b

UNIVERSITY
OF BERN

X-ray microtomography

9256-HS2024-0: Advanced Microscopy

David Haberthür
Institute of Anatomy, December 20, 2024

Grüessech mitenang!

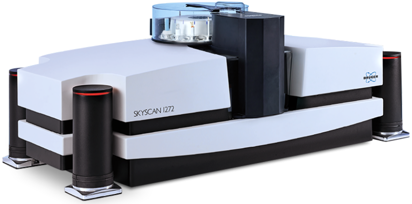
- David Haberthür
  - Physicist by trade
  - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
  - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
  - Post-Doc II: µCT group, Institute of Anatomy, University of Bern, Switzerland

Grüessech from the µCT group

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

µCT-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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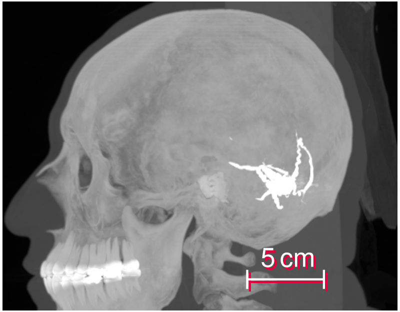
Contents

micro-Computed tomography

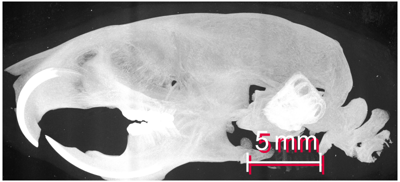
- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a very large range of sample sizes



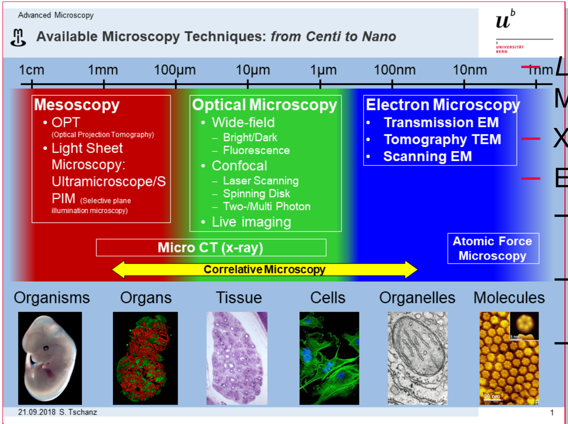
From [13], Subject *C3L-02465*

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



Advanced Microscopy Lecture 2018, Stefan Tschanz, Slide copied with permission

- *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

CT-Scanner

youtu.be/2CWpZKuy-NE

CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays

w.wiki/BHAN

CT History

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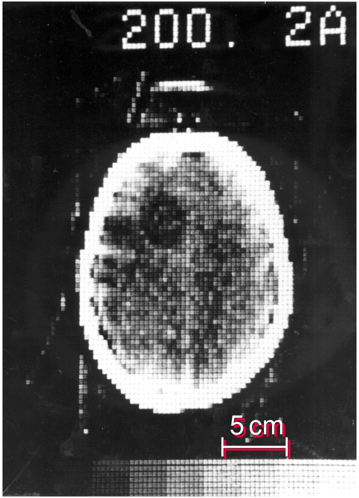
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [14]
- 1976: Hounsfield worked on first clinical scanner [15]



From [16], Figure 5

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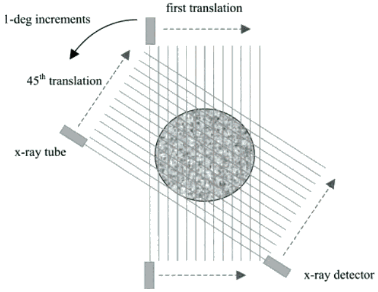
- CT scanner generations
  - First generation



From [17], Figure 1.12

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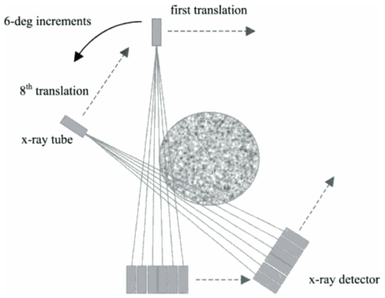

From [17], Figure 1.13

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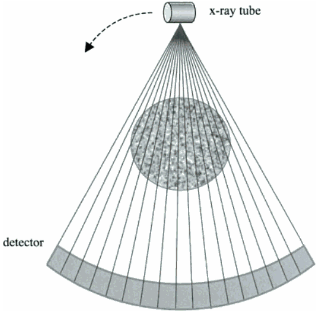
  - Second generation
  - Third generation



From [17], Figure 1.14

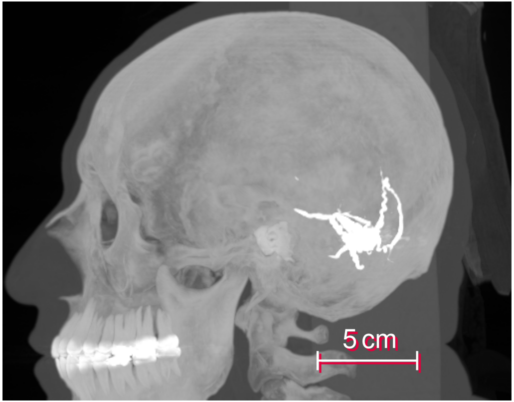
µCT History I

- 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 stagers, 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]
- µCT was first reported in the 1980s, for scanning gemstones to cut out the largest possible one
- Today: Nondestructive imaging for quantifying the (micros)tructure of organic materials

µCT History II

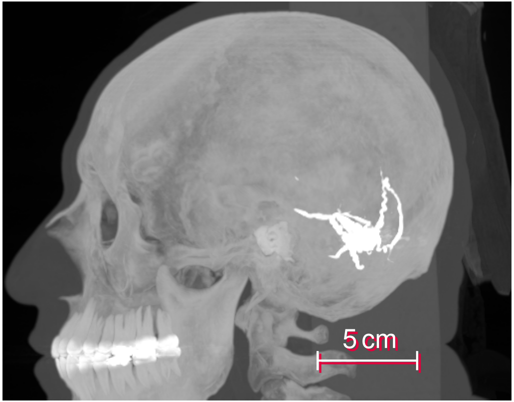
  - Mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
  - Teeth and their internal details
  - Soft tissues and vasculature using radio-opaque contrast agents
  - Characterization of anatomical details in high resolution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

Why µCT?



From [13], Subject *C3L-02465*

Why µCT?



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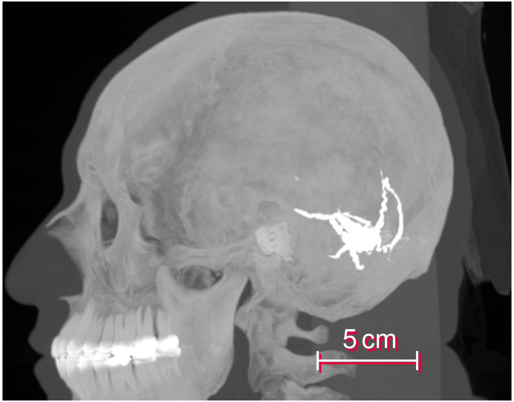

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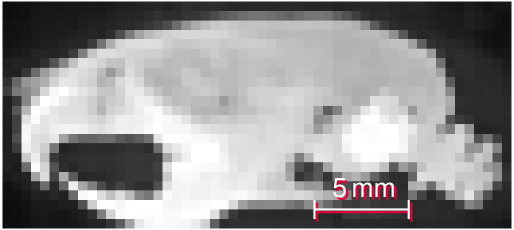

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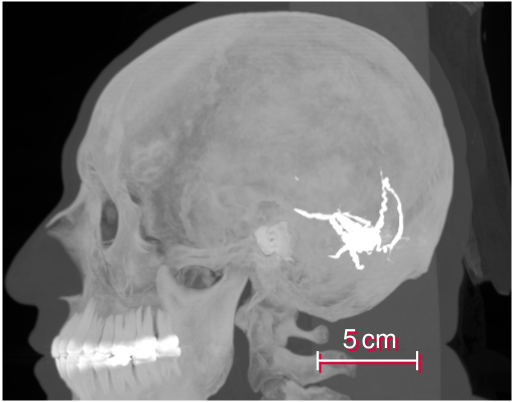

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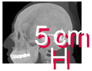

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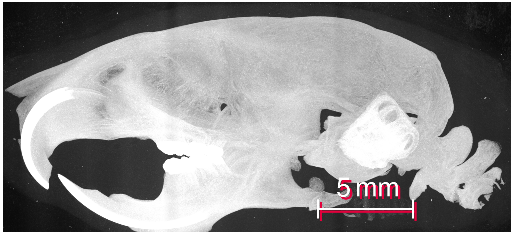
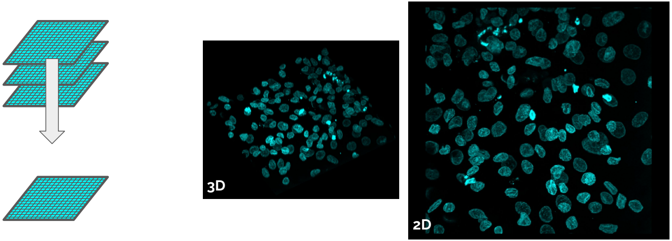


Maximum intensity projection

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.



Fundamentals of Digital Image Processing, Guillaume Witz, Slide 23

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

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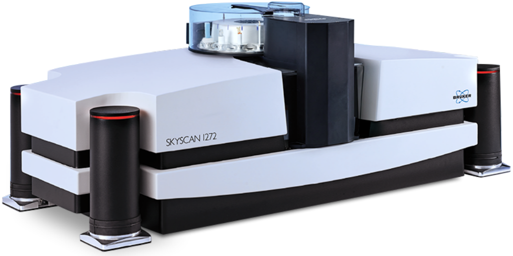

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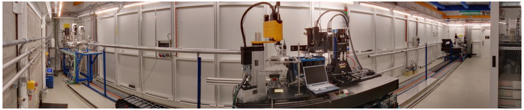

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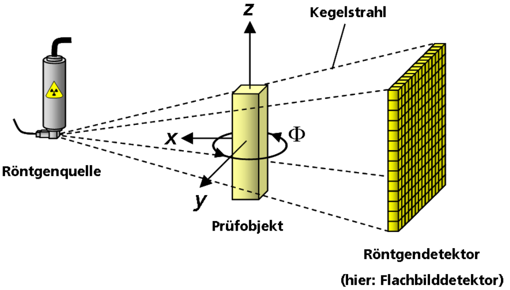
What is happening?

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

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



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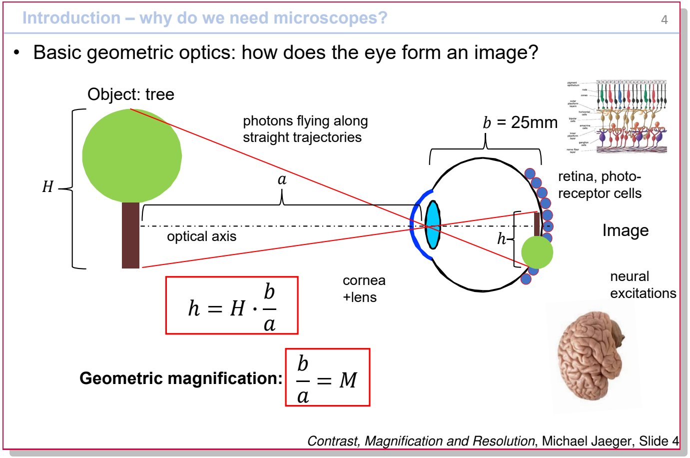
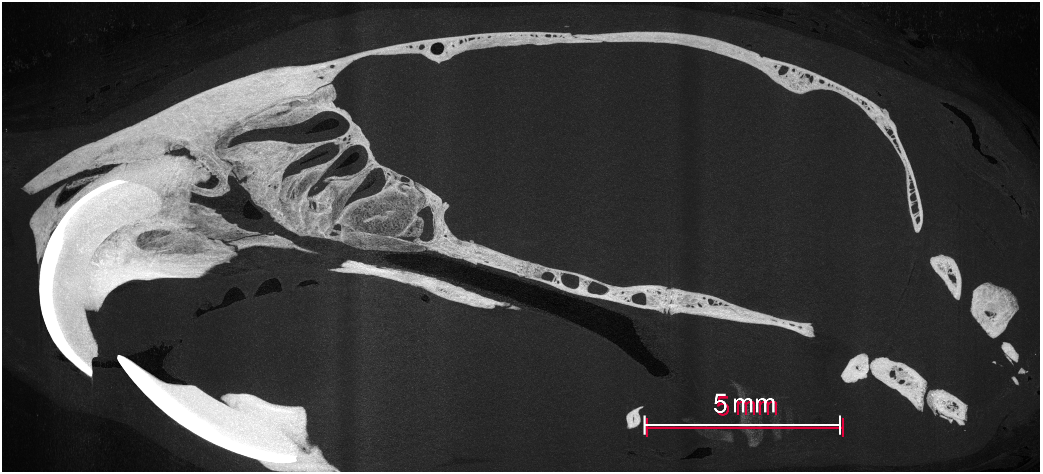
Magnification



Examples



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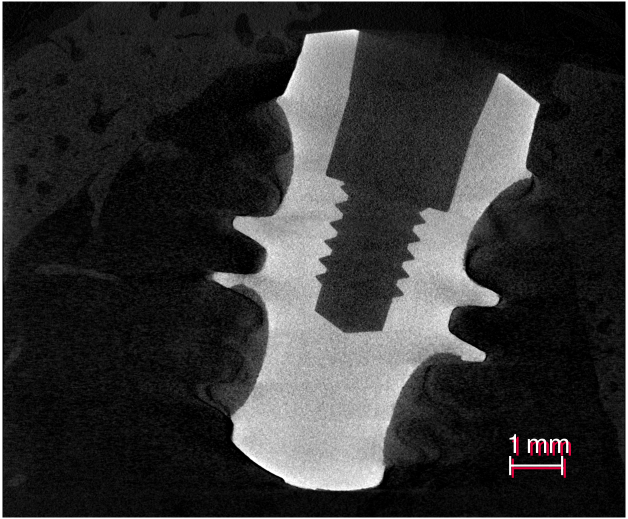

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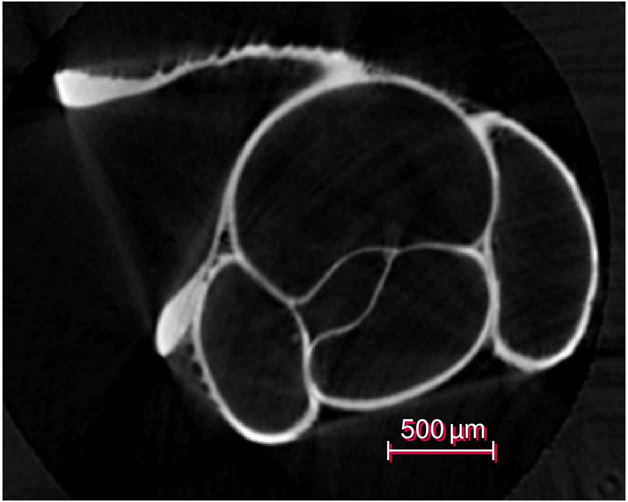
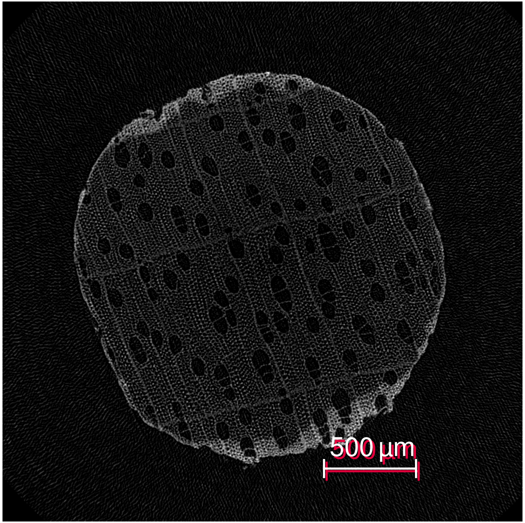


From [8], *Diancta phoenīx*

Examples



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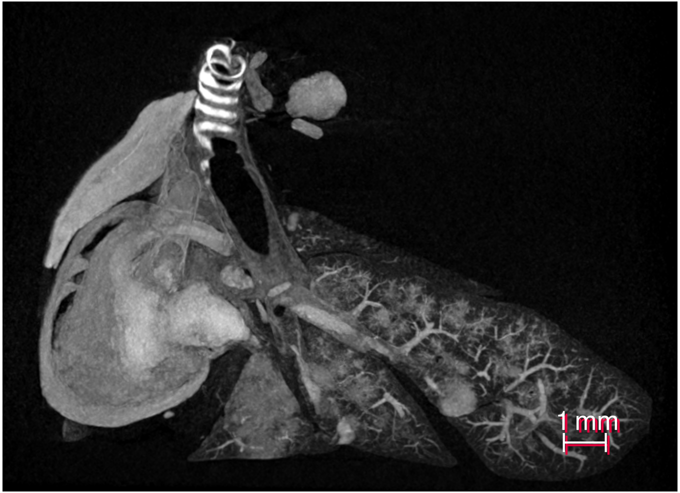


X-ray interaction

- "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

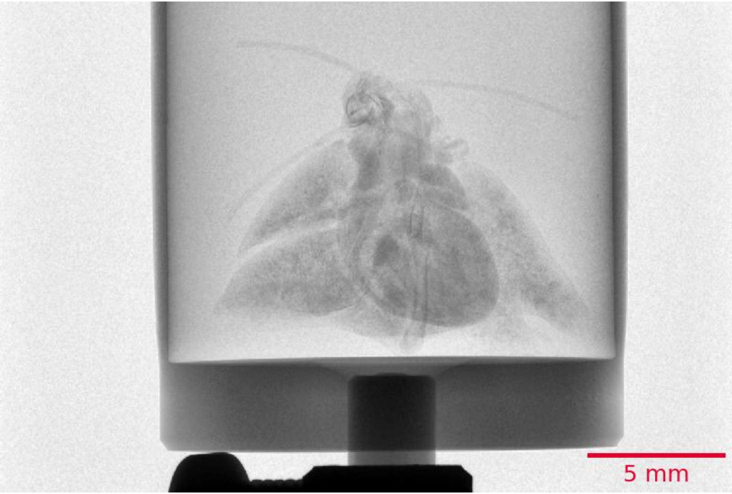
Tissue: content by mass percentage

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

Preparation

- Study design
- Sample preparation

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

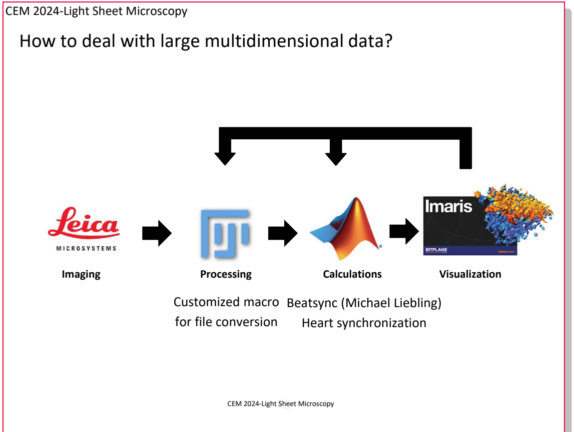
- Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.
- Radon Transformation
- Filtered back projection
- Cone beam reconstruction [19]
- Corrections (beam hardening, etc.)
- Writing to stack

Visualization

Visualization

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

Imaging



Light Sheet Microscopy, Nadia Mercader, Slide 43

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

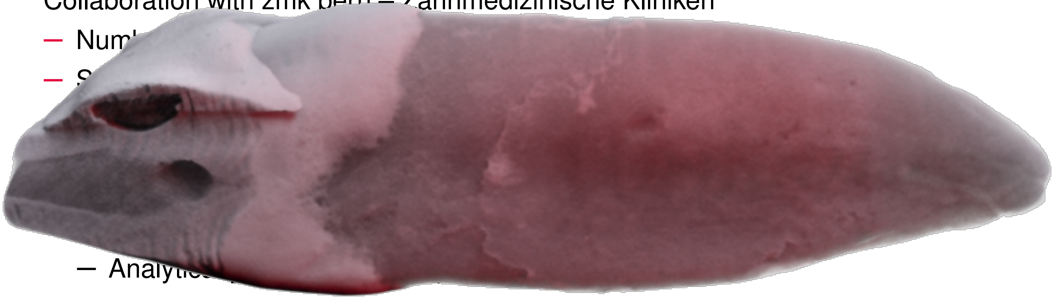
- Pretty images are nice, but we need quantitative numbers
- Segmentation
- Characterization

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

– Num

– S



– Analytic

How?

- 104 extracted human permanent mandibular canines
- µCT 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!

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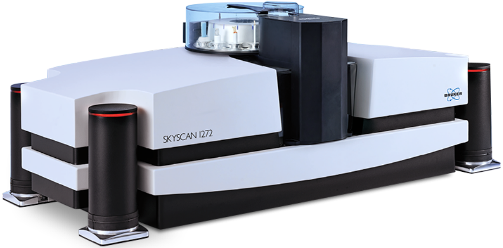

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```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files=  482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)=  80
Source Current (uA)= 125
Image Pixel Size (um)=9.999986
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* µCT 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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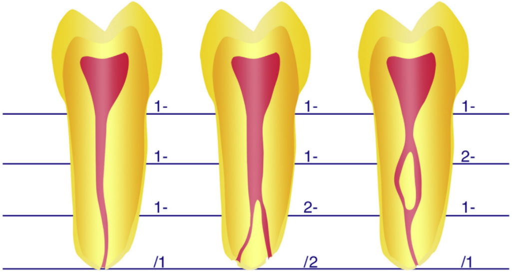

From [24], Fig. 2

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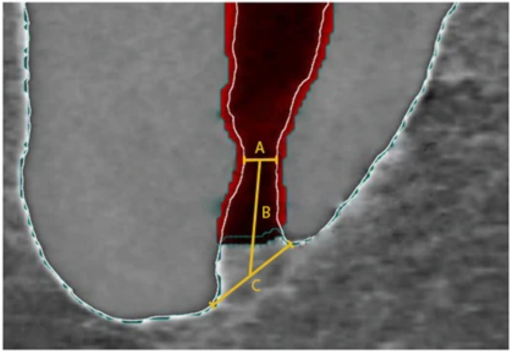

From [26], Fig. 1

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gph.is/2nqkple

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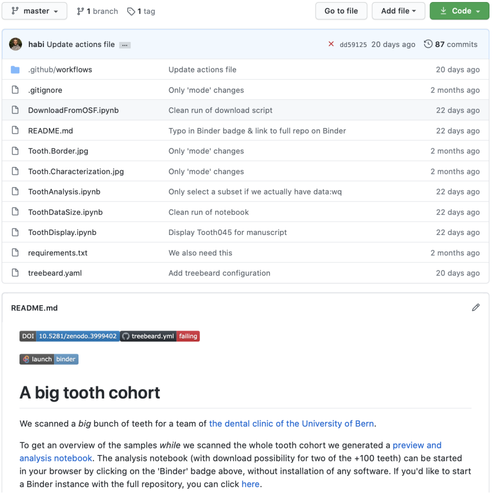
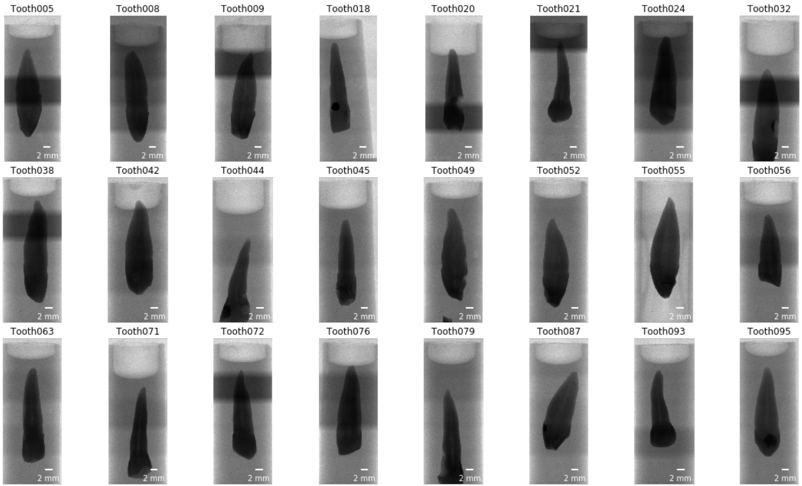


µCT imaging


Tooth005
Tooth008
Tooth009
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Tooth032
Tooth038
Tooth042
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Tooth045
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Tooth052
Tooth055
Tooth056
Tooth063
Tooth071
Tooth072
Tooth076
Tooth079
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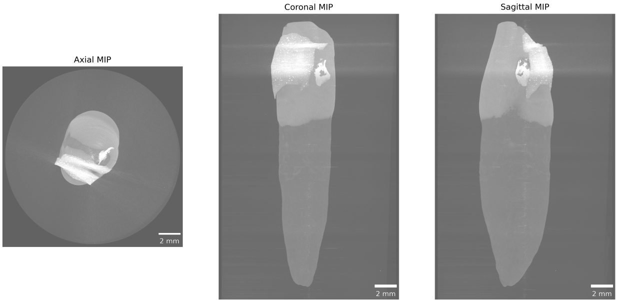
Tooth093
Tooth095
2 mm

Dataset cropping

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



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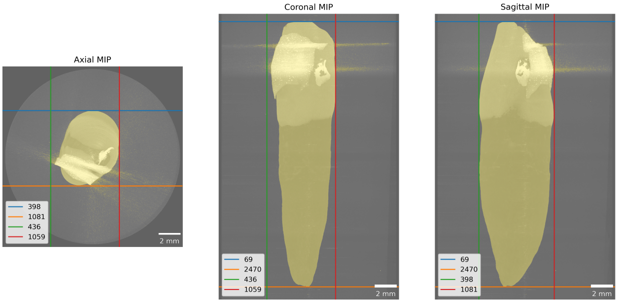
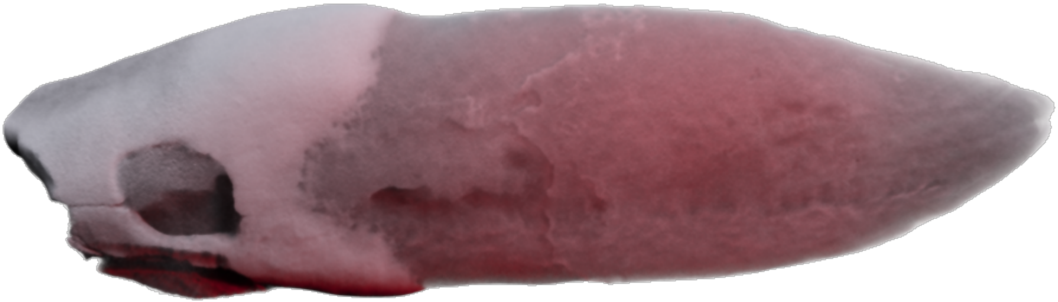


Tooth morphology



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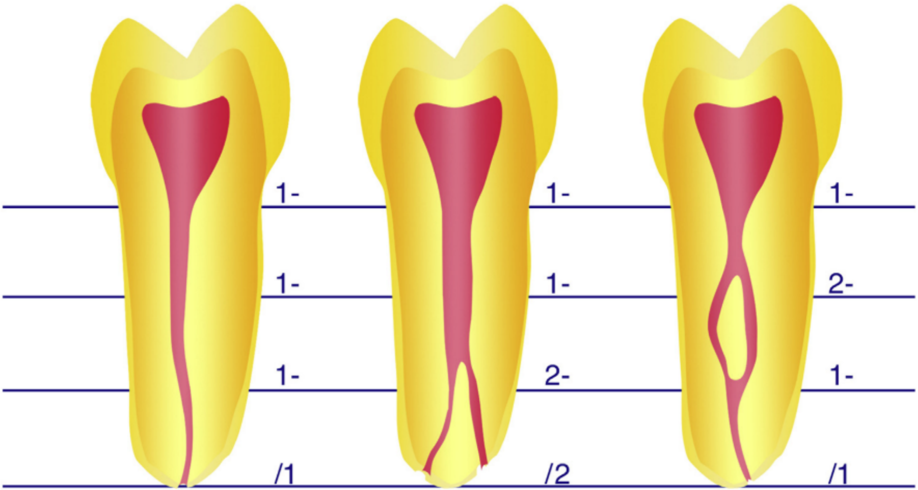

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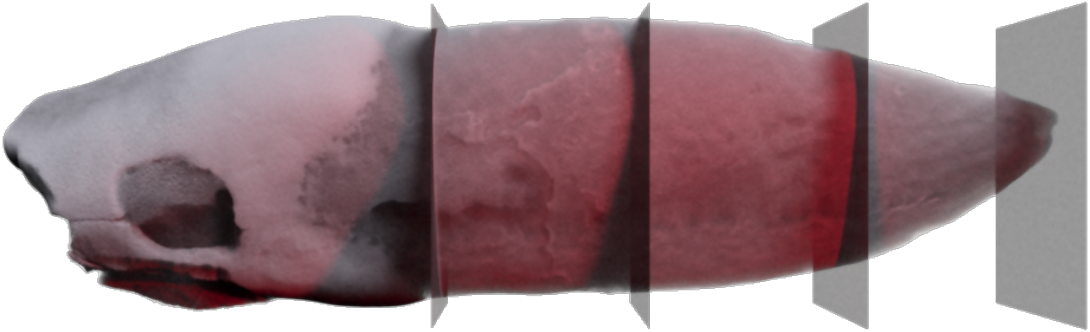
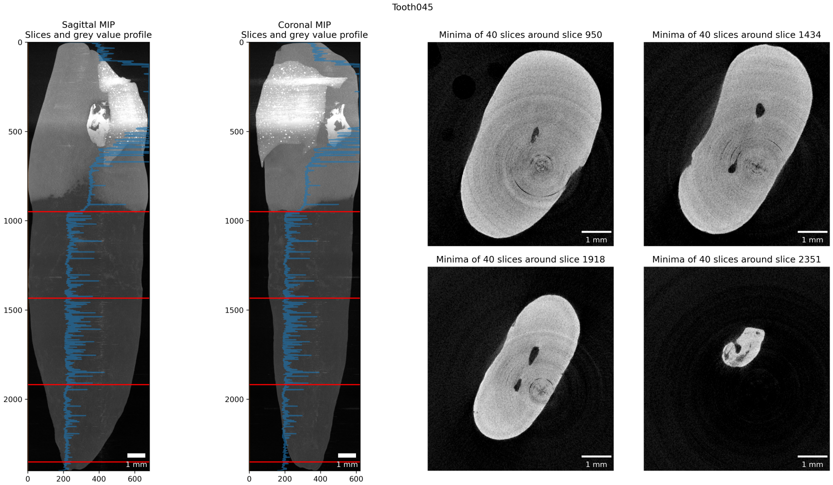


Detection of enamel-dentin border



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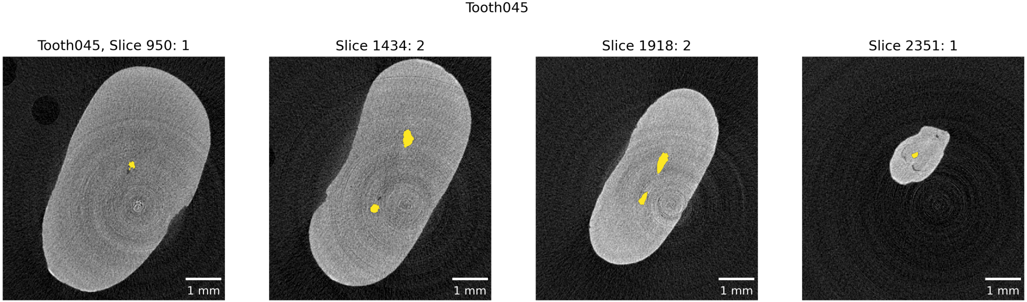
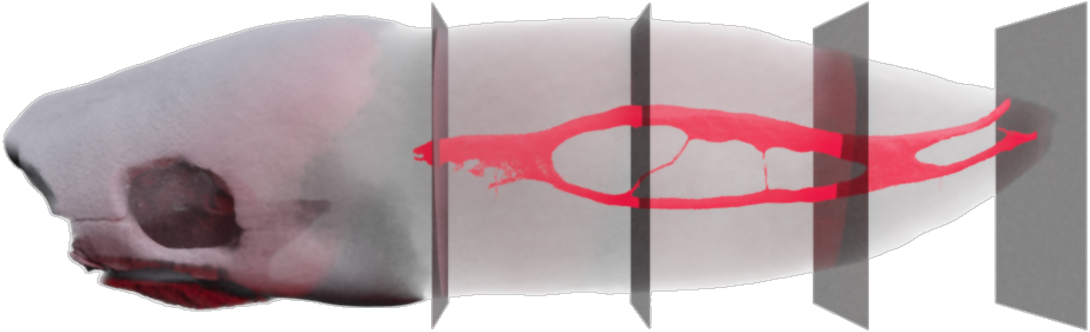


Outcome root canal configuration classification

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	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



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 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