Examples



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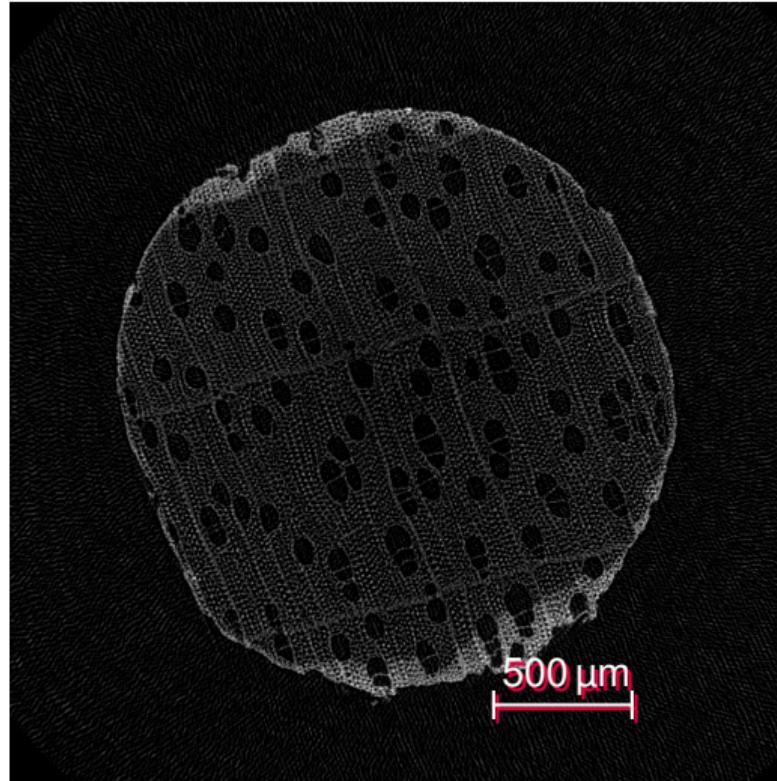
# Examples



From [8], *Diancta phoenix*

$u^b$

# Examples



*u*<sup>b</sup>

# 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.” ([22])
  - Photoelectric absorption ( $\tau$ ) 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 [23, i. e. Beer-Lamberts law]:  $I(t) = I_0 e^{-\alpha z}$

# 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

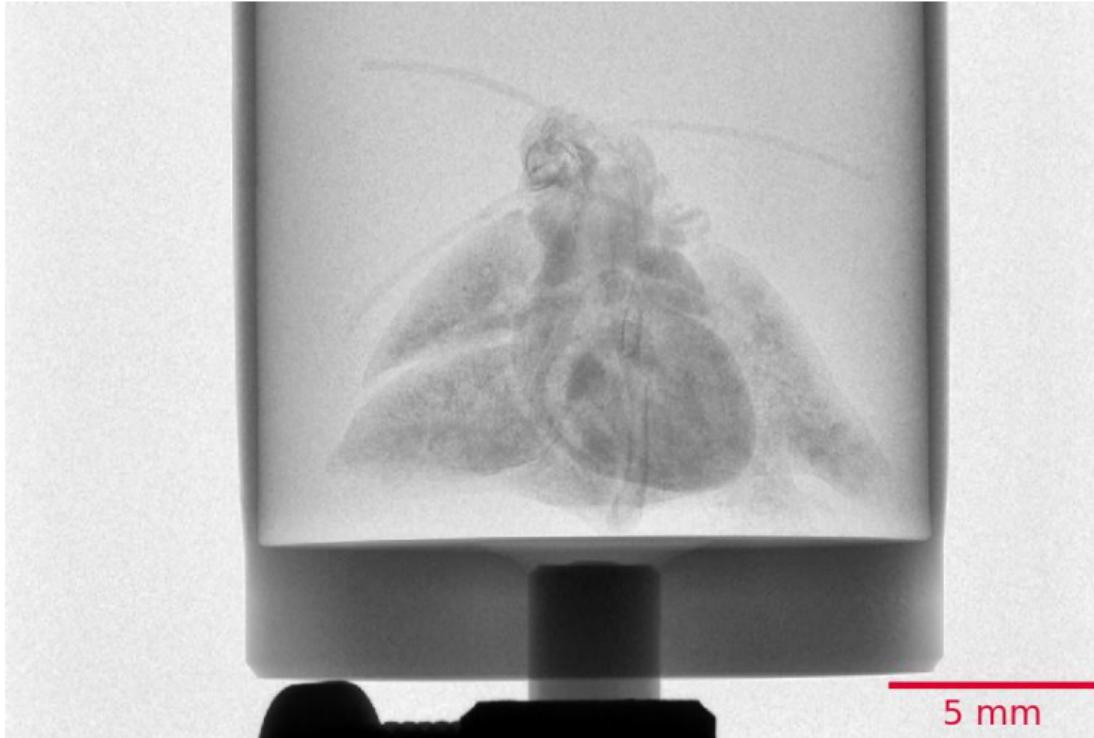
*u<sup>b</sup>*

# Preparation

- Study design
- Sample preparation

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# Projections

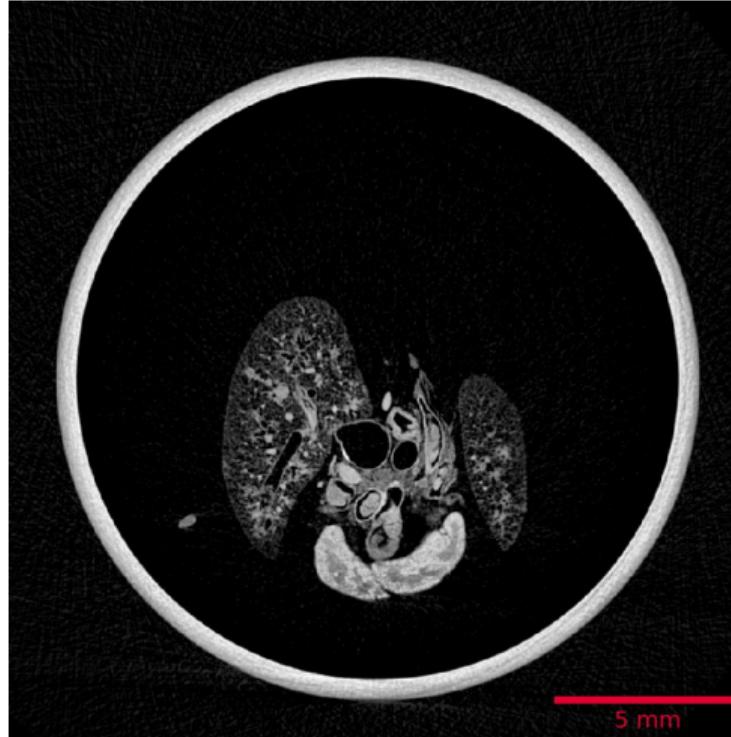


# Projections

- 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

*u*<sup>b</sup>

# 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 [20]
- Corrections (beam hardening, etc.)
- Writing to stack

$u^b$

# Visualization



# Visualization

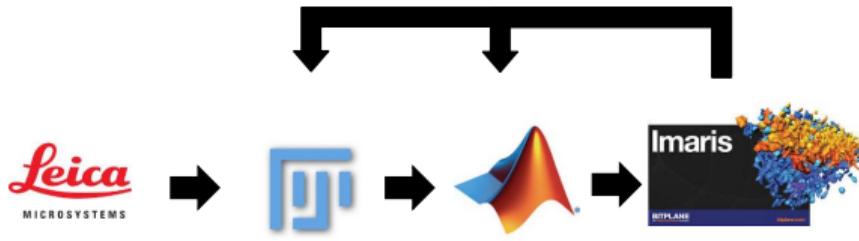
- Based on the calculated reconstructions, a computer synthesizes a (three-dimensional) visualization of the scanned sample

*u*<sup>b</sup>

# 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

*u*<sup>b</sup>

# What to use?

- ImageJ/Fiji [24]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research, i. e. Pretty images are nice, but we want quantitative numbers
  -  in Jupyter [25]
  - **git**
  - Script all your things!
  - Data repositories; i. e. sharing is caring!

*u<sup>b</sup>*

# Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number of teeth
- Structure of teeth

- Analytics



*u*<sup>b</sup>

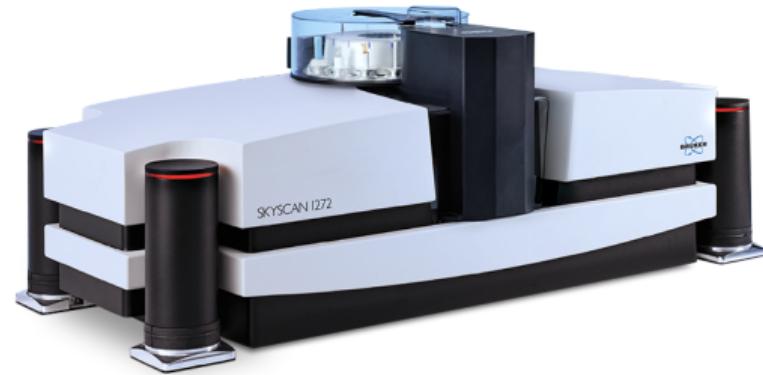
# How?

- 104 extracted human permanent mandibular canines
- $\mu$ CT imaging
- Root canal configuration, according to Briseño-Marroquín et al. [27]
- *Reproducible* analysis [28], e.g. you can click a button to double-check or recalculate the results yourself!



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[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

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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
08:23m:34s
Scan duration=0h:39m:51s
```

- 104 extracted human permanent mandibular canines
- $\mu$ CT imaging
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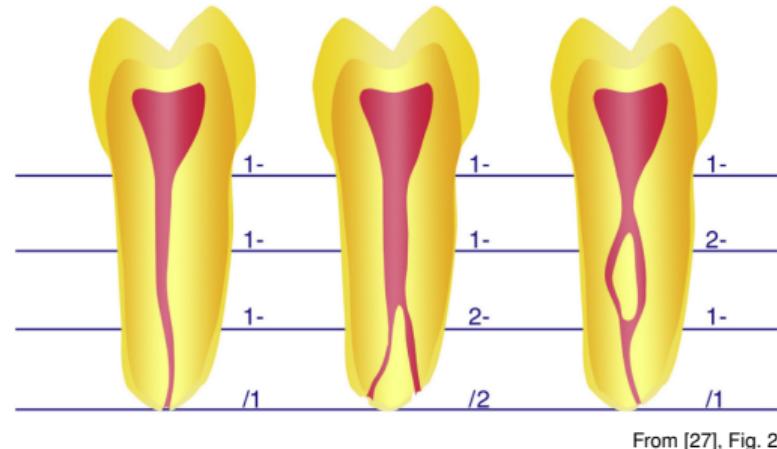
*Sample changer* on the SkyScan 1272

In total:

- 13 days of *continuous*  $\mu$ 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 [27], Fig. 2

*u*<sup>b</sup>

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[gph.is/2nqkple](https://gph.is/2nqkple)

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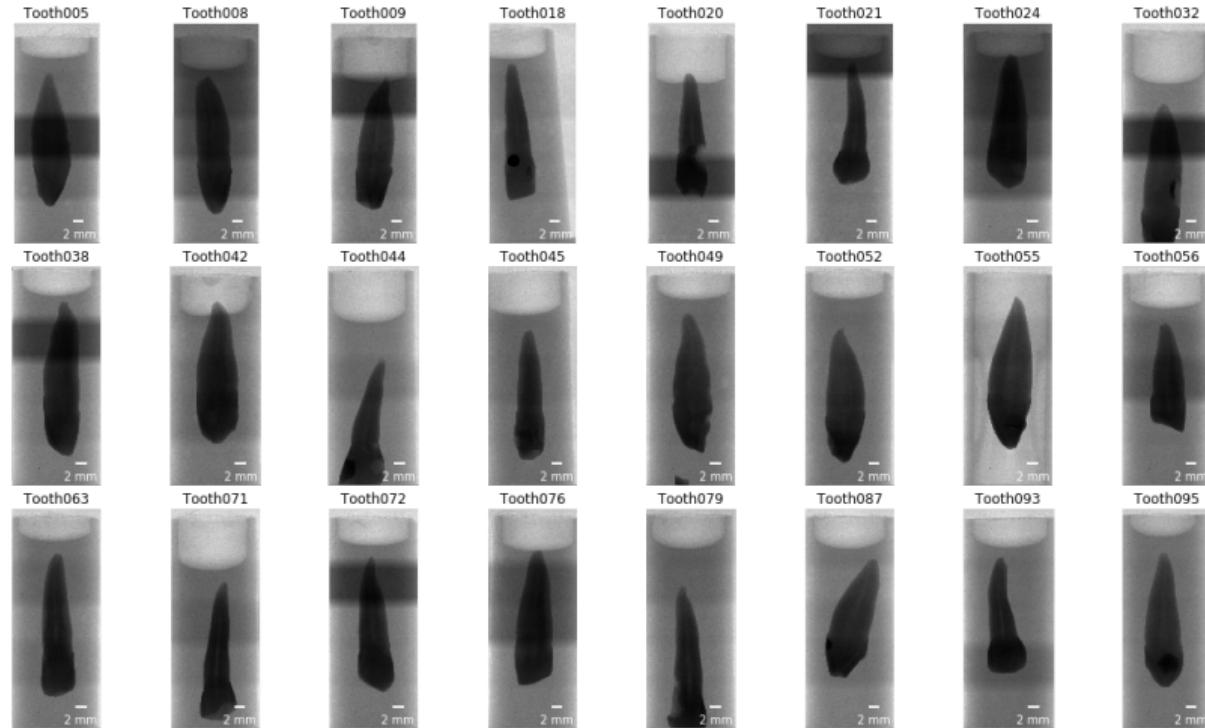
The screenshot shows a GitHub repository interface. At the top, there are buttons for 'master' (with a dropdown arrow), '1 branch' (with a dropdown arrow), '1 tag' (with a dropdown arrow), 'Go to file', 'Add file', and a green 'Code' button. Below this is a list of commits from a user named 'habi'. The commits are:

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 a section for 'README.md'. It contains the text 'A big tooth cohort'. Underneath this, it says 'We scanned a big bunch of teeth for a team of the dental clinic of the University of Bern.' and provides a preview and analysis notebook link. It also includes a 'launch binder' button.

$\mu$ b

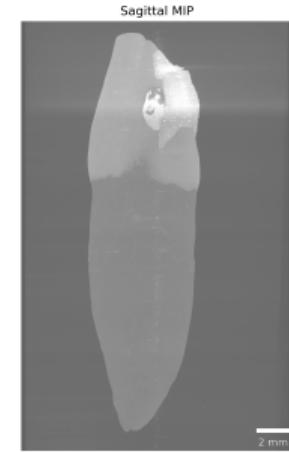
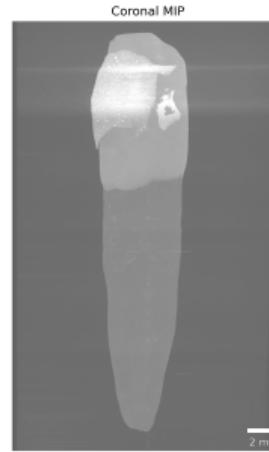
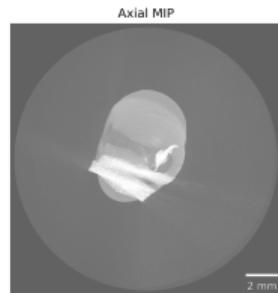
# $\mu$ CT imaging



*u*<sup>b</sup>

# Dataset cropping

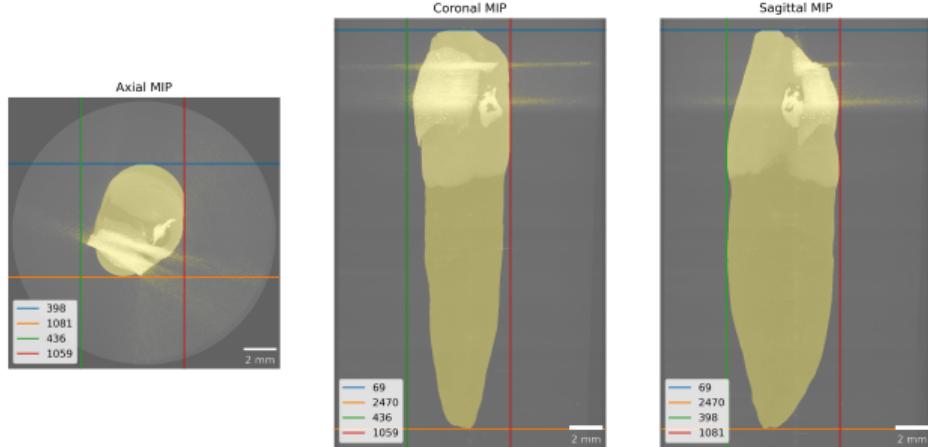
- Full datasets: 326 GB
- Cropped datasets: 115 GB



*u*<sup>b</sup>

# Dataset cropping

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



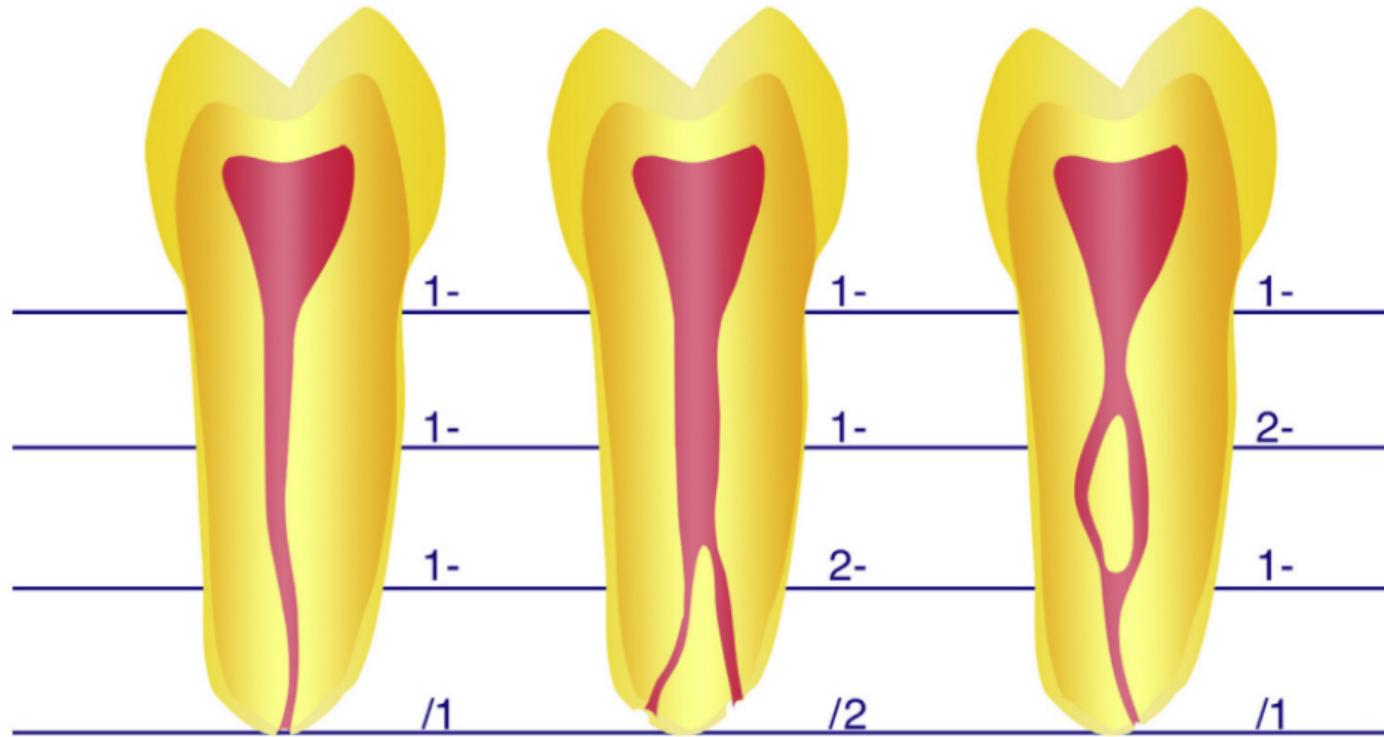
*u<sup>b</sup>*

# Tooth morphology



*u<sup>b</sup>*

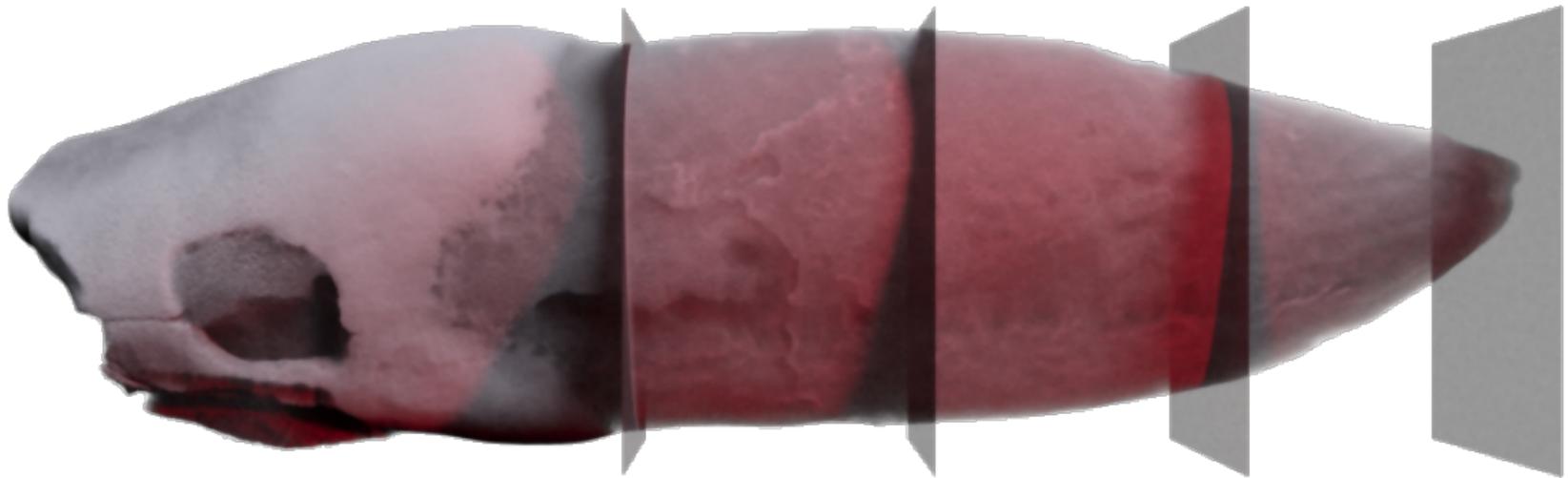
# Tooth morphology



From [27], Fig. 2

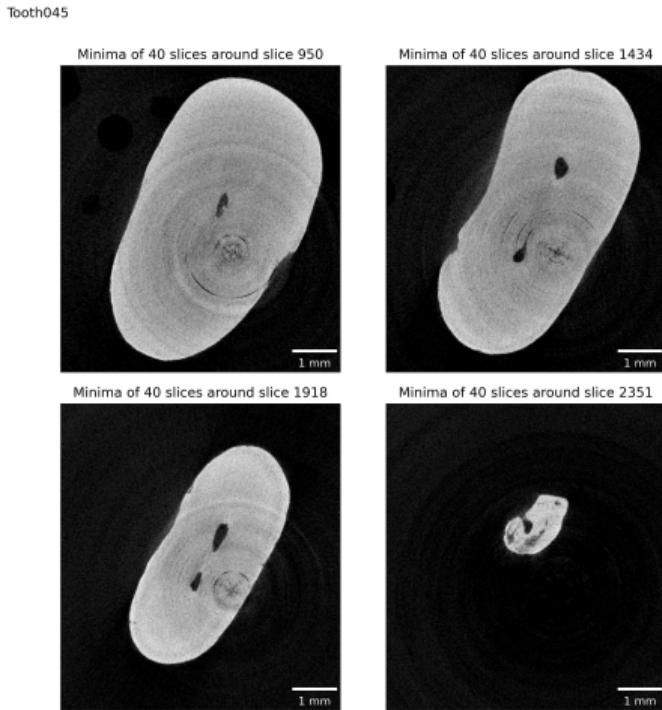
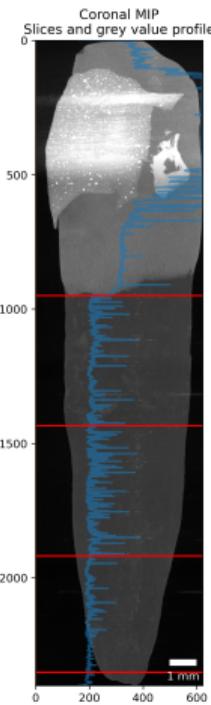
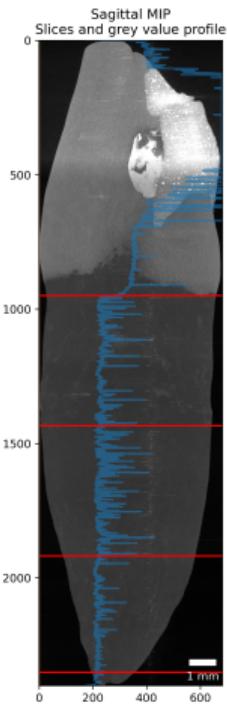
*u*<sup>b</sup>

# Tooth morphology



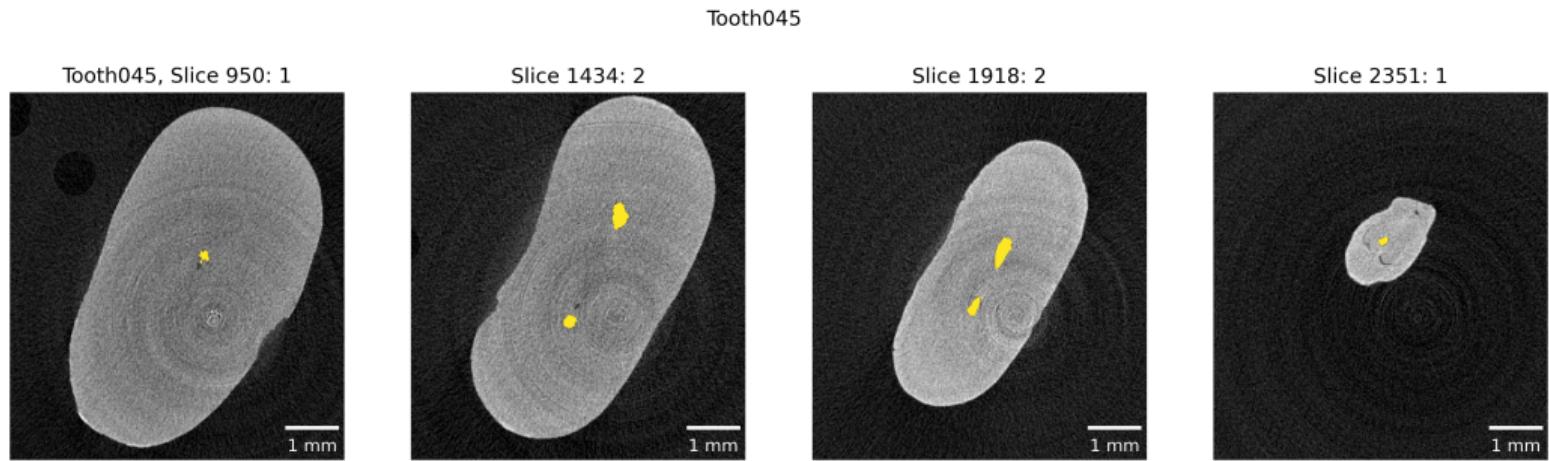
*u*<sup>b</sup>

# Detection of enamel-dentin border



*u*<sup>b</sup>

# Detection of enamel-dentin border



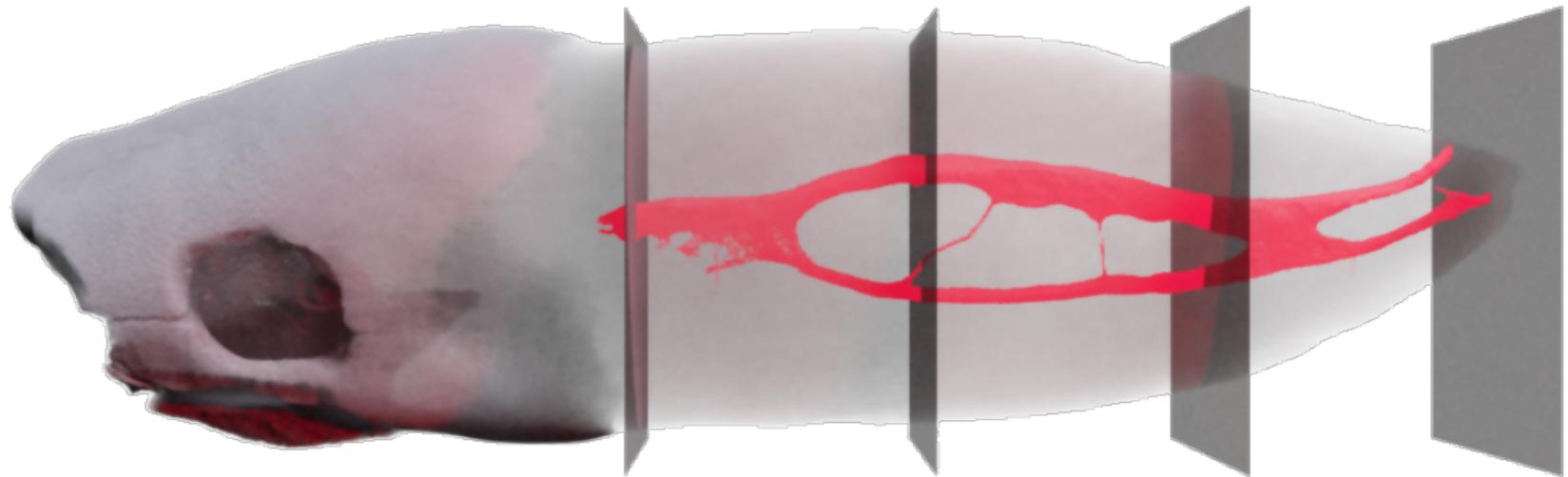
*u<sup>b</sup>*

# Classification of root canal configurations

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

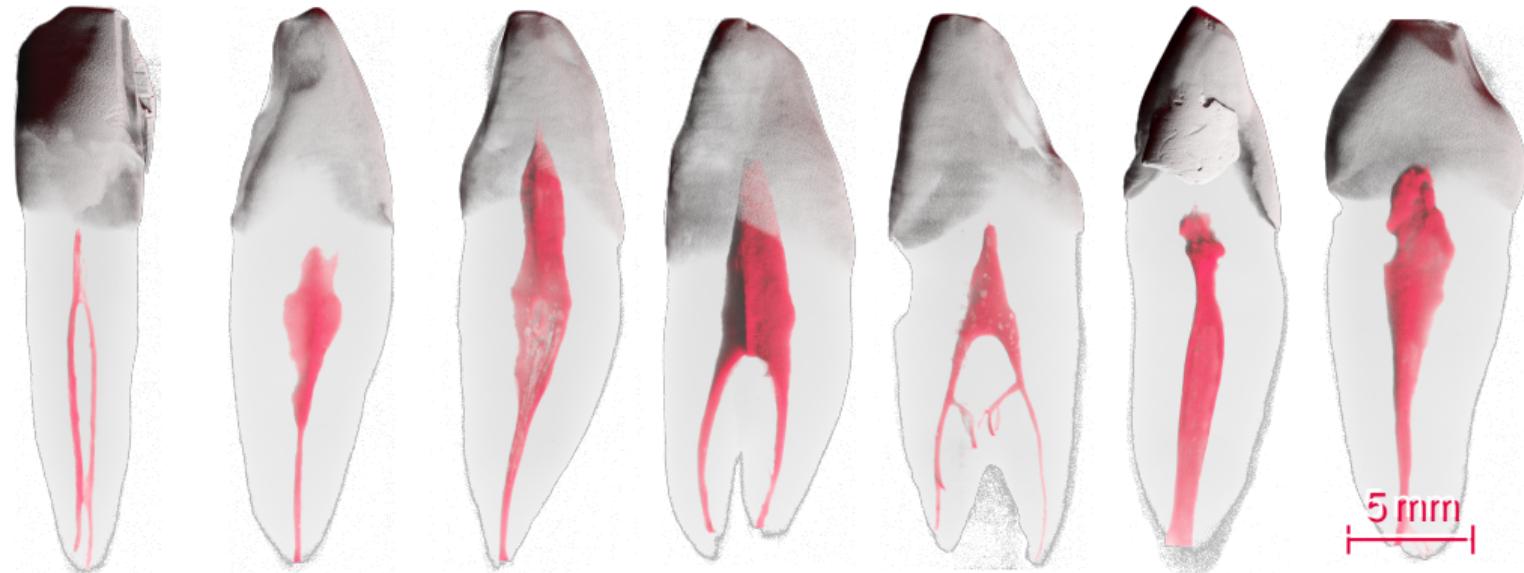
*u*<sup>b</sup>

# Extraction of root canal space



*u<sup>b</sup>*

# 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

*u<sup>b</sup>*

# Thanks!

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

# Colophon

- This BEAMER presentation was crafted in  $\text{\LaTeX}$  with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
  - Complete source code: [git.io/fjpP7](https://git.io/fjpP7)
  - The  $\text{\LaTeX}$  code is automatically compiled with a GitHub action to a (handout) PDF which you can access here: [git.io/JeQxO](https://git.io/JeQxO)
- Did you spot an error?
  - File an issue: [git.io/fjpPb](https://git.io/fjpPb)
  - Submit a pull request: [git.io/fjpPN](https://git.io/fjpPN)
  - Send me an email: [david.haberthuer@unibe.ch](mailto:david.haberthuer@unibe.ch)

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