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**UNIVERSITY
OF BERN**

u^b **X-ray microtomography**

485018-HS2025-0: Advanced Course II Ultraprecision Engineering

David Haberthür

Institute of Anatomy, September 16, 2025

u^b Grüessech mitenang!

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

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Grüessech from the μ CT group



David.Haberthuer@unibe.ch

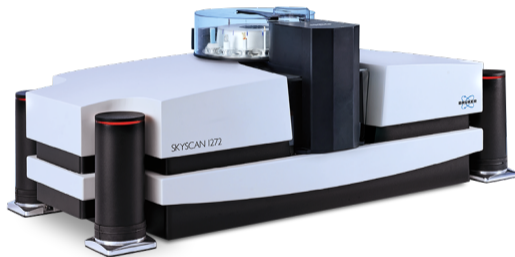
Ruslan.Hlushchuk@unibe.ch

Oleksiy.Khoma@unibe.ch

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μ CT-group

- microangioCT [1]
 - Angiogenesis: heart, musculature [2] and bones
 - Vasculature: (mouse) brain [3], (human) nerve scaffolds [4], (human) skin flaps [5] and tumors
- Zebrafish musculature and gills [6]
- (Lung) tumor detection and metastasis classification [7]
- Collaborations with museums [8] and scientist at UniBe [9] to scan a wide range of specimens
- Automate *all* the things! [10, 11]



bruker.com/skyscan1272

Contents

Overview & Imaging methods

Tomography

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Interaction of x-rays with matter

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Demo

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A study about teeth

Overview

Materials & Methods

Results

Metal foam analysis

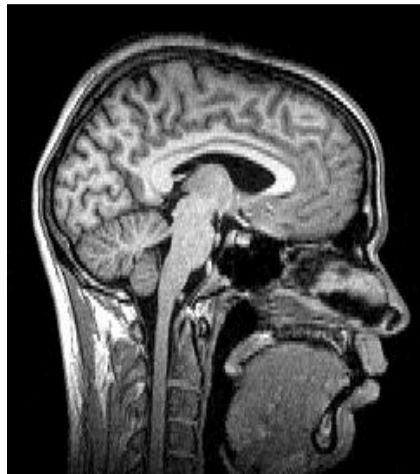
A study on fish

- Dense and/or non-transparent samples
- Calibrated & isotropic 3D images at micron resolutions
- Covers a very large range of sample sizes
- Gives information at different length scales
- Nondestructive imaging, thus compatible with routine sample preparation.
Enables correlative imaging pipelines, scanning of museum & collection material

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Biomedical imaging

- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples

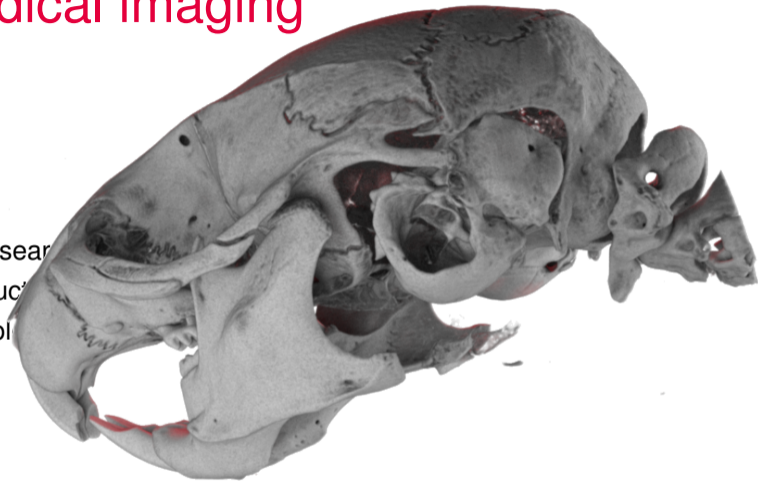


w.wiki/7g4 © ⓘ

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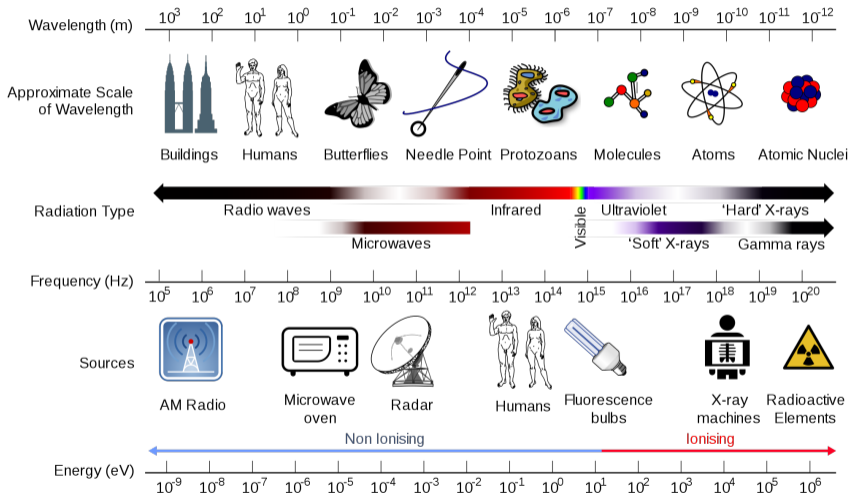
Biomedical imaging

- Medical research
- Non-destructive
- (Small) Biol



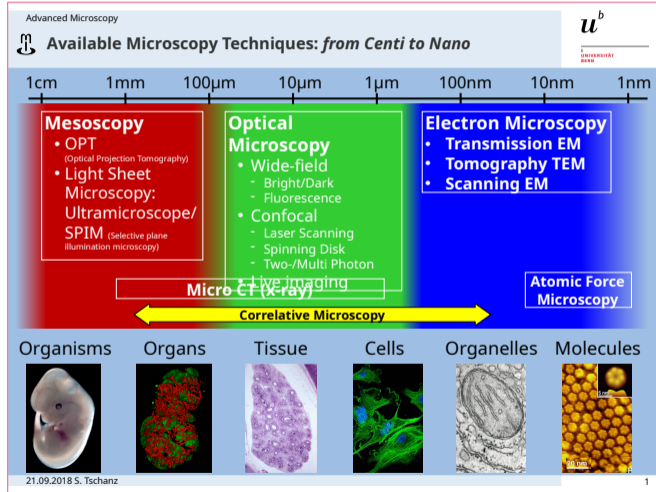
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Wavelengths & Scales



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Wavelengths & Scales



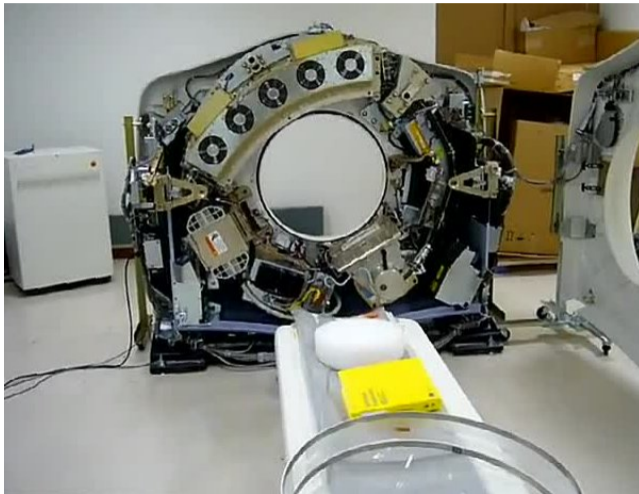
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Imaging methods

- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy
 - *Analytical electron microscopy* by Dimitri
 - *SEM Grundlagen* by Sabine Kässmeyer and Ivana Jaric
 - *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan

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CT-Scanner



youtu.be/2CWpZKuy-NE

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CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays

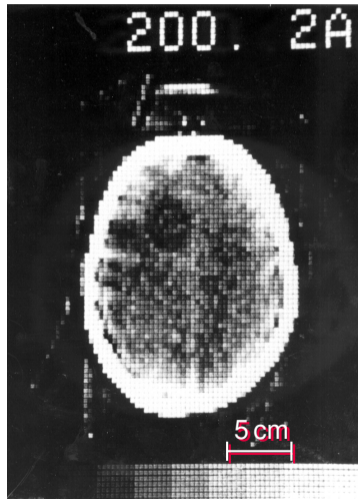


w.wiki/BHAN ©

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CT History

- 1895: Wilhelm Conrad Röntgen discovers X-rays
- 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [12]
- 1976: Hounsfield worked on first clinical scanner [13]

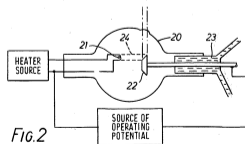
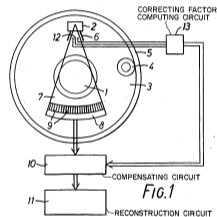


From [14], Figure 5

CT History

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

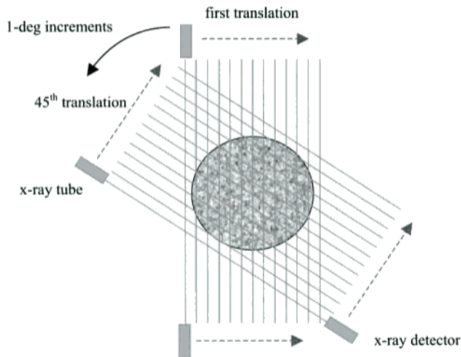
U.S. Patent Feb. 24, 1976 Sheet 1 of 2 3,940,625



From [15], p. 2

CT History

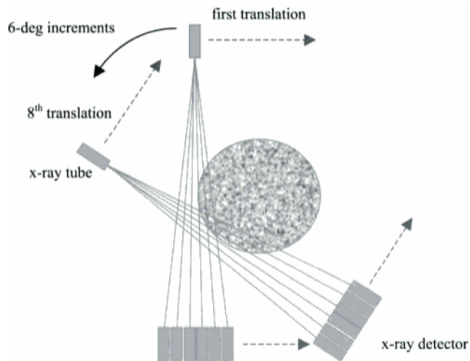
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- CT scanner generations
 - First generation



From [16], Figure 1.12

CT History

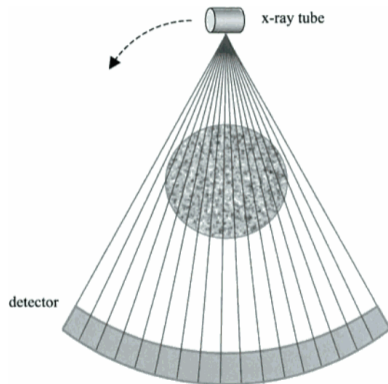
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- CT scanner generations
 - First generation
 - Second generation



From [16], 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 [12]
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- CT scanner generations
 - First generation
 - Second generation
 - Third generation



From [16], Figure 1.14

μ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s [17]
- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Lee Feldkamp [18] developed an early laboratory microCT system by assembling a micro-focus cone beam x-ray source, specimen holder and staggers, 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 [19]

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
- ≈ 2500 μ CT systems are in use worldwide with over 1000 publications annually

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.” ([20])
 - 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 [21, i. e. Beer-Lamberts law]: $I(t) = I_0 e^{-\alpha z}$

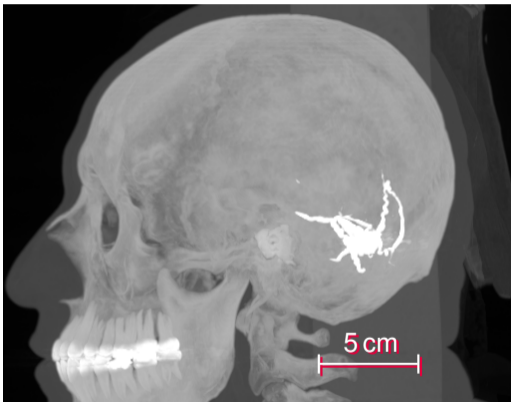
Composition of biological tissues

Tissue: content by mass percentage

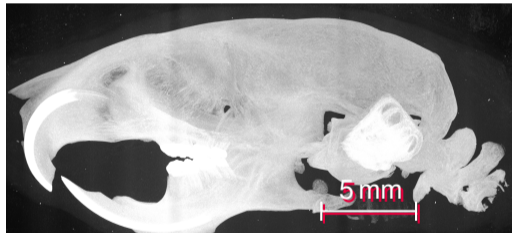
| Element | H | C | N | O | Na | P | S | Cl | K | Ca |
|---------------|------|------|-----|------|-----|------|-----|-----|-----|------|
| Atomic number | 1 | 6 | 7 | 8 | 11 | 15 | 16 | 17 | 19 | 20 |
| Fat | 11.4 | 59.8 | 0.7 | 27.8 | 0.1 | | 0.1 | 0.1 | | |
| Water | 11.2 | | | 88.8 | | | | | | |
| 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 |

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Why μ CT?

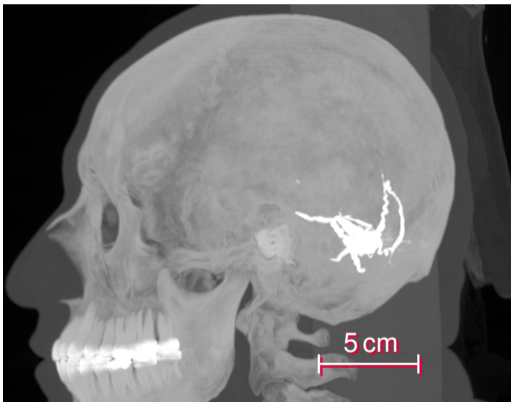


From [22], Subject C3L-02465



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Why μ CT?

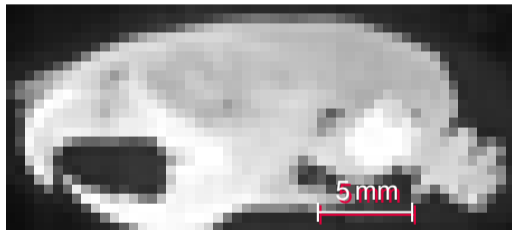
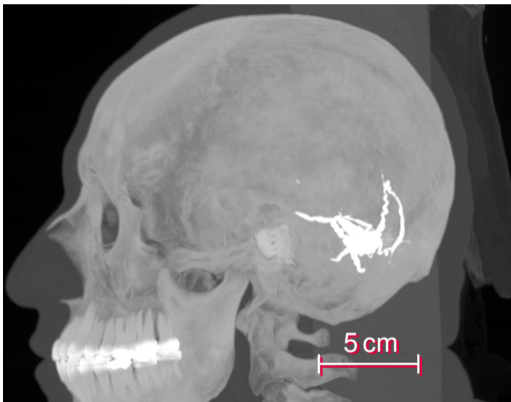


From [22], Subject C3L-02465



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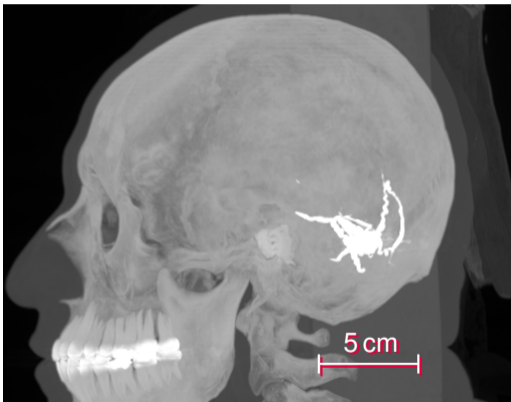
Why μ CT?



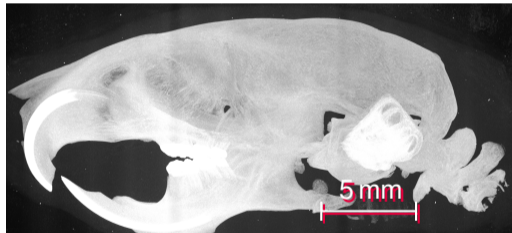
From [22], Subject C3L-02465

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Why μ CT?

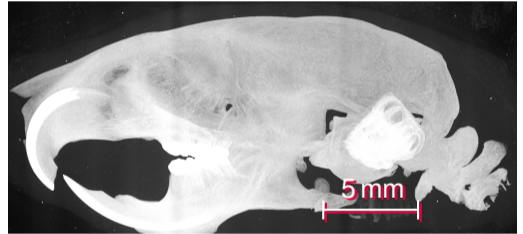


From [22], Subject C3L-02465



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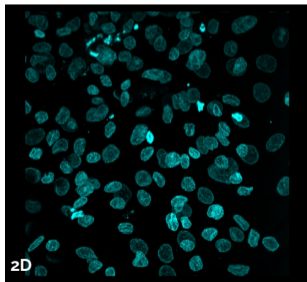
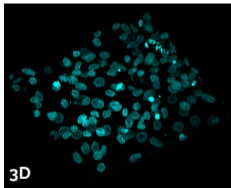
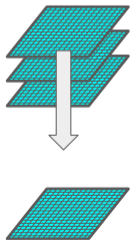
Why μ CT?



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.



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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



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Machinery

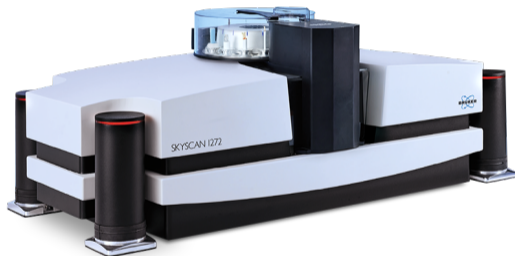
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Machinery

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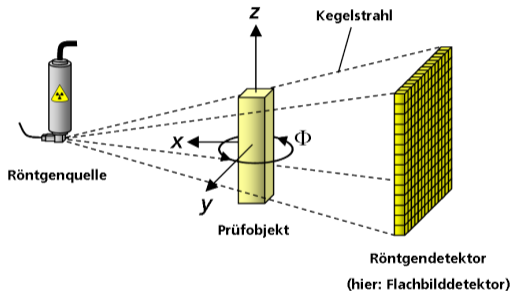
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What is happening?

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

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



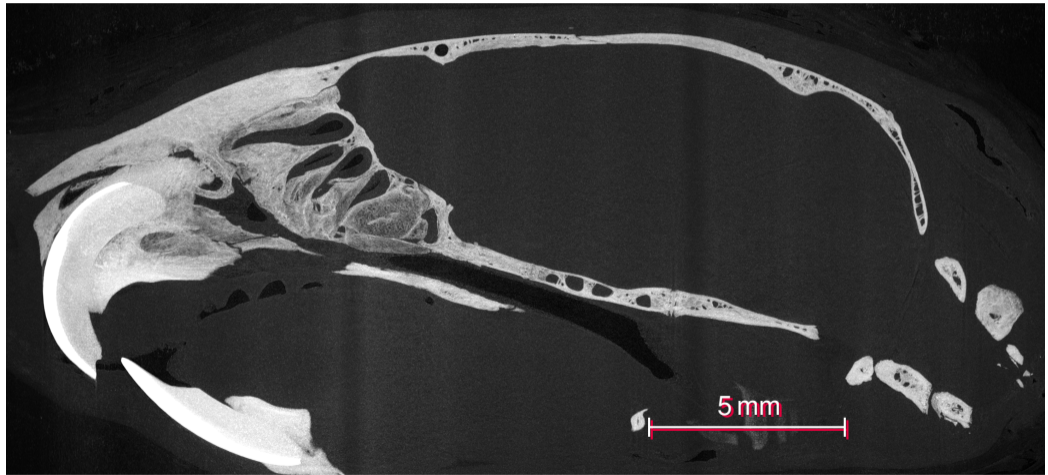
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Machinery

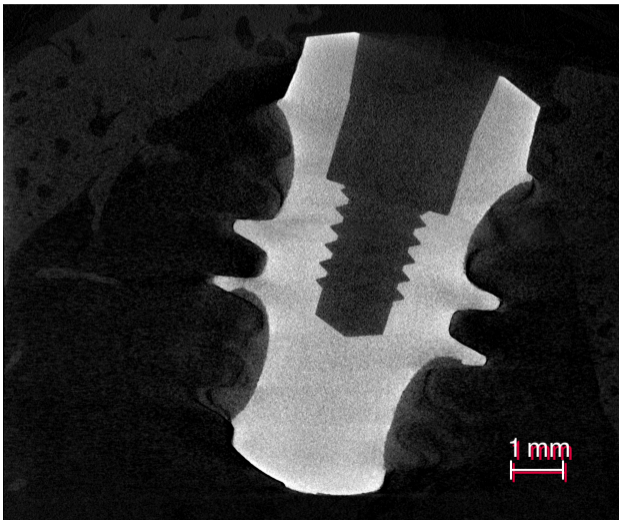
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Examples



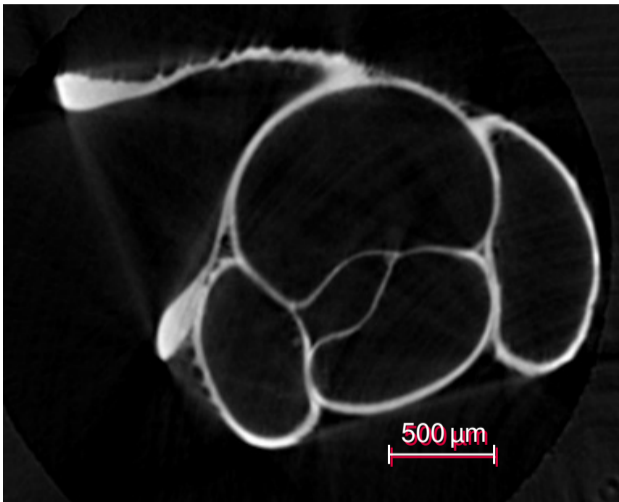
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Examples



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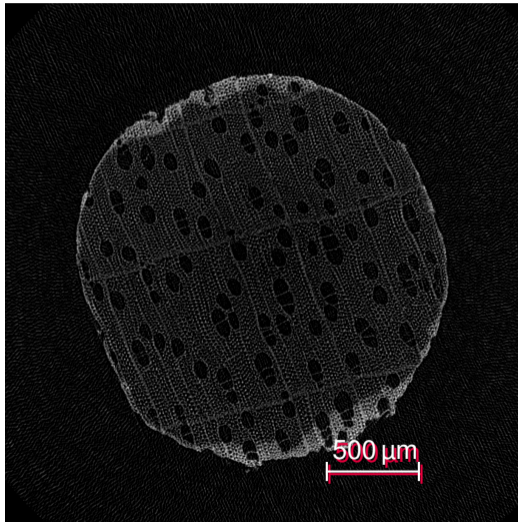
Examples



From [8], *Diantha phoenix*

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Examples



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Examples

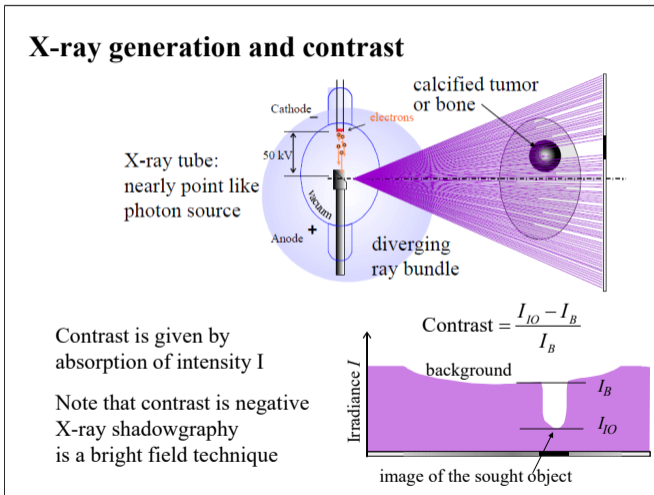


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Preparation

- Study design
- Sample preparation

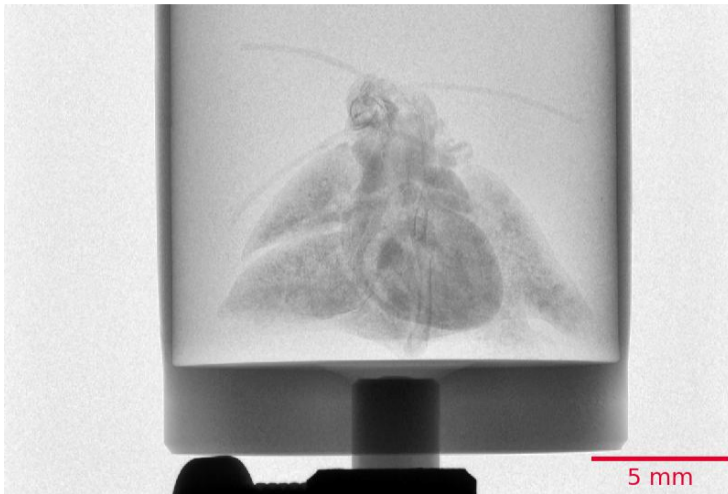
Projections



Contrast, Magnification and Resolution—Laws of Physics for Microscopists (1, 2022) by Martin Frenz, Slide 21

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Projections



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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

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Reconstructions



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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 [18]
- Corrections (beam hardening, etc.)
- Writing to stack

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Visualization



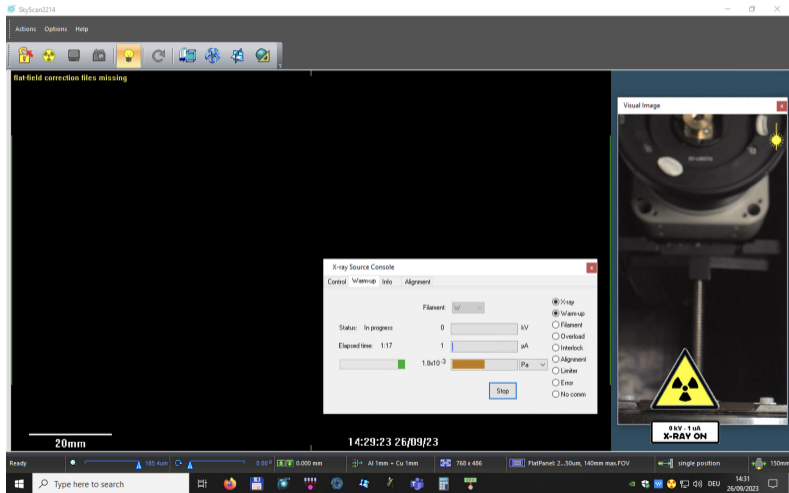
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Visualization

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

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Demo: Acquisition



Demo: Reconstruction

The screenshot displays the ANAMICOS software interface for 3D reconstruction. The main window shows a grayscale CT scan of a heart with a yellow bounding box. The 'Reconstruction' panel on the right shows settings for Position (1003), Top (3806), Bottom (345), and Step (1). The 'Batch manager' at the bottom shows a table of jobs.

| Start batch | Batch job | Status | Message | Description | Value |
|--------------|----------------------|--------|---------|---------------|--|
| Submit again | Limb04-0000000000... | done | | Scan prefix | D:\Results\ETS\JUSSE\Laminits\Limb04\proj\Limb04-... |
| Move up | Testtbaeplateu000... | done | | Scanned at | |
| Remove | | | | Scanned with | |
| Remove all | | | | Scan type | 2-part scan |
| | | | | Input folder | D:\Results\ETS\JUSSE\Laminits\Limb04\rec |
| | | | | Output format | A.HI.PMT. + DICOM |

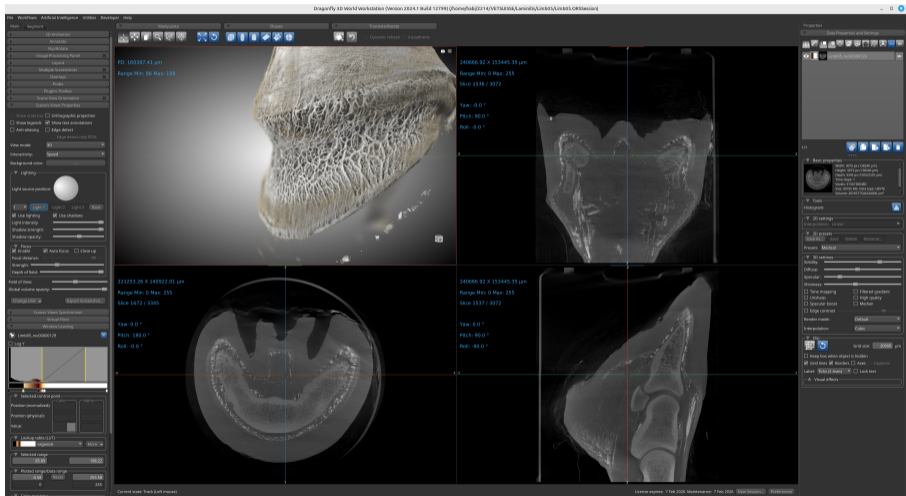
Demo: Reconstruction

Batch manager: listing batch jobs and the details

| Start batch | Batch job | Status | Message | Description | Value |
|--------------|----------------------|---------|---------|---------------|---|
| Submit again | Limb04-0000000000... | done | | Scan prefix | D:\Results\ETS\USSE\Laminits\Limb05\proj\Limb05-... |
| Move up | Teetbbaeateu000... | done | | Scanned at | |
| Remove | Limb05-0100000000... | pending | | Scanned with | |
| Remove all | | | | Scan type | 3-part scan |
| | | | | Input folder | D:\Results\ETS\USSE\Laminits\Limb05\proj |
| | | | | Output format | A.HI.PMT |

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Demo: Visualization



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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!

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Quantitative data

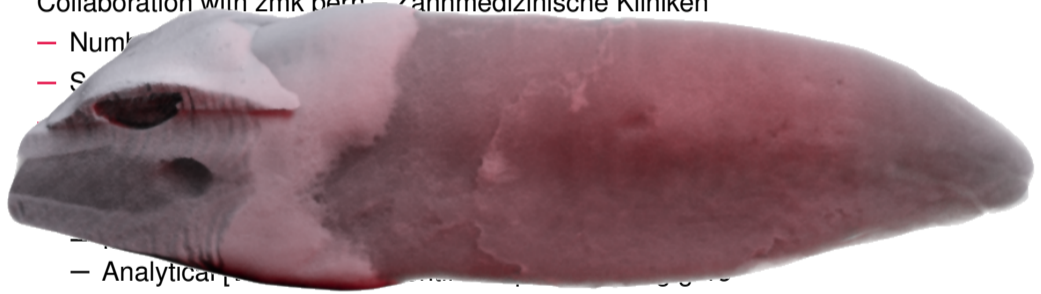
- Pretty images are nice to have, but science is built on quantitative data.
- Segmentation
- Characterization

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Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number
- S



- Analytical

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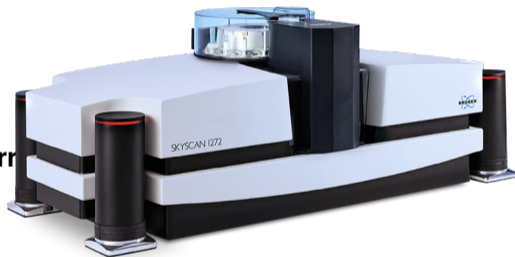
How?

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



How?

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```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files= 482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)= 80
Source Current (uA)= 125
Image Pixel Size (um)=9.999986
Exposure (ms)=950
Rotation Step (deg)=0.400
Frame Averaging=ON (3)
Filter=Al 1mm
Study Date and Time=02 Jul 2020 08h:23m:34
s
Scan duration=0h:39m:51s
```

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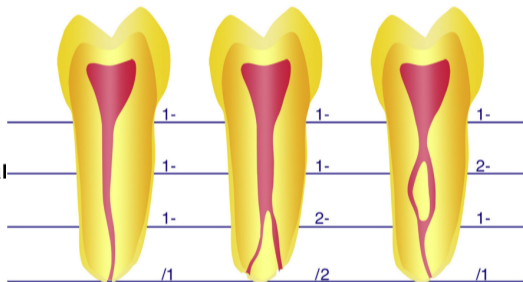
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- Sample changer* on the SkyScan 1272
In total:
- 13 days of *continuous* μ CT scanning
 - 819 GB of raw data
230 648 TIFF projections
 - 326 GB data as input for analysis
282 062 PNG reconstructions

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How?

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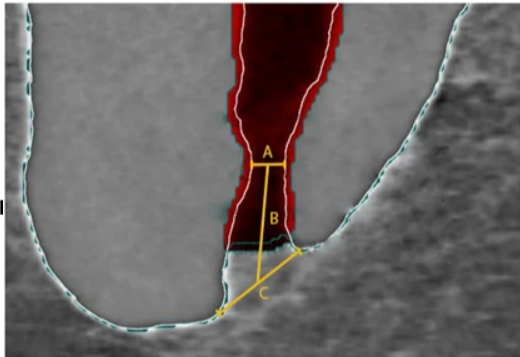


From [**Briseno-Marroquin2015**], Fig. 2

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How?

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- *Reproducible* analysis [**Haberthuer2020a**], e. g. you can click a button to double-check or recalculate the results yourself!



From [Wolf2017], Fig. 1

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How?

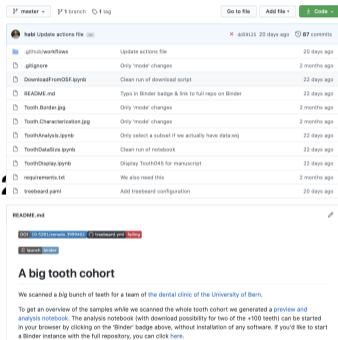
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gph.is/2nqkple

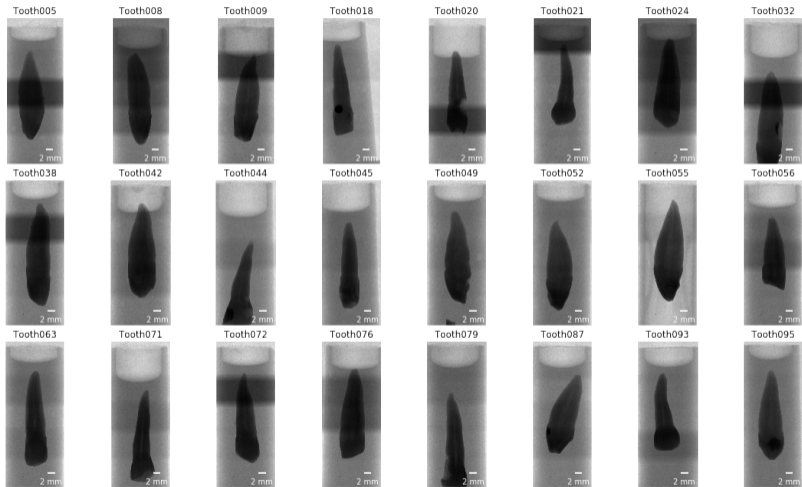
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- *Reproducible* analysis [Haberthuer2020a], e. g. you can click a button to double-check or recalculate the results yourself!



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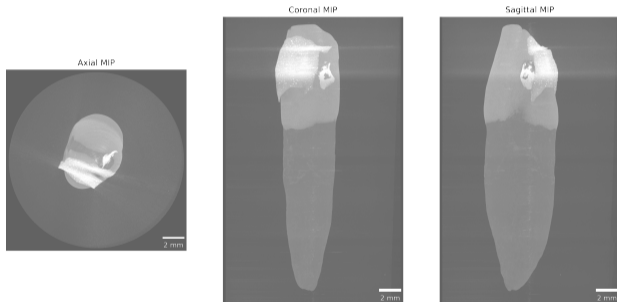
μ CT imaging



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Dataset cropping

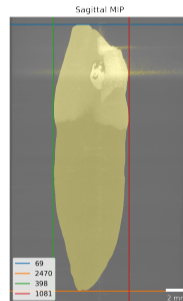
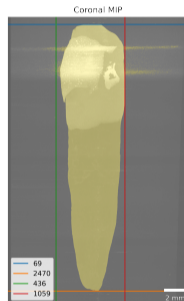
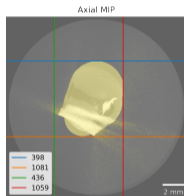
- Full datasets: 326 GB
- Cropped datasets: 115 GB



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Dataset cropping

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



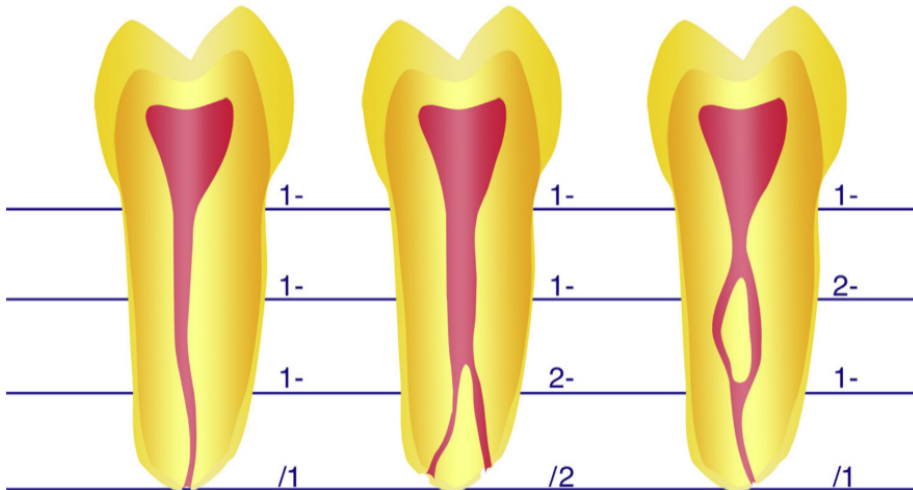
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Tooth morphology



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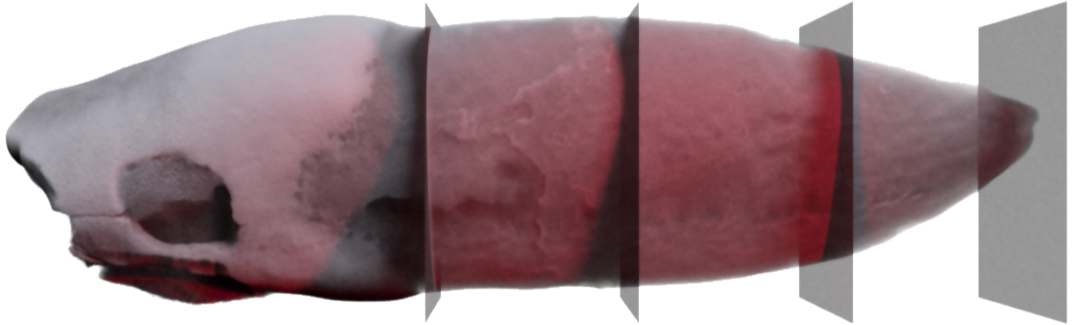
Tooth morphology



From [Briseno-Marroquin2015], Fig. 2

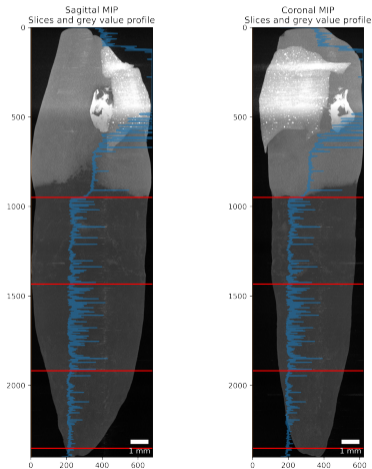
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Tooth morphology

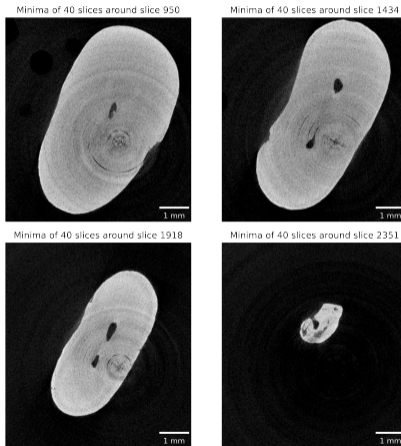


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Detection of enamel-dentin border



Tooth045

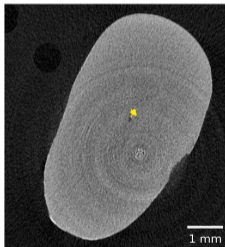


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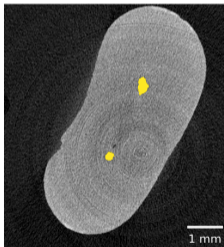
Detection of enamel-dentin border

Tooth045

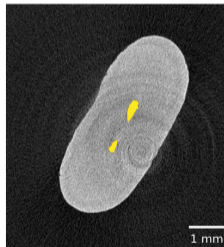
Tooth045, Slice 950: 1



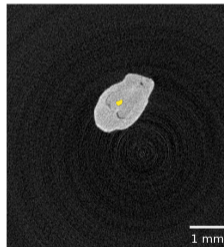
Slice 1434: 2



Slice 1918: 2



Slice 2351: 1

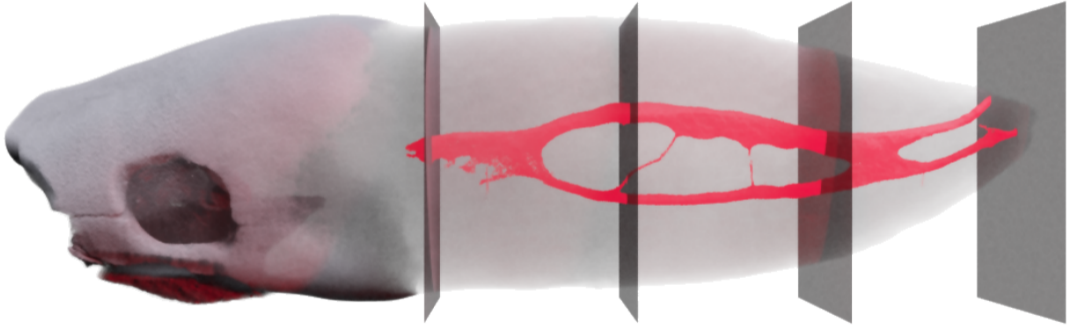


Classification of root canal configurations

| 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 | 66.6 |
| | | | 1-2-1/1 | 1 | 33.3 |
| | | Lingual | 1-1-1/1 | 2 | 66.6 |
| | | | 1-1-1/2 | 1 | 33.3 |

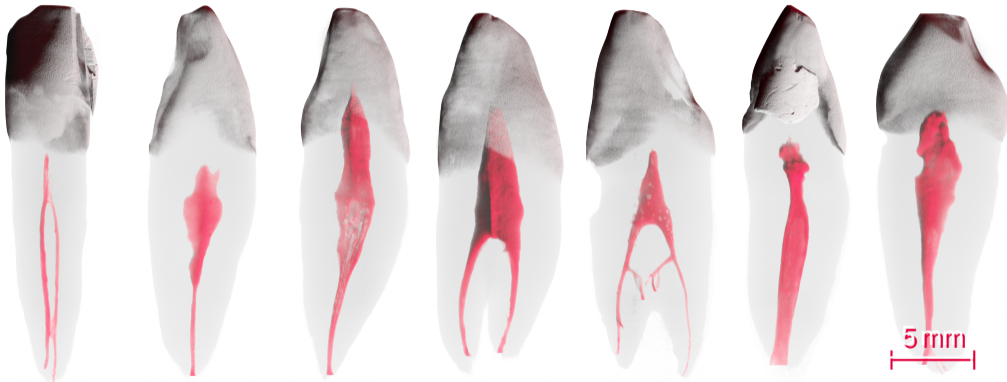
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Extraction of root canal space



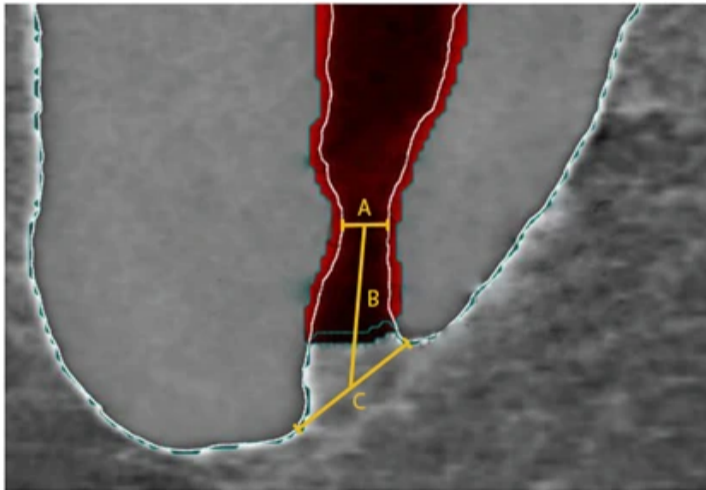
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Results of root canal space extraction



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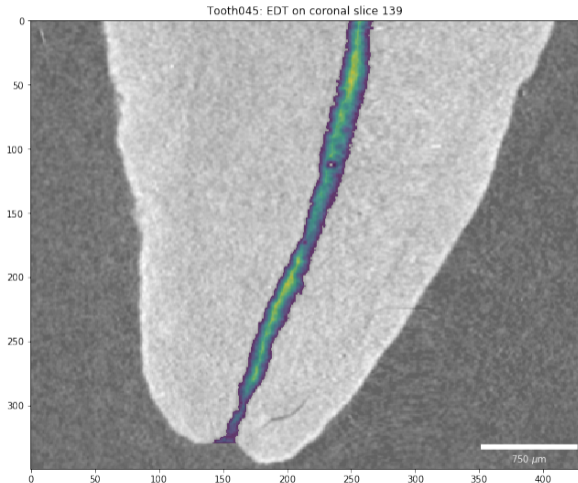
Physiological foramen geometry



From [Wolf2017], Fig. 1

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Physiological foramen geometry



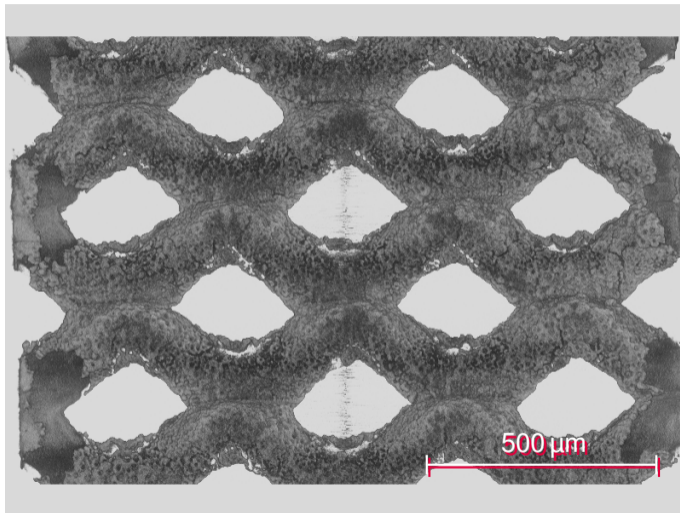
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Conclusion ZMK

- 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

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Metal foam

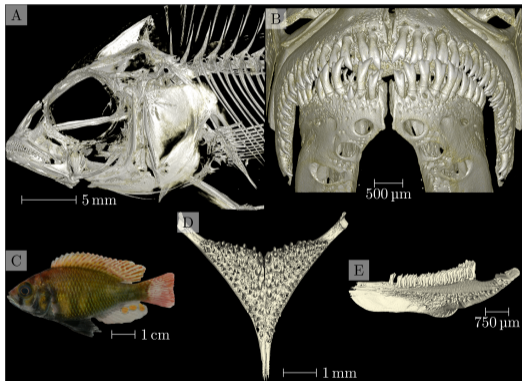


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Data wrangling by example: Cichlids

Collaboration with the Institute of Ecology and Evolution [11]

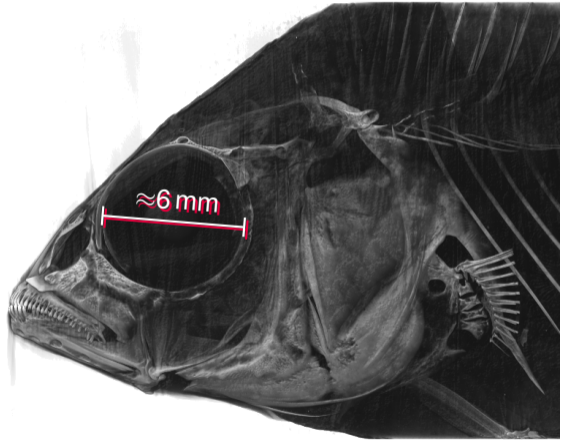
- 133 Cichlids from Lake Victoria, East Africa
 - Functional anatomy of the skulls and jaws
 - 6–18 cm in size
- 375 scans in total
 - Voxelsizes from 3.5–50 μm
 - 46 days of scanning time
 - 9.8 TB of raw data
 - 1.5 TB/+1 000 000 reconstructions



DOI:gsst8t, Fig. 1

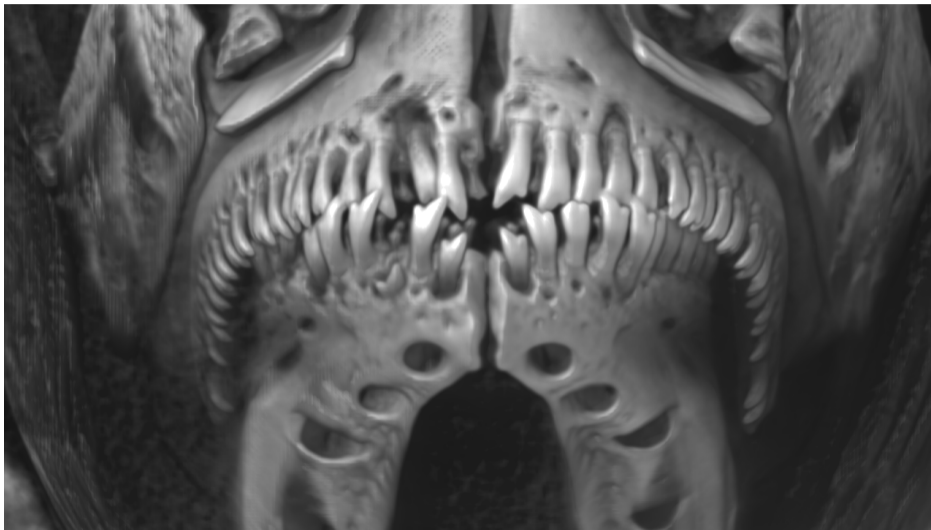
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Visualization of cichlid head



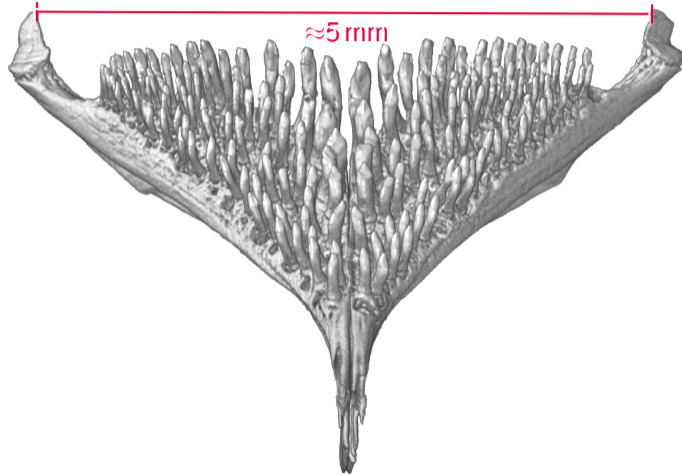
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Visualization of cichlid head



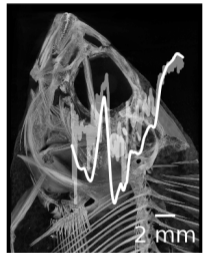
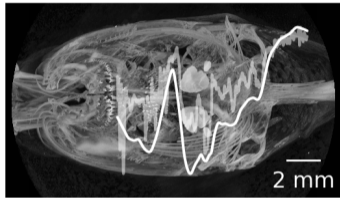
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Visualization of segmented pharyngeal jaw



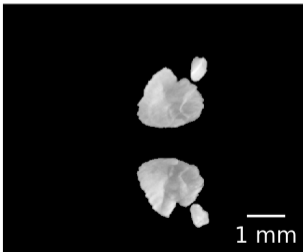
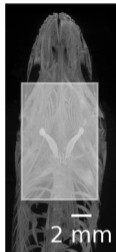
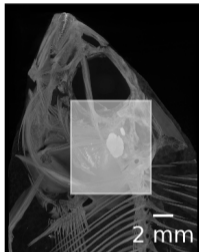
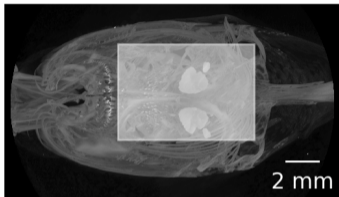
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Data wrangling by example: Cichlids



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Data wrangling by example: Cichlids



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Thanks!

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

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Colophon

- This BEAMER presentation was crafted in \LaTeX with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
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