

# X-ray microtomography

**David Haberthür**

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

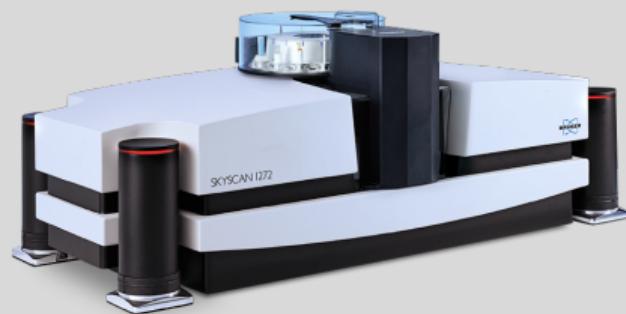
# Grüessech!

- 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:  $\mu$ CT group, Institute of Anatomy, University of Bern, Switzerland.  
Together with Ruslan Hlushchuk, Oleksiy-Zakhar Khoma and Tim Hoessly.

# $\mu$ 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 and scientist at our institute [8] to scan a wide range of specimens
- Automate *all* the things! [9]



[bruker.com/skyscan1272](http://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**

Overview

# Biomedical imaging

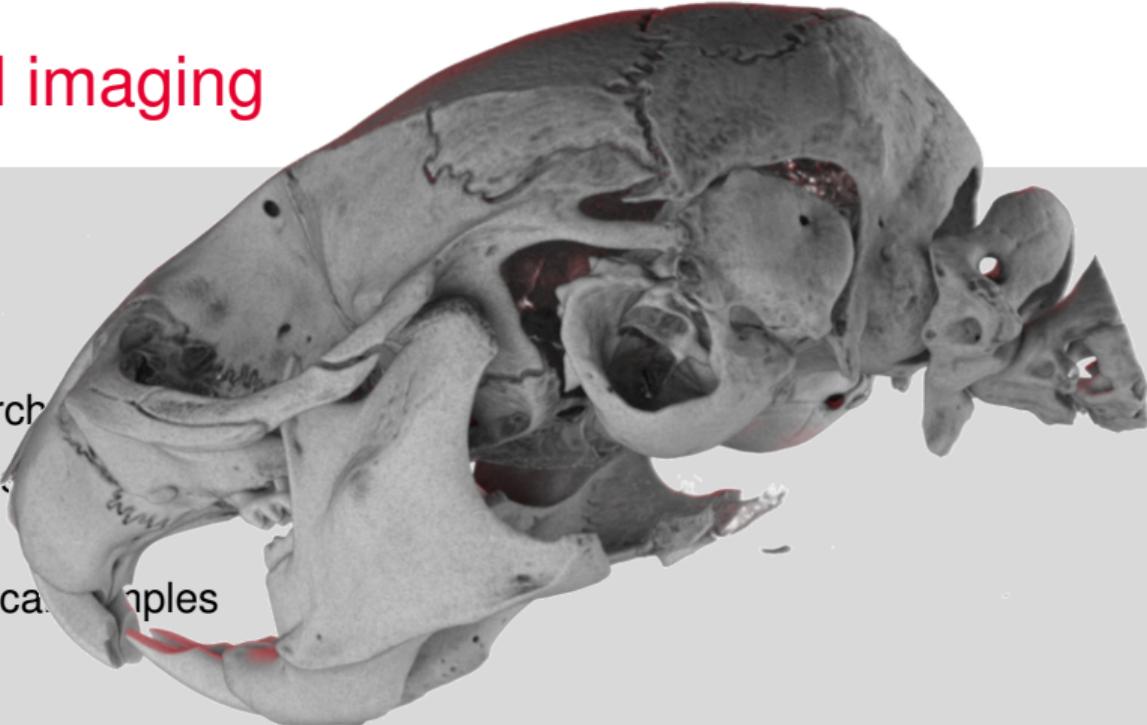


- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples

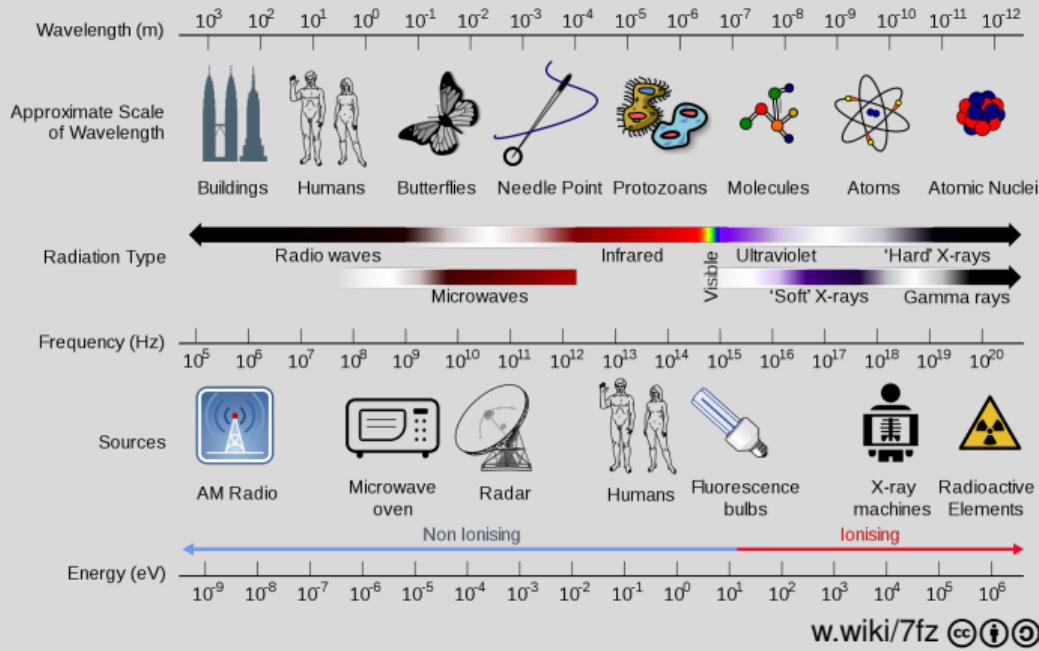
w.wiki/7g4 CC BY NC SA

# Biomedical imaging

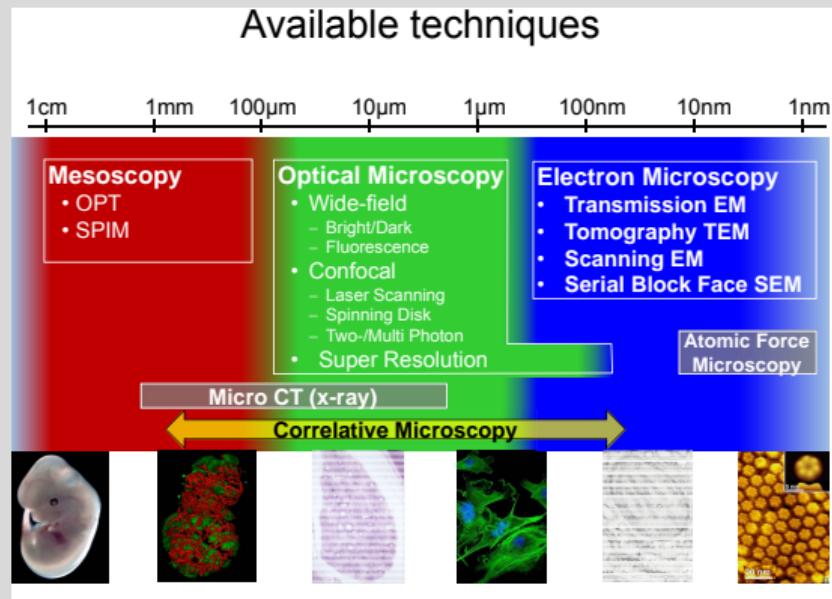
- Medical research
- Non-destructive analysis of the samples
- (Small) Biological samples



# 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.

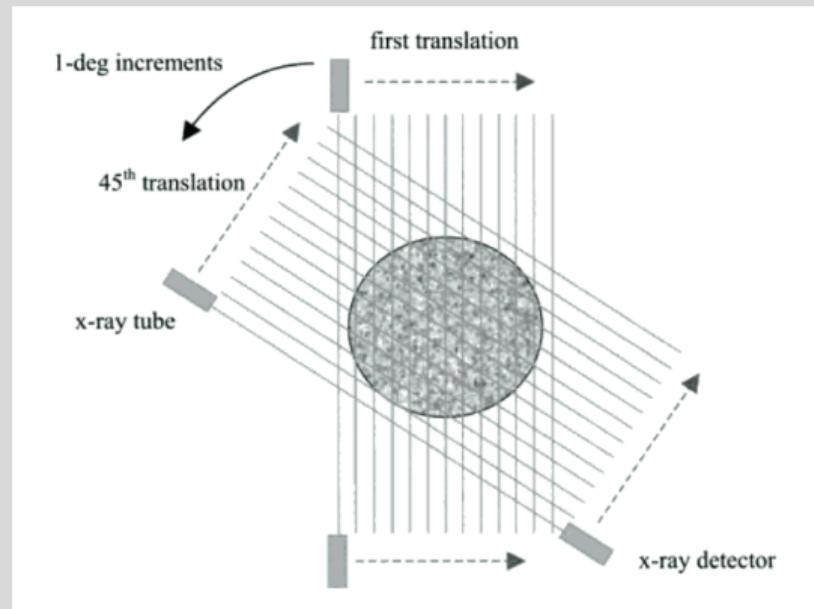
# CT-Scanner



[youtu.be/2CWpZKuy-NE](https://youtu.be/2CWpZKuy-NE)

# History

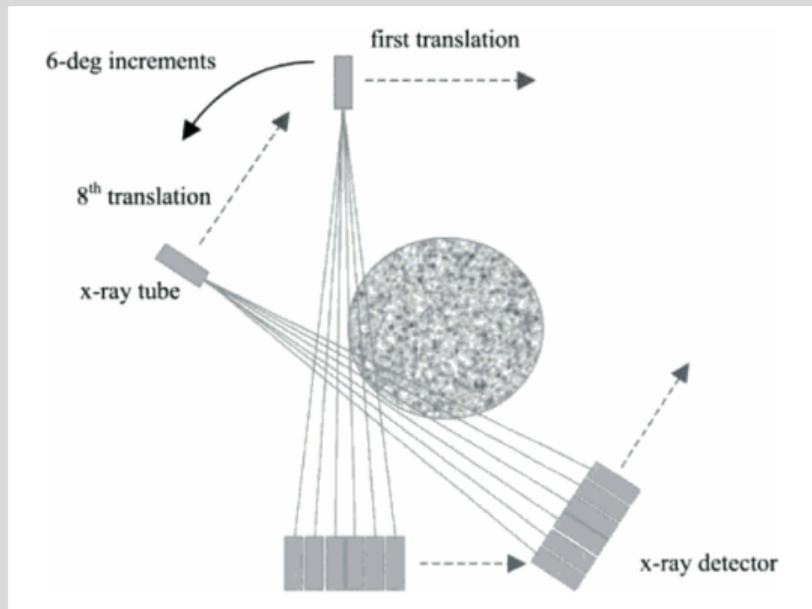
- Long history
  - 1963: Cormack used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector [10]
  - 1976: Hounsfield worked on first clinical scanner [11]
  - Nice overview by Hsieh [12]
- First, second and third generation of scanners



From [12], Figure 1.12

# History

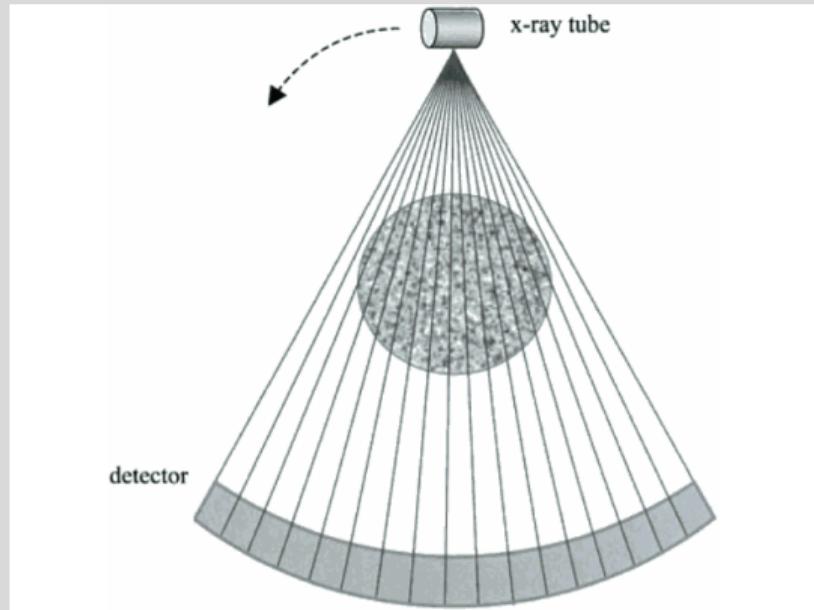
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From [12], Figure 1.13

# History

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From [12], Figure 1.14

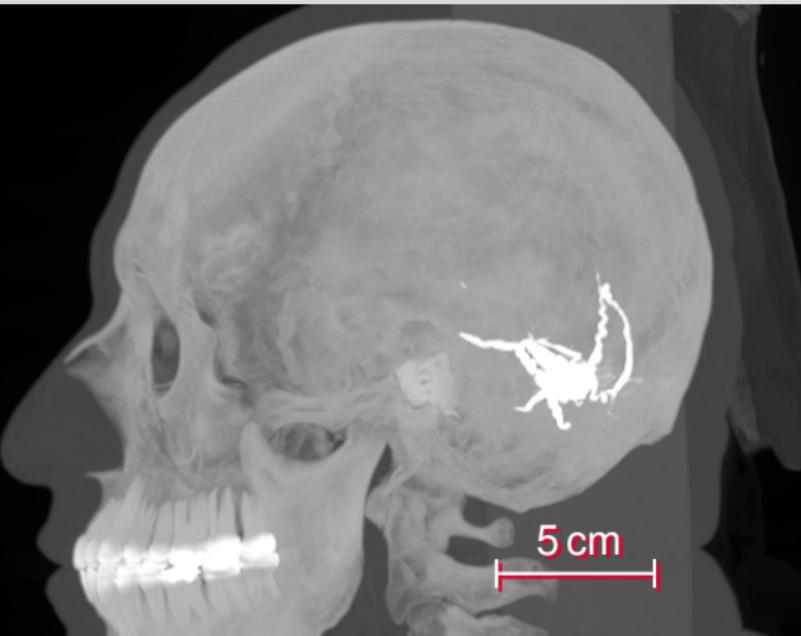
# X-ray interaction

- “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.” ([13])
  - Photoelectric absorption ( $\tau$ ) 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 ([14, i. e. Beer-Lamberts law]  $I(t) = I_0 e^{-\alpha z}$ )

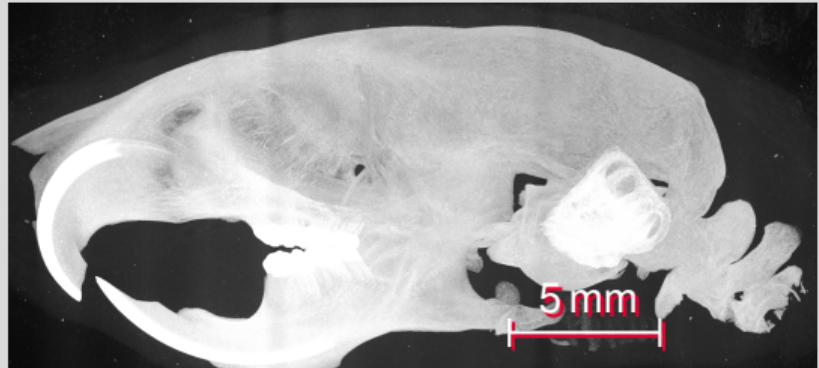
# Composition of biological tissues

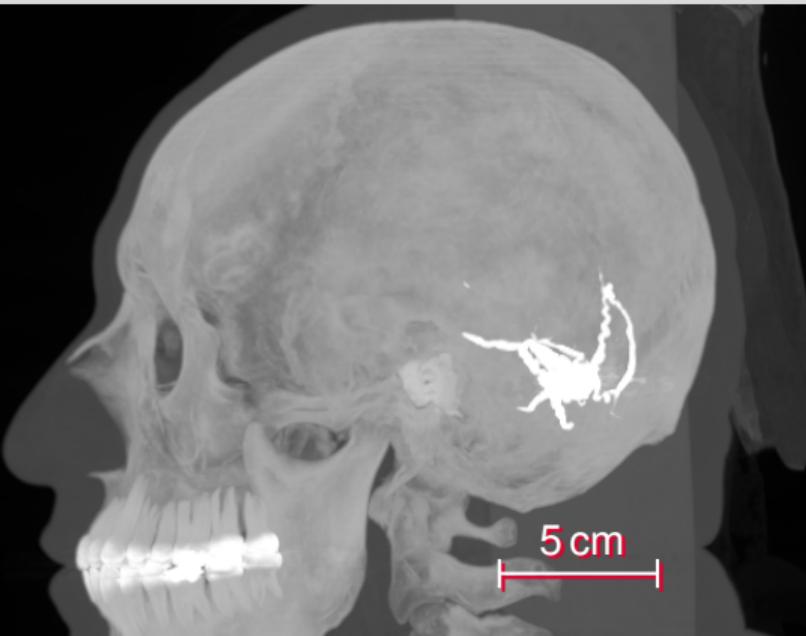
Tissue: content by mass percentage

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



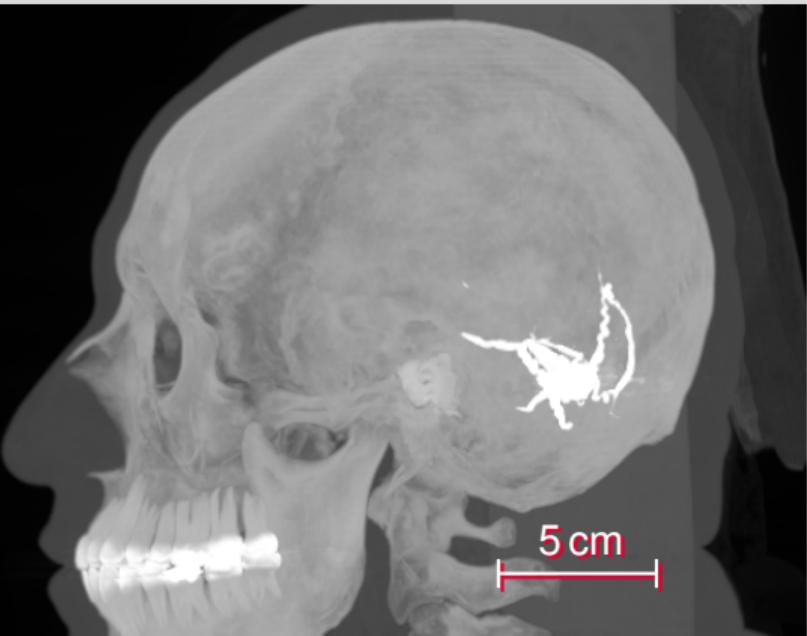
From [15], Subject C3L-02465



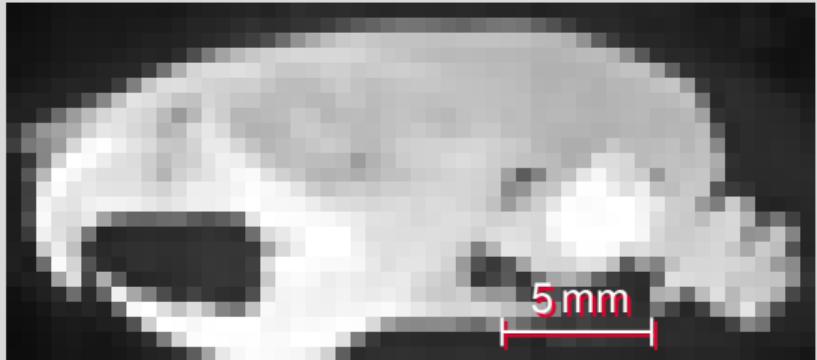


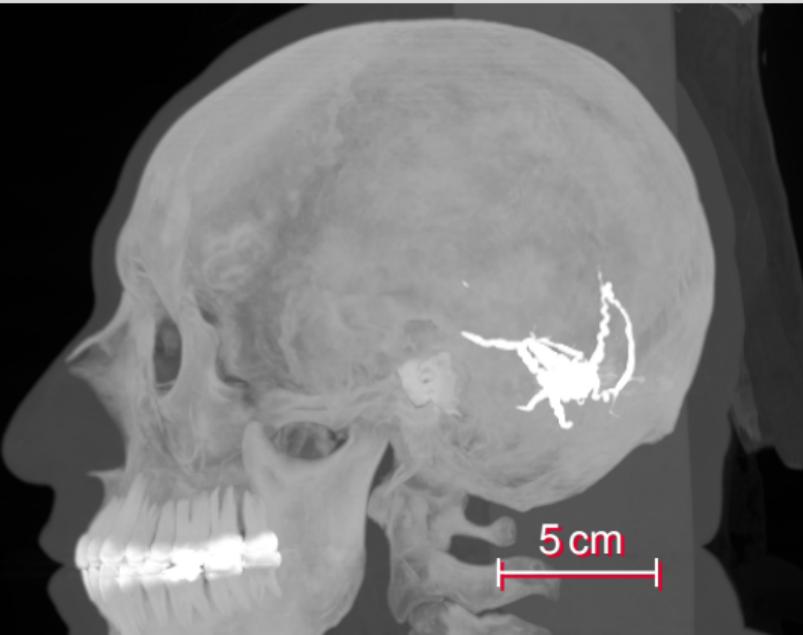
From [15], Subject C3L-02465



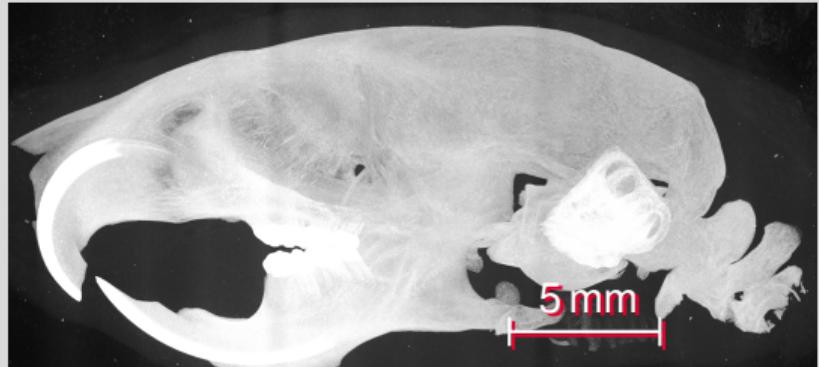


From [15], Subject C3L-02465





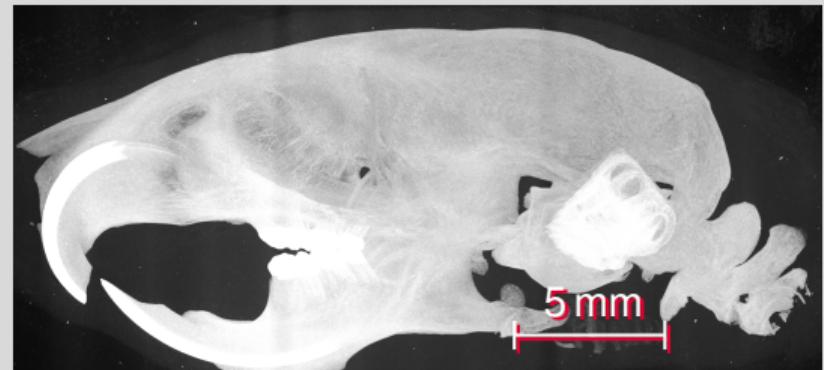
From [15], Subject C3L-02465



# Why $\mu$ CT?



From [15], 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.

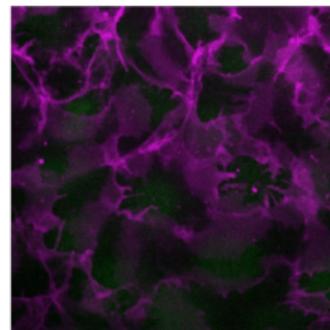
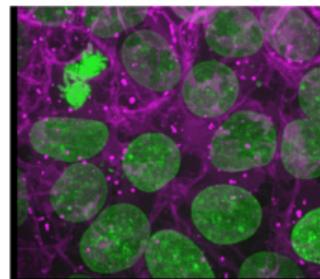


Image stack turned into transparent volume



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  $\mu\text{m}$  (*in vivo*) or 0.5  $\mu\text{m}$  (*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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[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

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flic.kr/p/7Xhk2Y

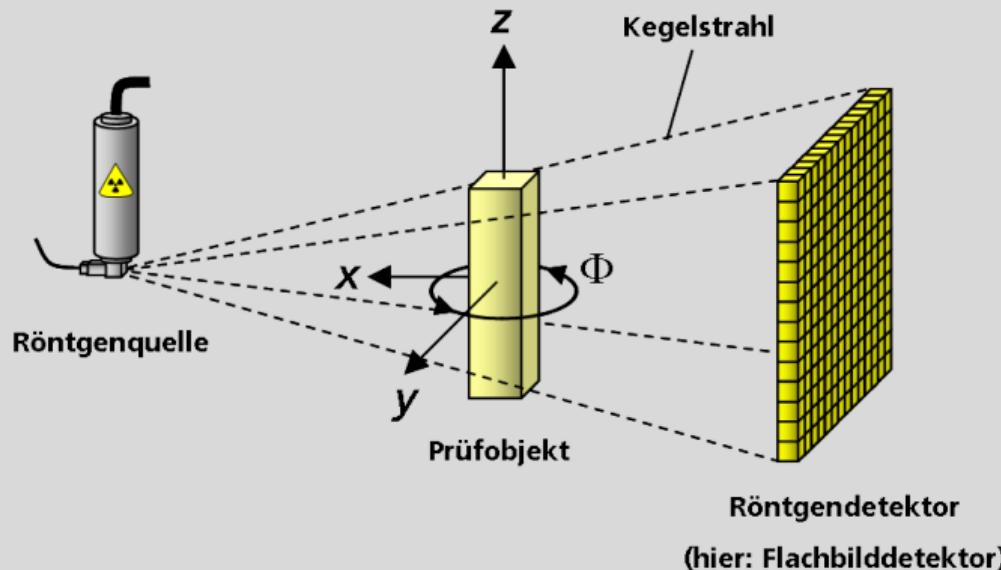
# Machinery

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

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

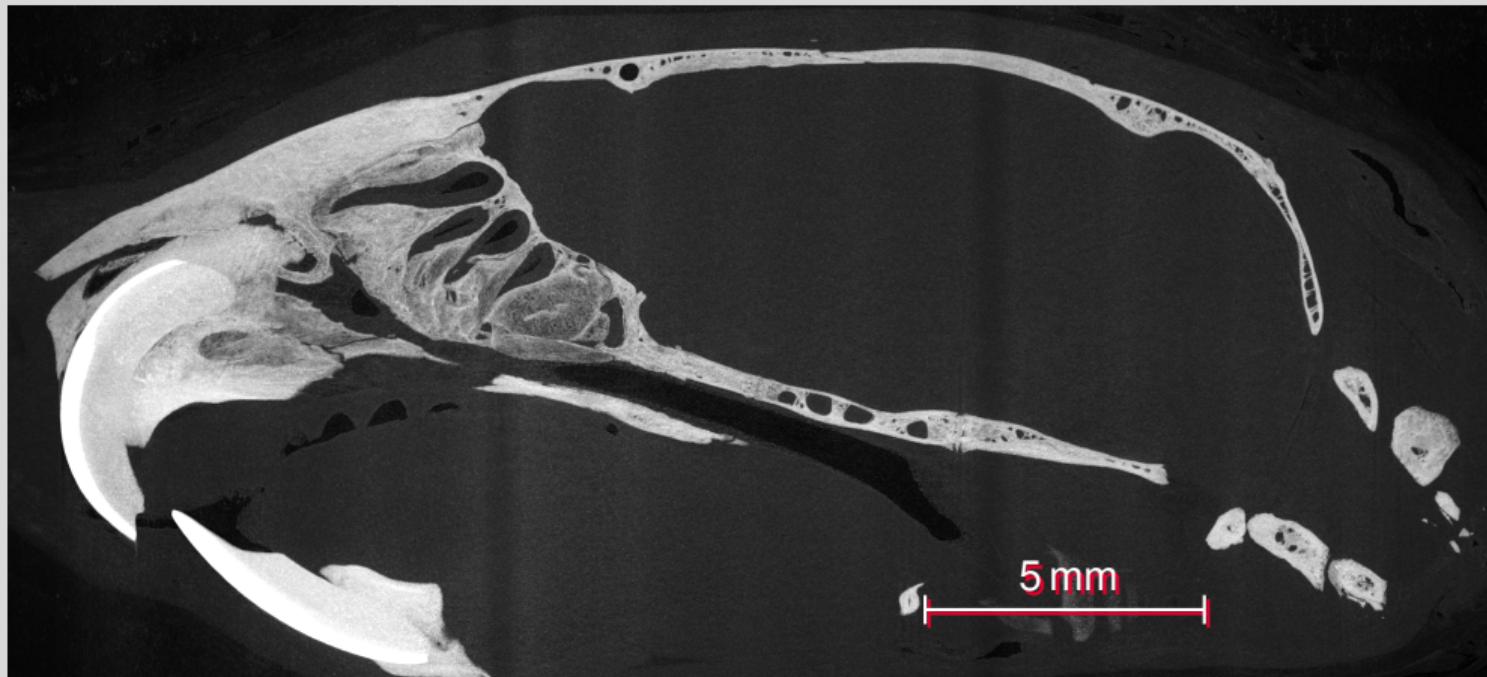
# Machinery

# What is happening?

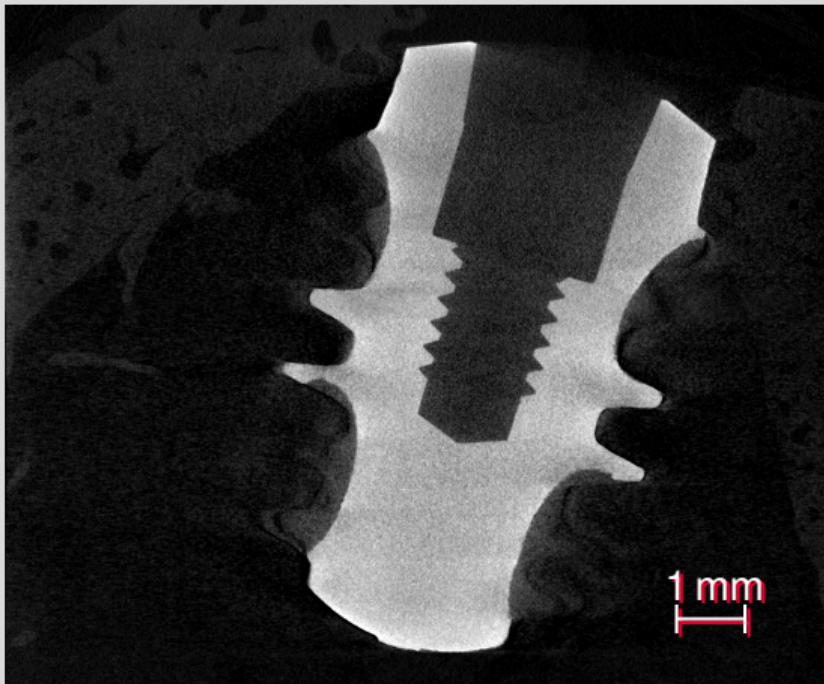


w.wiki/7g3

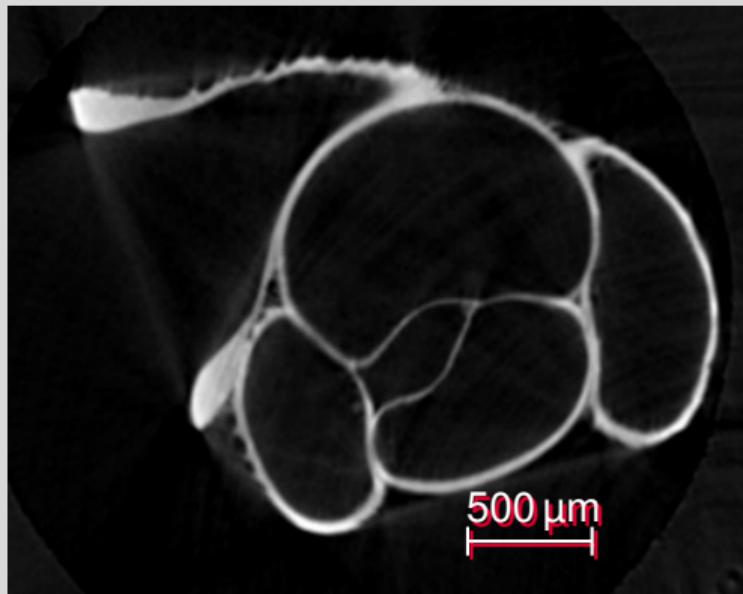
# Examples



# Examples

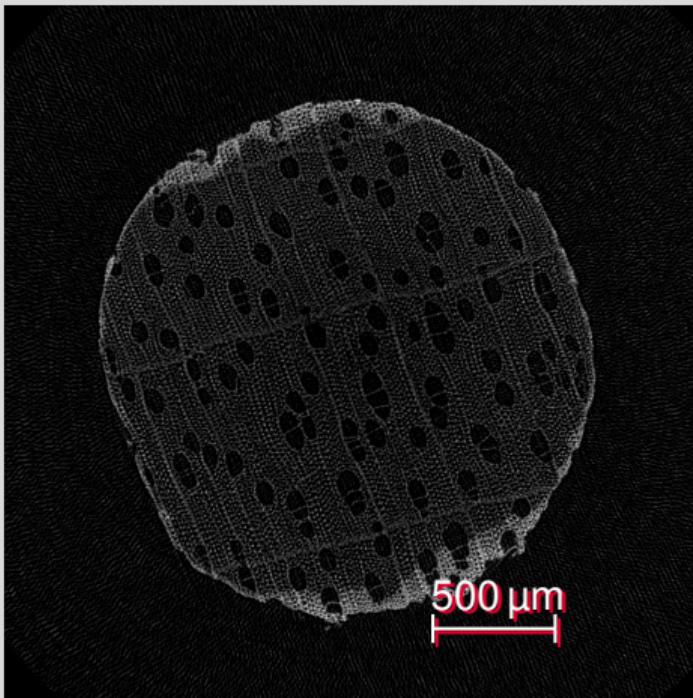


# Examples



From [16], *Diancta phoenix*

# Examples



# Examples



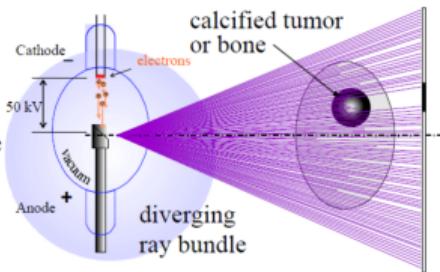
# Preparation

- Study design
- Sample preparation

# Projections

## X-ray generation and contrast

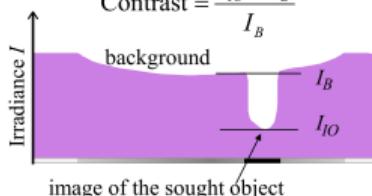
X-ray tube:  
nearly point like  
photon source



Contrast is given by  
absorption of intensity I

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

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



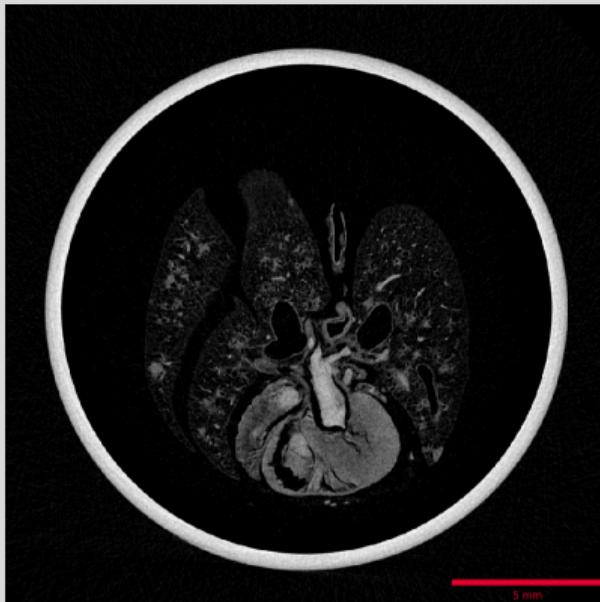
# Projections



# 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 [17]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  -  in Jupyter [18]
  - `git`
  - Script all your things!
  - Data repositories; i. e. sharing is caring!

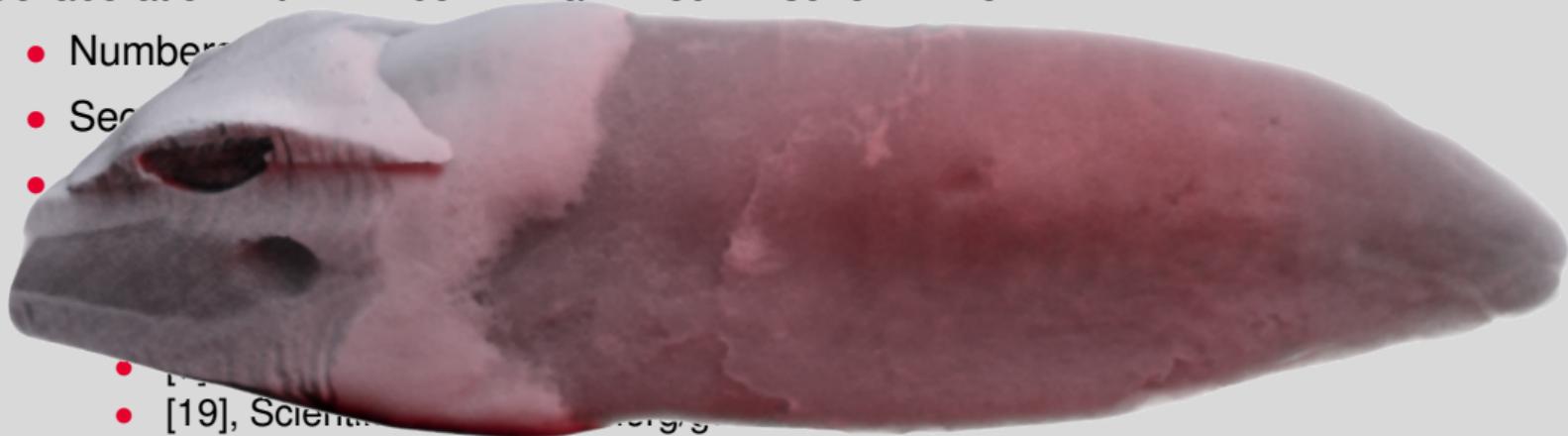
# Quantitative data

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

# Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Number of publications
- Second authorship
- First authorship
- [19], Scientific Reports 2019



# 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 [18])
- Two publications:
  - [9], BMC Oral Health, doi.org/gjpw2d
  - [19], Scientific Reports, doi.org/g7r8

# How?

- 104 extracted human permanent mandibular canines
- $\mu$ CT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [20]
  - Foramen geometry and size, according to Wolf et al. [21]
- *Reproducible* analysis [22], e. g. you can click a button to double-check or recalculate the results yourself!



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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
```

# How?

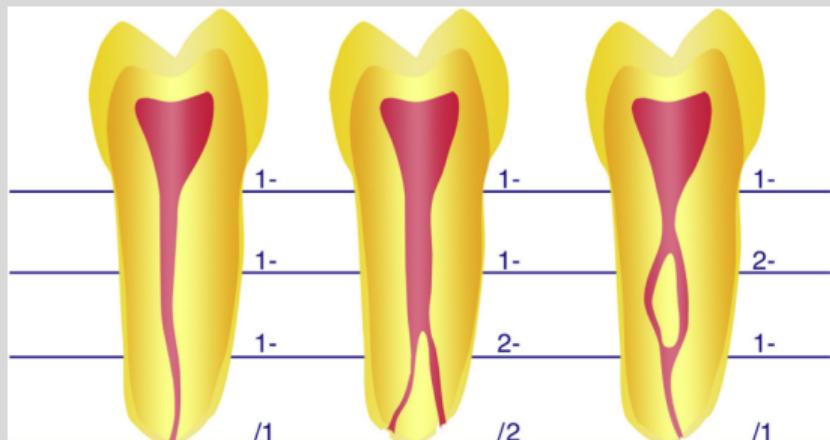
- 104 extracted human permanent mandibular canines
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*Sample changer on the SkyScan 1272*  
In total:

- 13 days of *continuous*  $\mu$ CT scanning
- 819 GB of raw data  
230 648 TIFF projections
- 326 GB data as input for analysis  
282 062 PNG reconstructions

# How?

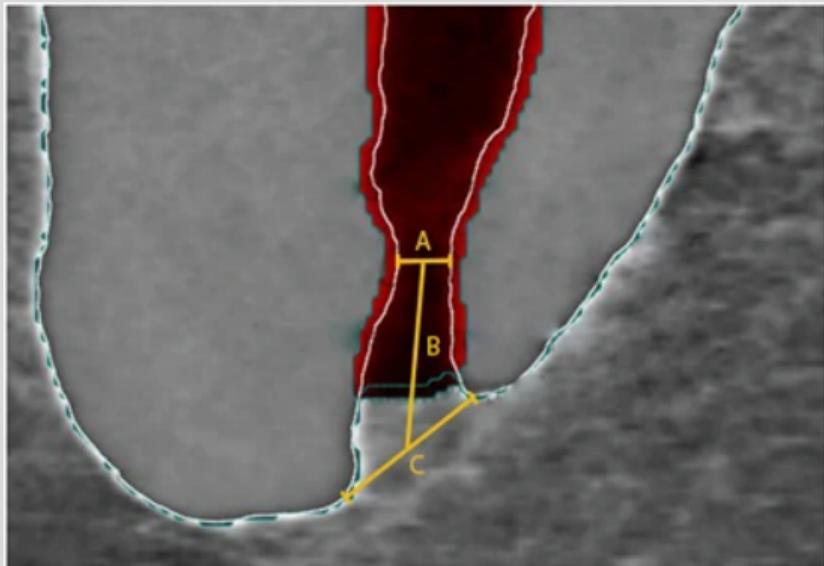
- 104 extracted human permanent mandibular canines
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From [20], Fig. 2

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From [21], Fig. 1

# How?

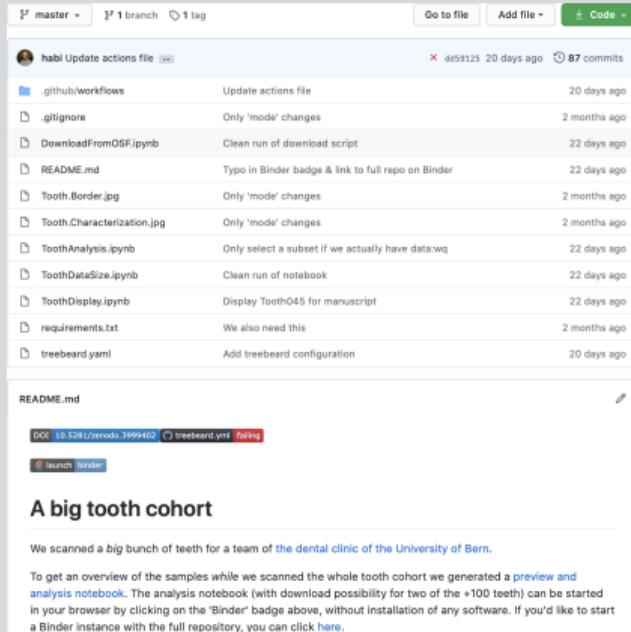
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[gph.is/2nqkple](http://gph.is/2nqkple)

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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 87 commits from user 'habi' with details like commit message, date, and time.

Commit Message	Date	Time
Update actions file	20 days ago	10:59 AM
.github/workflows	20 days ago	10:59 AM
.gitignore	2 months ago	10:59 AM
DownloadFromOSF.ipynb	22 days ago	10:59 AM
README.md	22 days ago	10:59 AM
Tooth.Border.jpg	2 months ago	10:59 AM
Tooth.Characterization.jpg	2 months ago	10:59 AM
ToothAnalysis.ipynb	22 days ago	10:59 AM
ToothDataSize.ipynb	22 days ago	10:59 AM
ToothDisplay.ipynb	22 days ago	10:59 AM
requirements.txt	2 months ago	10:59 AM
treebeard.yaml	20 days ago	10:59 AM

Below the commits is a 'README.md' file with a DOI link, a 'treebeard.yaml' status indicator, and a 'launch binder' button. The file content includes a section titled 'A big tooth cohort' and a note about the scanned teeth.

**README.md**

DOI: [10.5281/zenodo.3999402](https://doi.org/10.5281/zenodo.3999402) treebeard.yaml failing

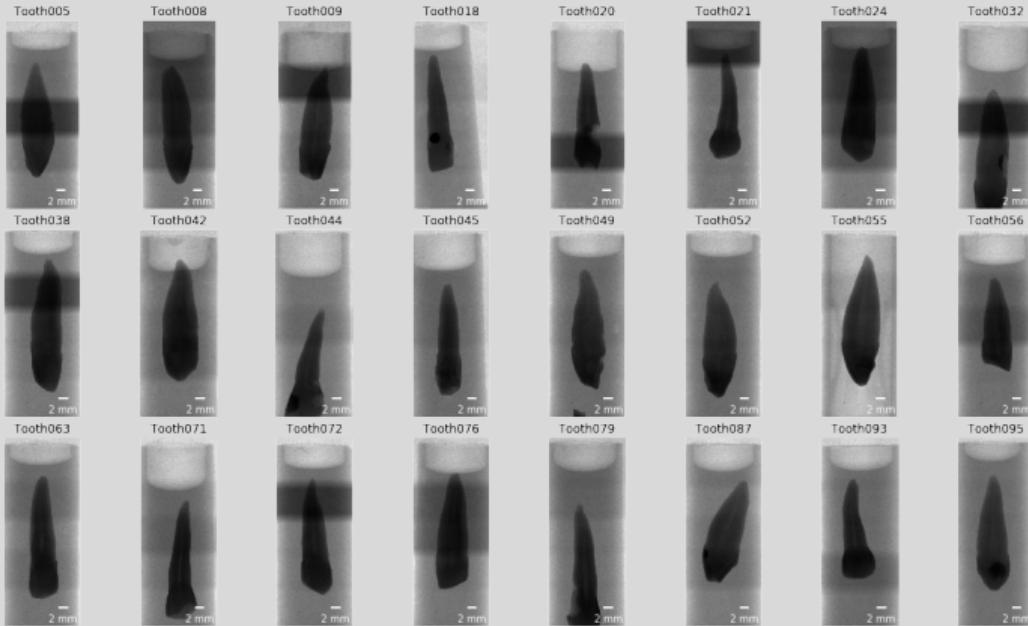
[launch binder](#)

**A big tooth cohort**

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

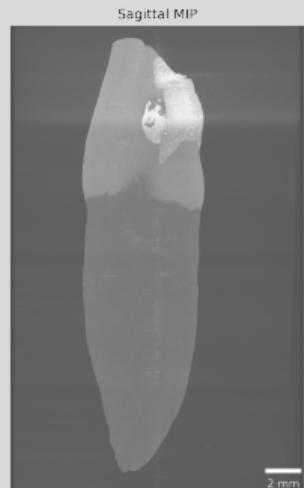
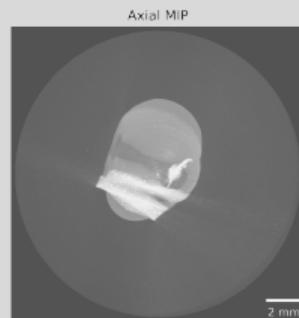
To get an overview of the samples while we scanned the whole tooth cohort we generated a [preview and analysis notebook](#). The analysis notebook (with download possibility for two of the +100 teeth) can be started in your browser by clicking on the 'Binder' badge above, without installation of any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

# $\mu$ CT imaging



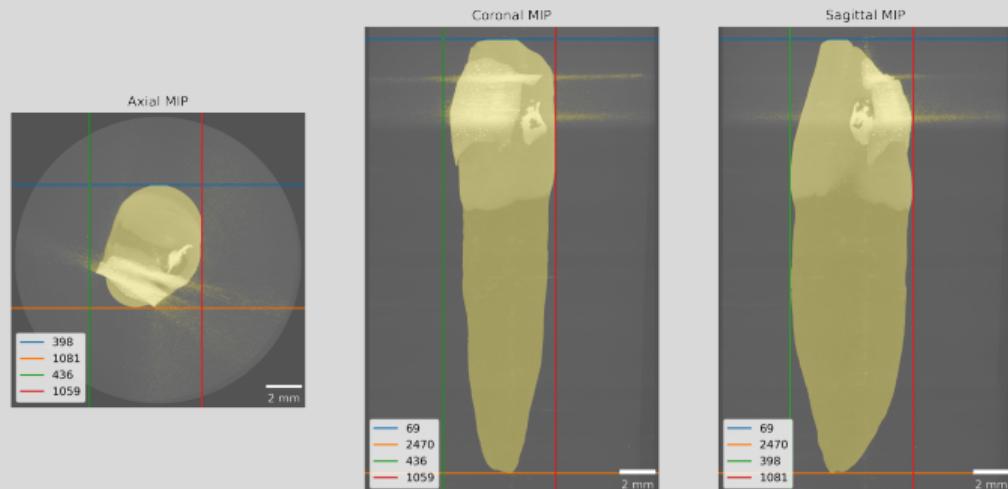
# Dataset cropping

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

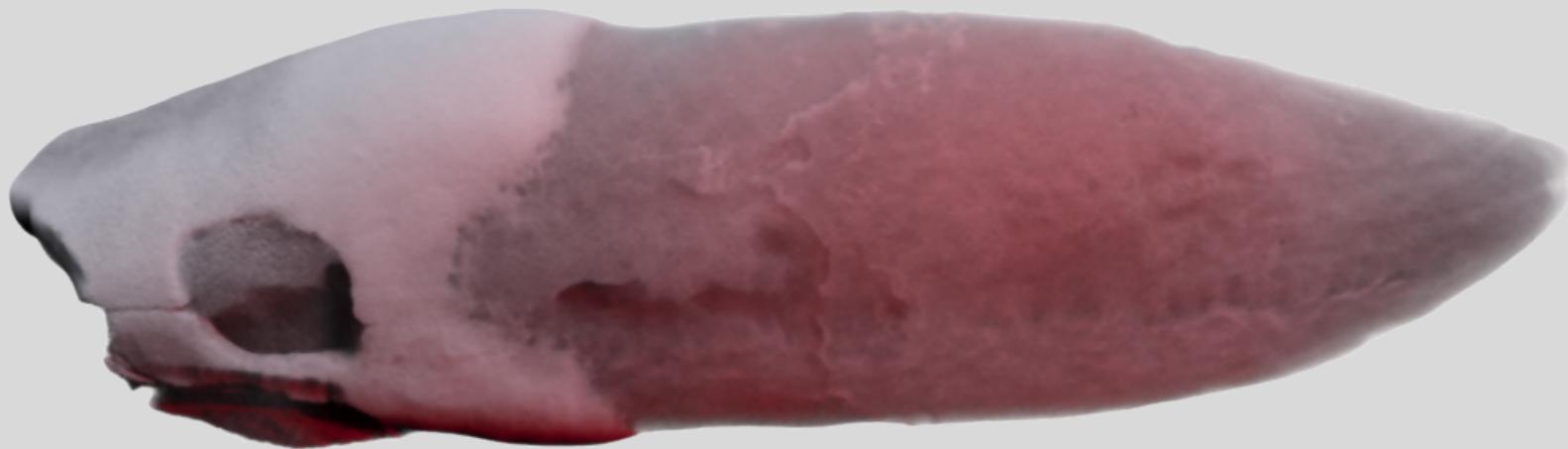


# Dataset cropping

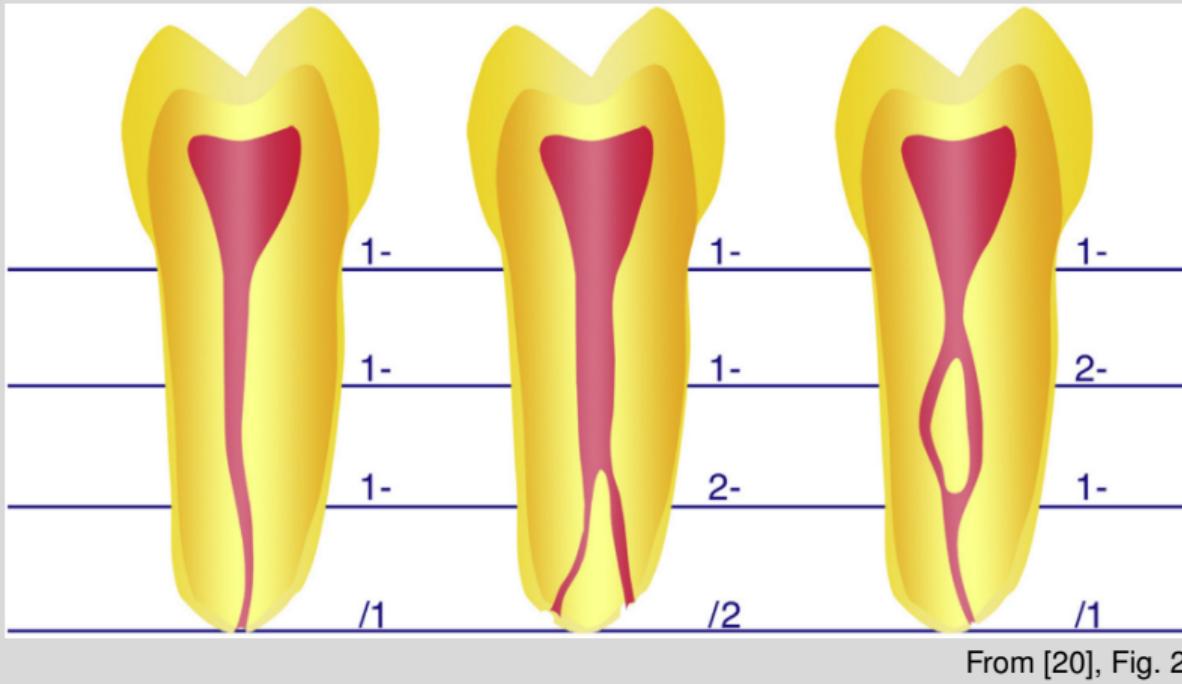
- Full datasets: 326 GB
- Cropped datasets: 115 GB



# Tooth morphology

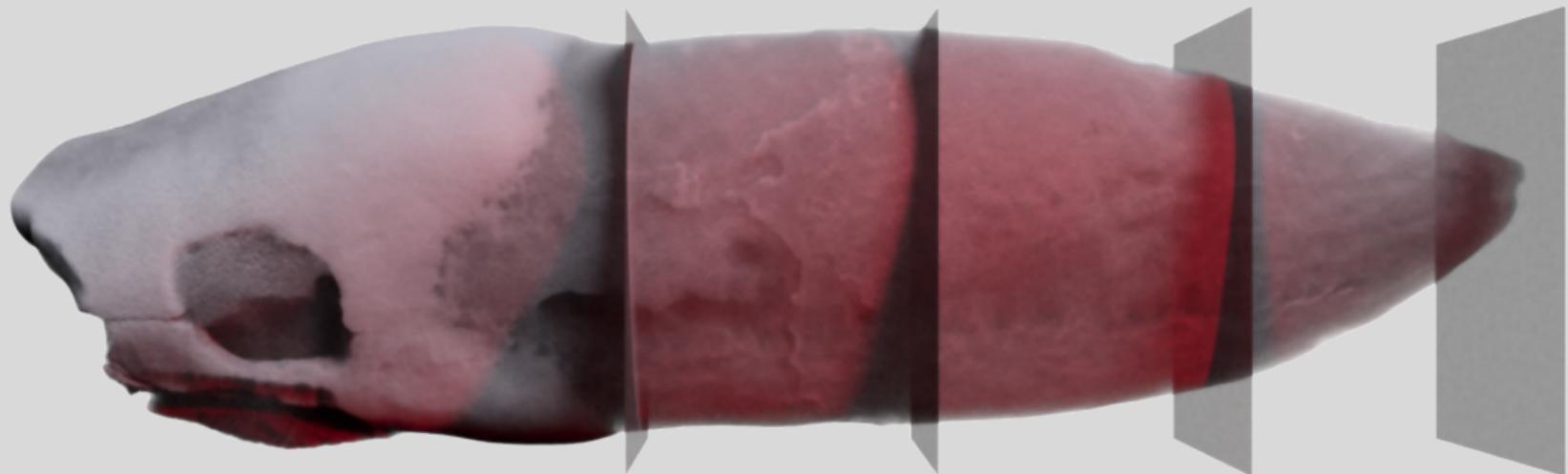


# Tooth morphology

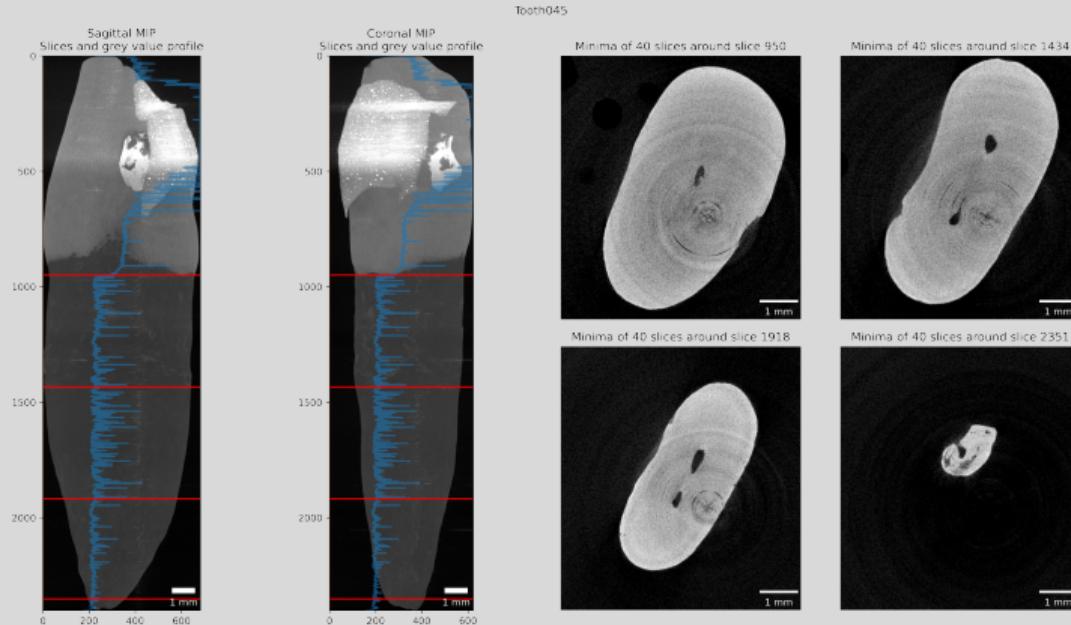


From [20], Fig. 2

# Tooth morphology



# Detection of enamel-dentin border



# Detection of enamel-dentin border

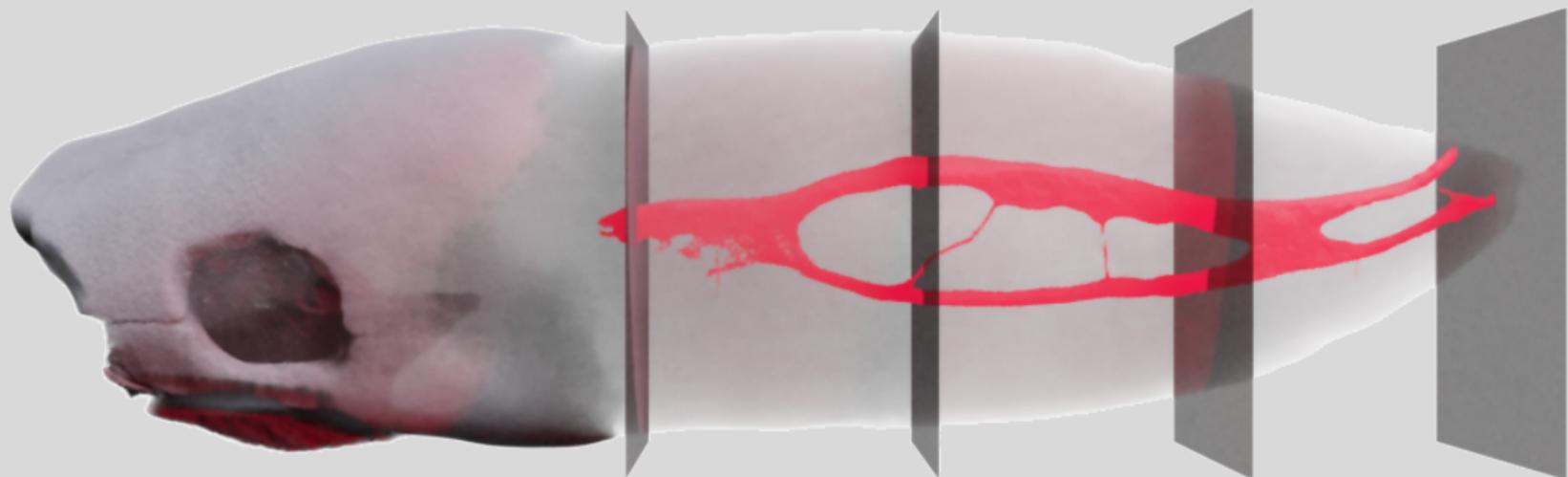
Tooth045



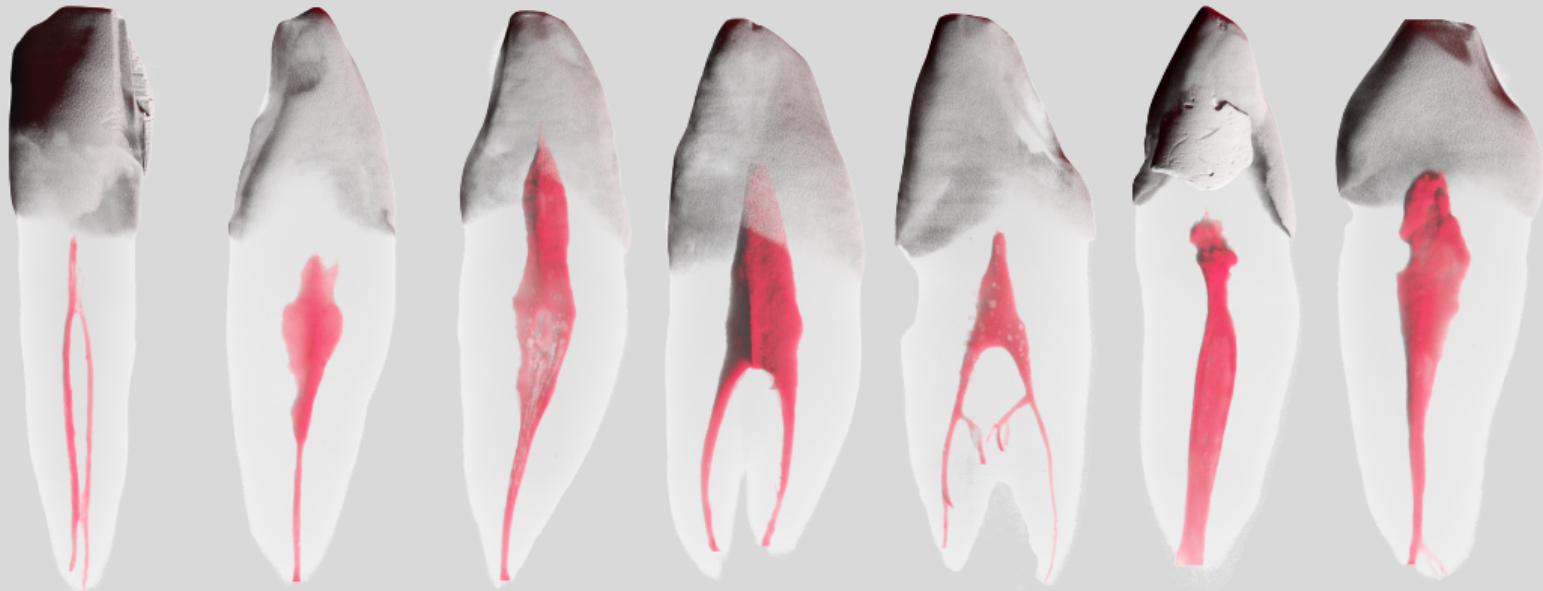
# Outcome root canal configuration classification

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

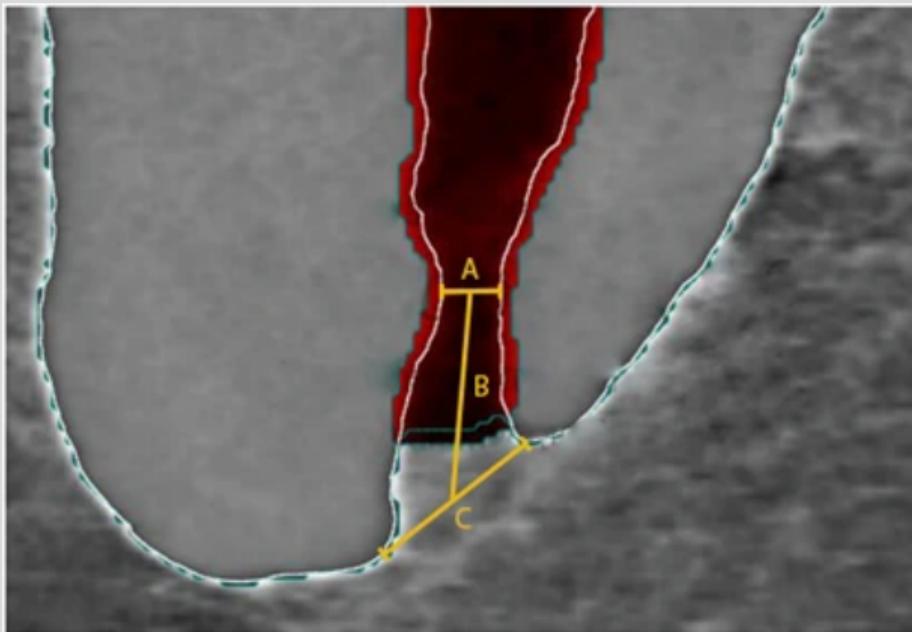
# Extraction of root canal space



# Outcome of root canal space extraction

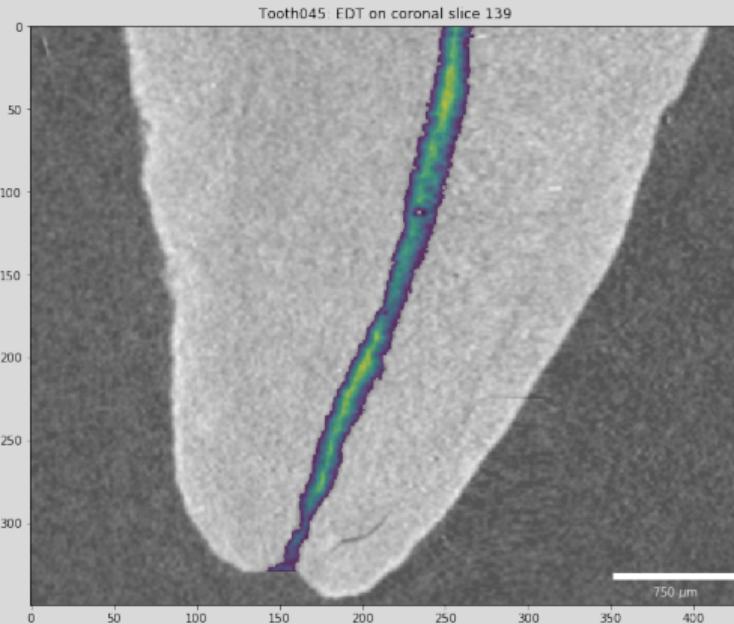


# Analysis of the physiological foramen geometry



From [21], 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  $\text{\LaTeX}$  with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
  - Complete source code: [git.io/fjpP7](https://git.io/fjpP7)
  - The  $\text{\LaTeX}$  code is automatically compiled with a GitHub action [1] to a (handout) PDF which you can access here: [git.io/JeQxO](https://git.io/JeQxO)
- Did you spot an error?
  - File an issue: [git.io/fjpPb](https://git.io/fjpPb)
  - Submit a pull request: [git.io/fjpPN](https://git.io/fjpPN)
  - Send me an email: [haberthuer@ana.unibe.ch](mailto:haberthuer@ana.unibe.ch)

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

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