

u^b

b

**UNIVERSITY
OF BERN**

u^b X-ray microtomography

9256-HS2024-0: Advanced Microscopy

David Haberthür

Institute of Anatomy, December 20, 2024

u^b

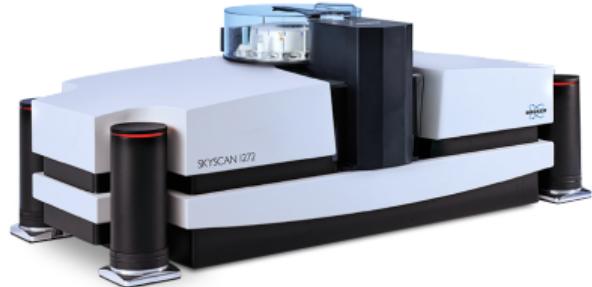
Grüessech mitenang!



David.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]



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

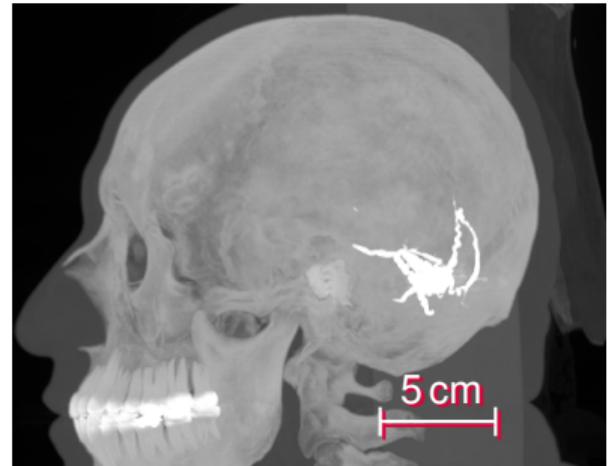
 Overview

 Materials & Methods

 Results

micro-Computed tomography

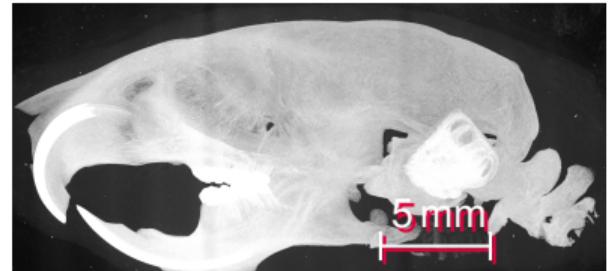
- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a large range of sample sizes



From [13], Subject C3L-02465

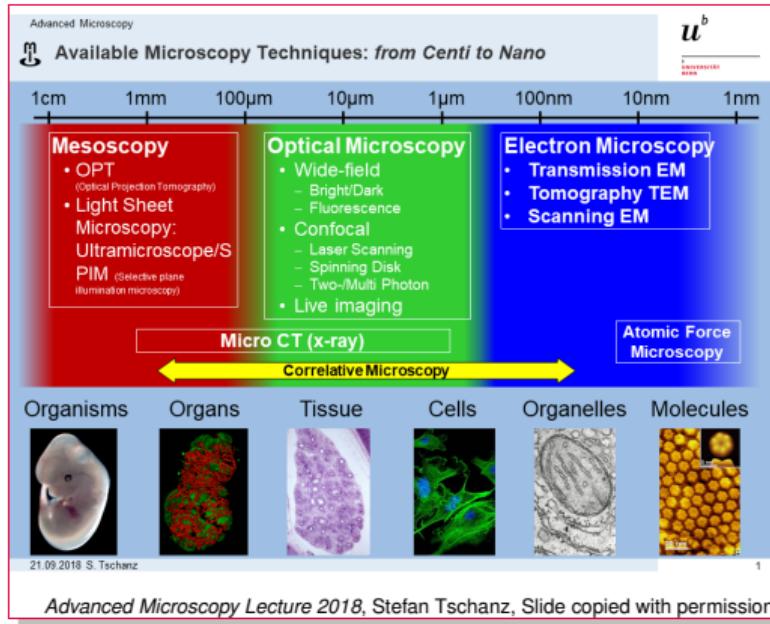
micro-Computed tomography

- Allows for imaging dense and non-transparent samples
- Non-destructive imaging, thus compatible with routine sample preparation
- Results in three-dimensional images with 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



u^b

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

u^b

CT-Scanner



youtu.be/2CWpZKuy-NE

u^b

CT History

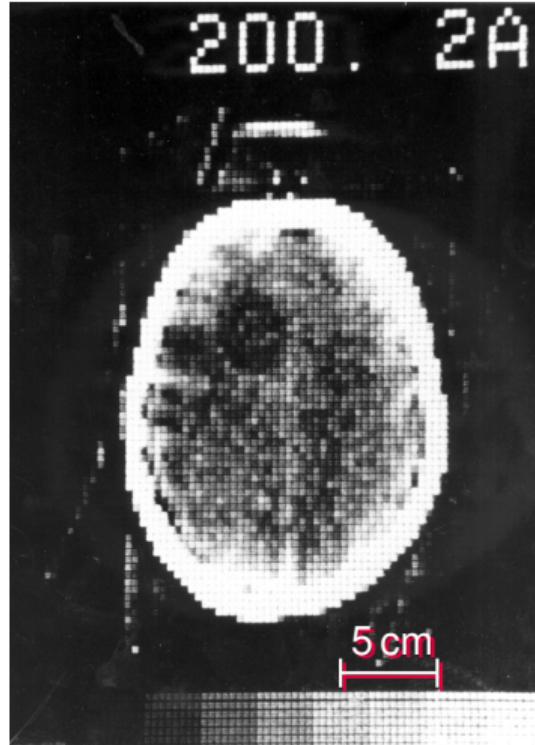
- 1895: Wilhelm Conrad Röntgen discovers X-rays



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]

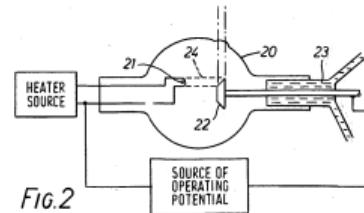
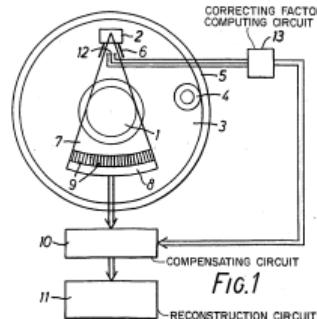


From [16], Figure 5

CT History

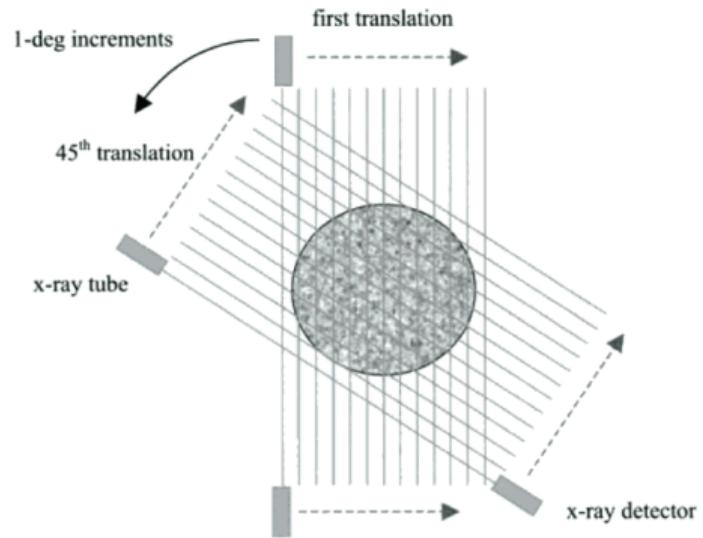
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- Nobel Prize in 1979, jointly for Allan Cormack and Godfrey Hounsfield

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



CT History

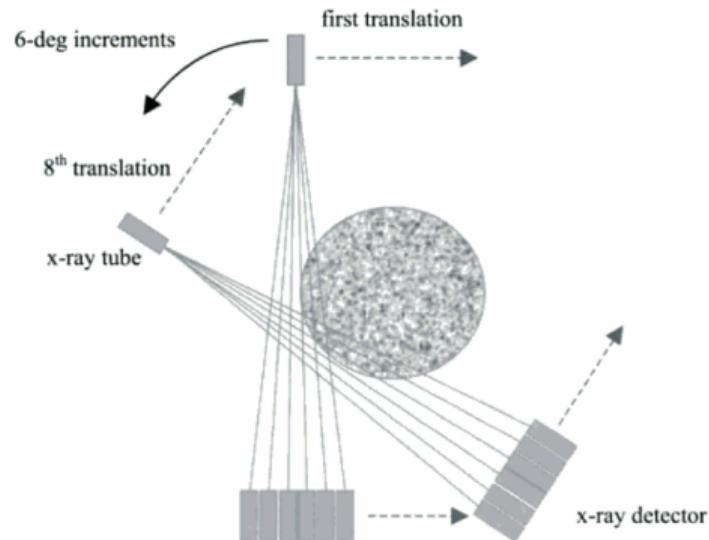
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- Nobel Prize in 1979, jointly for Allan Cormack and Godfrey Hounsfield
- CT scanner generations
 - First generation



From [18], Figure 1.12

CT History

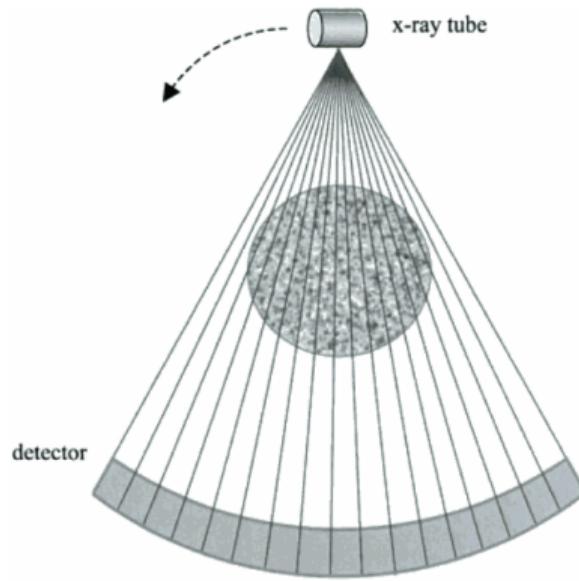
- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]
- Nobel Prize in 1979, jointly for Allan Cormack and Godfrey Hounsfield
- CT scanner generations
 - First generation
 - Second generation



From [18], 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]
- Nobel Prize in 1979, jointly for Allan Cormack and Godfrey Hounsfield
- CT scanner generations
 - First generation
 - Second generation
 - Third generation



From [18], Figure 1.14

μ CT History I

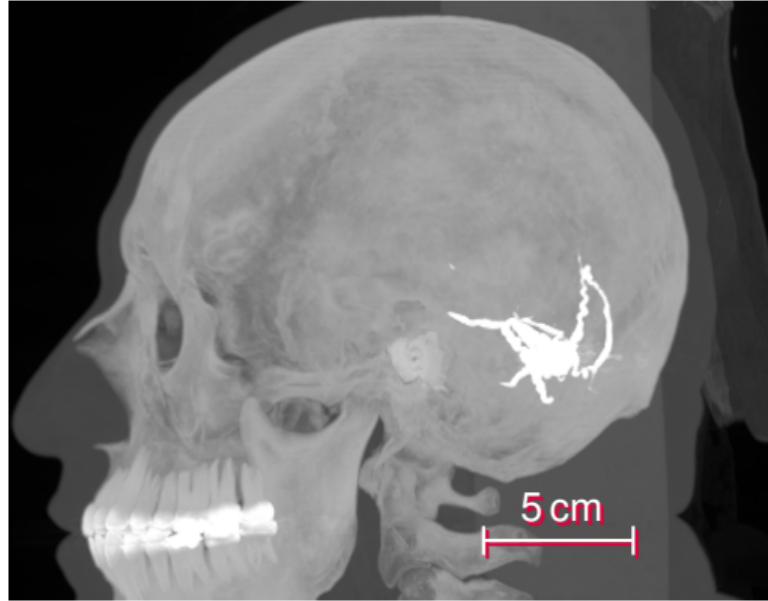
- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [19]
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp [20] developed an early laboratory microCT system by assembling a micro-focus cone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

μ CT History II

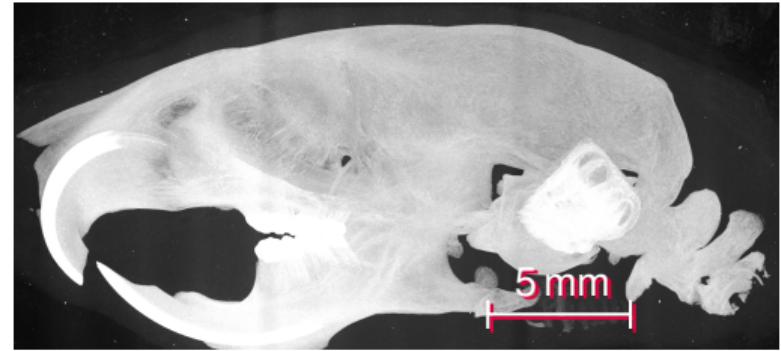
- μ CT was first reported in the 1980s, for scanning gemstones
- Early 1990s: Manufacturers like SkyScan and Scanco Medical made μ CT systems commercially available
- Today: Nondestructive imaging for quantifying the (micro)structure of (organic) materials
 - Mineralized bone tissue and the relationships 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 μ CT systems are in use worldwide with over 1000 publications annually

u^b

Why μ CT?



From [13], Subject C3L-02465



u^b

Why μ CT?

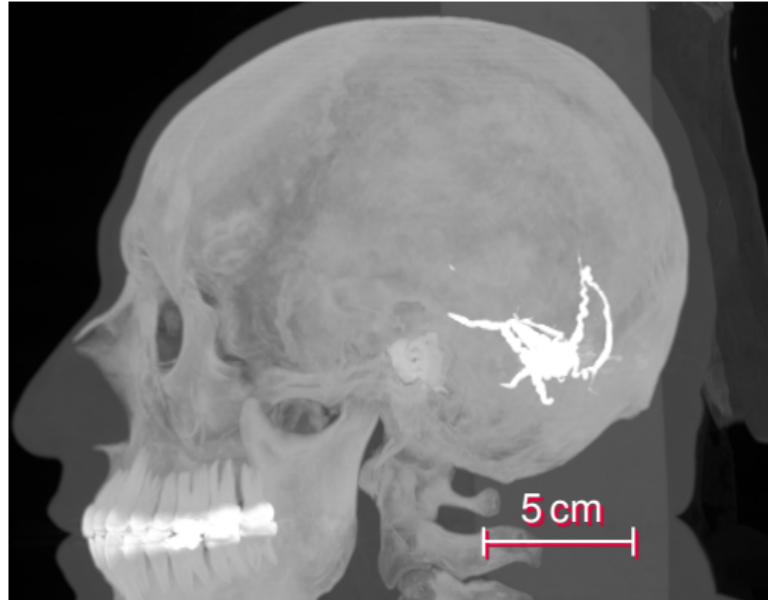


From [13], Subject C3L-02465

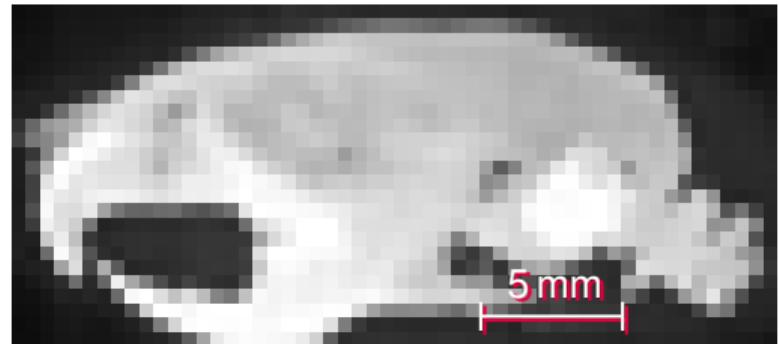


u^b

Why μ CT?

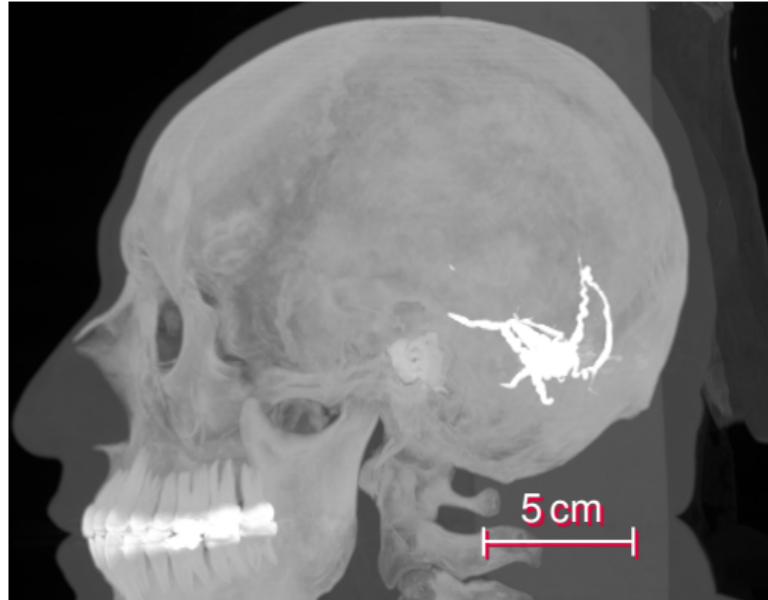


From [13], Subject C3L-02465

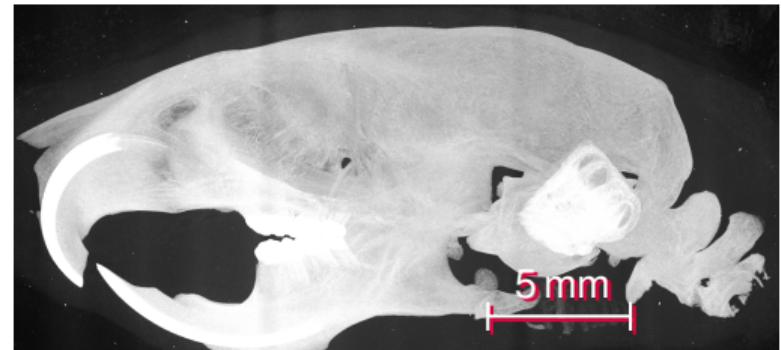


u^b

Why μ CT?

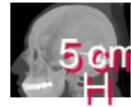


From [13], Subject C3L-02465

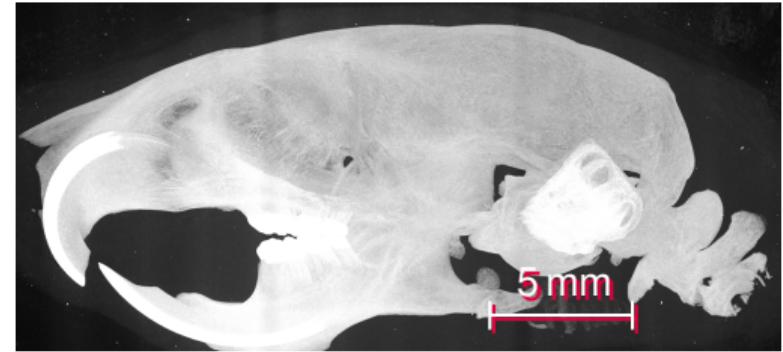


u^b

Why μ CT?



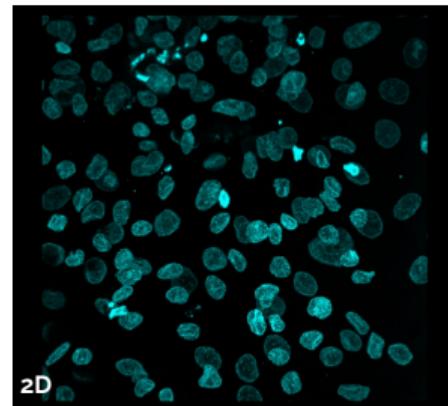
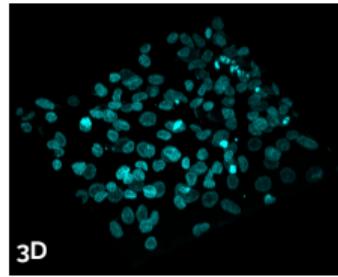
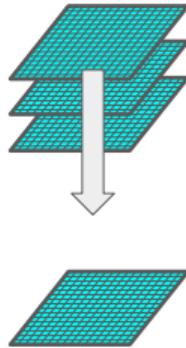
From [13], Subject C3L-02465



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

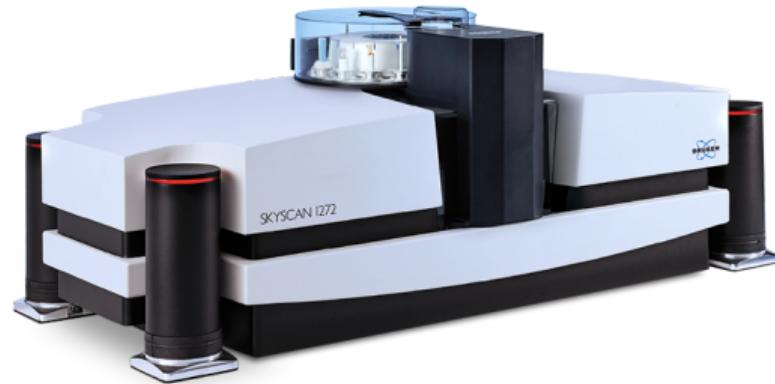
- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around 7 μm (*in vivo*)
 - Voxel size around 0.5 μm (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



flic.kr/p/fpTrGu @@ @@

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



bruker.com/skyscan1272

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



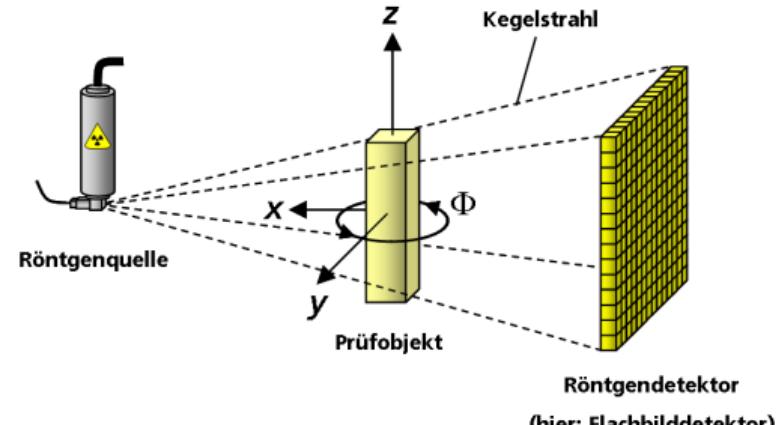
flic.kr/p/7Xhk2Y

u^b

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^b

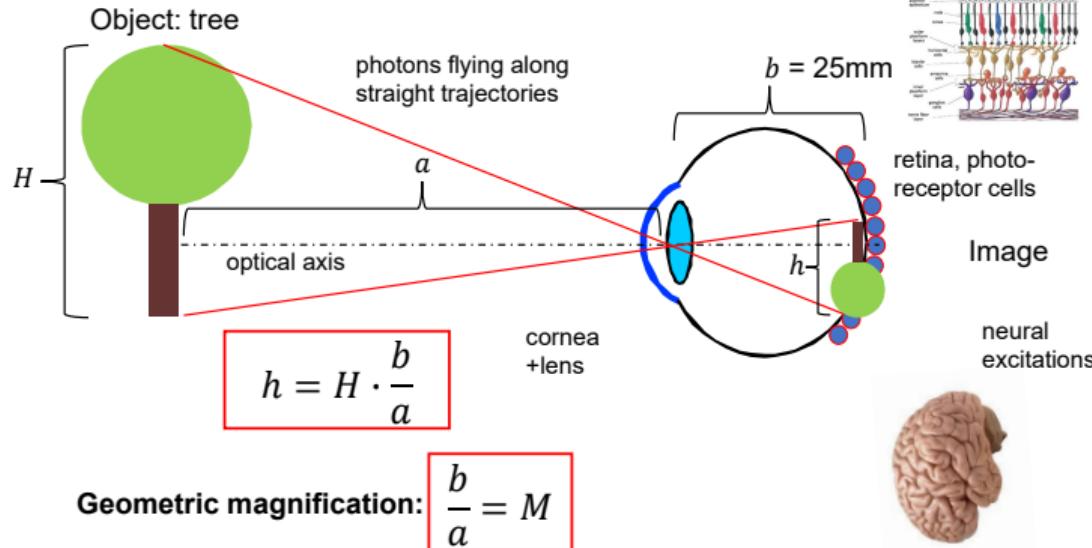
Machinery

Magnification

Introduction – why do we need microscopes?

4

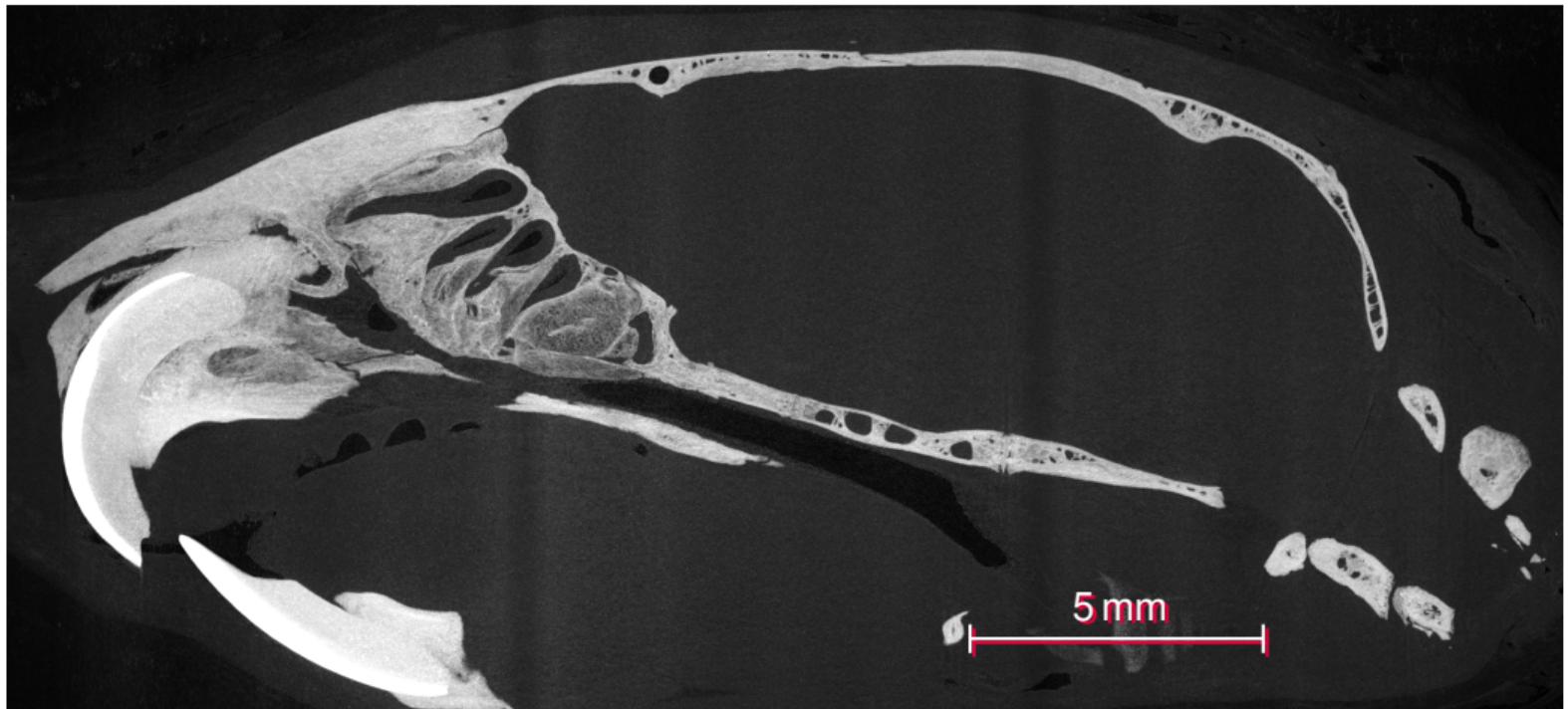
- Basic geometric optics: how does the eye form an image?



Contrast, Magnification and Resolution, Michael Jaeger, Slide 4

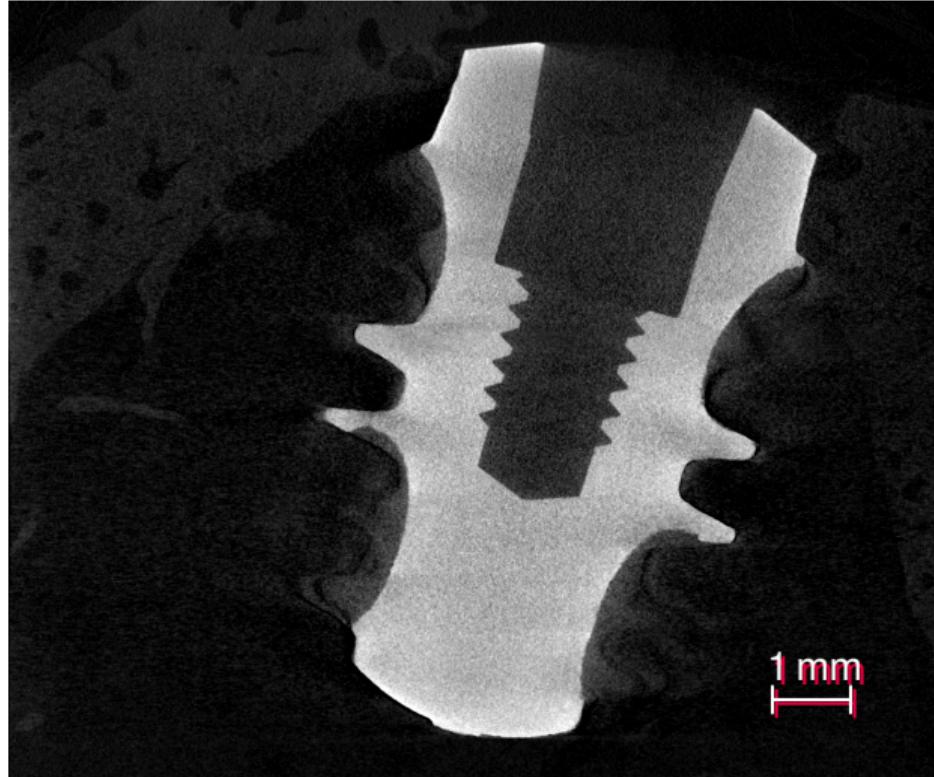
u^b

Examples



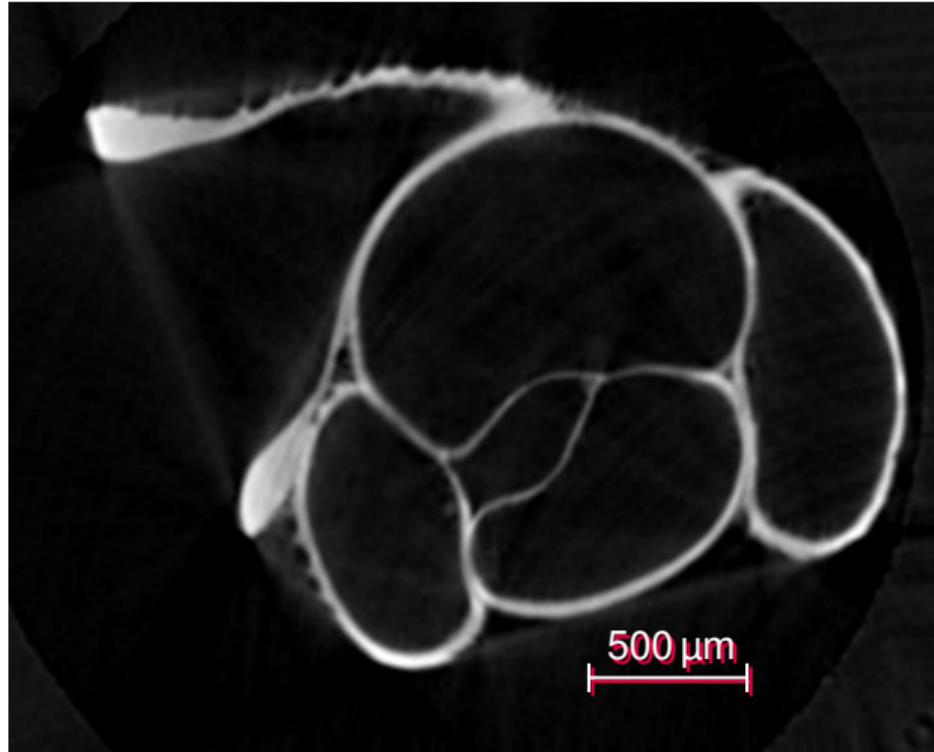
u^b

Examples



u^b

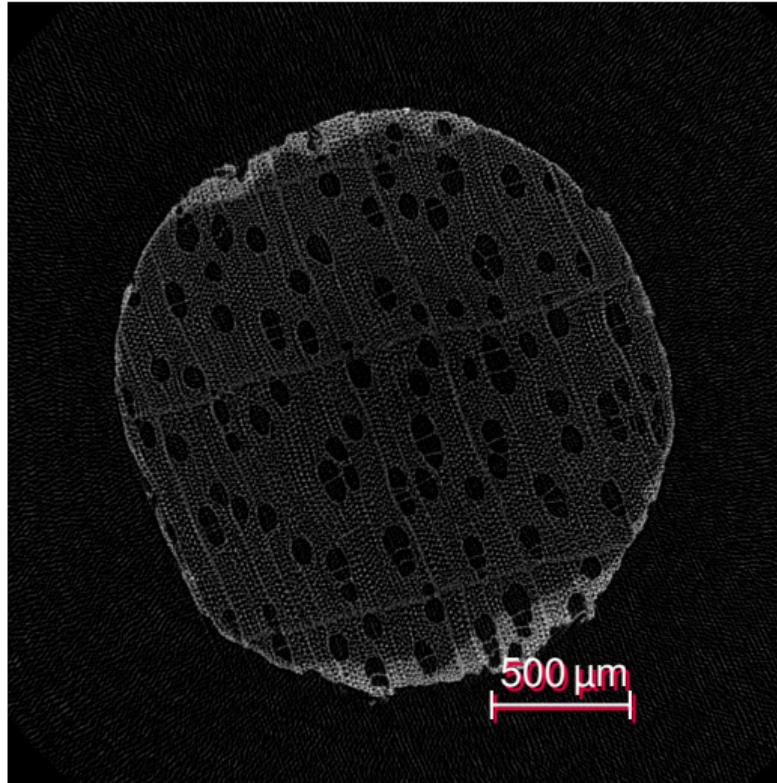
Examples



From [8], *Diancta phoenix*

u^b

Examples



u^b

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 (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 [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

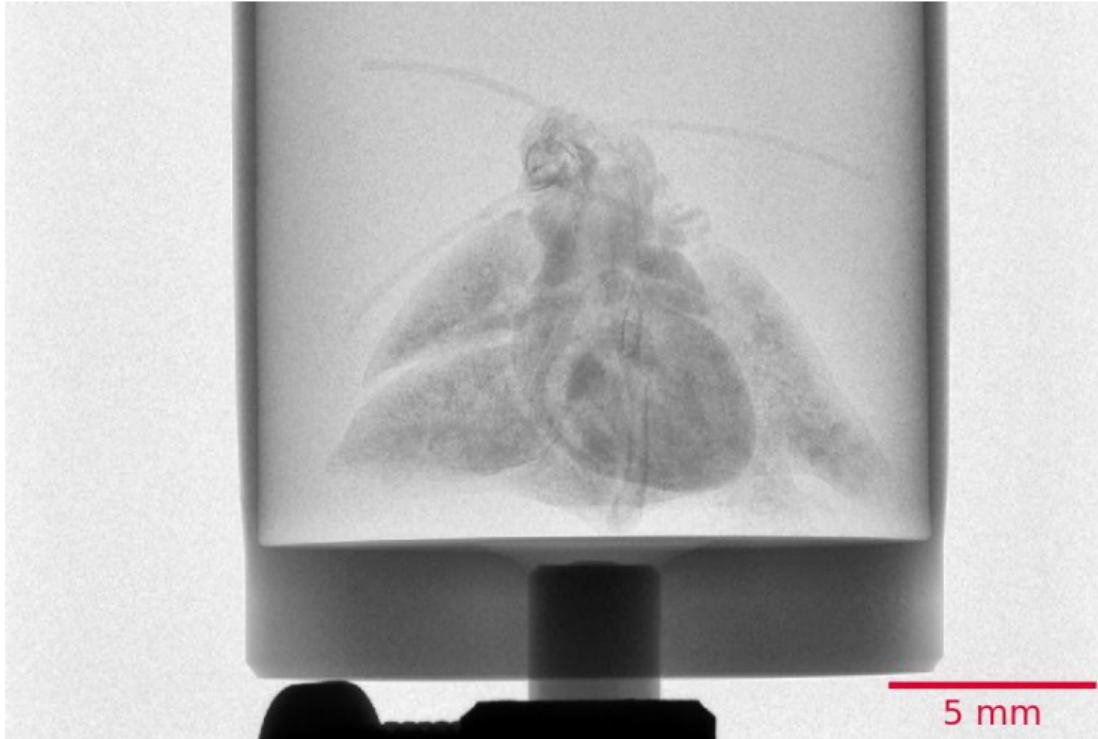
u^b

Preparation

- Study design
- Sample preparation

u^b

Projections

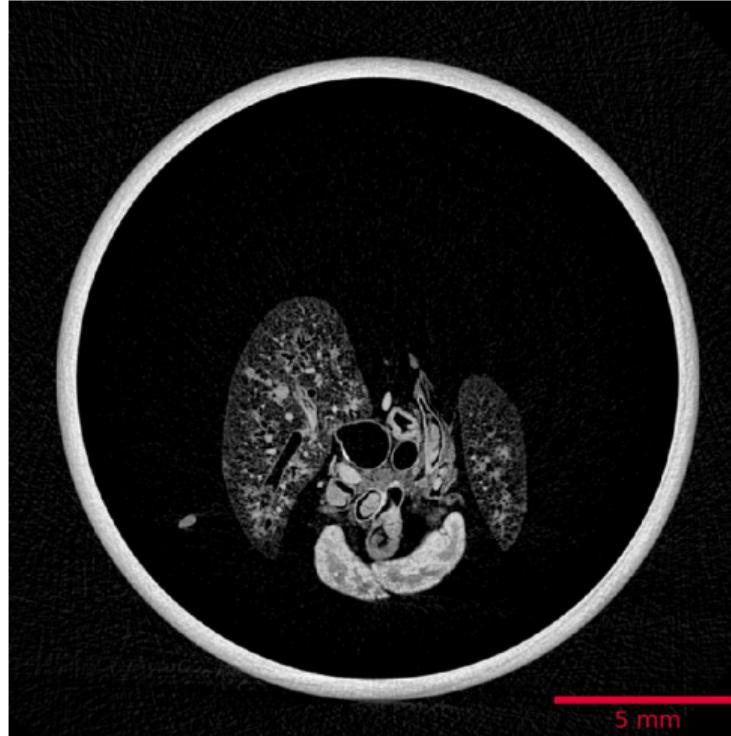


Projections

- 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^b

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

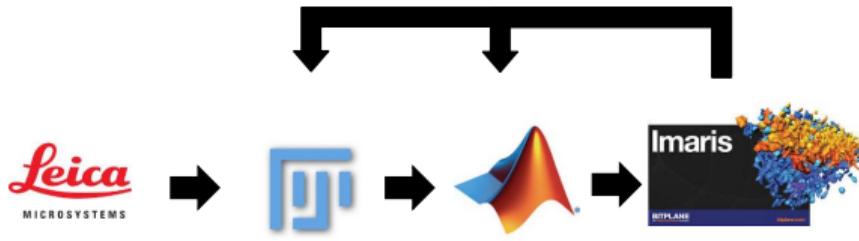
- Based on the calculated reconstructions, a computer synthesizes a (three-dimensional) visualization of the scanned sample

u^b

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^b

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^b

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number of teeth
- Structure of teeth



u^b

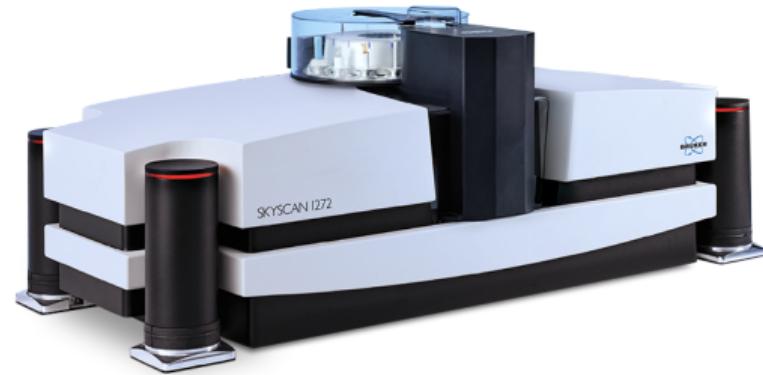
How?

- 104 extracted human permanent mandibular canines
- μ 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!



How?

- 104 extracted human permanent mandibular canines
- μ 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!



bruker.com/skyscan1272

- 104 extracted human permanent mandibular canines
- μ 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!

```
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
- μ 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!

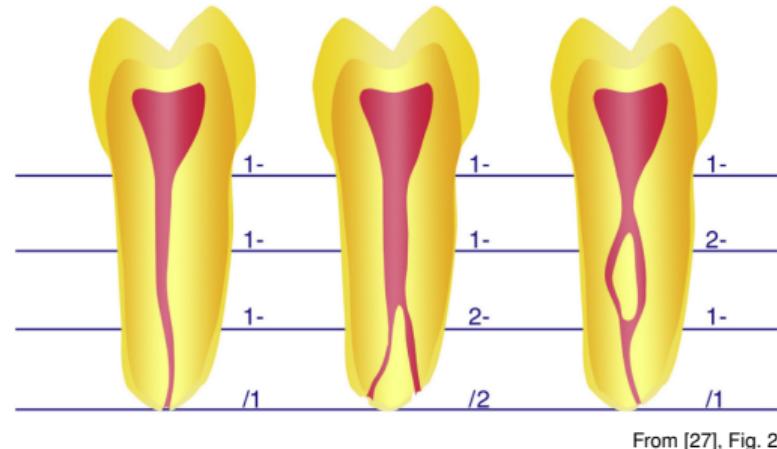
Sample changer on the SkyScan 1272

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?

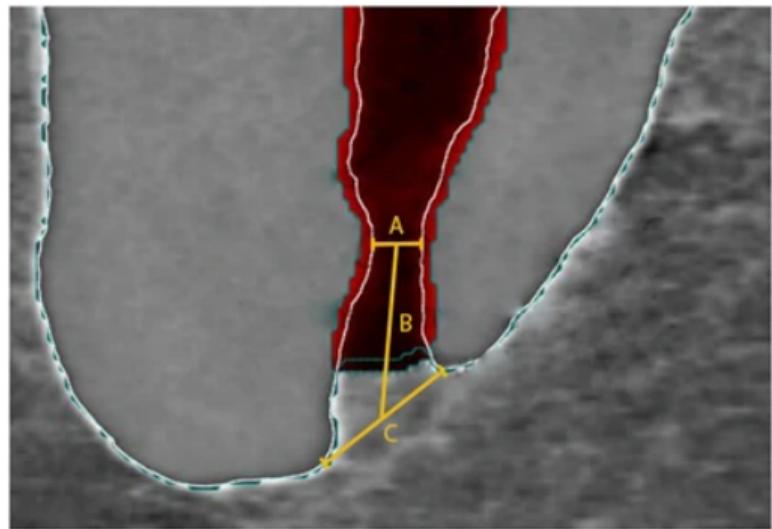
- 104 extracted human permanent mandibular canines
- μ CT imaging
- Root canal configuration, according to Briseño-Marroquín et al. [27]
- *Reproducible* analysis [28], e.g. you can click a button to double-check or recalculate the results yourself!



From [27], Fig. 2

How?

- 104 extracted human permanent mandibular canines
- μ 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!



From [29], Fig. 1

- 104 extracted human permanent mandibular canines
- μ 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!



gph.is/2nqkple

u^b

How?

- 104 extracted human permanent mandibular canines
- μ 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!

The screenshot shows a GitHub repository interface. At the top, there are buttons for 'master' (selected), '1 branch', '1 tag', '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 'README.md' file. It contains a DOI link (DOI 10.5281/zenodo.3999402) and a 'treebeard.yaml failing' status indicator. There is also a 'launch binder' button. The main text in the README is:

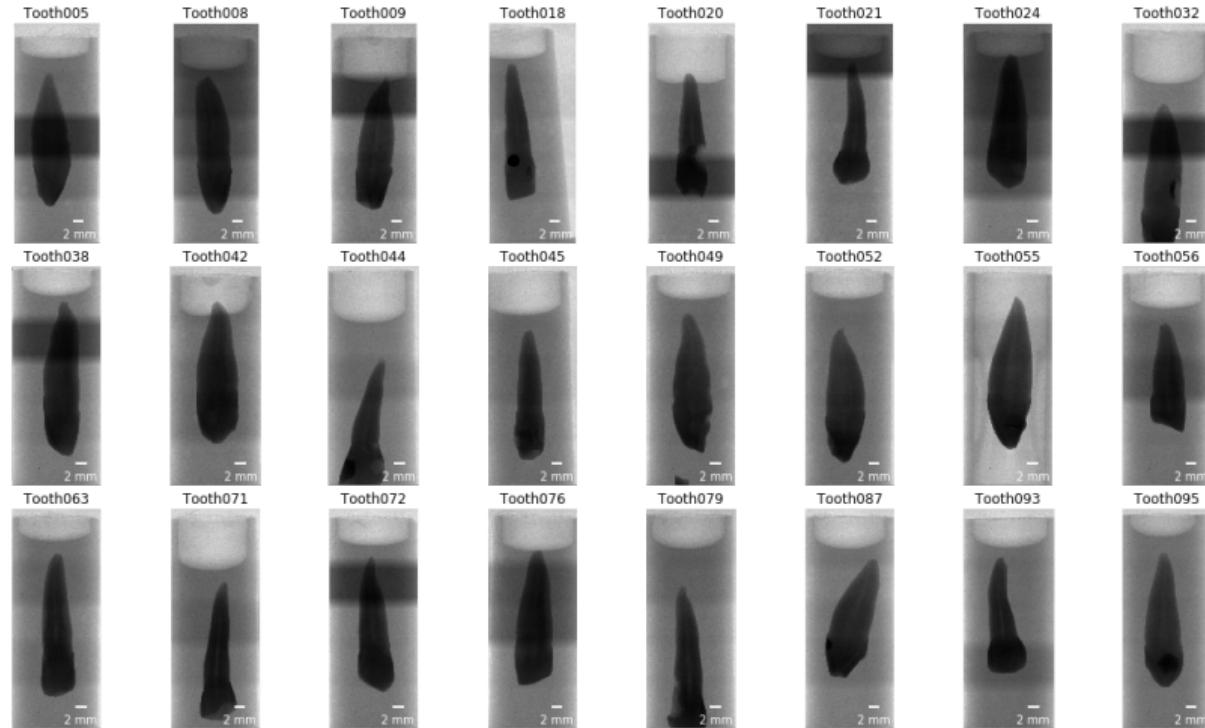
A big tooth cohort

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

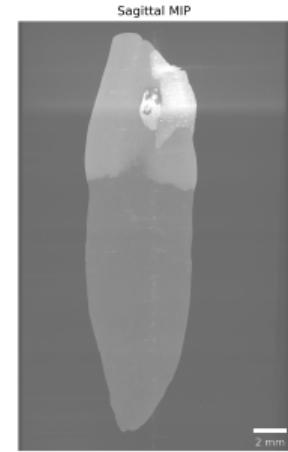
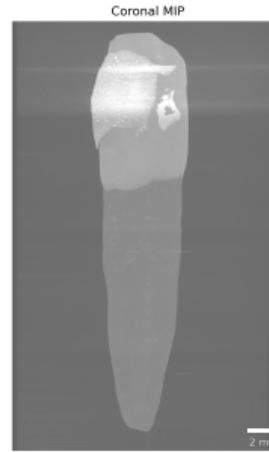
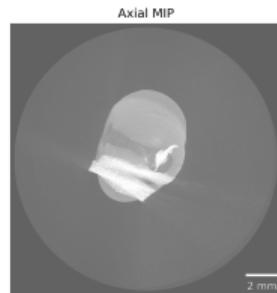
μ CT imaging



u^b

Dataset cropping

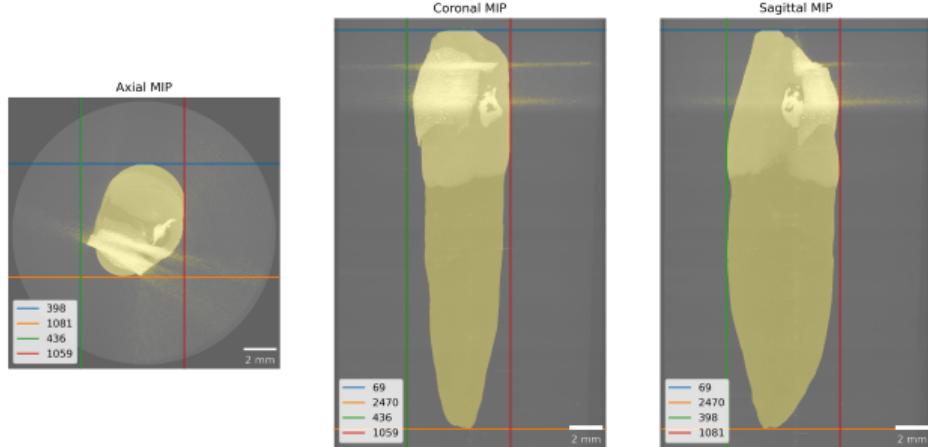
- Full datasets: 326 GB
- Cropped datasets: 115 GB



u^b

Dataset cropping

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



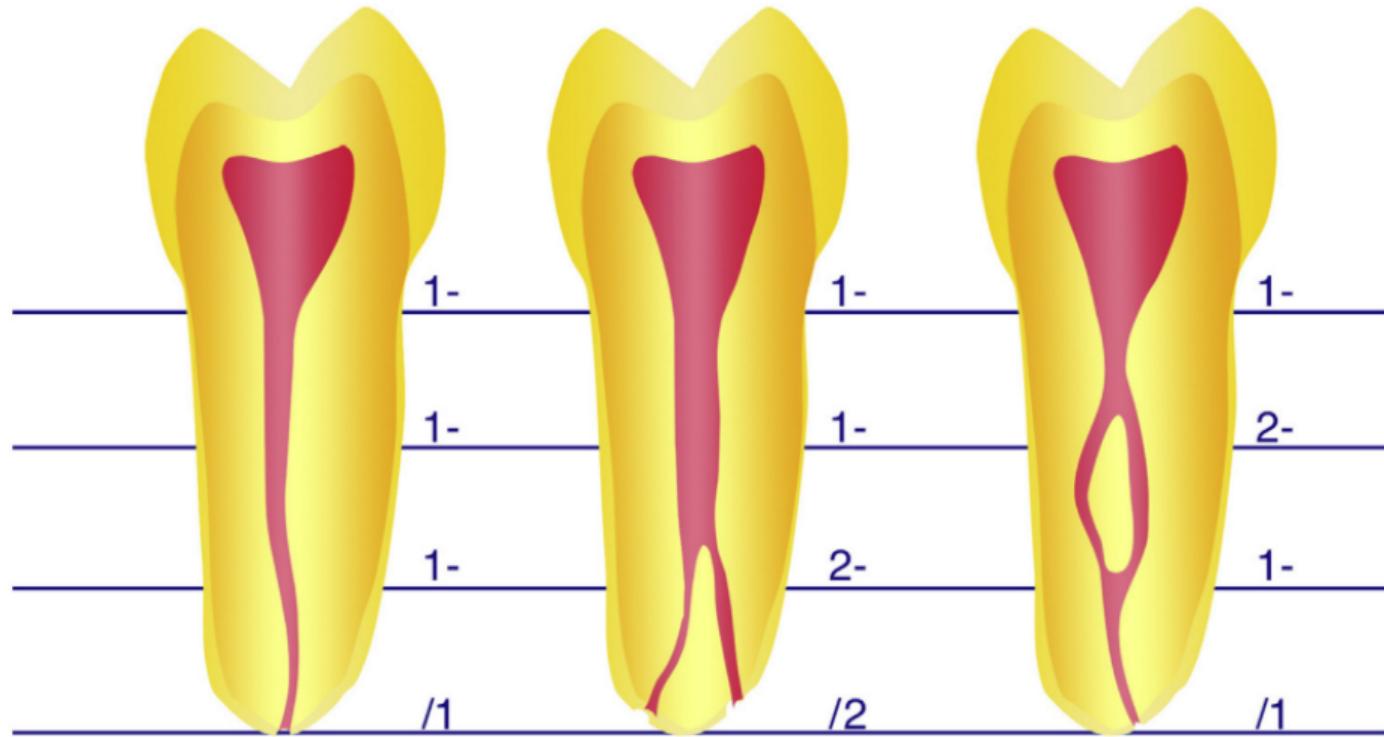
u^b

Tooth morphology



u^b

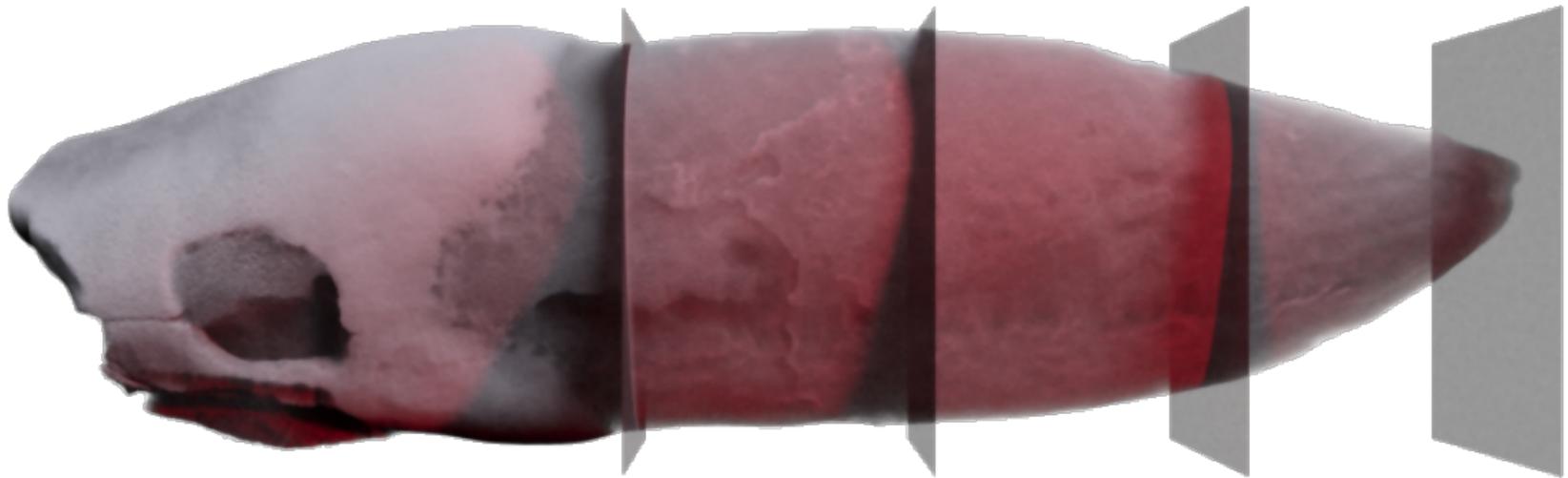
Tooth morphology



From [27], Fig. 2

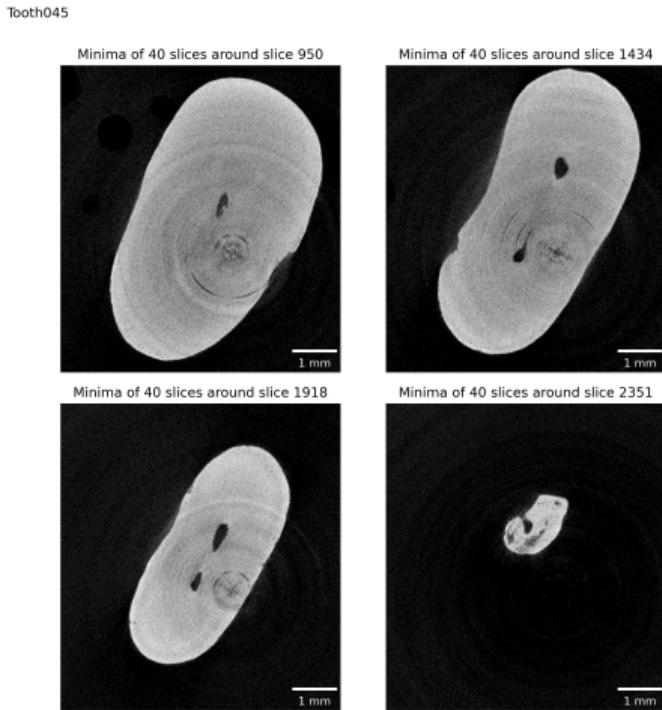
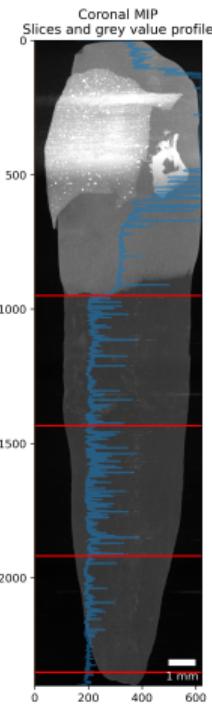
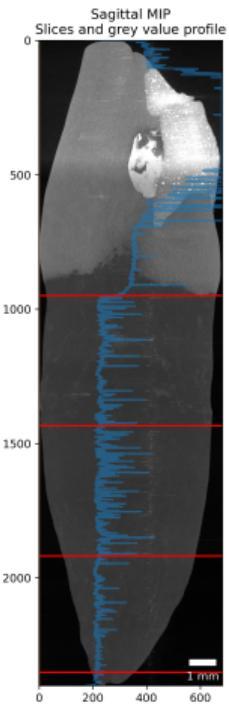
u^b

Tooth morphology



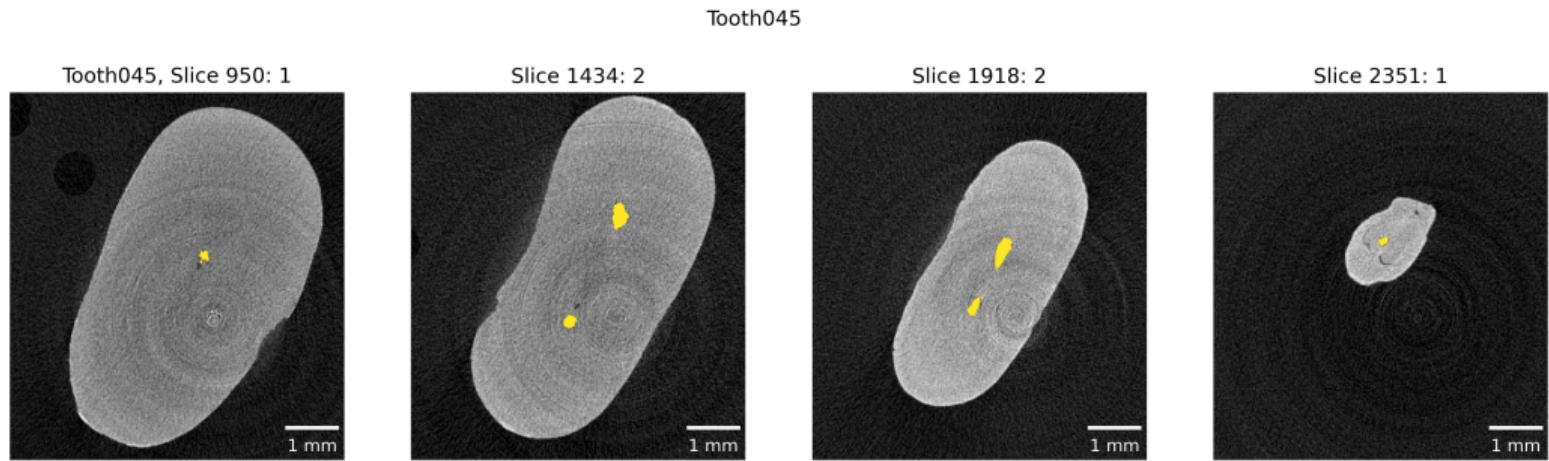
u^b

Detection of enamel-dentin border



u^b

Detection of enamel-dentin border

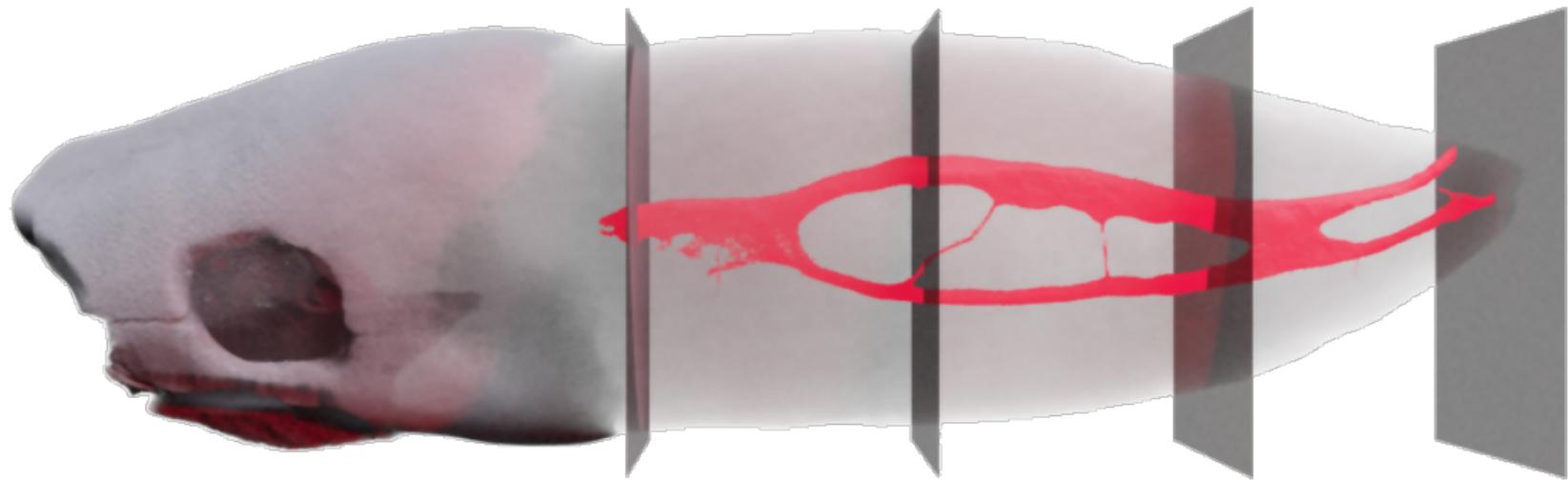


Classification of root canal configurations

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

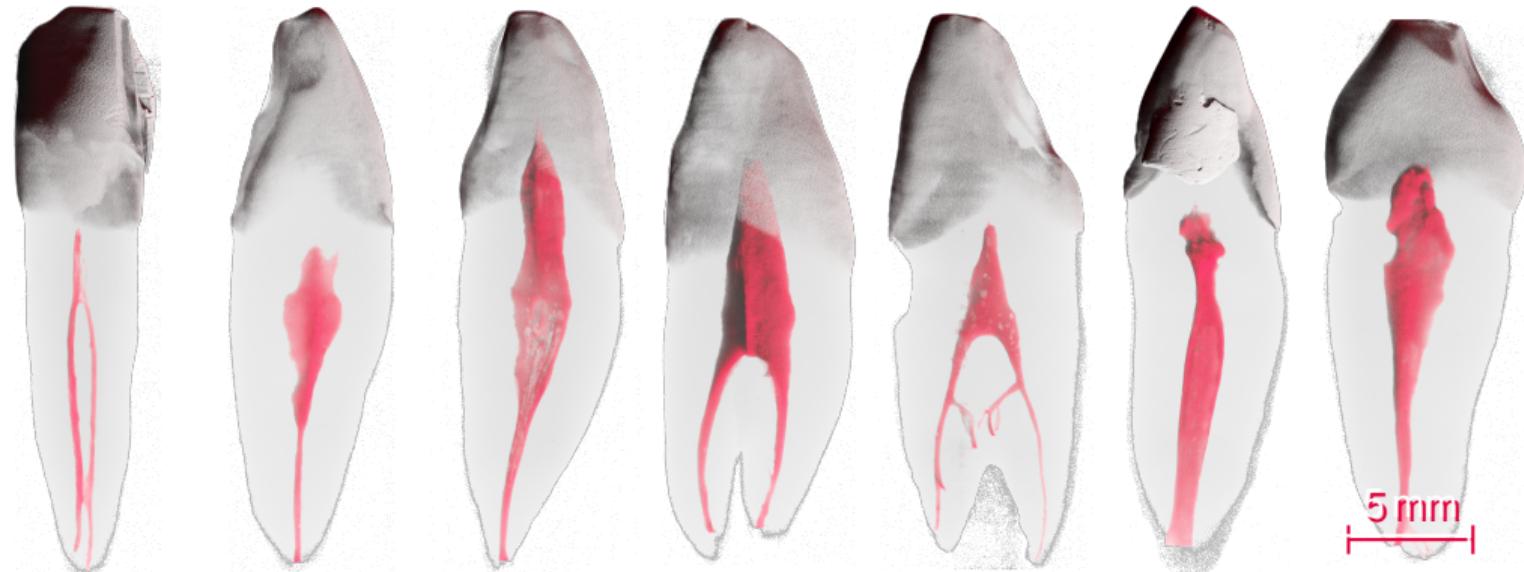
u^b

Extraction of root canal space



u^b

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^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

References I

- [1] Ruslan Hlushchuk et al. "Cutting-Edge Microangio-CT: New Dimensions in Vascular Imaging and Kidney Morphometry". In: (Mar. 2018). DOI: 10.1152/ajprenal.00099.2017.
- [2] Henry Nording et al. "The C5a/C5a Receptor 1 Axis Controls Tissue Neovascularization through CXCL4 Release from Platelets". In: (Dec. 2021). DOI: 10.1038/s41467-021-23499-w.
- [3] Ruslan Hlushchuk et al. "Innovative High-Resolution microCT Imaging of Animal Brain Vasculature". In: (Oct. 2020). DOI: 10.1007/s00429-020-02158-8.
- [4] Tsering Wüthrich et al. "Development of Vascularized Nerve Scaffold Using Perfusion-Decellularization and Recellularization". In: (Aug. 2020). DOI: 10.1016/j.msec.2020.111311.
- [5] Cédric Zubler et al. "The Anatomical Reliability of the Superficial Circumflex Iliac Artery Perforator (SCIP) Flap". In: (Mar. 2021). DOI: 10.1016/j.aanat.2020.151624.
- [6] Matthias Messerli et al. "Adaptation Mechanism of the Adult Zebrafish Respiratory Organ to Endurance Training". In: (Feb. 2020). DOI: 10.1371/journal.pone.0228333.
- [7] Verdiana Trappetti et al. "Synchrotron Microbeam Radiotherapy for the Treatment of Lung Carcinoma: A Pre-Clinical Study". In: (Aug. 2021). DOI: 10.1016/j.ijrobp.2021.07.1717.
- [8] Estée Bochud et al. "A New Diancta Species of the Family Diplommatinidae (Cyclophoroidea) from Vanua Levu Island, Fiji". In: (Nov. 2021). DOI: 10.3897/zookeys.1073.73241.

References II

- [9] Sebastian Halm et al. "Micro-CT Imaging of Thiel-embalmed and Iodine-Stained Human Temporal Bone for 3D Modeling". In: (2021). DOI: 10.1186/s40463-021-00522-0.
- [10] Yogita Kadlag et al. "Physical Properties and Average Atomic Numbers of Chondrules Using Computed Tomography". In: (Nov. 2023). DOI: 10.1016/j.pss.2023.105799.
- [11] David Haberthür et al. "Automated Segmentation and Description of the Internal Morphology of Human Permanent Teeth by Means of Micro-CT". In: (Apr. 2021). DOI: gjpw2d.
- [12] David Haberthür et al. "Microtomographic Investigation of a Large Corpus of Cichlids". In: (Sept. 2023). DOI: gsst8t.
- [13] Kenneth Clark et al. "The Cancer Imaging Archive (TCIA): Maintaining and Operating a Public Information Repository". In: (Dec. 2013). DOI: 10.1007/s10278-013-9622-7.
- [14] A. M. Cormack. "Representation of a Function by Its Line Integrals, with Some Radiological Applications". In: (Sept. 1963). DOI: 10.1063/1.1729798.
- [15] Godfrey Newbold Hounsfield. "Historical Notes on Computerized Axial Tomography". In: (1976).
- [16] E C Beckmann. "CT Scanning the Early Days.". In: (Jan. 2006). DOI: 10.1259/bjr/29444122.
- [17] *US3940625A - Apparatus for Examining Objects by Means of Penetrating Radiation - Google Patents.*
<https://patents.google.com/patent/US3940625A/>.

References III

- [18] J Hsieh. *Computed Tomography: Principles, Design, Artifacts, and Recent Advances*. Society of Photo Optical, 2003.
- [19] Angela S.P. Lin et al. "Microcomputed Tomography". In: *Springer Handbook of Microscopy*. Springer Handbooks. Cham: Springer International Publishing, 2019. DOI: 10.1007/978-3-030-00069-1_24.
- [20] L. A. Feldkamp et al. "Practical Cone-Beam Algorithm". In: (June 1984). DOI: 10.1364/JOSAA.1.000612.
- [21] LA Feldkamp et al. "Investigation of 3-Dimensional Structure of Trabecular Bone by Computed-Tomography of Iliac Biopsy Samples". In: *Calcified Tissue International*. SPRINGER VERLAG 175 FIFTH AVE, NEW YORK, NY 10010, 1983.
- [22] Mark Hammer. *X-Ray Physics: X-Ray Interaction with Matter, X-Ray Contrast, and Dose*. <http://xrayphysics.com/attenuation.html>.
- [23] Wikipedia contributors. *Beer–Lambert Law — Wikipedia, The Free Encyclopedia*. 2019.
- [24] Johannes Schindelin et al. "Fiji: An Open-Source Platform for Biological-Image Analysis". In: (July 2012). DOI: 10.1038/nmeth.2019.
- [25] Thomas Kluyver et al. "Jupyter Notebooks – a Publishing Format for Reproducible Computational Workflows". In: (2016). DOI: 10.3233/978-1-61499-649-1-87.
- [26] Thomas Gerhard Wolf et al. "Internal Morphology of 101 Mandibular Canines of a Swiss-German Population by Means of Micro-CT: An Ex Vivo Study". In: (2021). DOI: g7r8.

References IV

- [27] Benjamín Briseño-Marroquín et al. "Root Canal Morphology and Configuration of 179 Maxillary First Molars by Means of Micro-Computed Tomography: An Ex Vivo Study". In: (Dec. 2015). DOI: 10.1016/j.joen.2015.09.007.
- [28] David Haberthür. "Habi/Zmk-Tooth-Cohort: Used for Manuscript about Method". In: (Aug. 2020). DOI: 10.5281/ZENODO.3999402.
- [29] Thomas Gerhard Wolf et al. "Three-Dimensional Analysis of the Physiological Foramen Geometry of Maxillary and Mandibular Molars by Means of Micro-CT". In: (Sept. 2017). DOI: 10.1038/ijos.2017.29.