

X-ray microtomography

David Haberthür

December 3, 2021 | 9256-HS2021-0: Advanced Microscopy

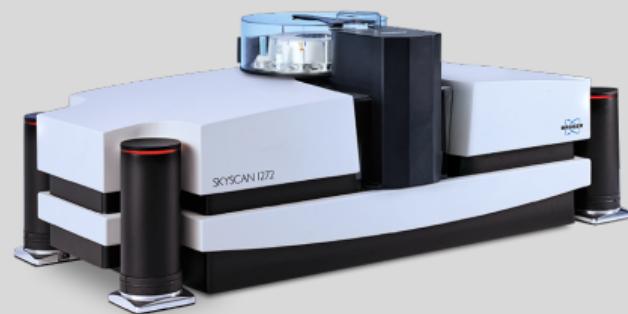
Grüessech!

- 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.
Together with Ruslan Hlushchuk, Oleksiy-Zakhar Khoma and Tim Hoessly.

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 our institute [9] to scan a wide range of specimens
- Automate *all* the things! [10]



bruker.com/skyscan1272

Contents

Overview

Imaging

Tomography

History

Interaction of x-rays with matter

Tomography today

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

Example of a complete study (about teeth)

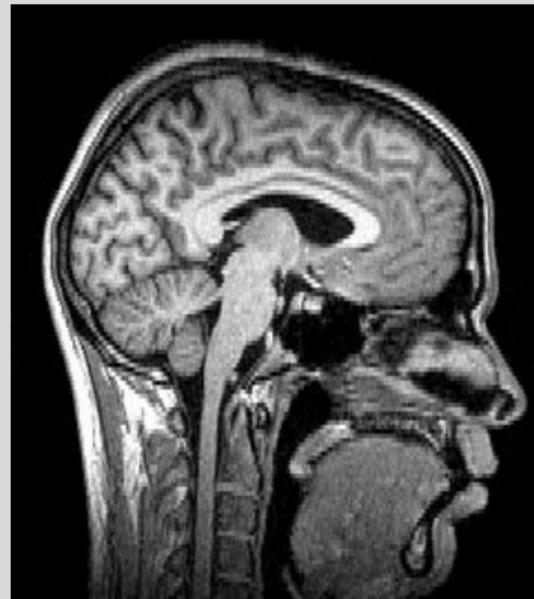
Overview

Materials & Methods

Results

Biomedical imaging

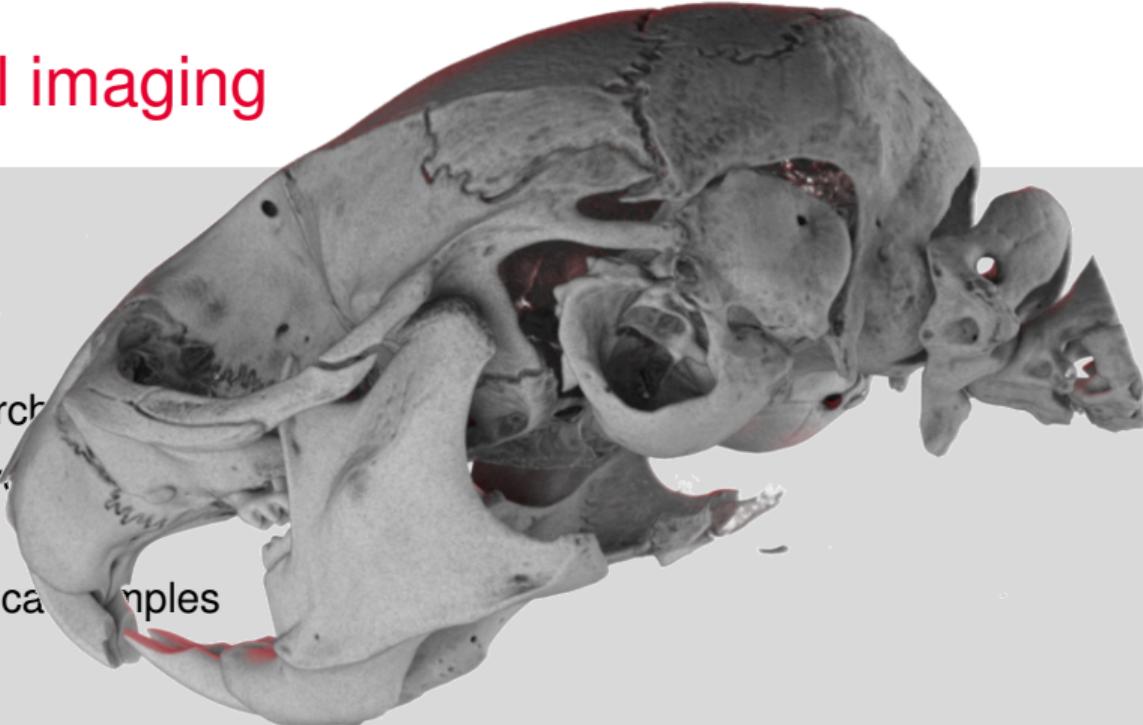
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



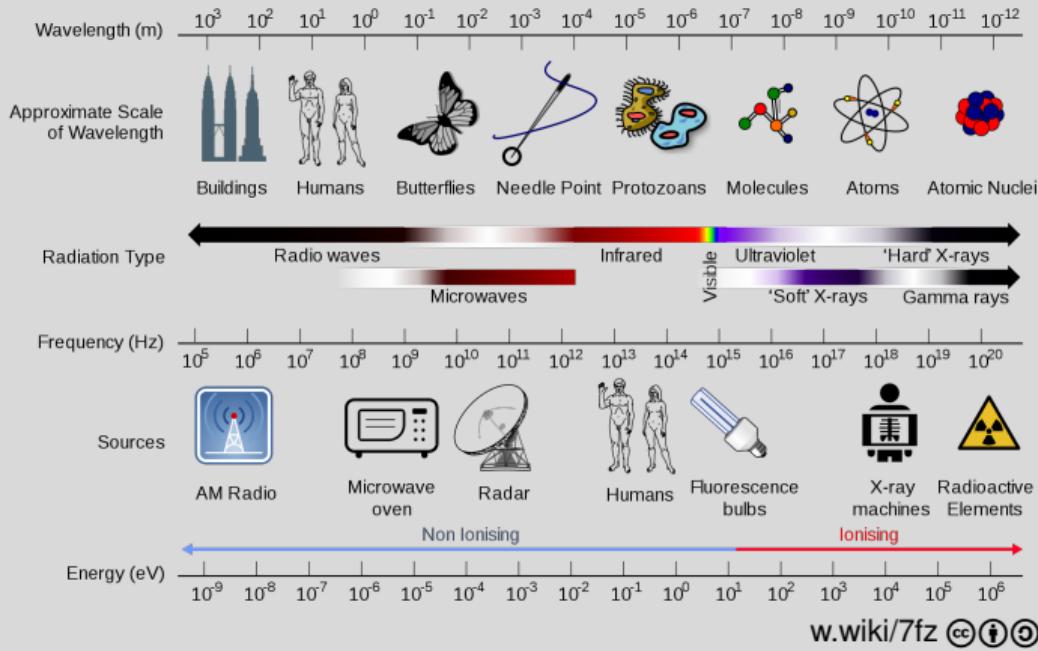
w.wiki/7g4 

Biomedical imaging

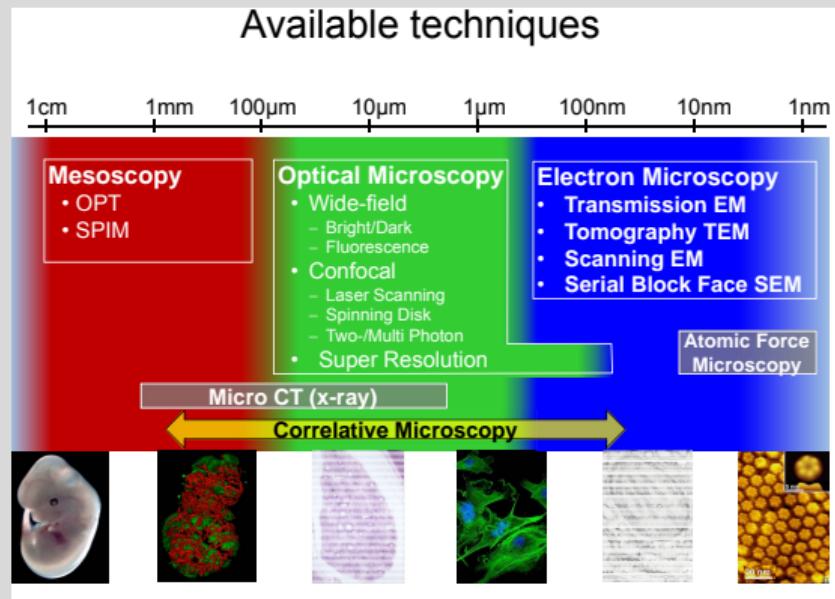
- Medical research
- Non-destructive analysis of the samples
- (Small) Biological samples



Wavelength & Scale



Wavelength & Scale



Yury Belyaev, MIC, slide from internal seminar presentation

Imaging methods

- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy: see lectures *Transmission Electron Microscopy* by Dimitri Vanhecke, *Scanning Electron Microscopy* by Michael Stoffel and *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

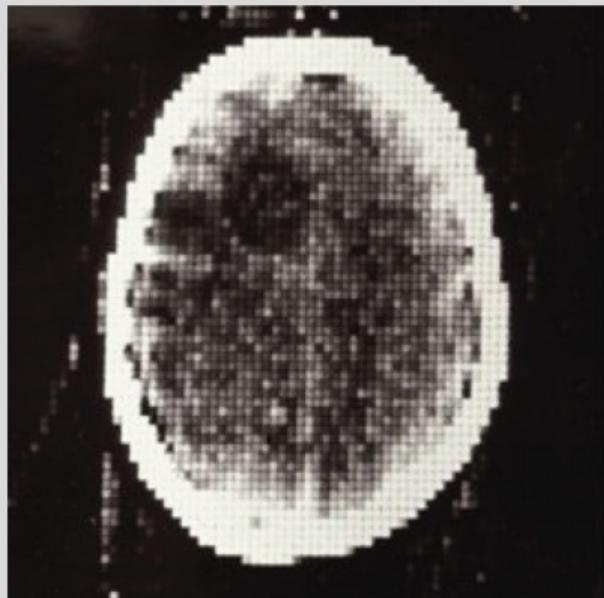
CT-Scanner



youtu.be/2CWpZKuy-NE

History

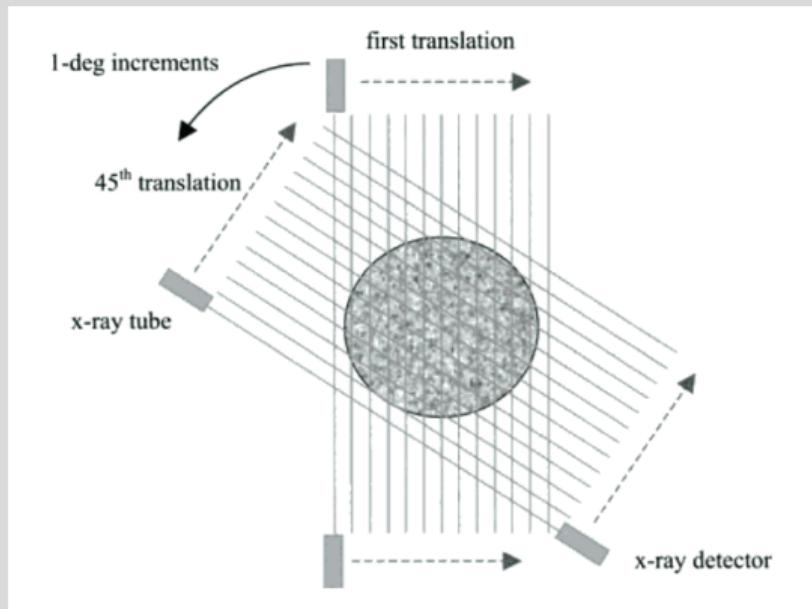
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]



From [14], Figure 8.2

History

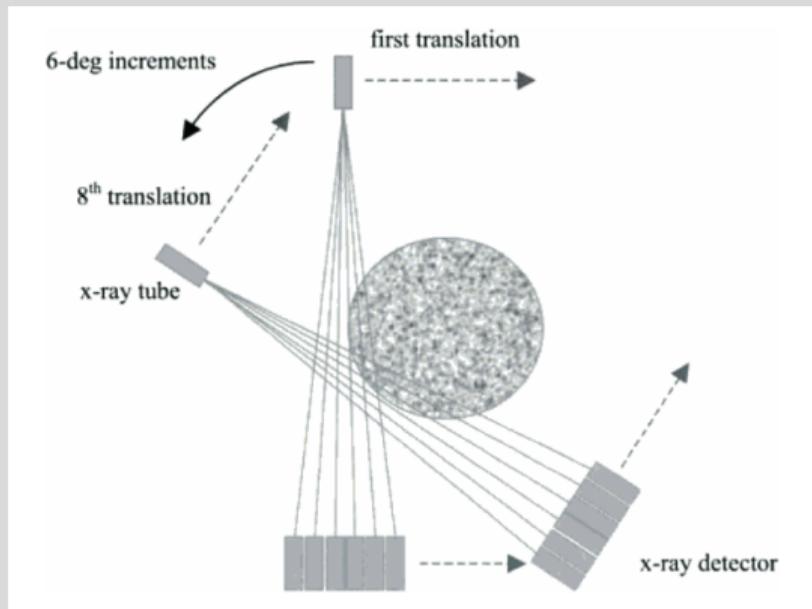
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]
- CT scanner generations: First



From [13], Figure 1.12

History

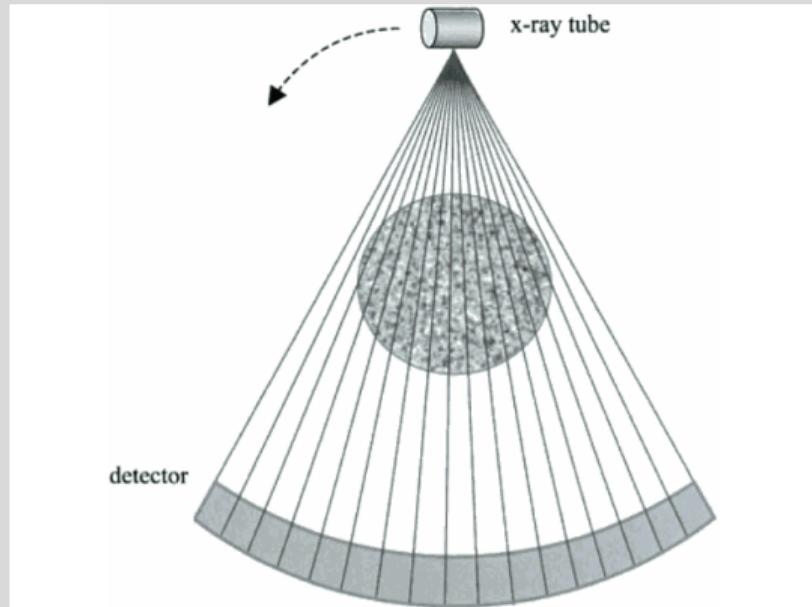
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]
- CT scanner generations: First, second



From [13], Figure 1.13

History

- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
 - 1976: Hounsfield worked on first clinical scanner [12]
 - Nice overview by Hsieh [13]
- CT scanner generations: First, second and third



From [13], Figure 1.14

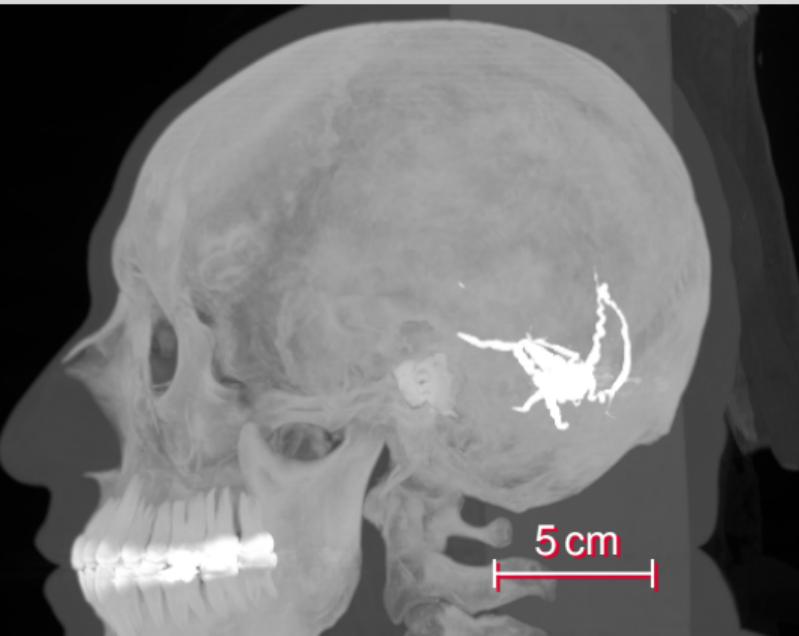
X-ray interaction

- “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.” ([15])
 - 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 ([16, i. e. Beer-Lamberts law] $I(t) = I_0 e^{-\alpha z}$)

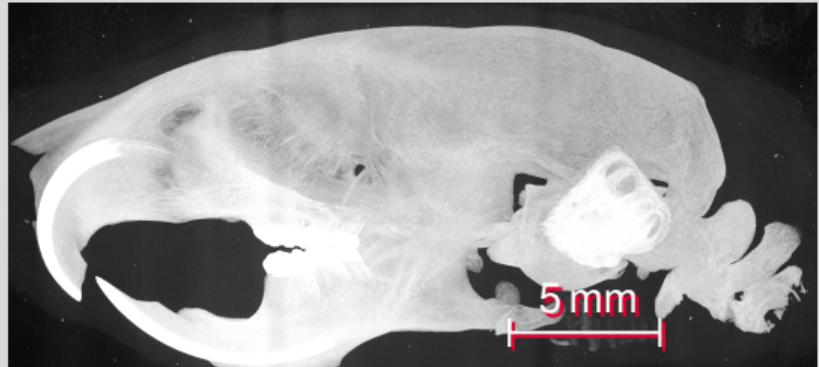
Composition of biological tissues

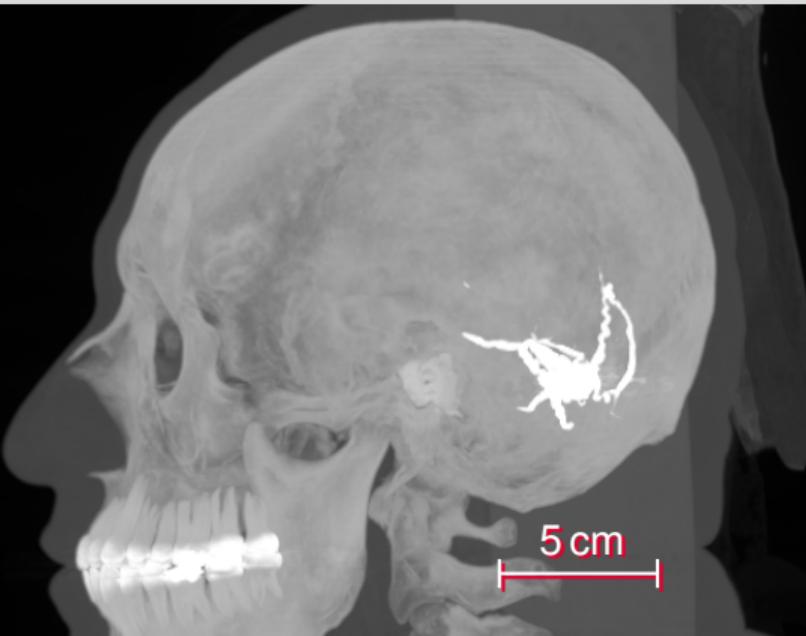
Tissue: content by mass percentage

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



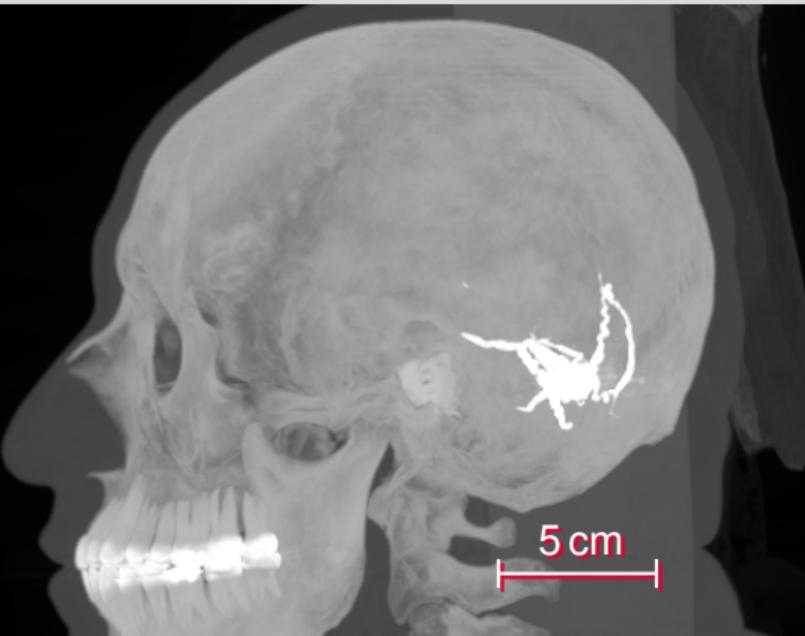
From [17], Subject C3L-02465



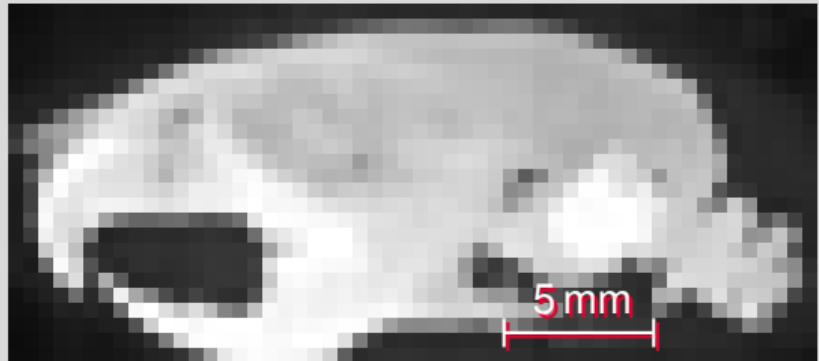


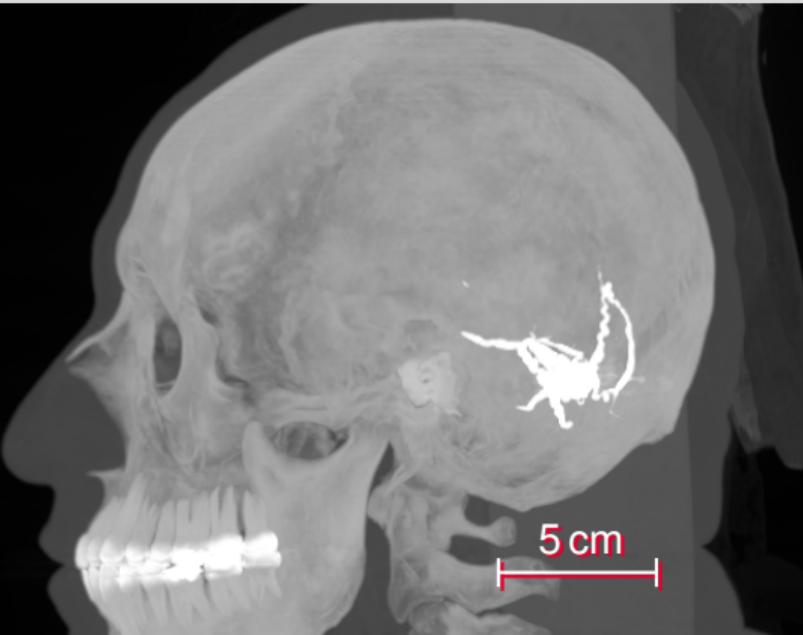
From [17], Subject C3L-02465



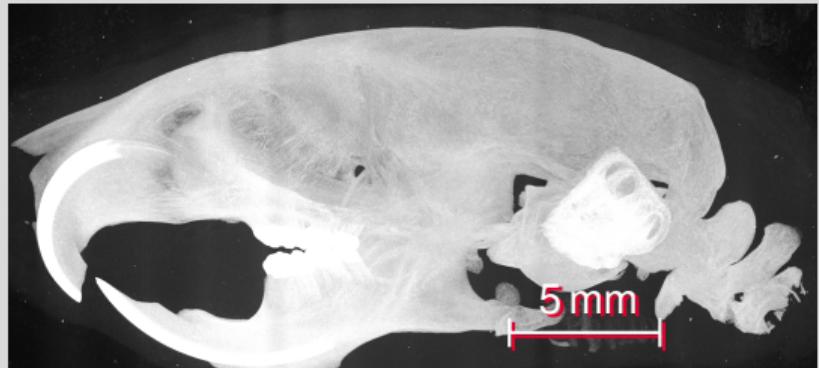


From [17], Subject C3L-02465





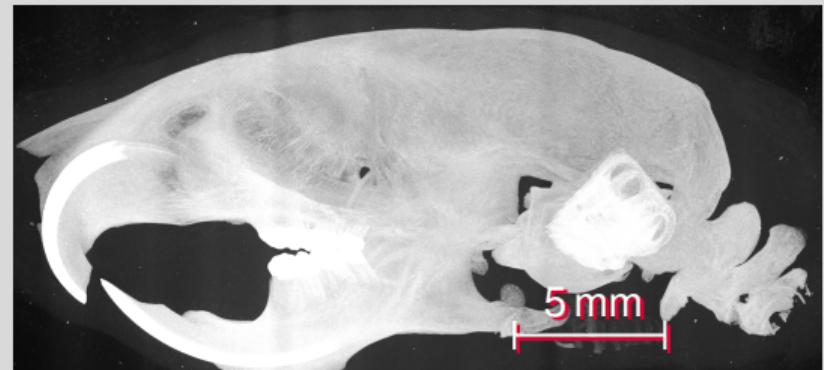
From [17], Subject C3L-02465



Why μ CT?



From [17], Subject C3L-02465



Volumetric representation

3D data can also be represented in 3D. Different strategies exist to show the depth e.g. projection of the brightest pixel along the viewing axis, surface representation etc.

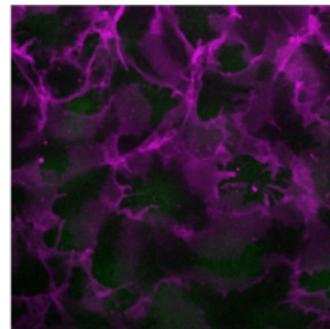
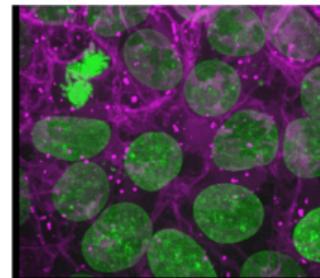


Image stack turned into transparent volume



Example dataset from scikit-image.
Origin: Allen Institute for Cell Science

Fundamentals of Digital Image Processing by Guillaume Witz, Slide 20

Machinery

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



flic.kr/p/D4rbom

Machinery

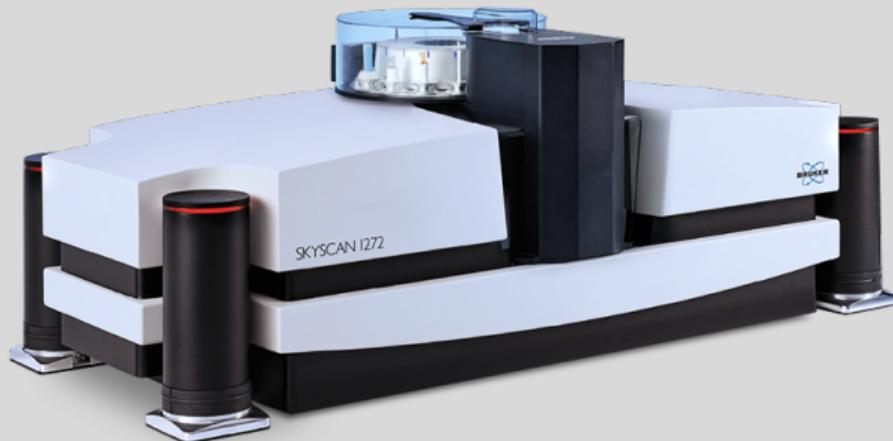
- Hospital CT
 - Voxel size around 0.5 mm
- Lab/Desktop CT
 - Voxel size around 7 μm (*in vivo*) or 0.5 μm (*ex vivo*)
- Synchrotron CT
 - Voxel size down to 160 nm



flic.kr/p/fpTrGu

Machinery

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

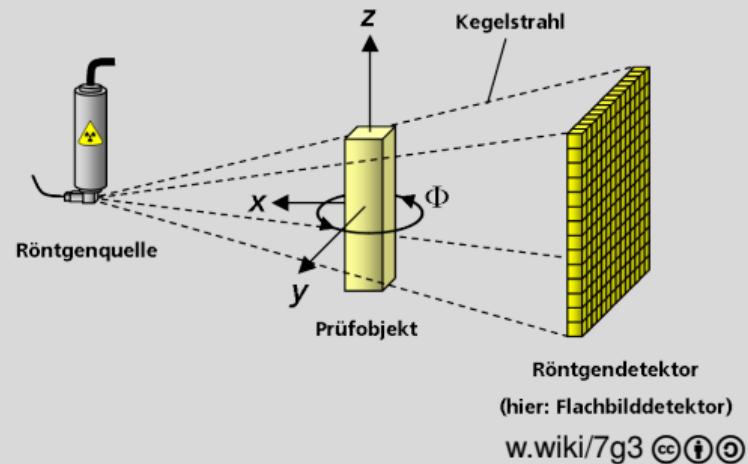


flic.kr/p/7Xhk2Y

What is happening?

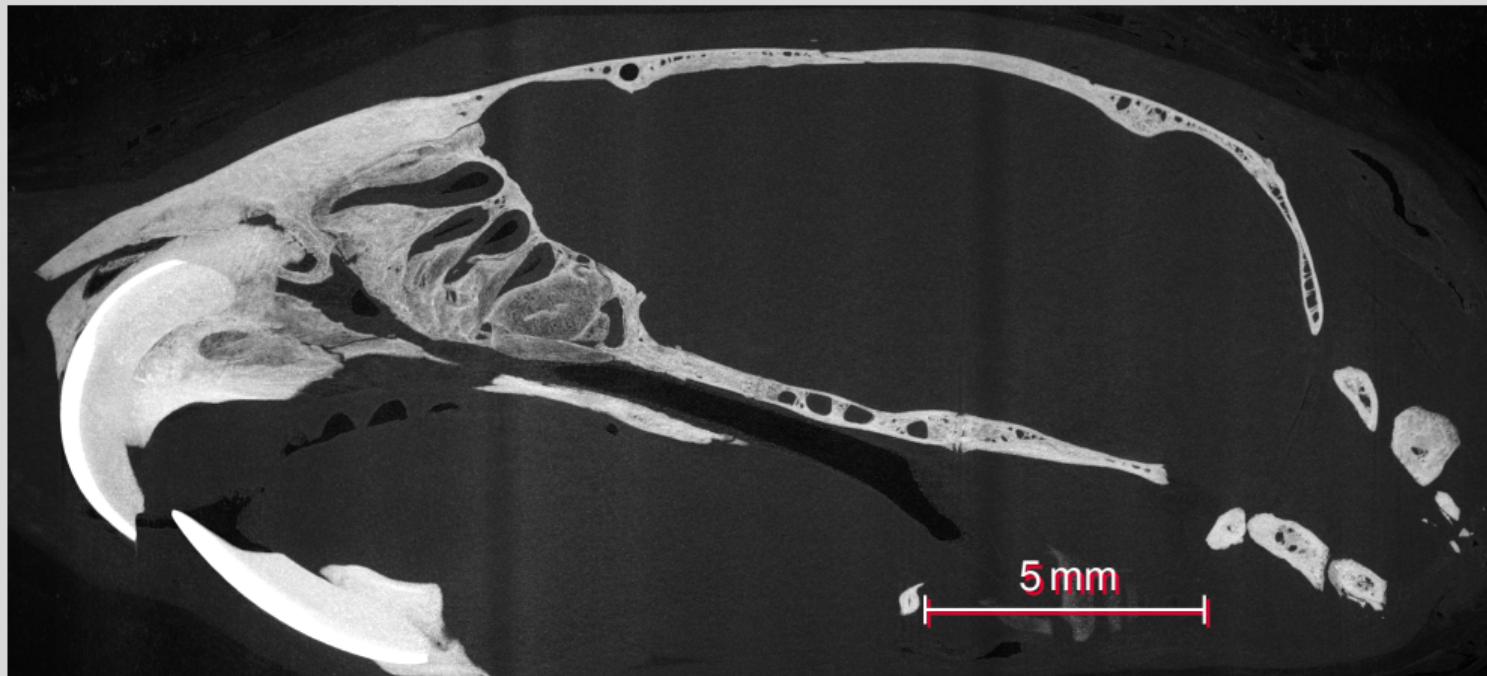
No matter what kind of machine, the basic principle is always the same

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

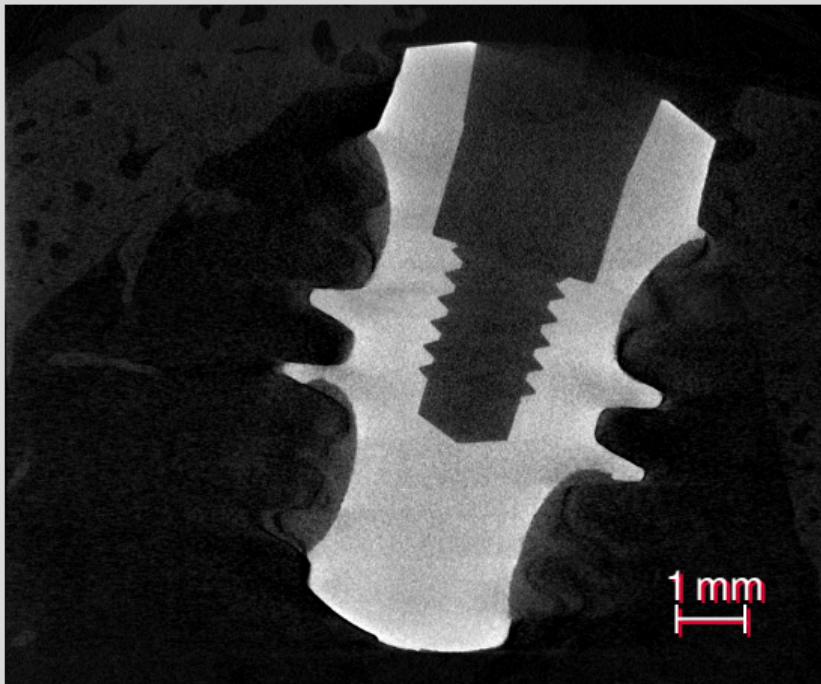


Machinery

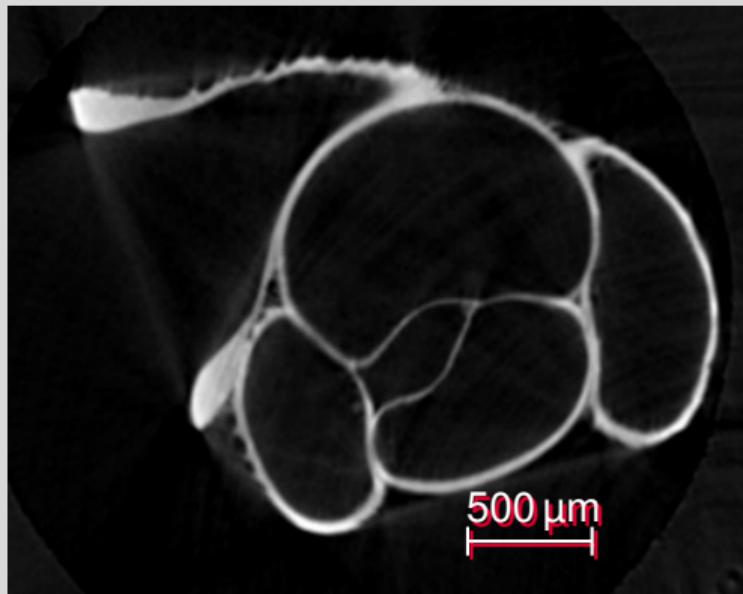
Examples



Examples

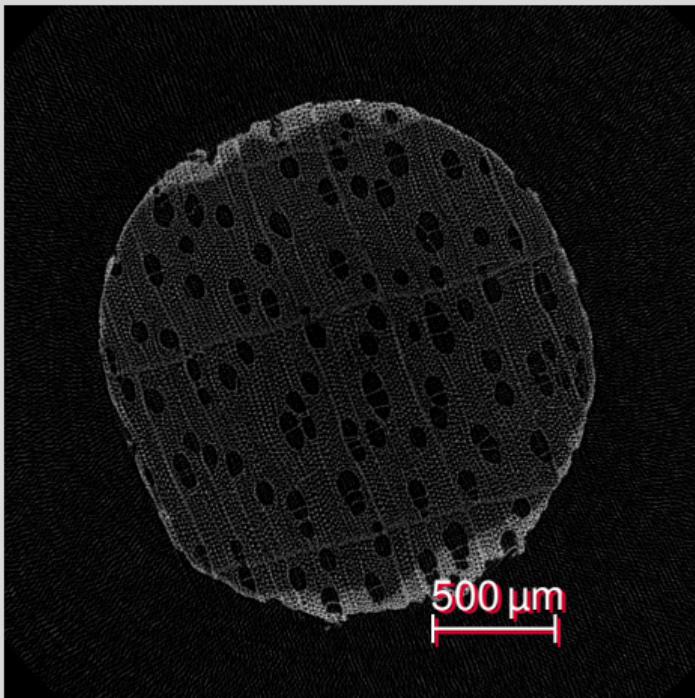


Examples



From [8], *Diancta phoenix*

Examples



Examples



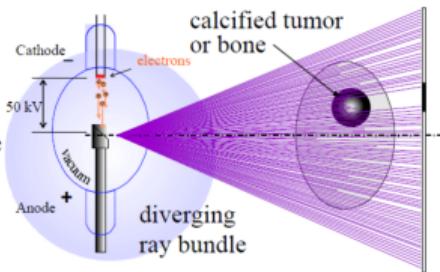
Preparation

- Study design
- Sample preparation

Projections

X-ray generation and contrast

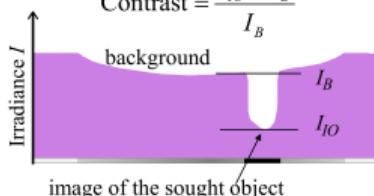
X-ray tube:
nearly point like
photon source



Contrast is given by
absorption of intensity I

Note that contrast is negative
X-ray shadowgraphy
is a bright field technique

$$\text{Contrast} = \frac{I_{IO} - I_B}{I_B}$$



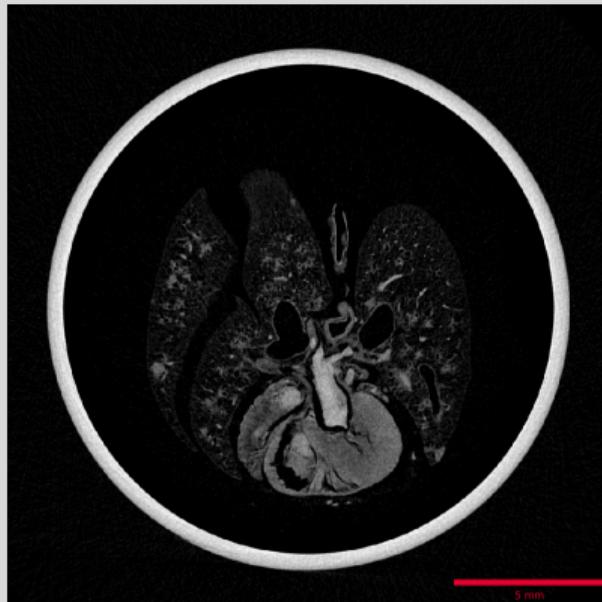
Projections



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

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
- Fan beam reconstruction
- Corrections (beam hardening, etc.)
- Writing to stack

Visualization



Visualization

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

What to use?

- ImageJ/Fiji [18]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
 -  in Jupyter [19]
 - `git`
 - Script all your things!
 - Data repositories; i. e. sharing is caring!

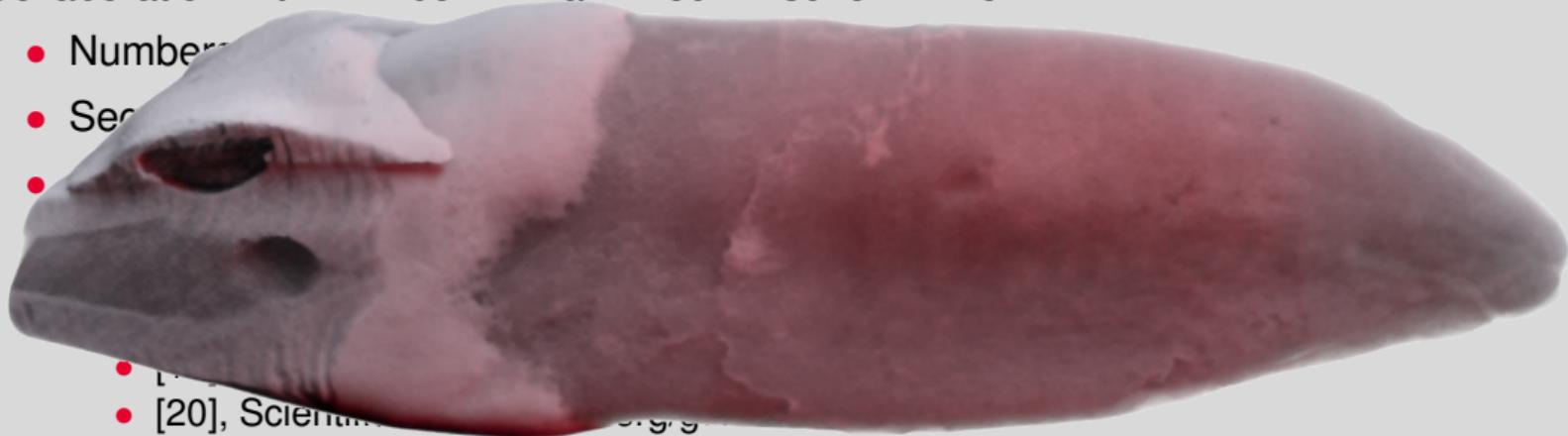
Quantitative data

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

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number of teeth
- Secondary dentin
- Root canal system
- [20], Scientific publications



Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers instead of just pretty images
- Segmentation of teeth and root canal
- (Unbiased) Characterization
- Reproducible and automated image analysis ( in Jupyter [19])
- Two publications:
 - [10], BMC Oral Health, doi.org/gjpw2d
 - [20], Scientific Reports, doi.org/g7r8

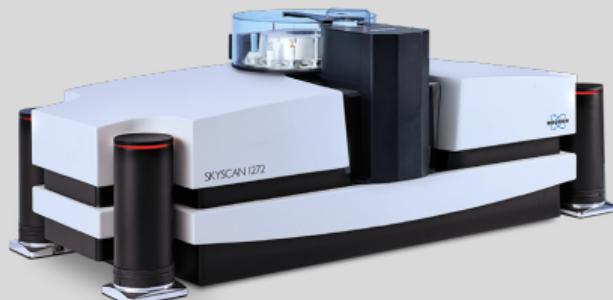
How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], 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
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!



bruker.com/skyscan1272

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], 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 08h:23m:34s
Scan duration=0h:39m:51s
```

How?

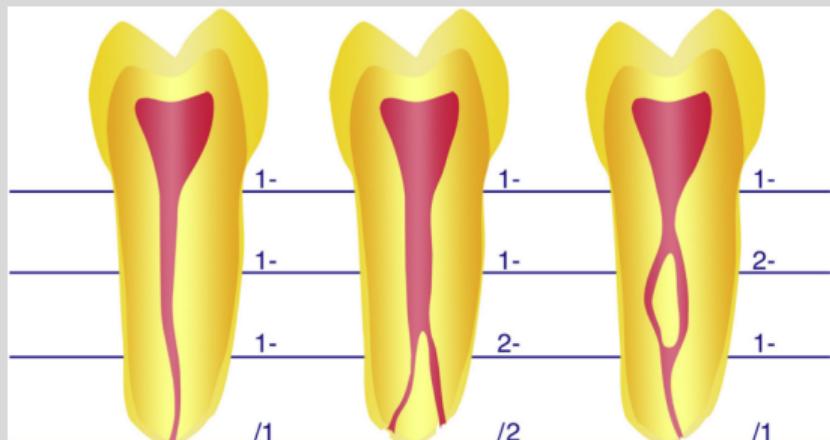
- 104 extracted human permanent mandibular canines
- μ CT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate 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?

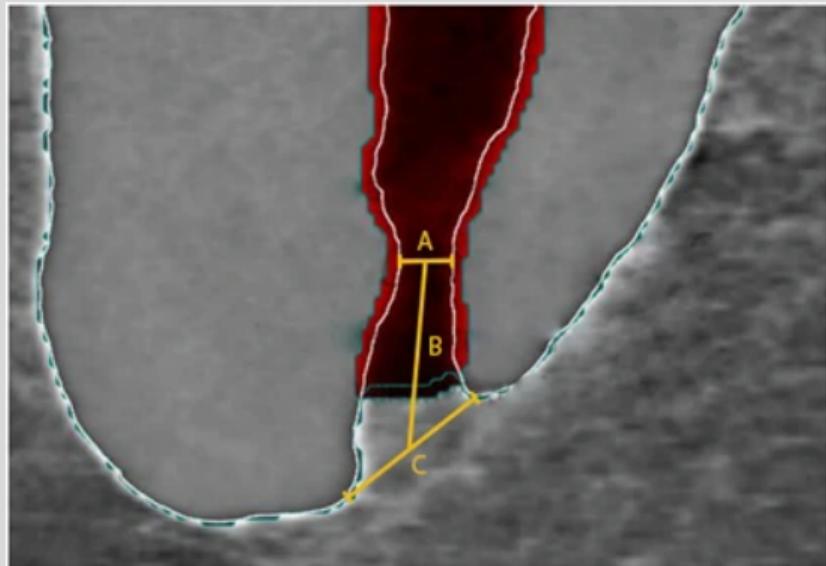
- 104 extracted human permanent mandibular canines
- μ CT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!



From [21], Fig. 2

How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!



From [22], Fig. 1

How?

- 104 extracted human permanent mandibular canines
- μ CT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!

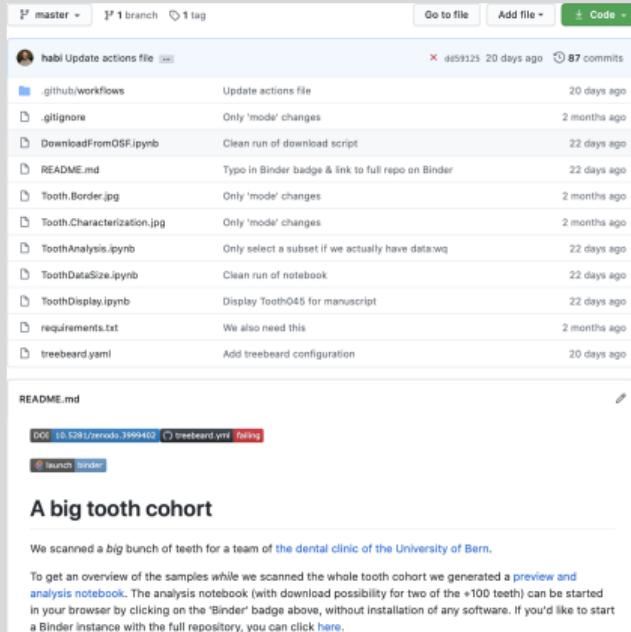


18.44 Jarę PEKA. Zarząd Transportu Miejskiego zachęca do wymiany KOM-kart na Po

gph.is/2nqkple

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
 - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
 - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], 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', '1 branch', '1 tag', 'Go to file', 'Add file', and 'Code'. Below this is a list of commits from a user named 'habi' with 87 commits, all made 20 days ago. The commits include actions like updating workflows, .gitignore, and README files, as well as running scripts and notebooks. Below the commits is a 'README.md' file with a DOI link (10.5281/zenodo.3999402) and a 'treebeard.yaml' file. A 'launch binder' button is also present. The main content area features the heading 'A big tooth cohort' and a paragraph explaining the dataset.

habi Update actions file · 87 commits

Update actions file · 20 days ago

.github/workflows · 20 days ago

.gitignore · 2 months ago

DownloadFromOSF.ipynb · 22 days ago

README.md · 22 days ago

Tooth.Border.jpg · 2 months ago

Tooth.Characterization.jpg · 2 months ago

ToothAnalysis.ipynb · 22 days ago

ToothDataSize.ipynb · 22 days ago

ToothDisplay.ipynb · 22 days ago

requirements.txt · 2 months ago

treebeard.yaml · 20 days ago

README.md

DOI: 10.5281/zenodo.3999402 treebeard.yaml failing

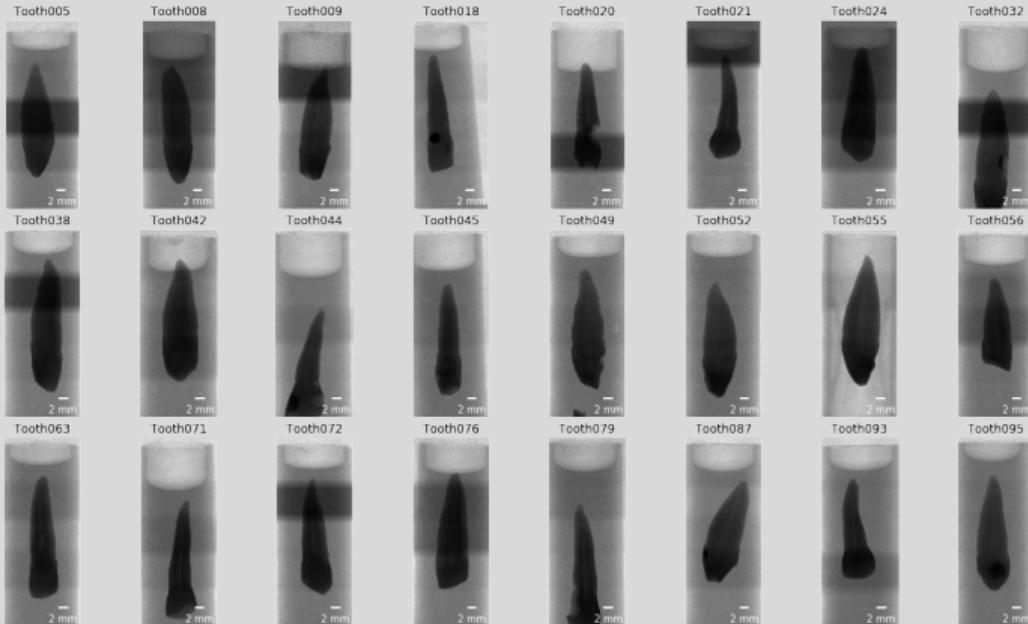
launch binder

A big tooth cohort

We scanned a big bunch of teeth for a team of [the dental clinic of the University of Bern](#).

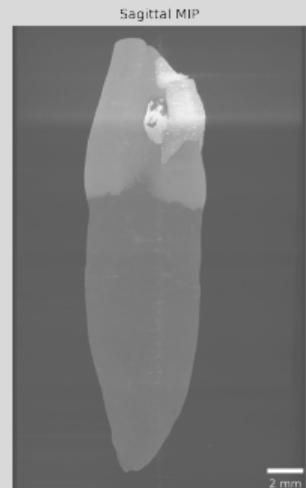
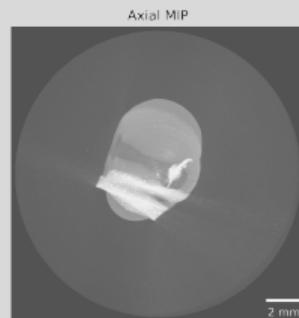
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview](#) and [analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of any software. If you'd like to start a Binder instance with the full repository, you can [click here](#).

μ CT imaging



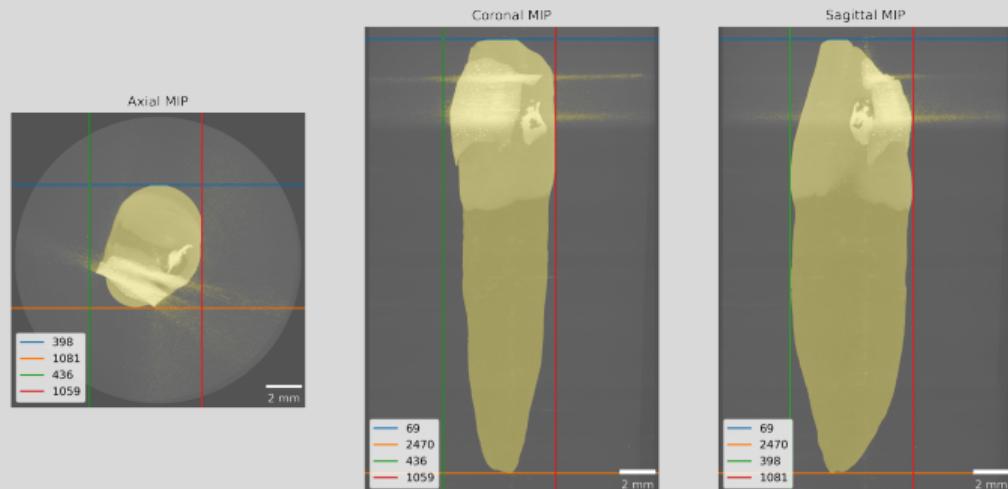
Dataset cropping

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



Dataset cropping

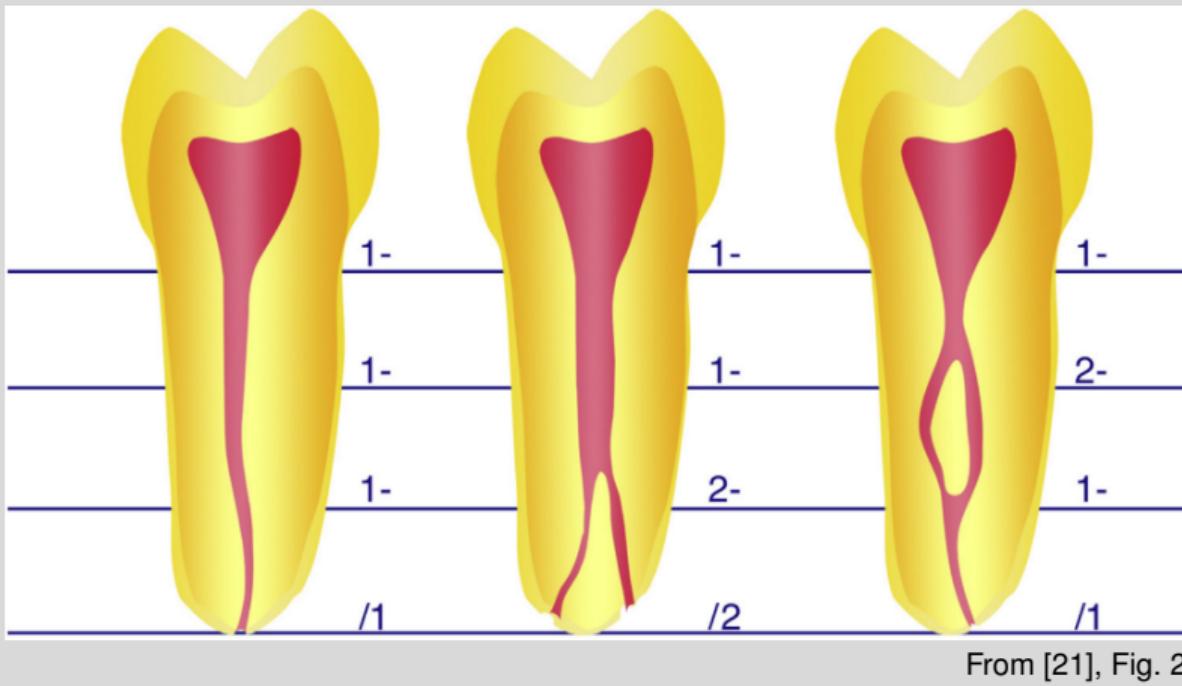
- Full datasets: 326 GB
- Cropped datasets: 115 GB



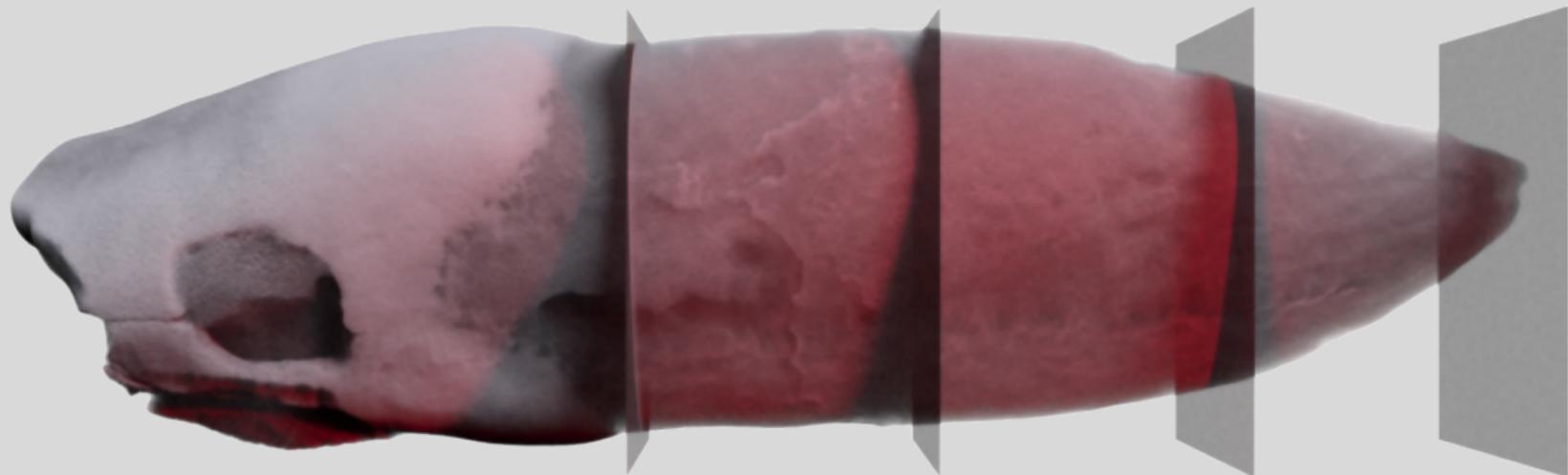
Tooth morphology



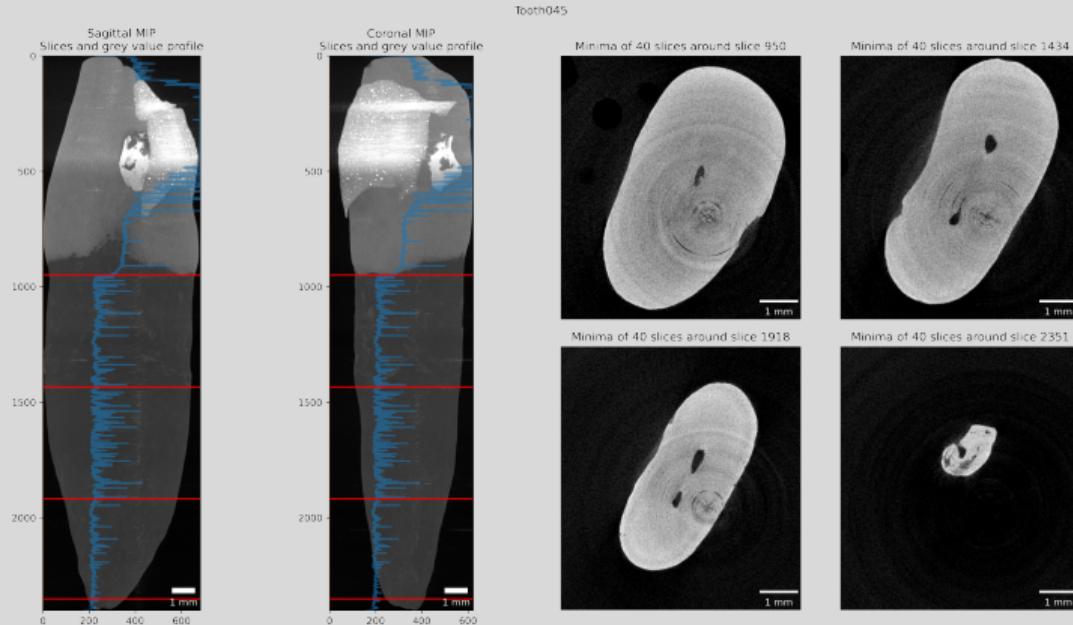
Tooth morphology



Tooth morphology

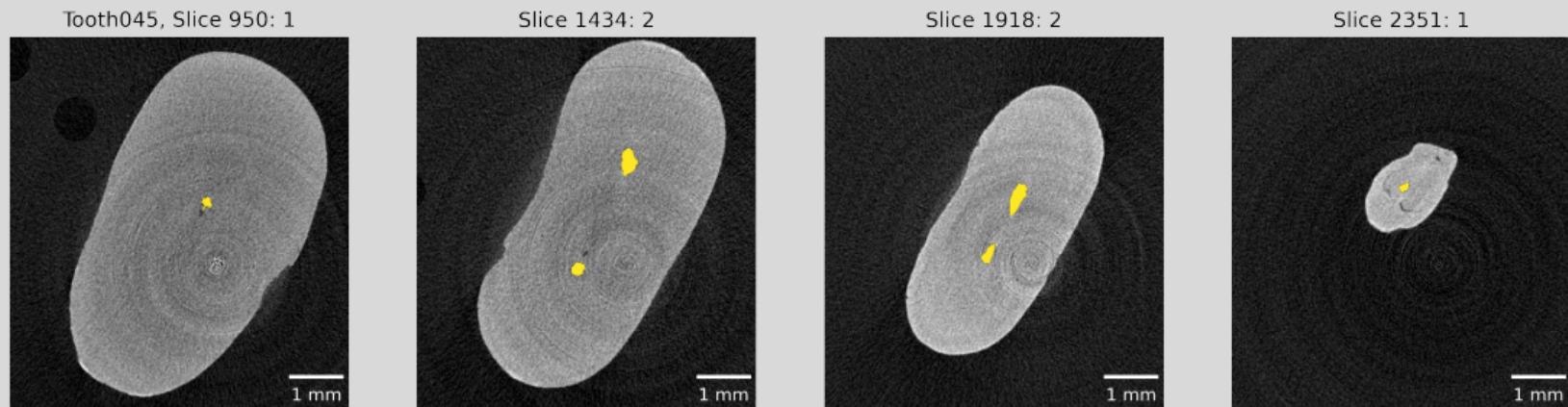


Detection of enamel-dentin border



Detection of enamel-dentin border

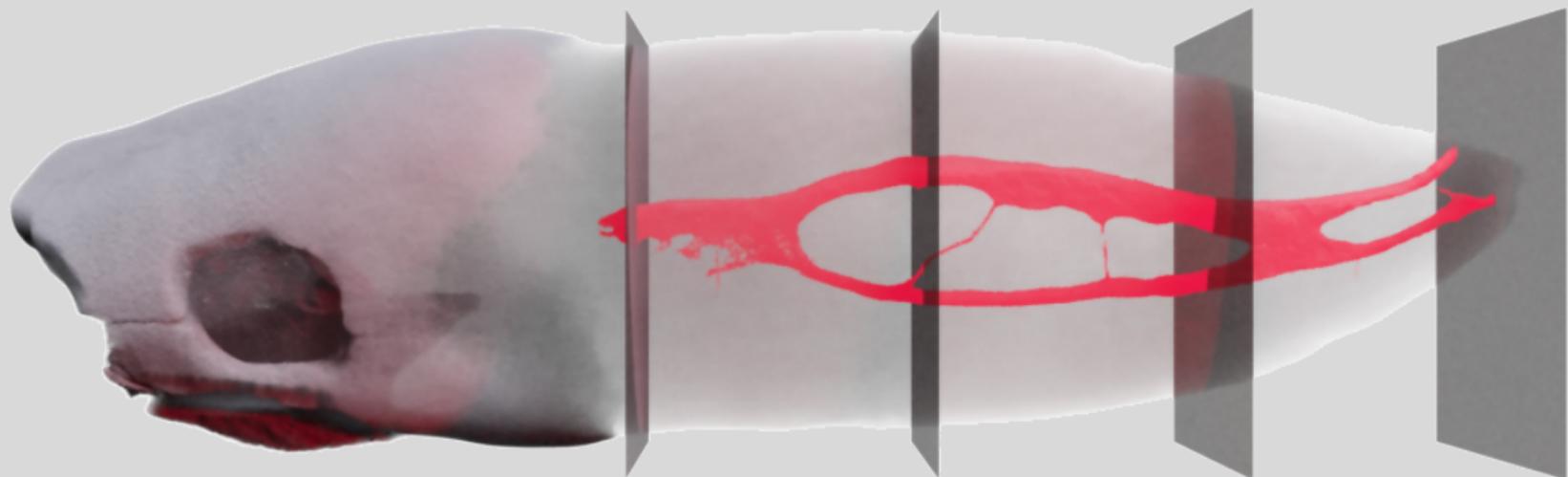
Tooth045



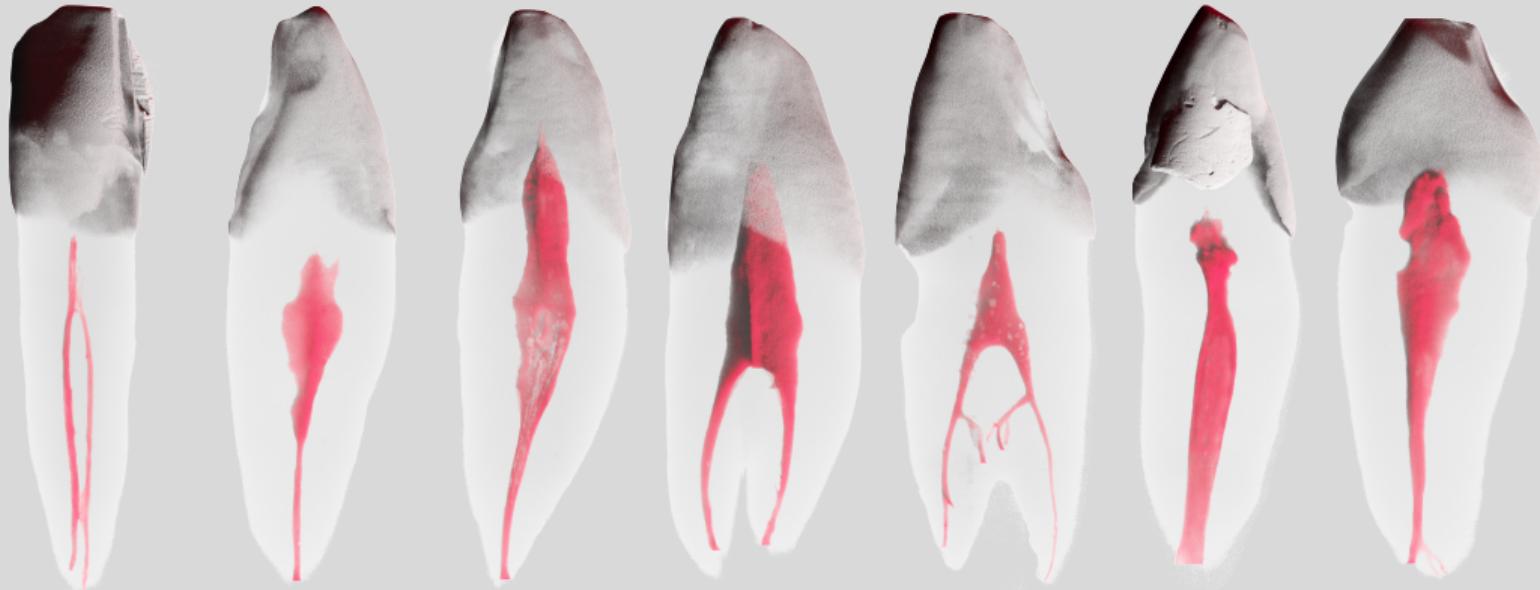
Outcome root canal configuration classification

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
		1-2-1/1	1
	Lingual	1-1-1/1	2
		1-1-1/2	1

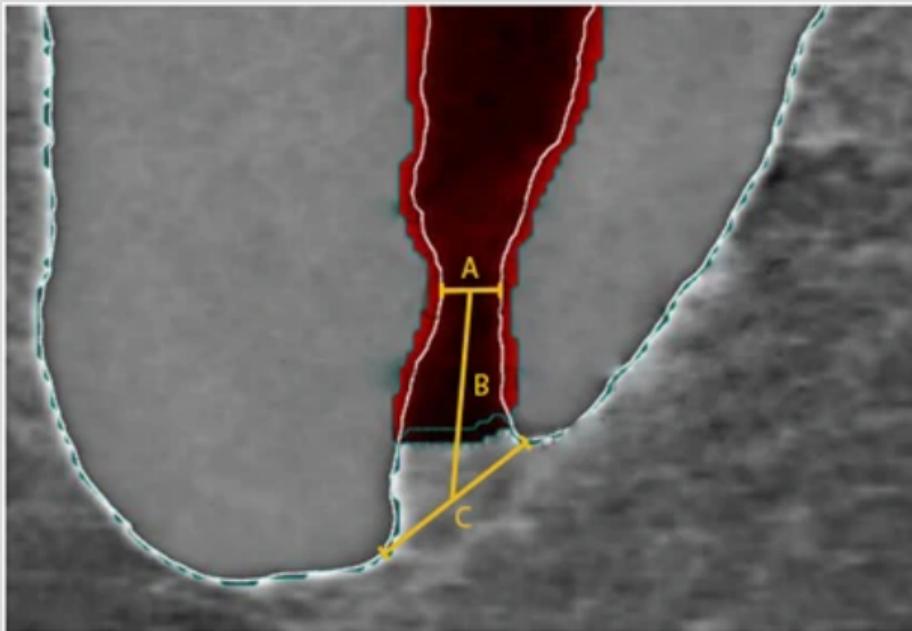
Extraction of root canal space



Outcome of root canal space extraction

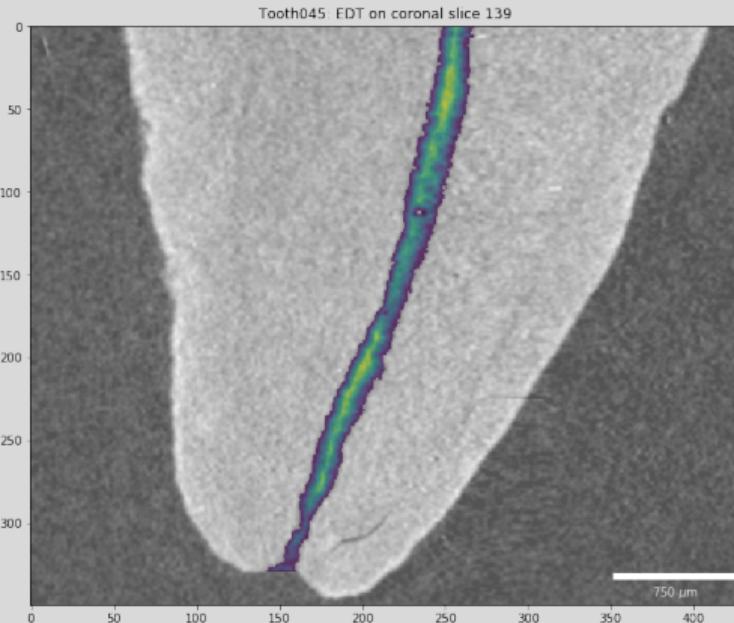


Analysis of the physiological foramen geometry



From [22], Fig. 1

Analysis of the physiological foramen geometry



Conclusion

- 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 [1] 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: haberthuer@ana.unibe.ch

[1] Details on how this works are specified in a small test repository here: git.io/JeOOj

References I

- [1] Ruslan Hlushchuk et al. "Ex vivo microangioCT: Advances in microvascular imaging". DOI: 10.1016/j.vph.2018.09.003.
- [2] Henry Nording et al. "The C5a/C5a receptor 1 axis controls tissue neovascularization through CXCL4 release from platelets". DOI: 10.1038/s41467-021-23499-w.
- [3] Ruslan Hlushchuk et al. "Innovative high-resolution microCT imaging of animal brain vasculature". DOI: 10.1007/s00429-020-02158-8.
- [4] Tsering Wüthrich et al. "Development of vascularized nerve scaffold using perfusion-decellularization and recellularization". DOI: 10.1016/j.msec.2020.111311.
- [5] Cédric Zubler et al. "The anatomical reliability of the superficial circumflex iliac artery perforator (SCIP) flap". DOI: 10.1016/j.aanat.2020.151624.
- [6] Matthias Messerli et al. "Adaptation mechanism of the adult zebrafish respiratory organ to endurance training". DOI: 10.1371/journal.pone.0228333.
- [7] Verdiana Trappetti et al. "Synchrotron Microbeam Radiotherapy for the treatment of lung carcinoma: a pre-clinical study". DOI: 10.1016/j.ijrobp.2021.07.1717.

References II

- [8] Estée Bochud et al. "A new Diancta species of the family Diplommatinidae (Cyclophoroidea) from Vanua Levu Island, Fiji". DOI: [10.3897/zookeys.1073.73241](https://doi.org/10.3897/zookeys.1073.73241).
- [9] Sebastian Halm et al. "Micro-CT imaging of Thiel-embalmed and iodine-stained human temporal bone for 3D modeling". DOI: [10.1186/s40463-021-00522-0](https://doi.org/10.1186/s40463-021-00522-0).
- [10] David Haberthür et al. "Automated segmentation and description of the internal morphology of human permanent teeth by means of micro-CT". DOI: [10.1186/s12903-021-01551-x](https://doi.org/10.1186/s12903-021-01551-x).
- [11] A. M. Cormack. "Representation of a Function by Its Line Integrals, with Some Radiological Applications". DOI: [10.1063/1.1729798](https://doi.org/10.1063/1.1729798).
- [12] Godfrey Newbold Hounsfield. "Historical notes on computerized axial tomography.".
- [13] J Hsieh. *Computed tomography: principles, design, artifacts, and recent advances*. Society of Photo Optical.
- [14] Oliver Taubmann et al. "Computed Tomography". DOI: [10.1007/978-3-319-96520-8_8](https://doi.org/10.1007/978-3-319-96520-8_8).
- [15] Mark Hammer. *X-Ray Physics: X-Ray Interaction with Matter, X-Ray Contrast, and Dose*.
- [16] Wikipedia contributors. *Beer–Lambert law — Wikipedia, The Free Encyclopedia*.

References III

- [17] Kenneth Clark et al. "The Cancer Imaging Archive (TCIA): Maintaining and Operating a Public Information Repository". DOI: [10.1007/s10278-013-9622-7](https://doi.org/10.1007/s10278-013-9622-7).
- [18] Johannes Schindelin et al. "Fiji: an open-source platform for biological-image analysis". DOI: [10.1038/nmeth.2019](https://doi.org/10.1038/nmeth.2019).
- [19] Thomas Kluyver et al. "Jupyter Notebooks – a publishing format for reproducible computational workflows". DOI: [10.3233/978-1-61499-649-1-87](https://doi.org/10.3233/978-1-61499-649-1-87).
- [20] 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". DOI: [10.1038/s41598-021-00758-w](https://doi.org/10.1038/s41598-021-00758-w).
- [21] 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". DOI: [10.1016/j.joen.2015.09.007](https://doi.org/10.1016/j.joen.2015.09.007).
- [22] Thomas Gerhard Wolf et al. "Three-dimensional analysis of the physiological foramen geometry of maxillary and mandibular molars by means of micro-CT". DOI: [10.1038/ijos.2017.29](https://doi.org/10.1038/ijos.2017.29).
- [23] David Haberthür. "habi/zmk-tooth-cohort: Used for manuscript about method". DOI: [10.5281/ZENODO.3999402](https://doi.org/10.5281/ZENODO.3999402).