b
UNIVERSITY
OF BERN

X-ray microtomography
9256-HS2024-0: Advanced Microscopy

David Haberthür
Institute of Anatomy, December 20, 2024

Grüessech mitenang!

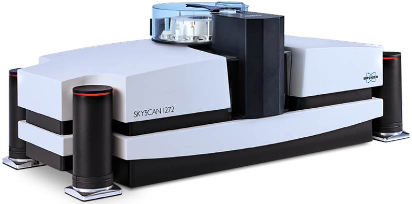
- David Haberthür
  - Physicist by trade
  - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
  - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
  - Post-Doc II: µCT group, Institute of Anatomy, University of Bern, Switzerland

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]



bruker.com/skyscan1272

Contents

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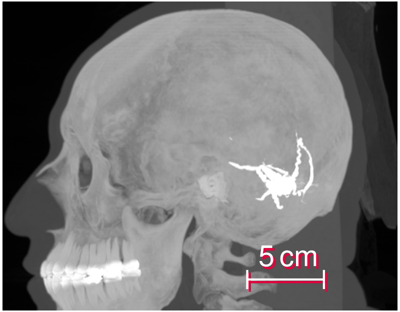
- Interaction of x-rays with matter

A scan, from *getting started* to *nice image*

Example: A study about teeth
- Overview
- Materials & Methods
- Results

micro-Computed tomography

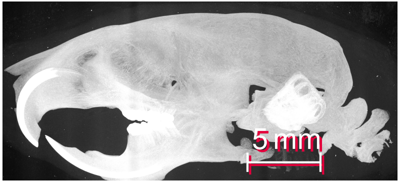
- Allows for imaging dense and non-transparent samples
- Non-destructive imaging
- Results in three-dimensional images
- Covers a very large range of sample sizes



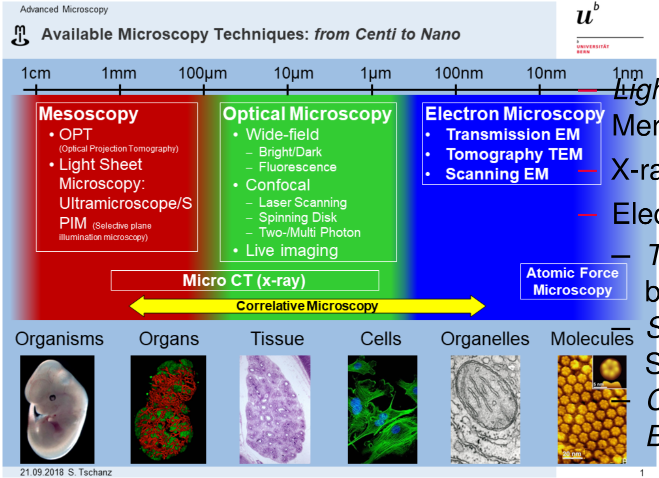
From **Clark2013**, Subject *C3L-02465*

micro-Computed tomography

- Allows for imaging dense and non-transparent samples
- Non-destructive imaging, thus compatible with routine sample preparation
- Results in three-dimensional images with µm resolution
- Covers a very large range of sample sizes
- (Small) biological samples
- Enables correlative imaging pipelines, scanning of precious biological samples, as well as museum & collection material



Imaging methods



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

Stefan Tschanz, with permission

CT-Scanner

youtu.be/2CWpZKuy-NE

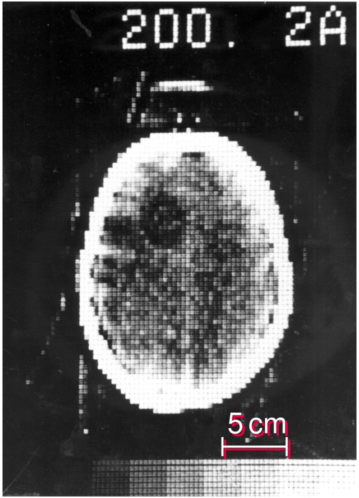
CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays

w.wiki/BHAN

CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays
- **Cormack1963**: **Cormack1963** used a collimated ^{60}Co source and a Geiger counter as a detector **Cormack1963**
- **Hounsfield1976a**: **Hounsfield1976a** worked on first clinical scanner **Hounsfield1976a**



From **Beckmann2006**, Figure 5

CT History

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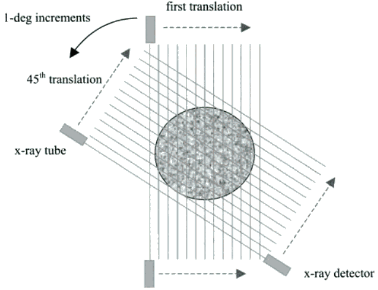
- CT scanner generations
  - First generation



From **Hsieh2003**, Figure 1.12

CT History

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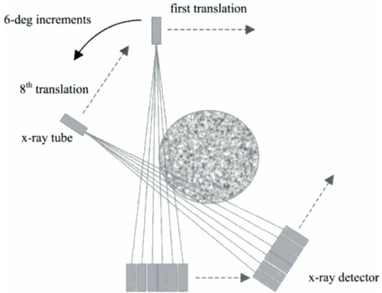
- CT scanner generations
  - First generation
  - Second generation



From **Hsieh2003**, Figure 1.13

CT History

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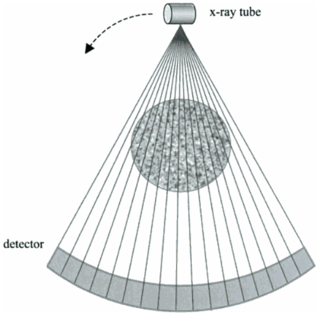
  - First generation
  - Second generation
  - Third generation



From **Hsieh2003**, Figure 1.14

µCT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s **Lin2019**
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp **Feldkamp1984** 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 **Feldkamp1983**

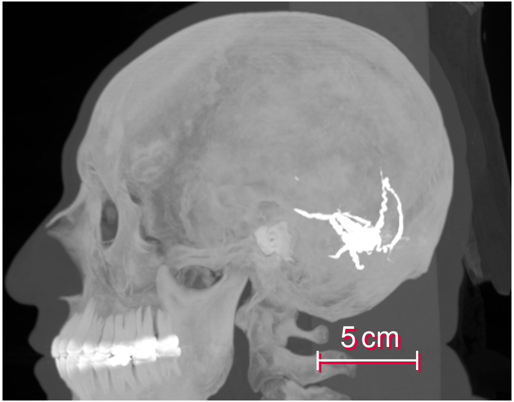
µCT History II

- 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
  - 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

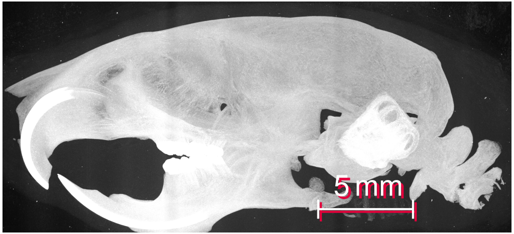
µCT History

- 1984: Lee Feldkamp **Feldkamp1984** developed an early laboratory microCT system at Ford Motor Company's Scientific Research Laboratory (to nondestructively detect damage in ceramic automobile parts). Then collaborated with scientists at Henry Ford Hospital and University of Michigan interested in understanding bone microstructure **Feldkamp1989** and osteoarthritis **Layton1988**.
- 1987: The first commercially available µCT scanner was developed by SkyScan (now Bruker)
- Today: µCT is widely used for non-destructive imaging in
  - Material Science: internal structure of composites, foams, and other materials. Understand the relationship between structure and properties.
  - Biomedical Research: microarchitecture of bone tissue, dental research, and small animal imaging. Since the 1990s, µCT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
  - Paleontology and Archaeology: study of fossils and archaeological artifacts
- $\approx$2500 µCT systems are in use worldwide with over 1000 publications annually

Why µCT?



From **Clark2013**, Subject *C3L-02465*



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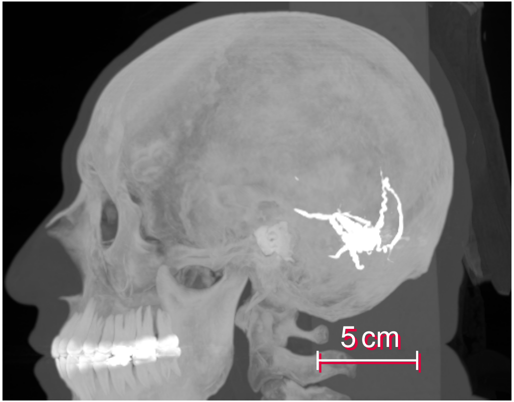

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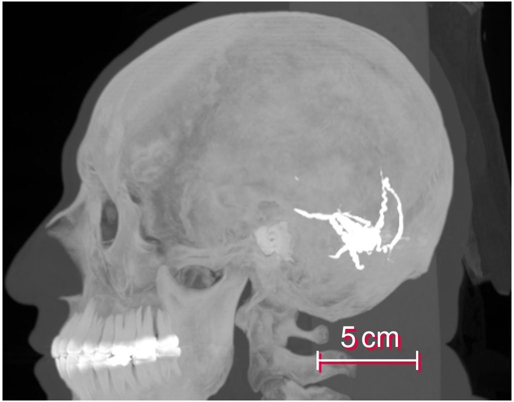

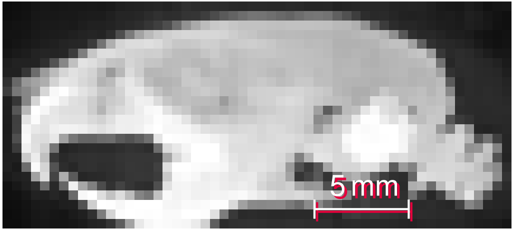

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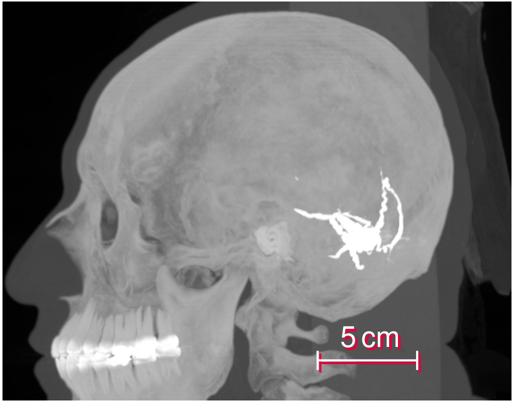

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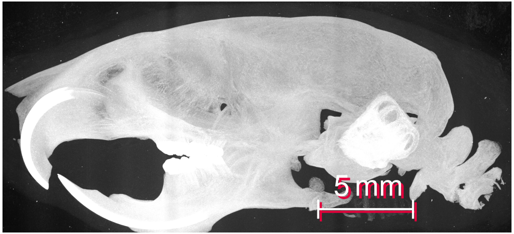

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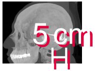

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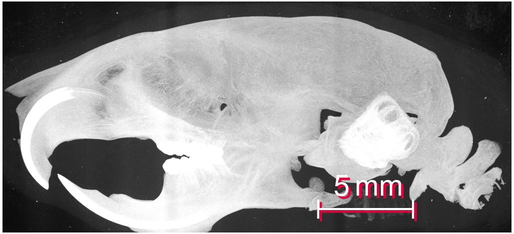
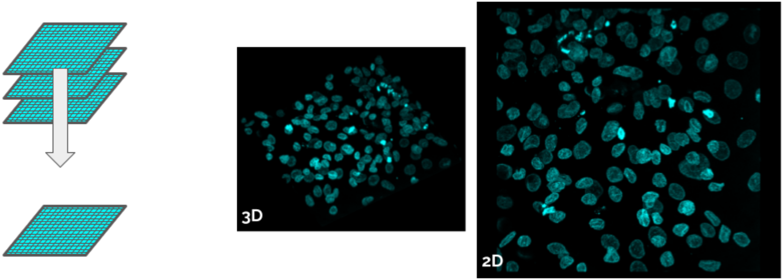


Maximum intensity projection

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 (2023) by Guillaume Witz, Slide 24

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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flic.kr/p/fpTrGu

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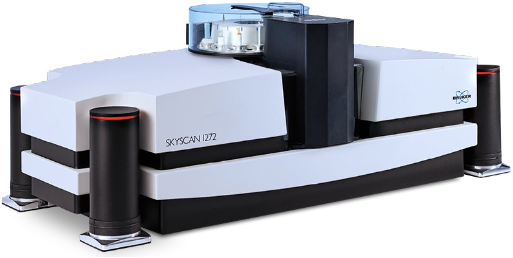

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Machinery

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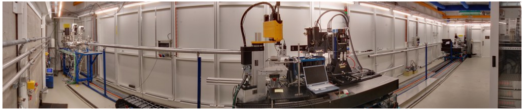

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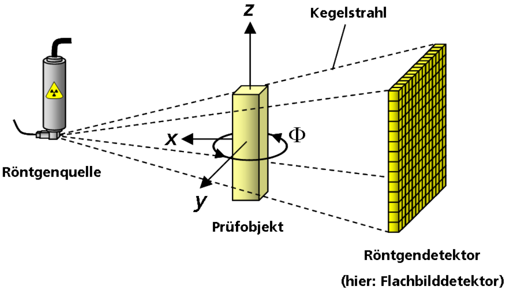
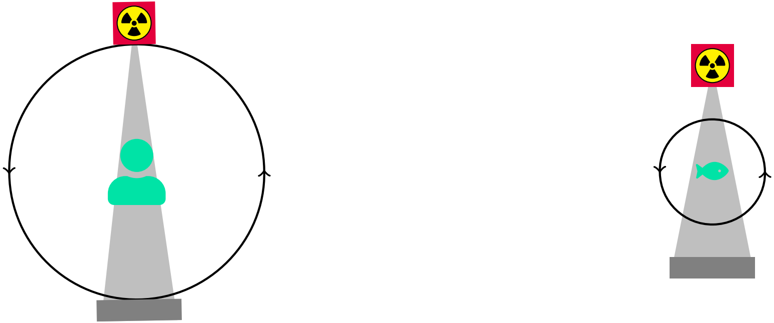
What is happening?

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

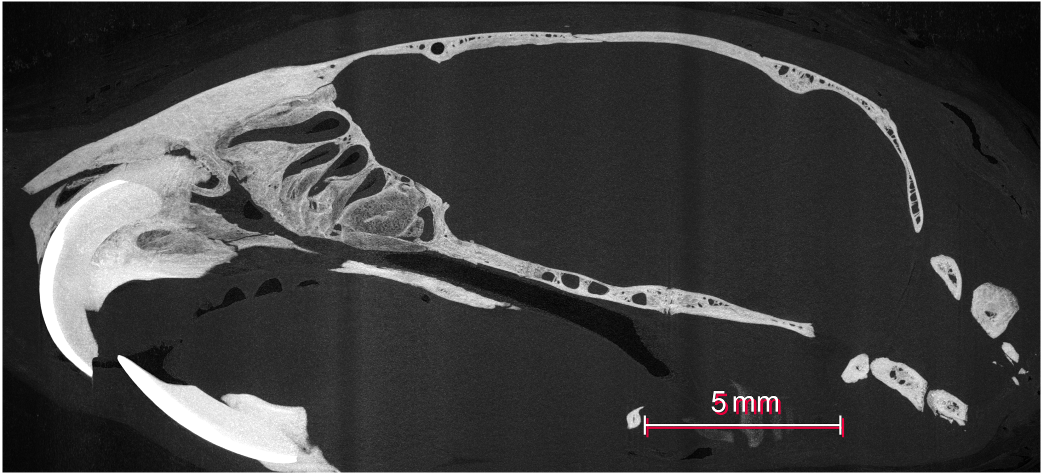
- an x-ray source
- a sample
- a detector



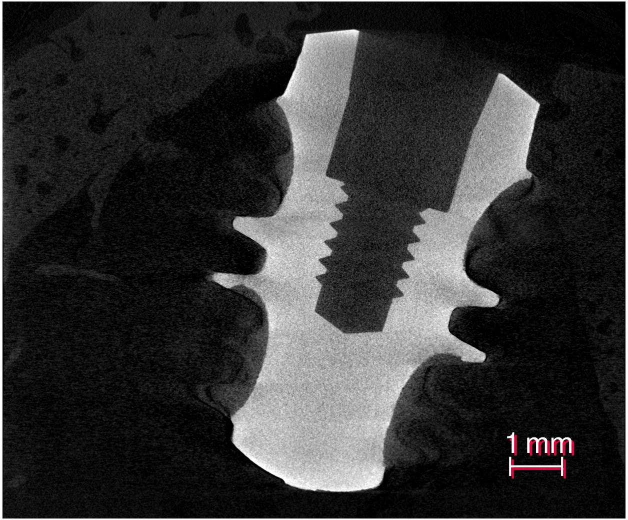
Machinery



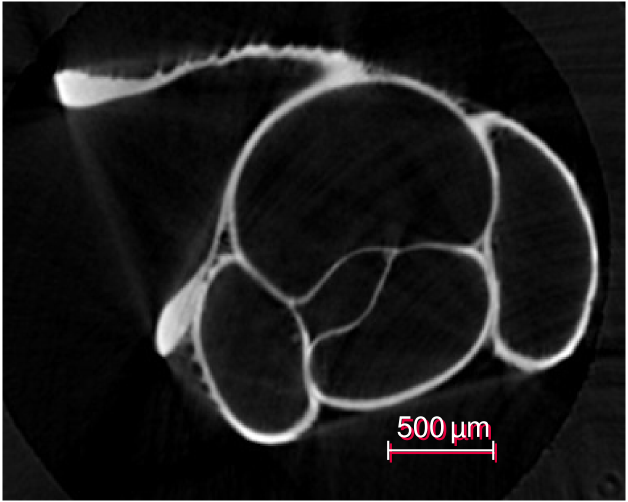
Examples



Examples

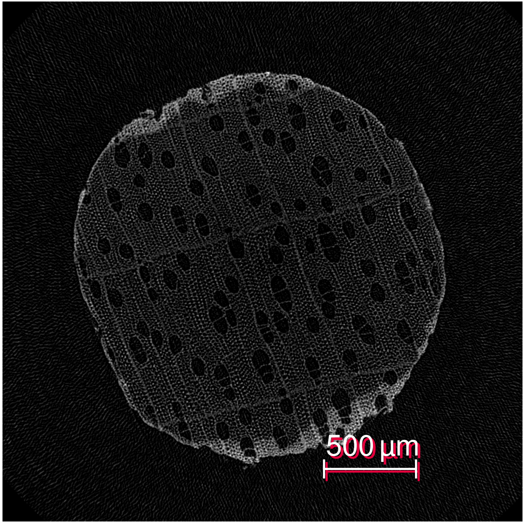


Examples

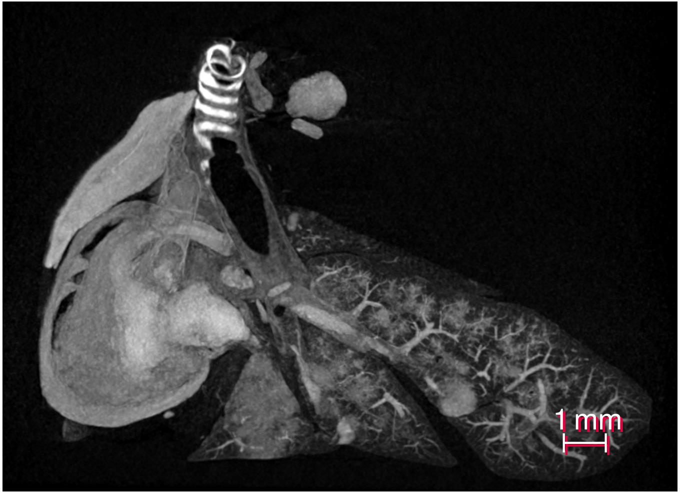


From [8], *Diancta phoenīx*

Examples



Examples



X-ray interaction

- "X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose." (**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

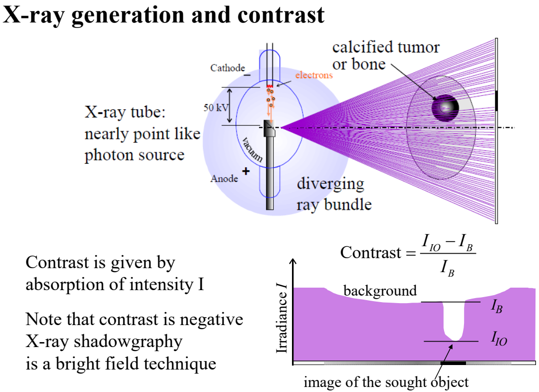
Tissue: content by mass percentage

Element	H	C	N	O	Na	P	S	Cl	K	Ca
Atomic number	1	6	7	8	11	15	16	17	19	20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3			22.5

Preparation

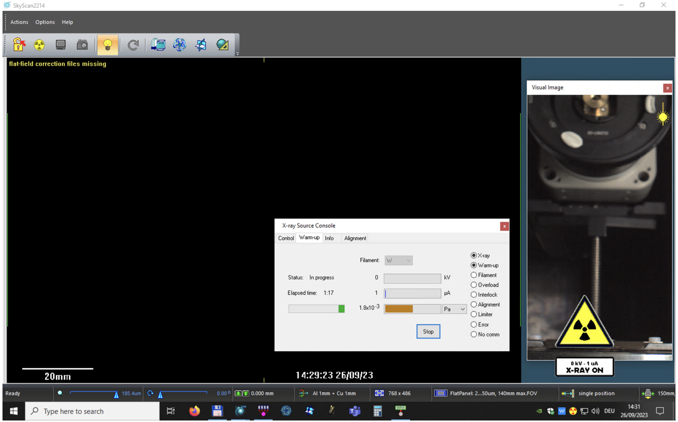
- Study design
- Sample preparation

Projections



Contrast, Magnification and Resolution—Laws of Physics for Microscopists (1, 2023) by Martin Frenz, Slide 21

Projection acquisition



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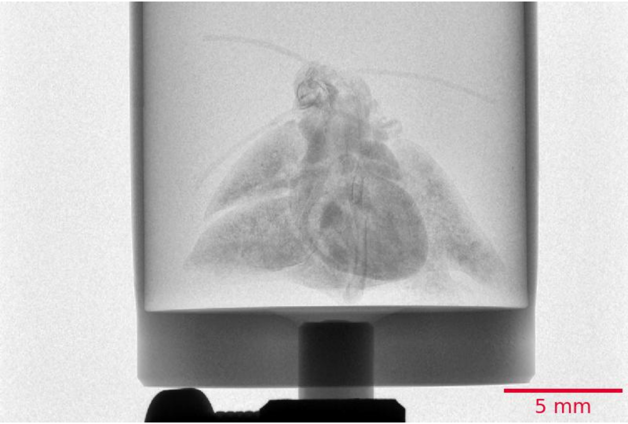
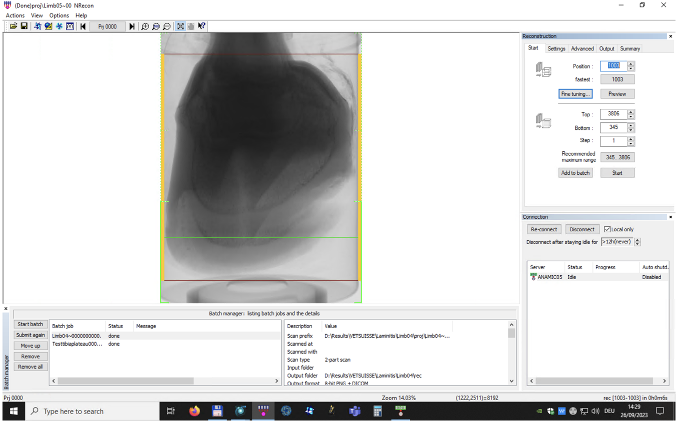


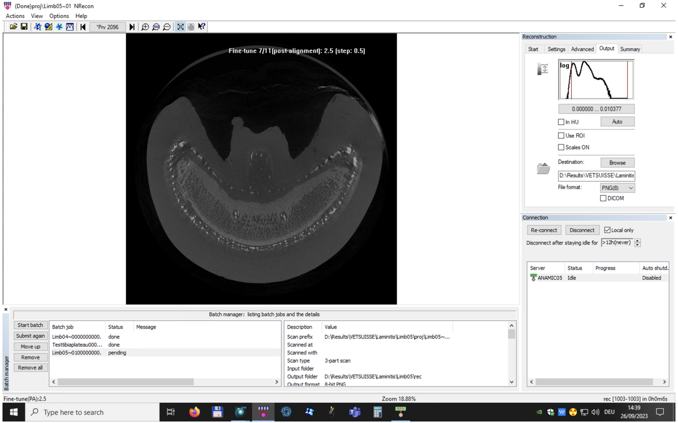
Projections

– A (micro-focus) x-ray source illuminates the object
– The x-rays penetrate the sample and are attenuated
– A scintillator converts the x-rays to visible light
– A (planar) x-ray detector collects (magnified) projection images.
– The projections are recorded on disk

Reconstructions



Reconstructions



Reconstructions

Reconstructions

- Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.
- Radon Transformation
- Filtered back projection
- Cone beam reconstruction **Feldkamp1984**
- Corrections (beam hardening, etc.)
- Writing to stack

Visualization

Visualization

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

What to use?

- ImageJ/Fiji **Schindelin2012**
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  - in Jupyter **Kluyver2016**
  - git
  - Script all your things!
  - Data repositories; i. e. sharing is caring!

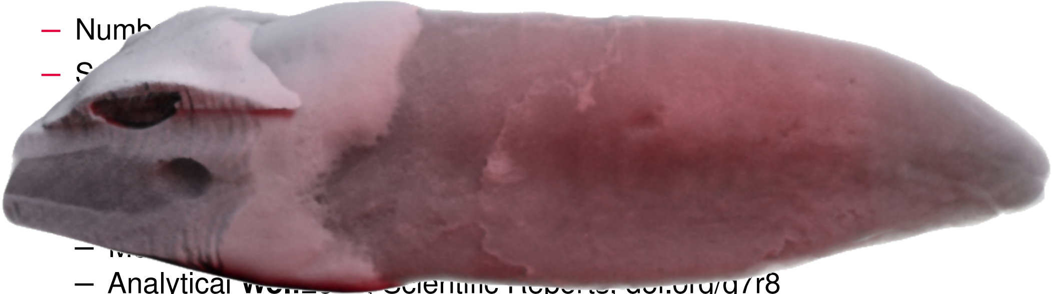
Quantitative data

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

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numb
- S



  - M
  - Analytical ..., Scientific Reports, doi.org/g7r8

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Root canal configuration, according to **Briseno-Marroquin2015 Briseno-M**
- *Reproducible* analysis **Haberthuer2020a**, e. g. you can click a button to double-check or recalculate the results yourself!

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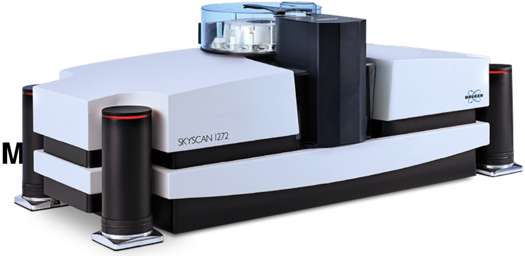

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```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files=  482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)=  80
Source Current (uA)= 125
Image Pixel Size (um)=9.999986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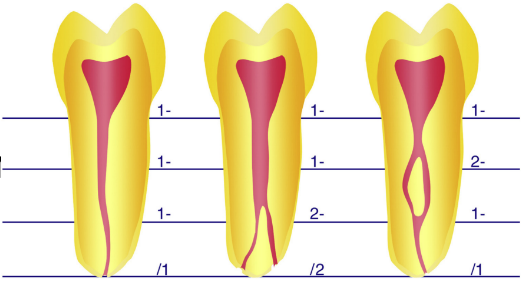

From **Briseno-Marroquin2015**, Fig. 2

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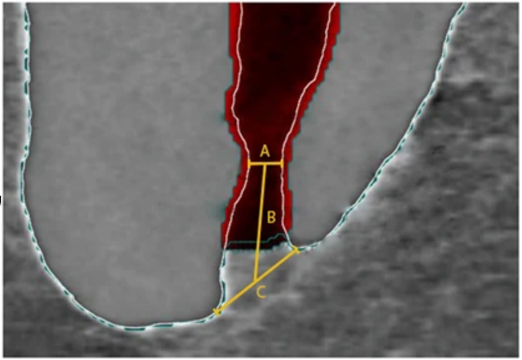

From **Wolf2017**, Fig. 1

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gph.is/2nqkple

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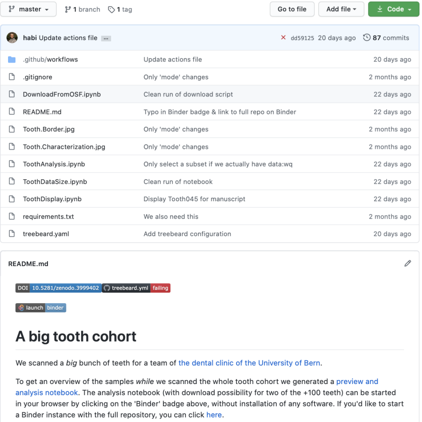
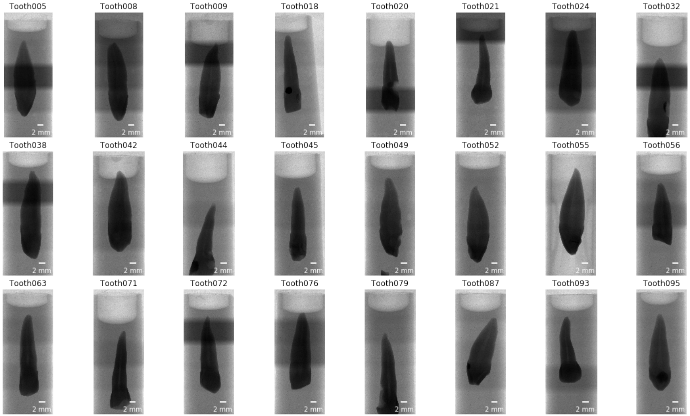
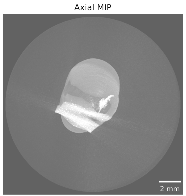


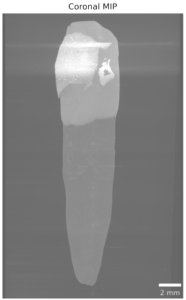
µCT imaging

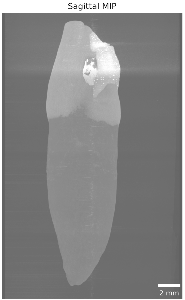

Tooth005
Tooth008
Tooth009
Tooth018
Tooth020
Tooth021
Tooth024
Tooth032
Tooth038
Tooth042
Tooth044
Tooth045
Tooth049
Tooth052
Tooth055
Tooth056
Tooth063
Tooth071
Tooth072
Tooth076
Tooth079
Tooth087
Tooth093
Tooth095
2 mm

Dataset cropping

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







Dataset cropping

- Full datasets: 326 GB
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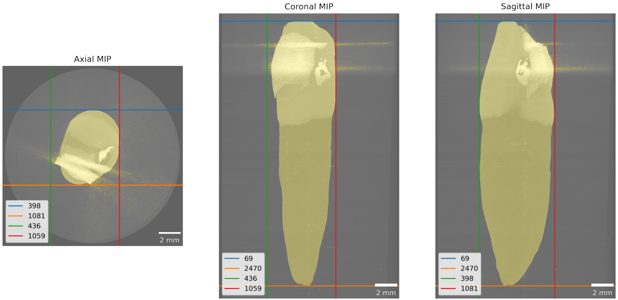
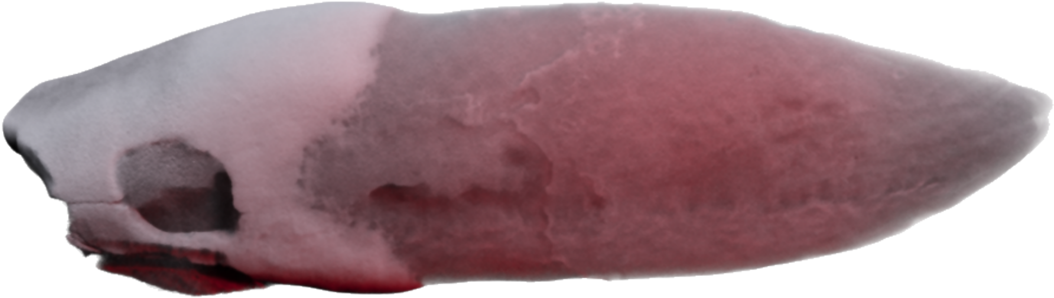
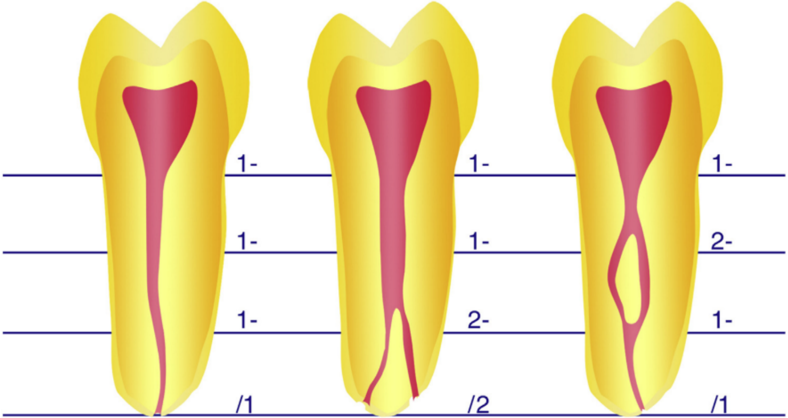


Tooth morphology

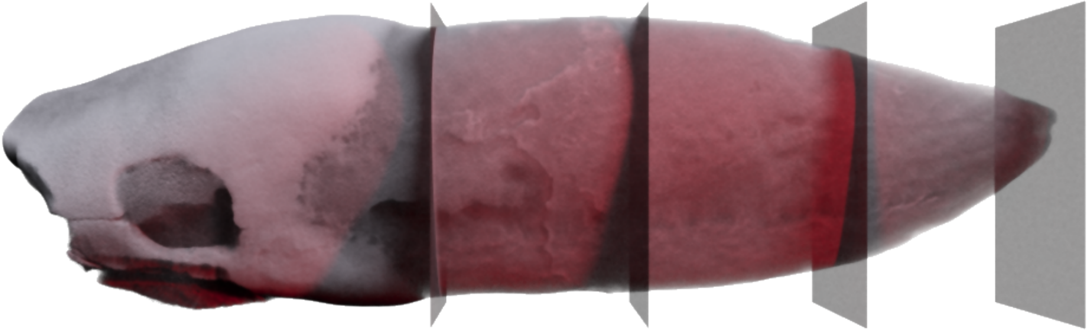


Tooth morphology

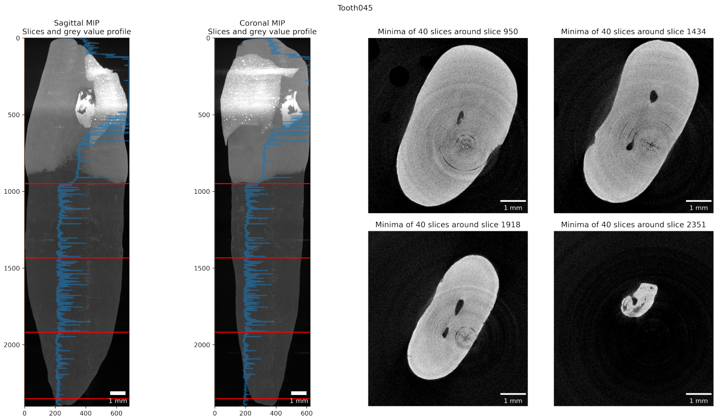


From **Briseno-Marroquin2015**, Fig. 2

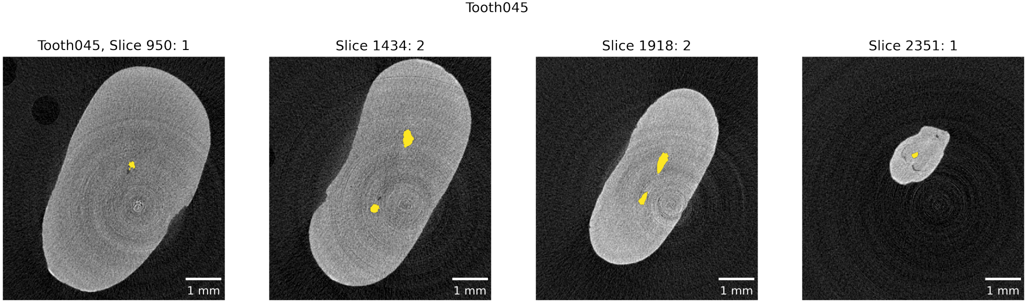
Tooth morphology



Detection of enamel-dentin border



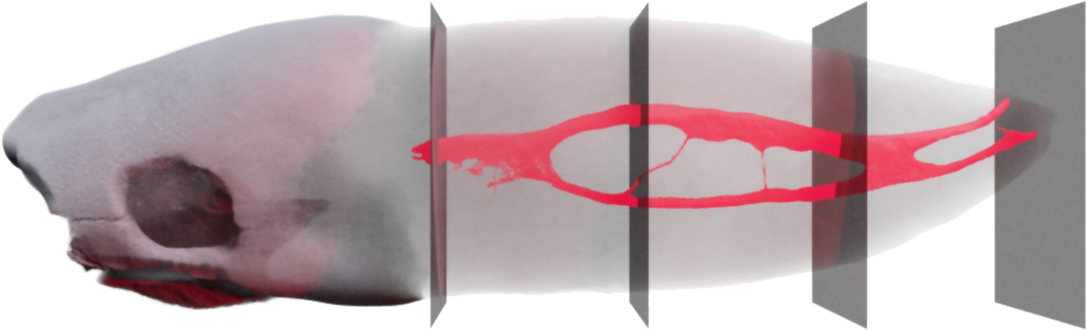
Detection of enamel-dentin border



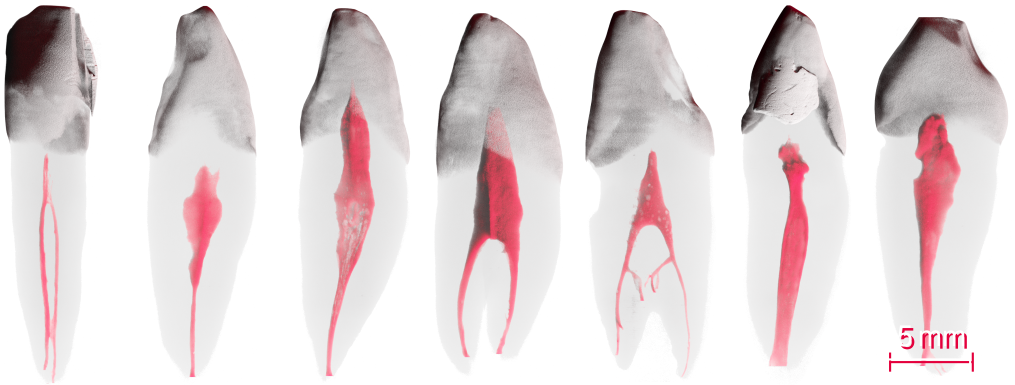
Outcome root canal configuration classification

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

Extraction of root canal space



Results of root canal space extraction



Conclusion ZMK

- Efficient use of time, e. g. more teeth does not mean more (human) work
- Reproducible analysis with *free and open-source* software, usable by *anyone*
- Objective analysis, e. g. no operator bias

Thanks!

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

Colophon

- This BEAMER presentation was crafted in LaTeX with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
  - Complete source code: git.io/fjpP7
  - The LaTeX code is automatically compiled with a GitHub action to 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

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