

*w*<sup>b</sup>

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*b*

**UNIVERSITY  
OF BERN**

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giveni=T. G.hash=7aef301479a84587c1fa1fac608150bcfamily=Paqué, familyi=P.,  
given=Frank,  
giveni=F.hash=1f9fd8925e24d25d3aef11d313378c2afamily=Sven Patyna,

*u*<sup>b</sup>

familyi=S. P., given=Michael,  
giveni=M.hash=023b0e41ddb46e953e5d493dc3c263bafamily=Willershausen,  
familyi=W., given=Brita,  
giveni=B.hash=7a41b26f38c6ace1e4182a108e47e7b1family=Briseño-  
Marroquín, familyi=B., given=Benjamín, giveni=B.

# *u<sup>b</sup>* X-ray microtomography

485018-HS2024-0: Advanced Course II  
Ultraprecision Engineering

**David Haberthür**

, September 25, 2024

*u<sup>b</sup>* 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.

$u^b$

# Grüessech from the $\mu$ CT-group

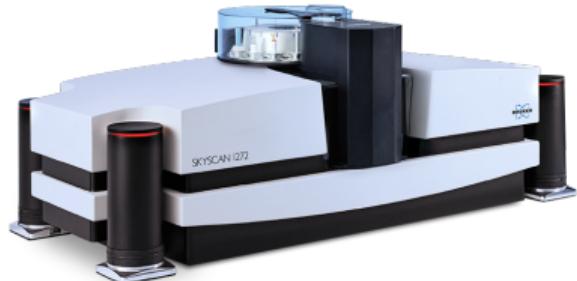


[David.Haberthuer@unibe.ch](mailto:David.Haberthuer@unibe.ch) [Ruslan.Hlushchuk@unibe.ch](mailto:Ruslan.Hlushchuk@unibe.ch) [Oleksiy.Khoma@unibe.ch](mailto:Oleksiy.Khoma@unibe.ch)

*u*<sup>b</sup>

# μCT-group

- microangioCT **Hlushchuk2018**
  - Angiogenesis: heart, musculature **Nording2021** and bones
  - Vasculature: (mouse) brain **Hlushchuk2020**, (human) nerve scaffolds **Wuthrich2020**, (human) skin flaps **Zubler2021** and tumors
- Zebrafish musculature and gills **MesserliAaldijk2020**
- (Lung) tumor detection and metastasis classification **Trappetti2021**
- Collaborations with museums **Bochud2021** and scientist at UniBe **Halm2021** to scan a wide range of specimens
- Automate *all* the things! **Haberthuer2021**, **Haberthuer2022**



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

*u*<sup>b</sup>

# Contents

Overview

Imaging

Tomography

    History

    Interaction of x-rays with matter

    Tomography today

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

Examples

    A study about teeth

        Overview

        Materials & Methods

        Results

    Metal foam analysis

    A study on fish

$u^b$

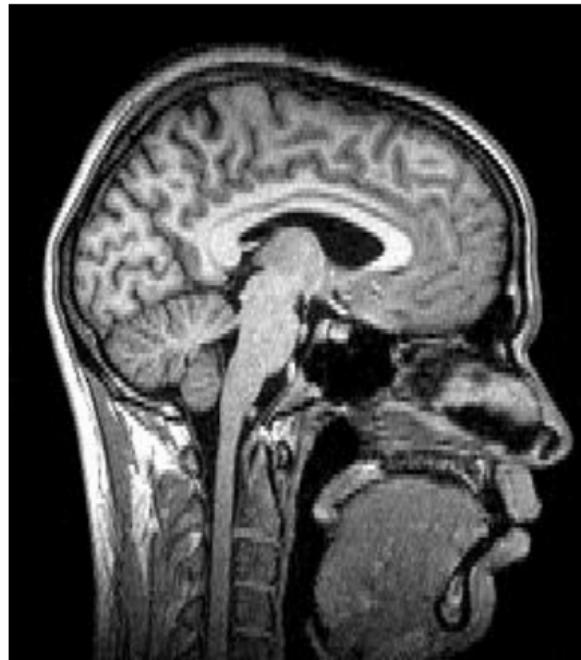
# $\mu$ CT

- 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

*u<sup>b</sup>*

# Biomedical imaging

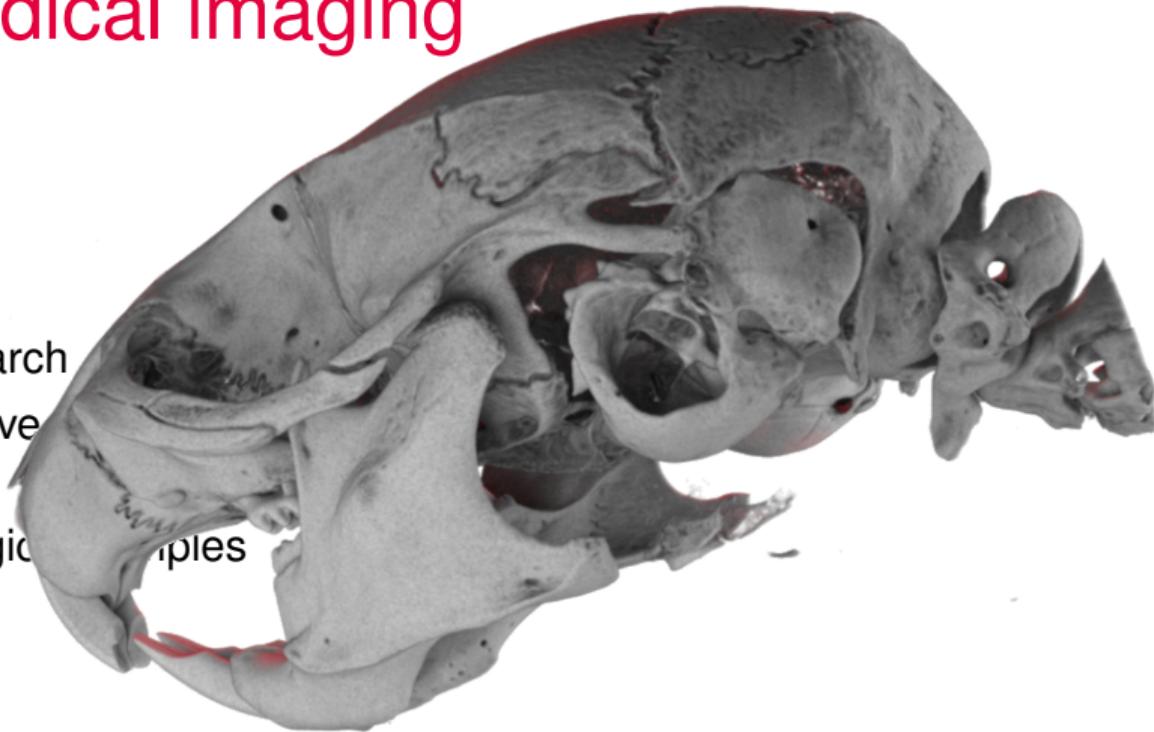
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



w.wiki/7g4 CC BY-NC-SA

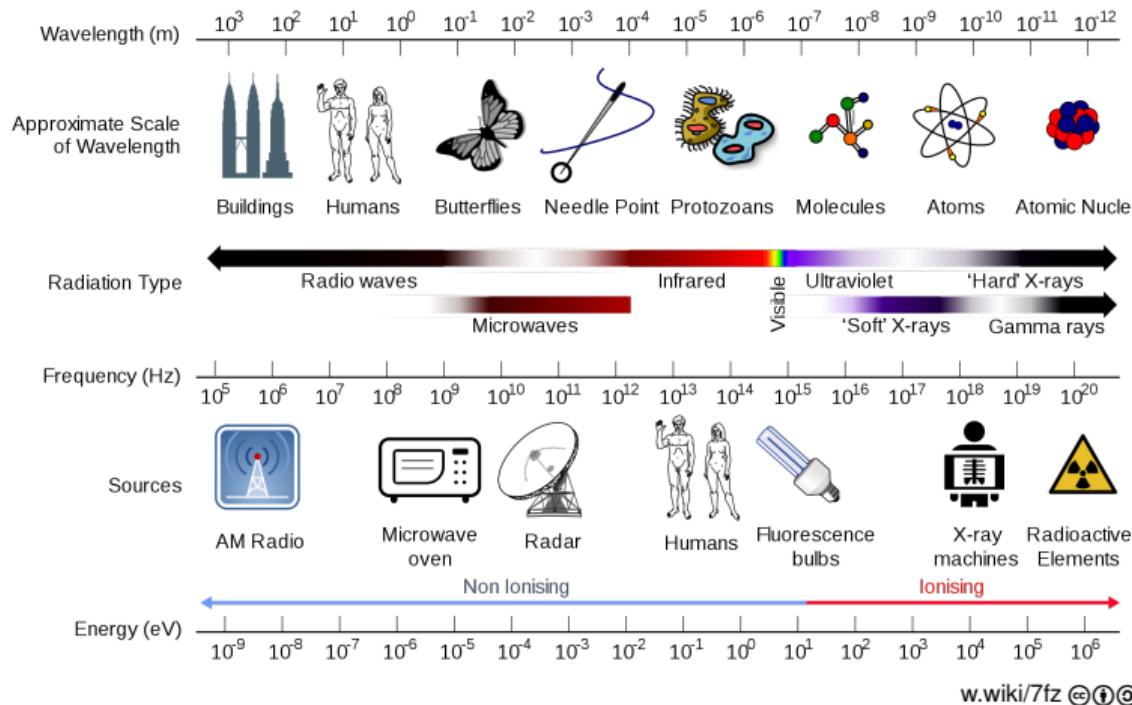
# Biomedical imaging

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



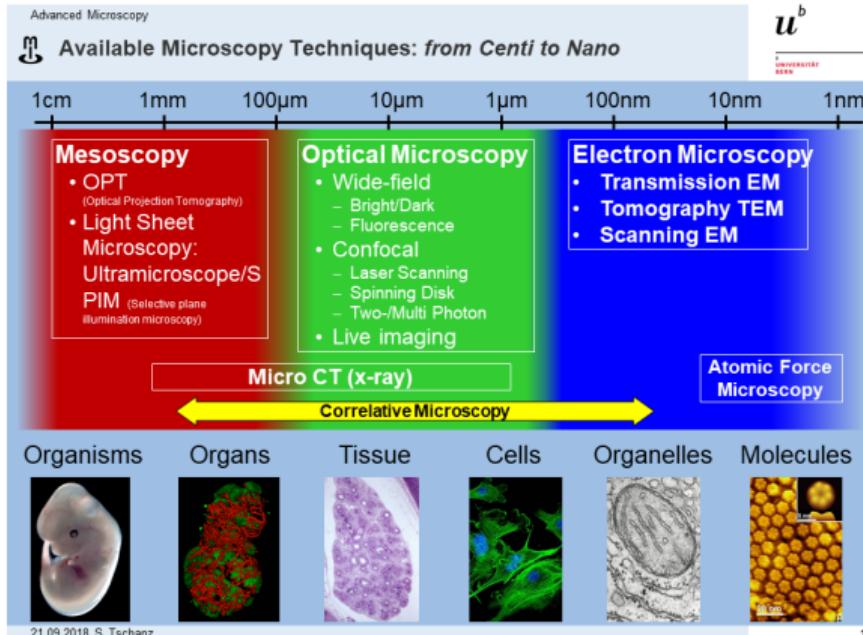
*u<sup>b</sup>*

# Wavelength & Scale



*u*<sup>b</sup>

# Wavelength & Scale



Stefan Tschanz, with permission

# 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 Iwan

$u^b$

# CT-Scanner

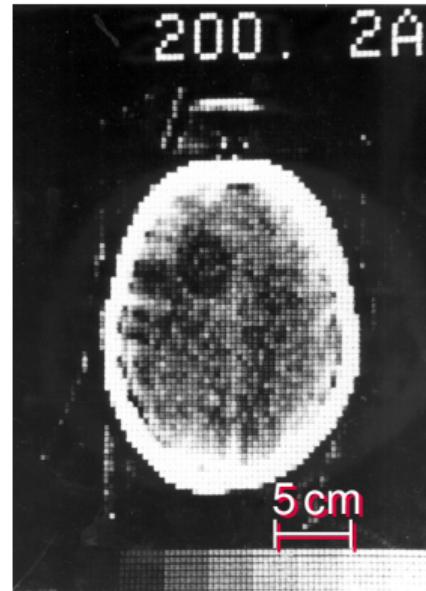


[youtu.be/2CWpZKuy-NE](https://youtu.be/2CWpZKuy-NE)

*u*<sup>b</sup>

# CT History

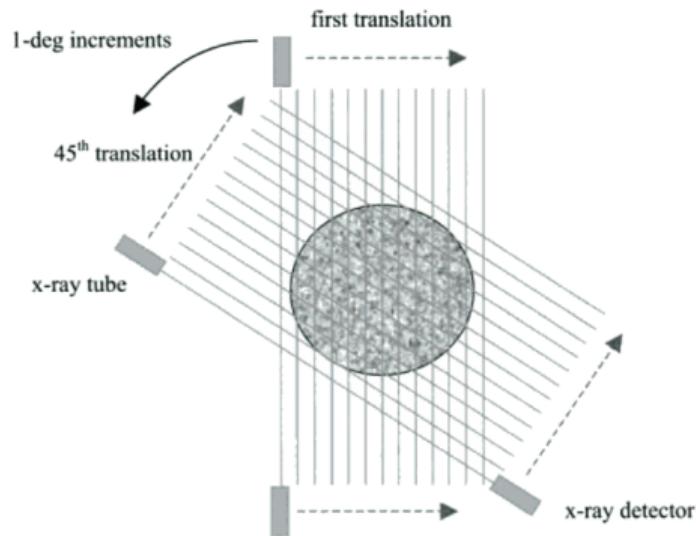
- Long history
  - Cormack1963: Cormack1963 used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector Cormack1963
  - 1976: Hounsfield1976a worked on first clinical scanner Hounsfield1976a
  - Nice overview by Hsieh2003 Hsieh2003



From Beckmann2006, Figure 5

# CT History

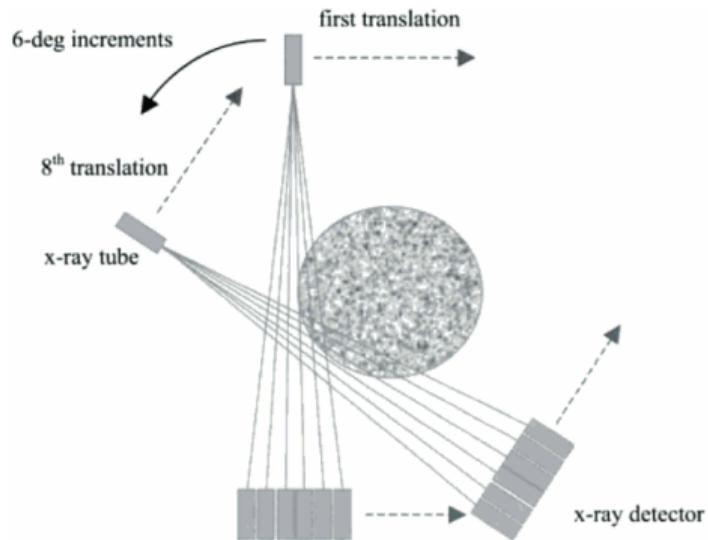
- Long history
  - Cormack1963: Cormack1963 used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector Cormack1963
  - 1976: Hounsfield1976a worked on first clinical scanner Hounsfield1976a
  - Nice overview by Hsieh2003 Hsieh2003
- CT scanner generations: First



From Hsieh2003, Figure 1.12

# CT History

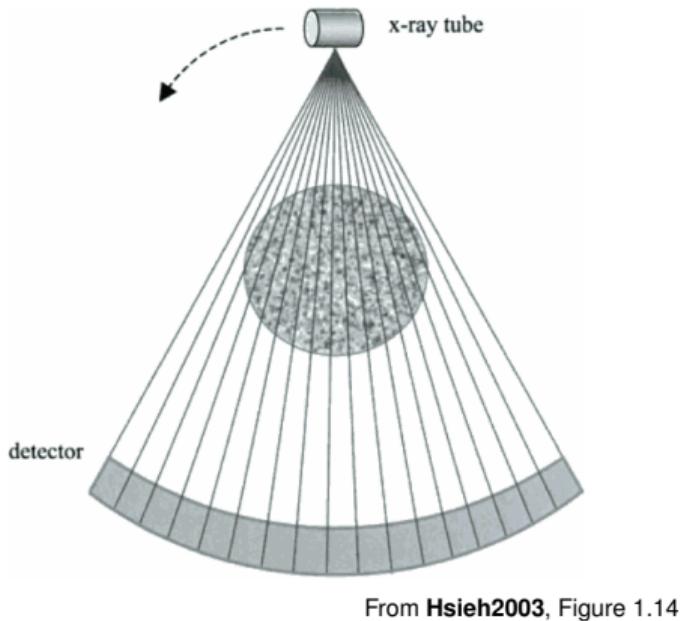
- Long history
  - Cormack1963: Cormack1963 used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector Cormack1963
  - 1976: Hounsfield1976a worked on first clinical scanner Hounsfield1976a
  - Nice overview by Hsieh2003 Hsieh2003
- CT scanner generations: First, second



From Hsieh2003, Figure 1.13

# CT History

- Long history
  - Cormack1963: Cormack1963 used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector Cormack1963
  - 1976: Hounsfield1976a worked on first clinical scanner Hounsfield1976a
  - Nice overview by Hsieh2003 Hsieh2003
- CT scanner generations: First, second and third



From Hsieh2003, Figure 1.14

# $\mu$ CT History I

- X-ray computed tomography began to replace analog focal plane tomography in the early 1970s **Lin2019**
- $\mu$ CT was first reported in the 1980s, for scanning gemstones
- Lee Feldkamp **Feldkamp1984** developed one of the early laboratory microCT systems by assembling a micro-focus cone beam x-ray source, specimen holder and stages, and an image intensifier at Ford Motor Company's Scientific Research Laboratory to nondestructively detect damage in ceramic manufactured automobile parts
- 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**
- CT scanners in medical diagnostics, beginning in the early 1970s

# $\mu$ CT History II

- Non-medical use in the late 1970s, for detection of internal defects in fabricated parts and equipment
- Today: Nondestructive imaging for quantifying the microstructure of organic materials, particularly mineralized bone tissue and the relationships between the mechanical behavior of bone to its structural and compositional properties
- Since the 1990s,  $\mu$ CT includes imaging of soft tissues and vasculature using radio-opaque contrast agents
- $\approx$ 2500  $\mu$ 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.”  
**(xrayphysics)**
  - 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 **wiki:beer-lambert**:  $I(t) = I_0 e^{-\alpha z}$

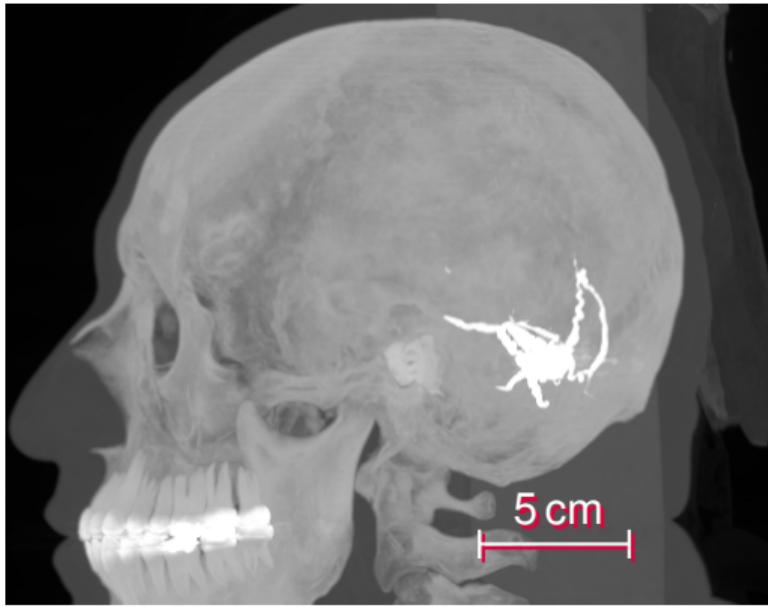
# Composition of biological tissues

Tissue: content by mass percentage

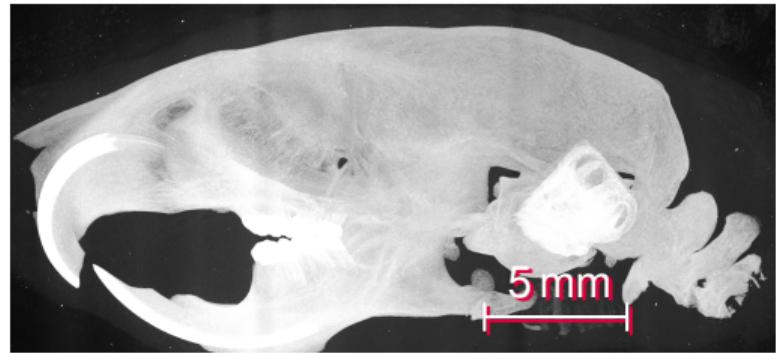
Element	H	C	N	O	Na	P	S	Cl	K	Ca
Atomic number	1	6	7	8	11	15	16	17	19	20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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

*u*<sup>b</sup>

# Why $\mu$ CT?

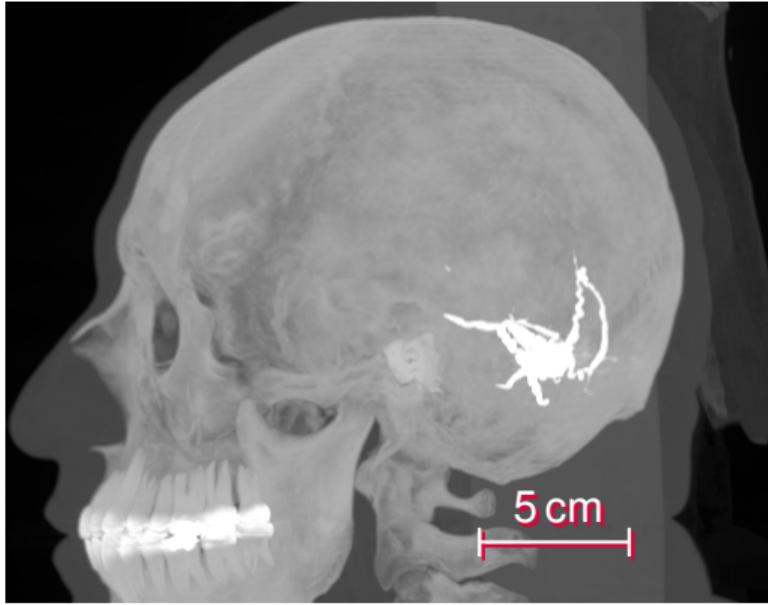


From **Clark2013**, Subject *C3L-02465*



$u^b$

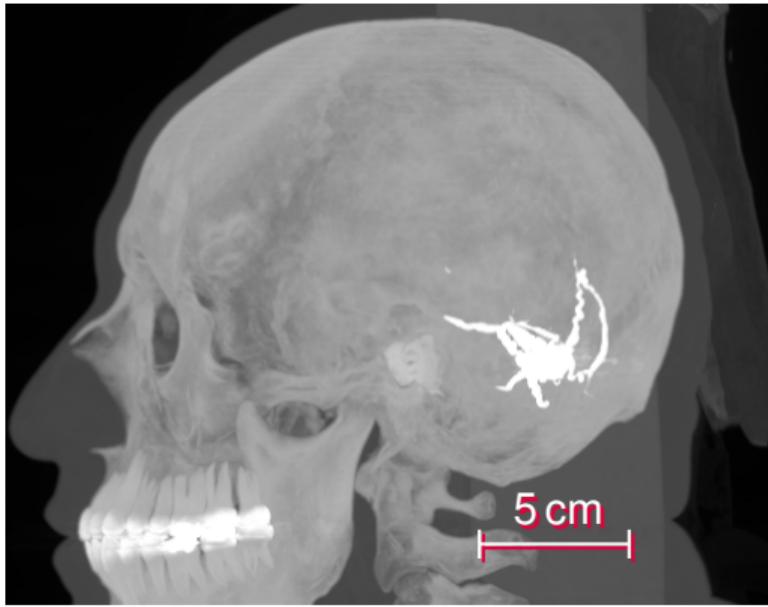
# Why $\mu$ CT?



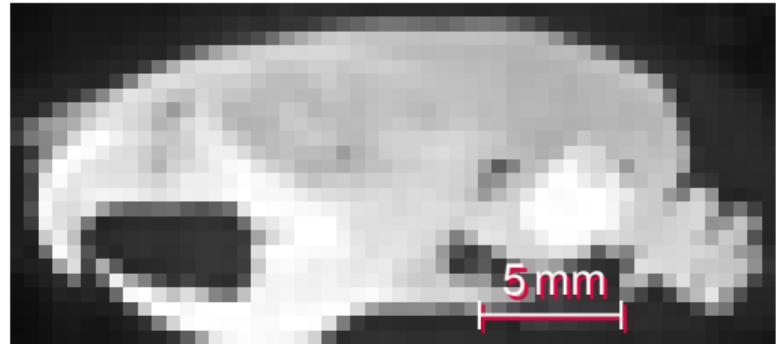
From **Clark2013**, Subject *C3L-02465*

$u^b$

# Why $\mu$ CT?

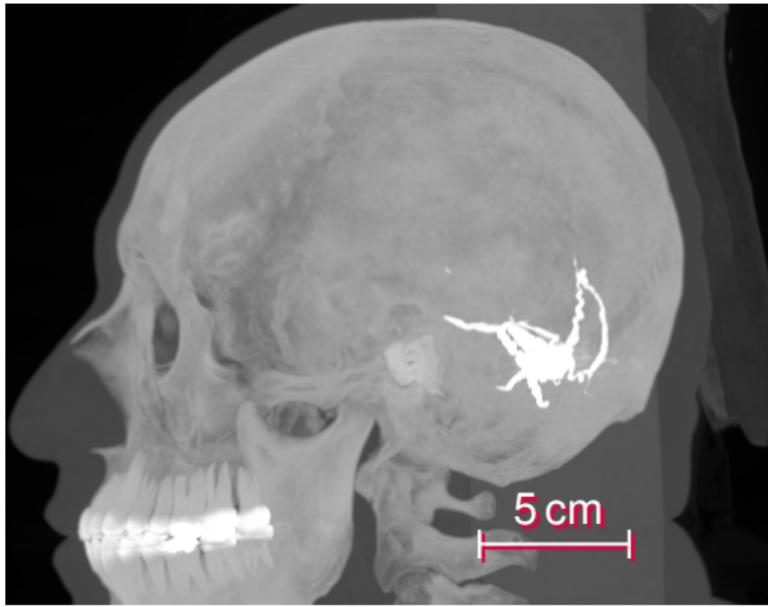


From **Clark2013**, Subject *C3L-02465*

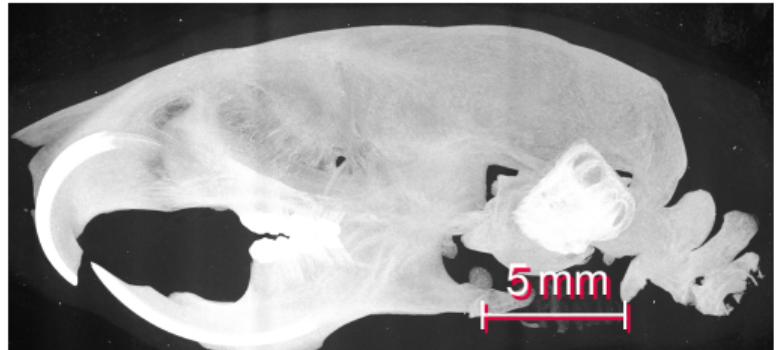


$u^b$

# Why $\mu$ CT?

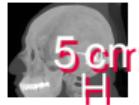


From **Clark2013**, Subject *C3L-02465*

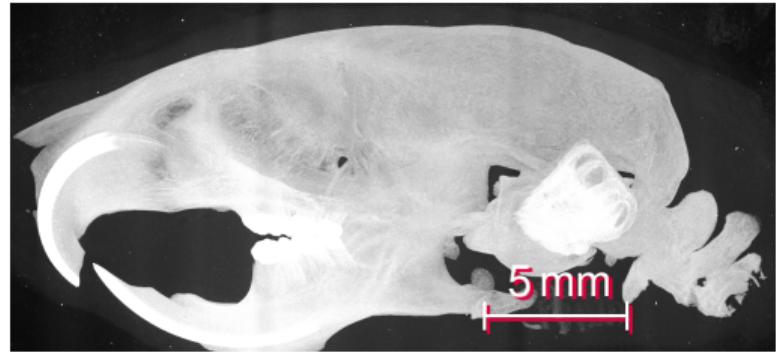


$u^b$

# Why $\mu$ CT?



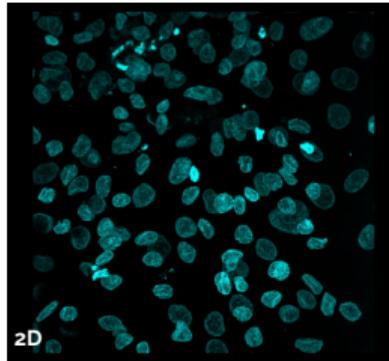
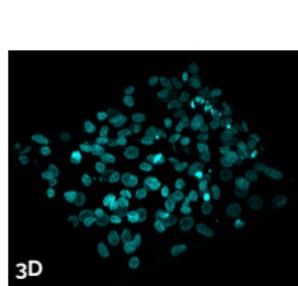
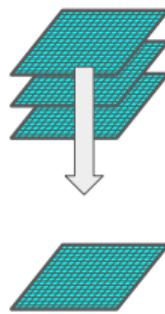
From Clark2013, Subject C3L-02465



# 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 (2022) by Guillaume Witz, Slide 23*

*u*<sup>b</sup>

# 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



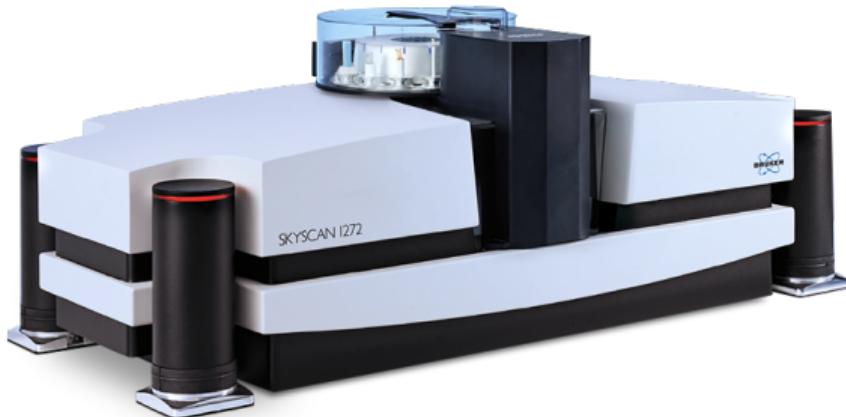
flic.kr/p/D4rbom

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[flic.kr/p/fpTrGu](https://flic.kr/p/fpTrGu)

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

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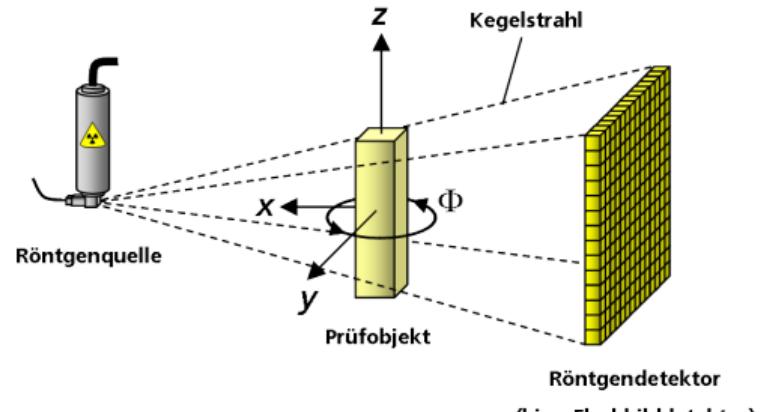


flic.kr/p/7Xhk2Y © ⓘ ⓘ ⓘ

*u<sup>b</sup>*

# What is happening?

- No matter what kind of machine, the basic principle is always the same
- an x-ray source
  - a sample
  - a detector



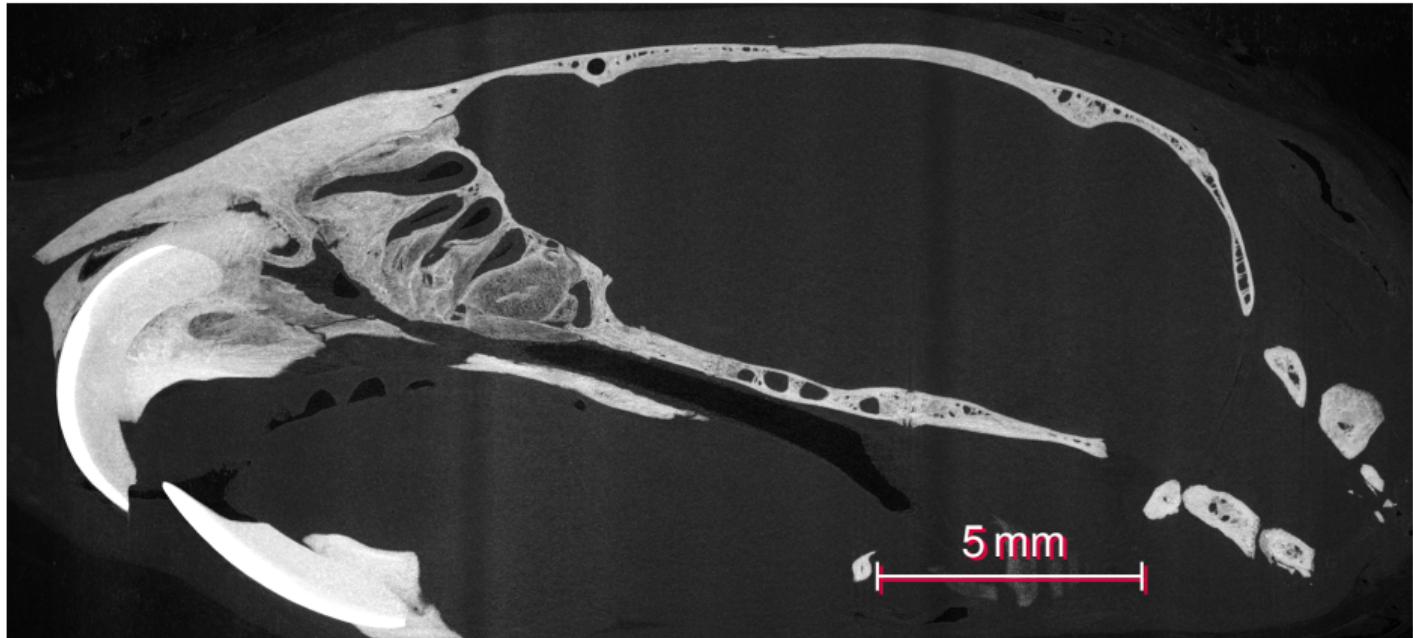
w.wiki/7g3 @①②

*u*<sup>b</sup>

# Machinery

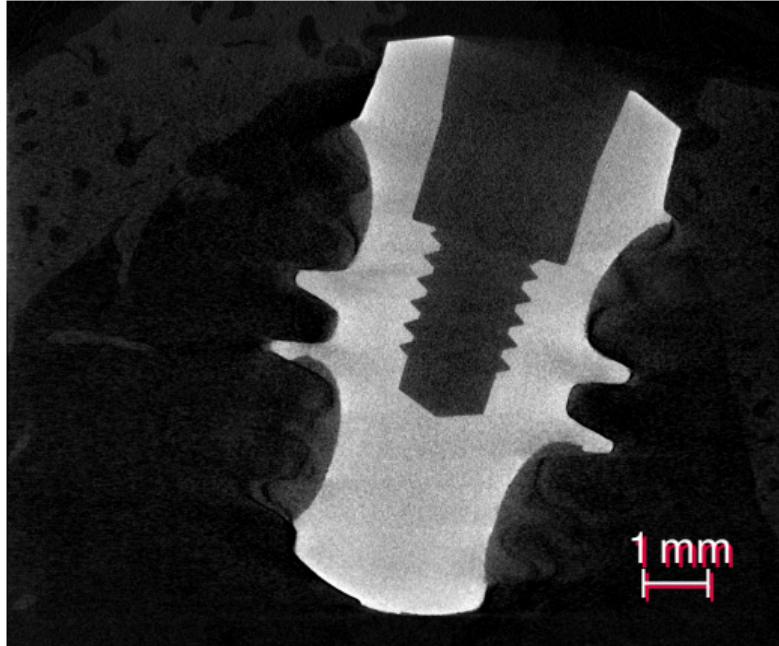
*u*<sup>b</sup>

# Examples



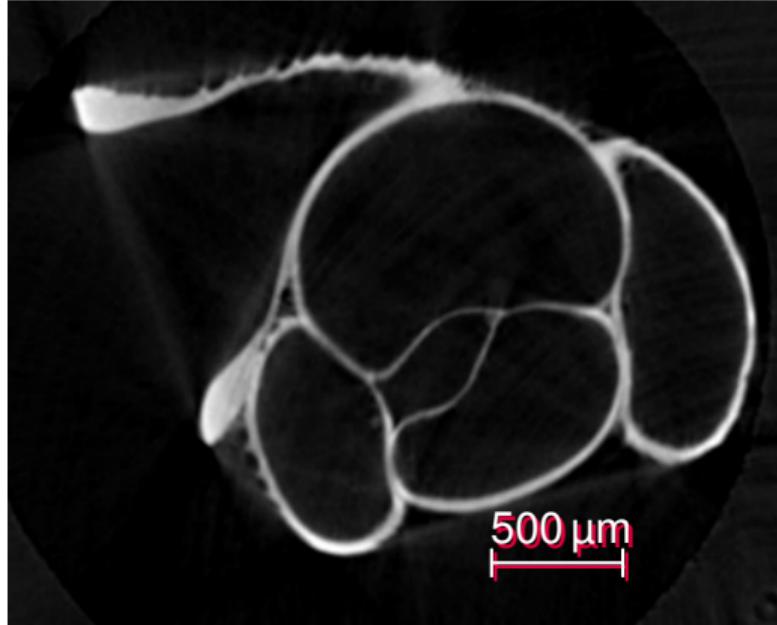
$u^b$

# Examples



*u*<sup>b</sup>

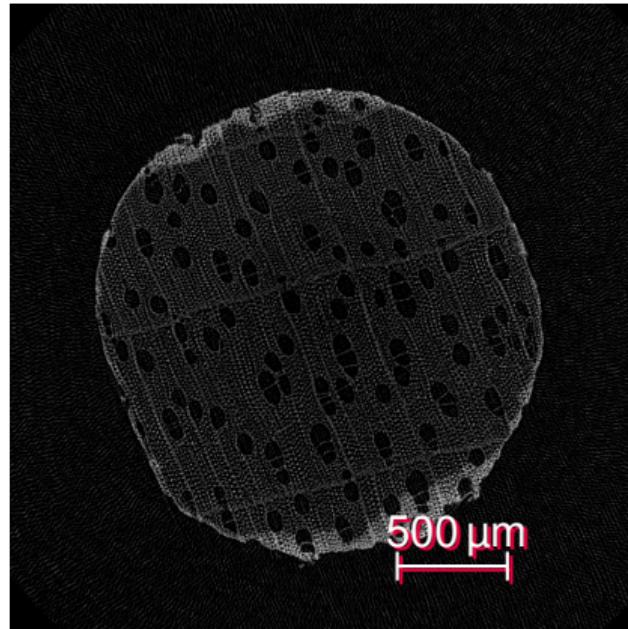
# Examples



From Bochud2021, *Diancta phoenix*

$u^b$

# Examples



$u^b$

# Examples



*u*<sup>b</sup>

# Preparation

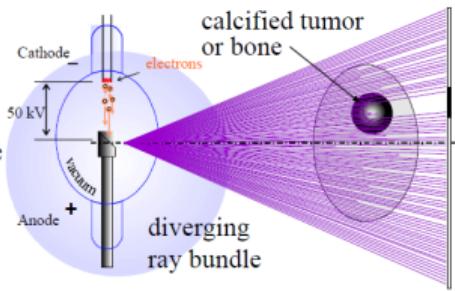
- Study design
- Sample preparation

*u*<sup>b</sup>

# Projections

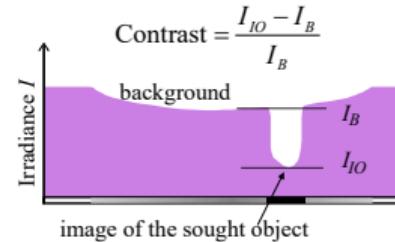
## X-ray generation and contrast

X-ray tube:  
nearly point like  
photon source



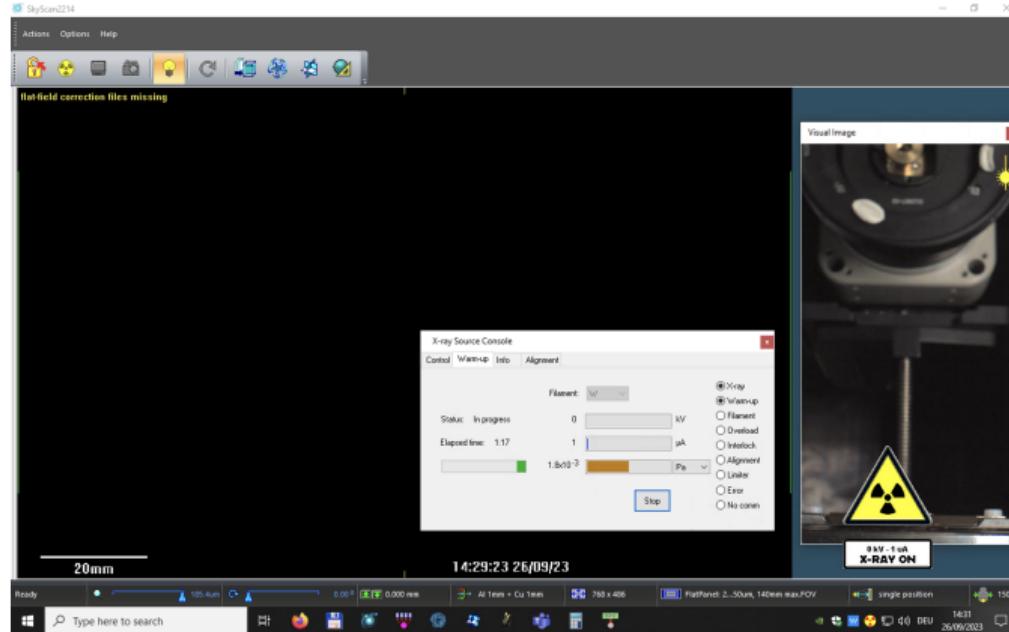
Contrast is given by  
absorption of intensity I

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



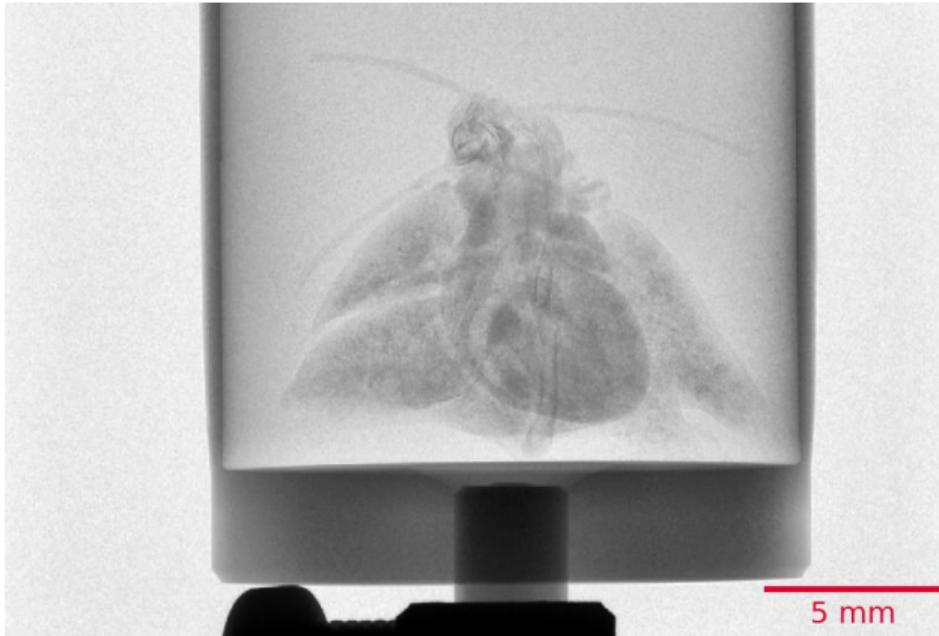
*Contrast, Magnification and Resolution—Laws of Physics for Microscopists (1, 2022) by Martin Frenz, Slide 21*

# $u^b$ Projection acquisition



$u^b$

# Projections

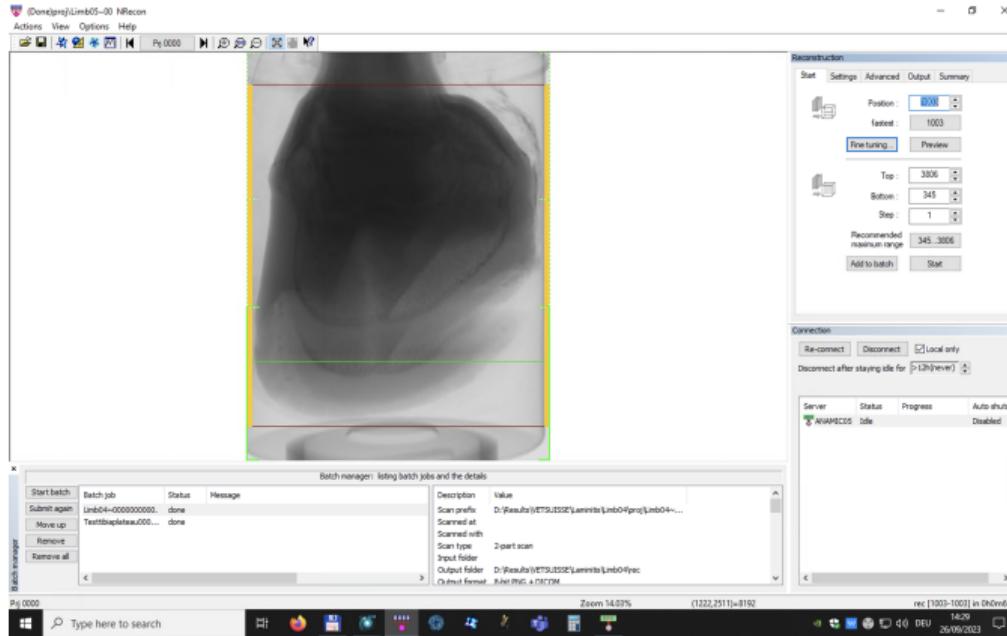


# Projections

- 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

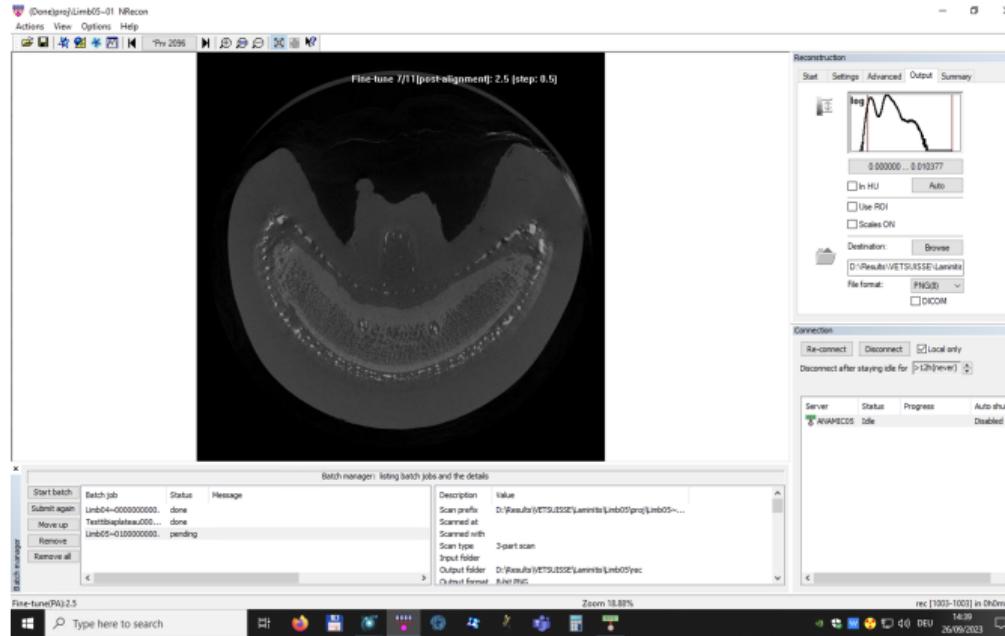
$u^b$

# Reconstructions



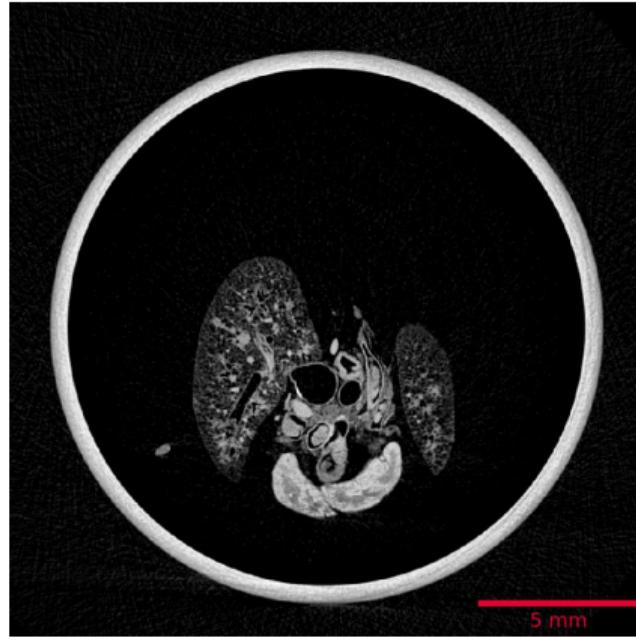
$u^b$

# Reconstructions



$u^b$

# 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

$u^b$

# Visualization



# Visualization

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

*u*<sup>b</sup>

# 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

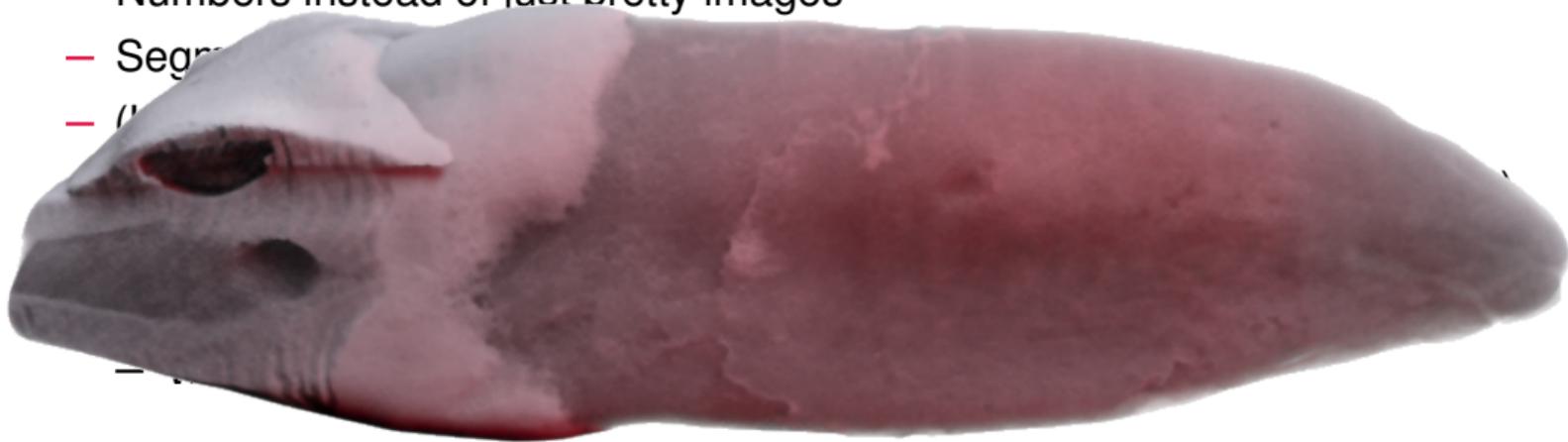
- Pretty images are nice, but we need quantitative numbers
- Segmentation
- Characterization

*u*<sup>b</sup>

# Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers instead of just pretty images
- Segments
- Labels



# 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 **Kluyver2016**)
- Two publications:
  - **Haberthuer2021**, BMC Oral Health, doi.org/gjpw2d
  - **Wolf2021**, Scientific Reports, doi.org/g7r8

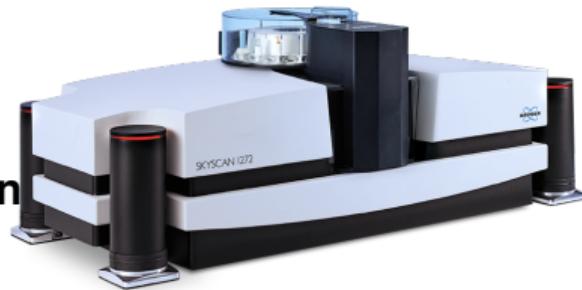
*u*<sup>b</sup>

# How?

- 104 extracted human permanent mandibular canines
- $\mu$ 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!



- 104 extracted human permanent mandibular canines
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[bruker.com/skyscan1272](http://bruker.com/skyscan1272)

*u*<sup>b</sup>

# 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:34s
Scan duration=0h:39m:51s
```

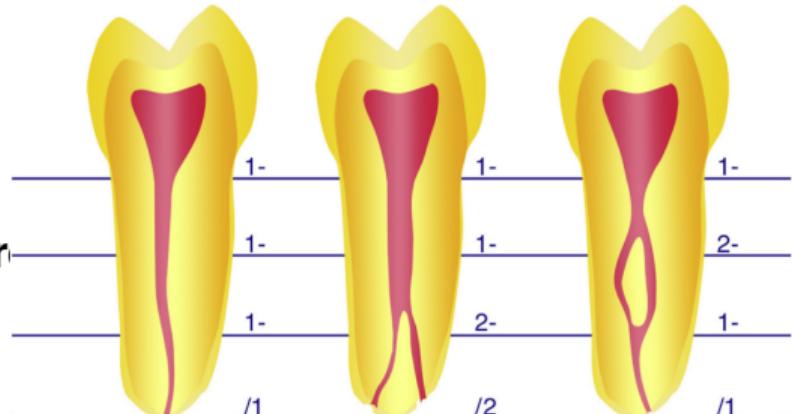
- 104 extracted human permanent mandibular canines
- µCT imaging
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- *Reproducible* analysis **Haberthuer2020a**, e.g. you can click a button to double-check or recalculate the results yourself!

*Sample changer* on the SkyScan 1272  
In total:

- 13 days of *continuous* µCT 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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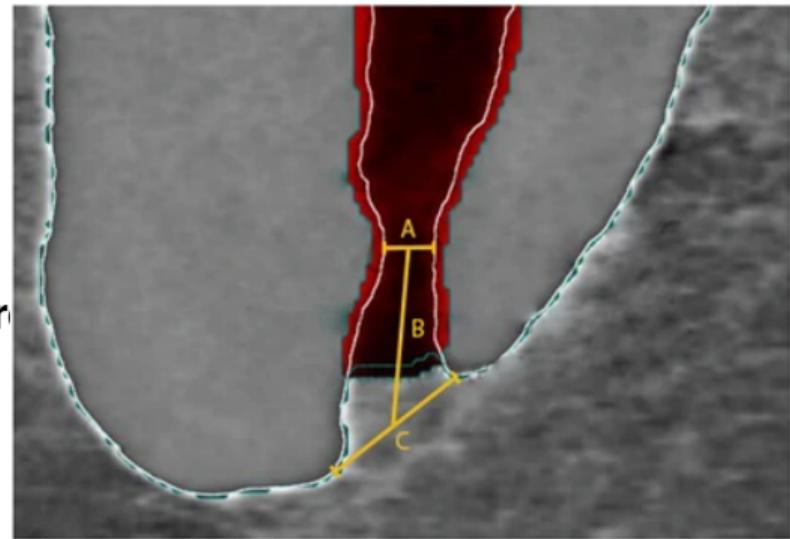


From **Briseno-Marroquin2015**, Fig. 2

*u*<sup>b</sup>

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From **Wolf2017**, Fig. 1

*u*<sup>b</sup>

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- Root canal configuration, according to **Briseno-Marroquin2015** **Briseno-Marr**
- *Reproducible* analysis **Haberthuer2020a**, e.g. you can click a button to double-check or recalculate the results yourself!



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

*u*<sup>b</sup>

# How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Root canal configuration, according to **Briseno-Marroquin2015** **Briseno-Marroquin2020a**
- *Reproducible* analysis **Haberthuer2020a**, 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 a 'Code' dropdown. Below this is a list of commits from a user named 'habli'. The commits are:

File	Description	Date
.github/workflows	Update actions file	20 days ago
.gitignore	Only 'mode' changes	2 months ago
DownloadFromOSF.ipynb	Clean run of download script	22 days ago
README.md	Typo in Binder badge & link to full repo on Binder	22 days ago
Tooth.Border.jpg	Only 'mode' changes	2 months ago
Tooth.Characterization.jpg	Only 'mode' changes	2 months ago
ToothAnalysis.ipynb	Only select a subset if we actually have data.wq	22 days ago
ToothAxisSize.ipynb	Clean run of notebook	22 days ago
ToothDisplay.ipynb	Display Tooth045 for manuscript	22 days ago
requirements.txt	We also need this	2 months ago
treebeard.yaml	Add treebeard configuration	20 days ago

Below the commits is a 'README.md' file. It contains the following content:

```
README.md

[![DOI](https://zenodo.4999402/treebeard.yaml)](https://zenodo.4999402/treebeard.yaml) [![treebeard.yaml](https://zenodo.4999402/treebeard.yaml)](https://zenodo.4999402/treebeard.yaml)

[![Launch Binder](https://zenodo.4999402/binder/badge.svg)](https://zenodo.4999402/binder/)

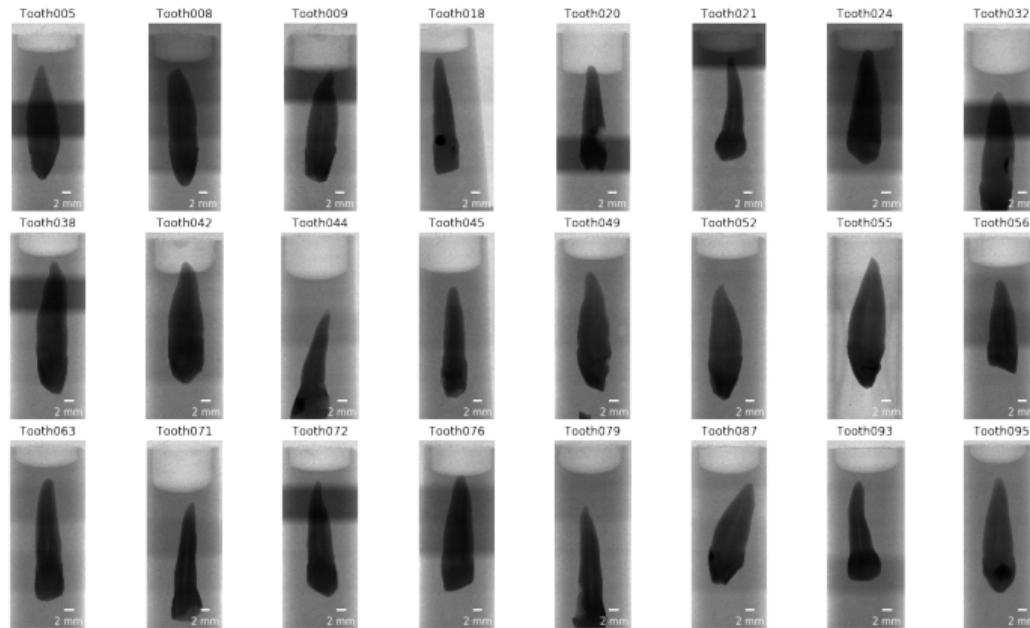
A big tooth cohort

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.
```

$u^b$

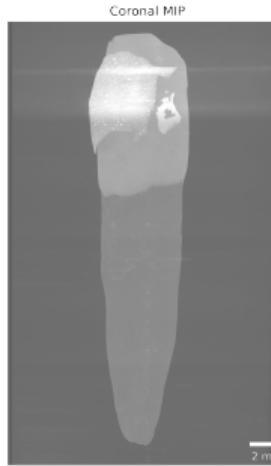
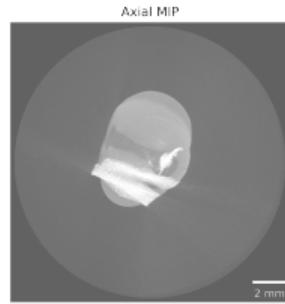
# $\mu$ CT imaging



*u*<sup>b</sup>

# Dataset cropping

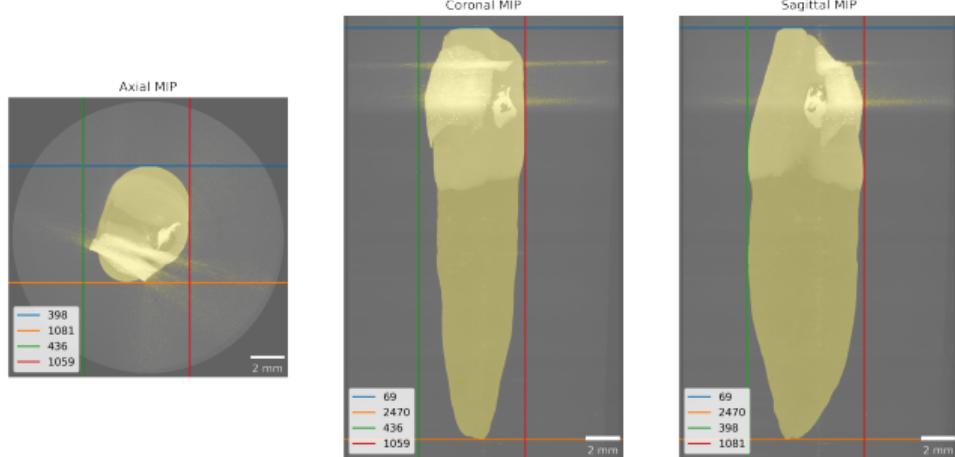
- Full datasets: 326 GB
- Cropped datasets: 115 GB



$u^b$

# Dataset cropping

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



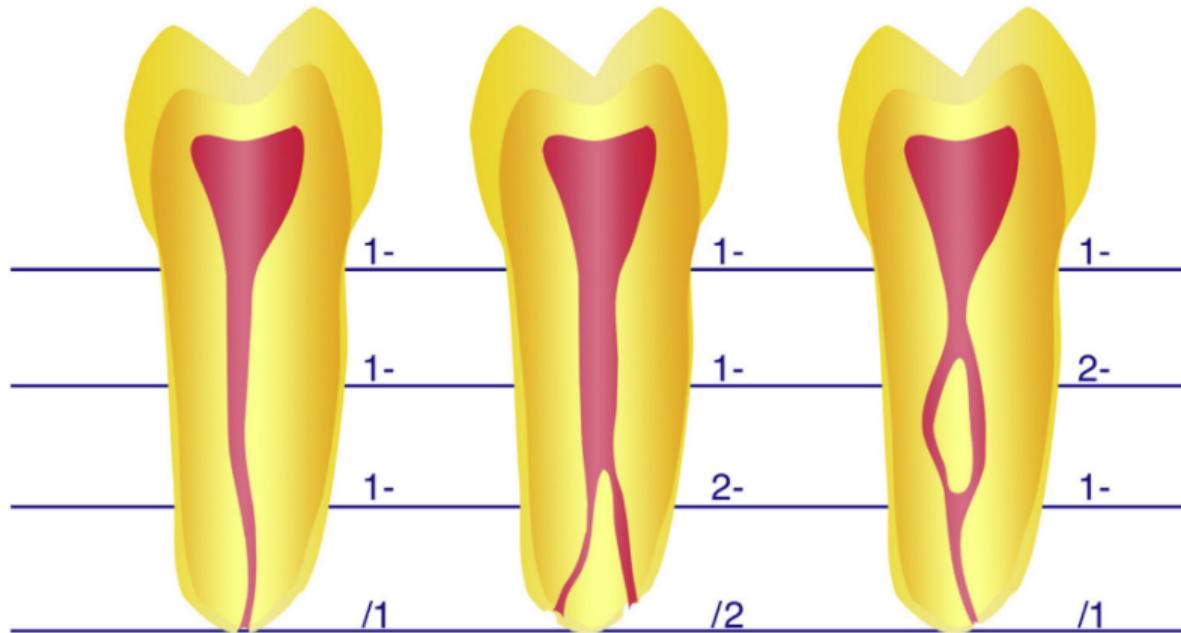
*u*<sup>b</sup>

# Tooth morphology



*u<sup>b</sup>*

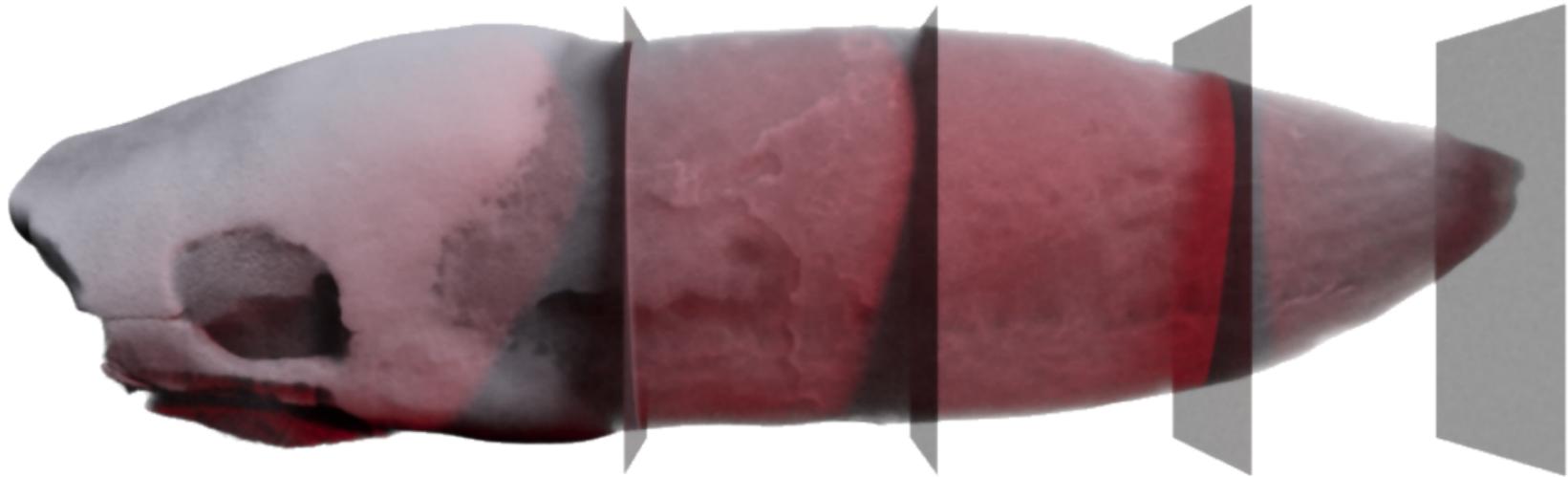
# Tooth morphology



From Briseno-Marroquin 2015, Fig. 2

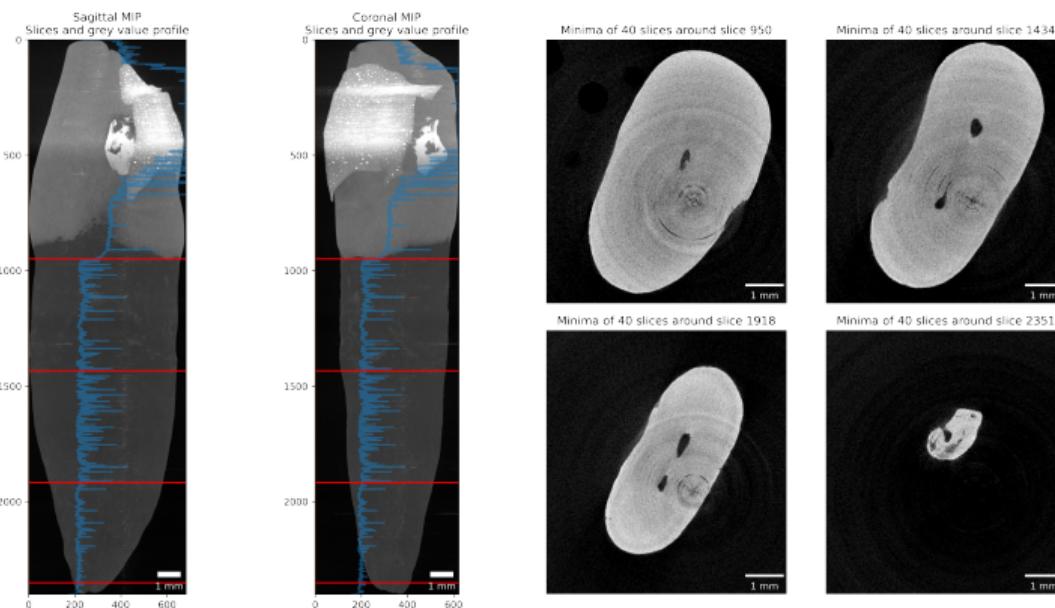
*u<sup>b</sup>*

# Tooth morphology



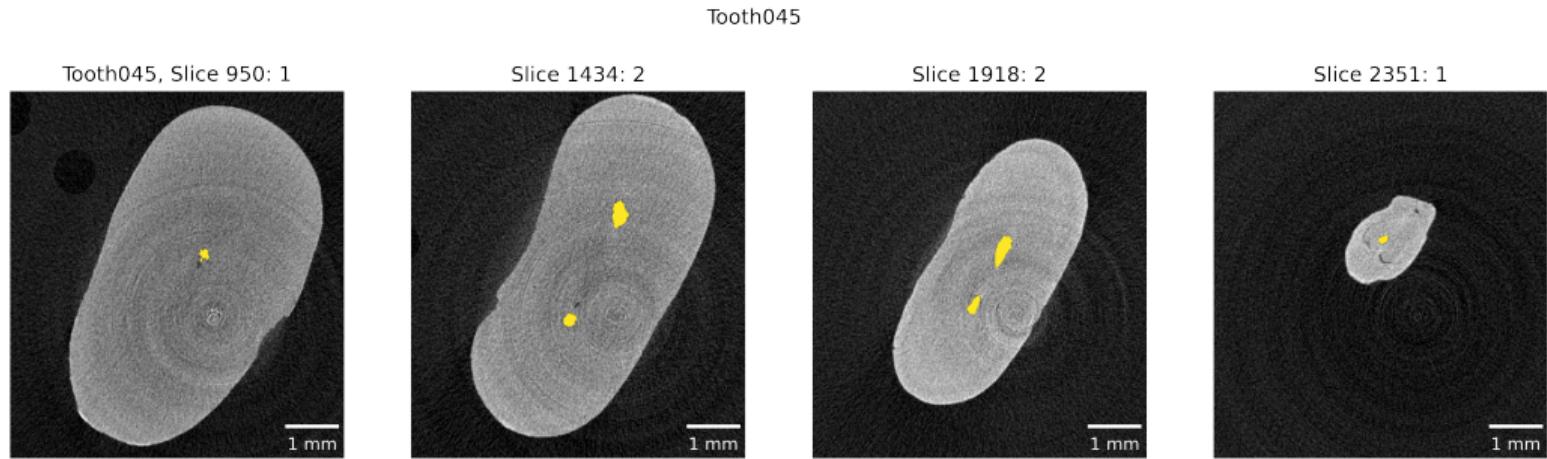
*u<sup>b</sup>*

# Detection of enamel-dentin border



*u*<sup>b</sup>

# Detection of enamel-dentin border



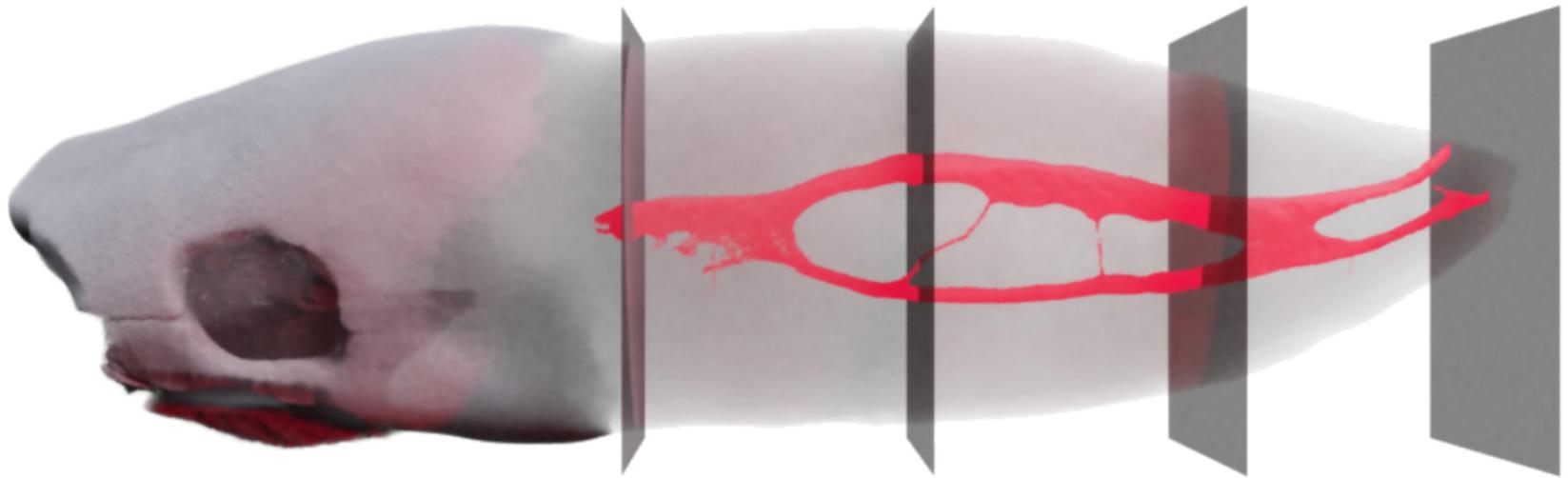
*u<sup>b</sup>*

# 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

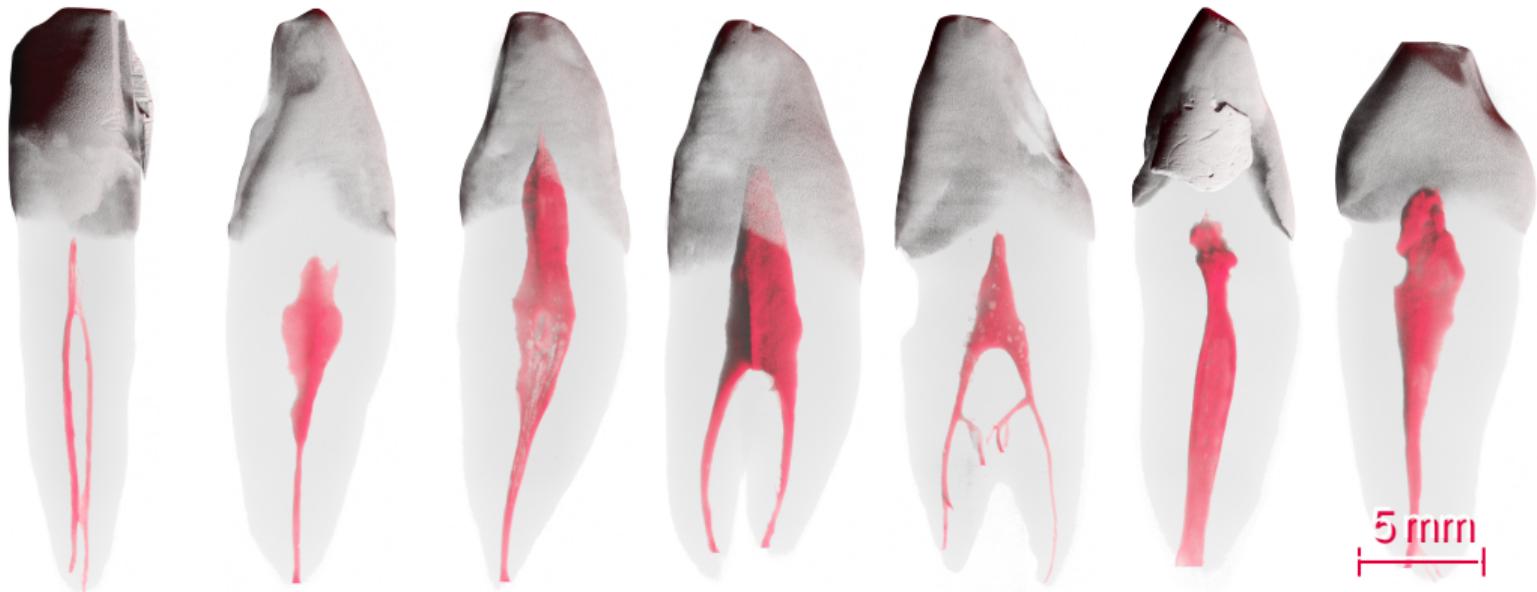
*u<sup>b</sup>*

# Extraction of root canal space



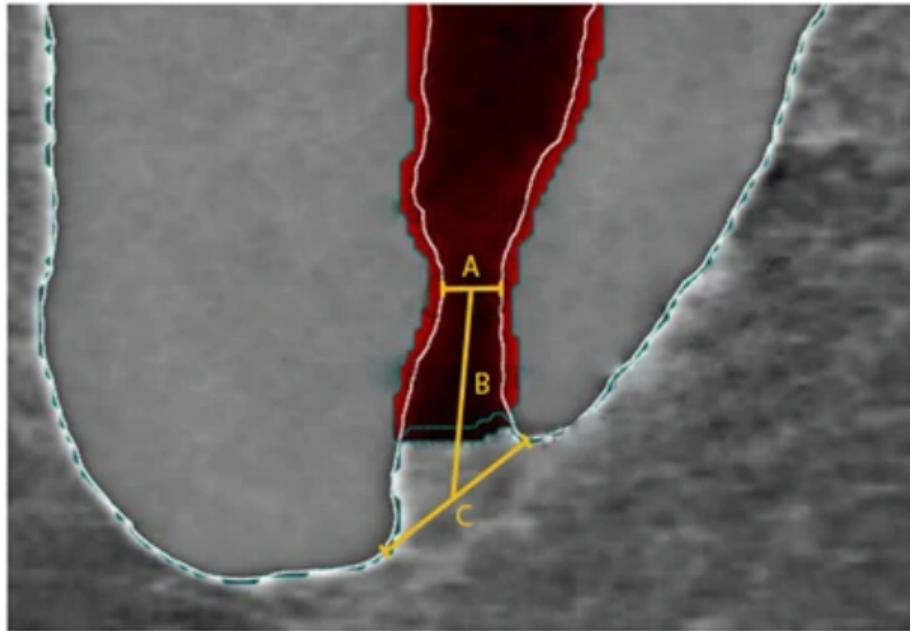
*u<sup>b</sup>*

# Results of root canal space extraction



*u<sup>b</sup>*

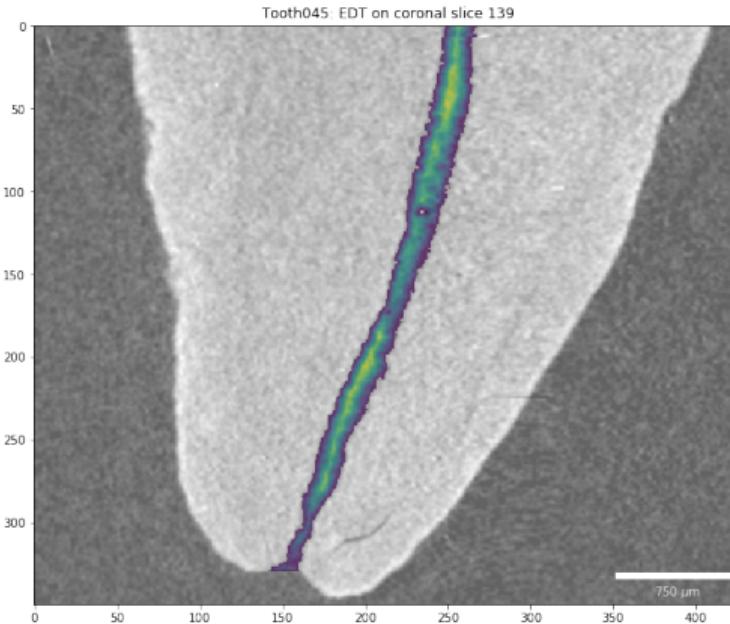
# Analysis of the physiological foramen geometry



From Wolf2017, Fig. 1

*u*<sup>b</sup>

# Analysis of the physiological foramen geometry

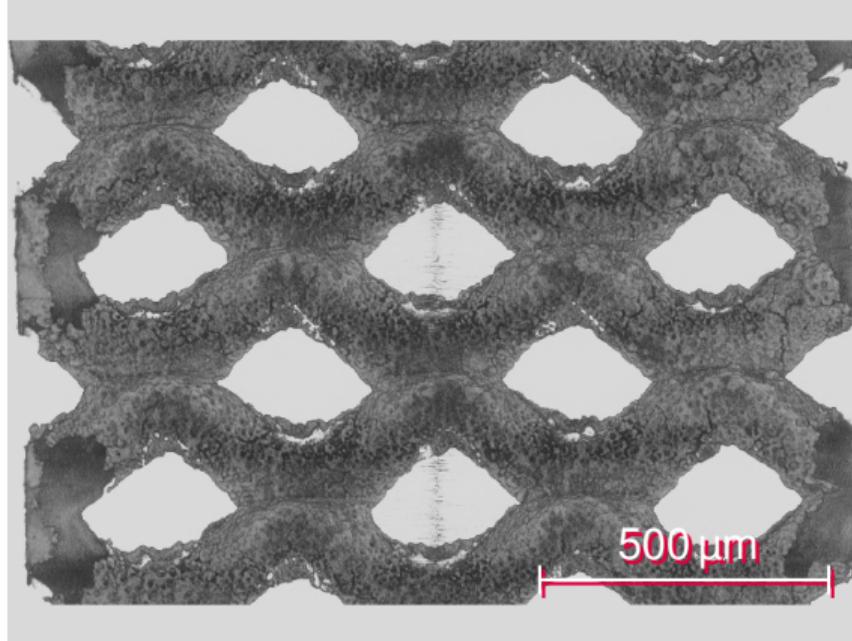


# Conclusion ZMK

- 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

$u^b$

# Metal foam

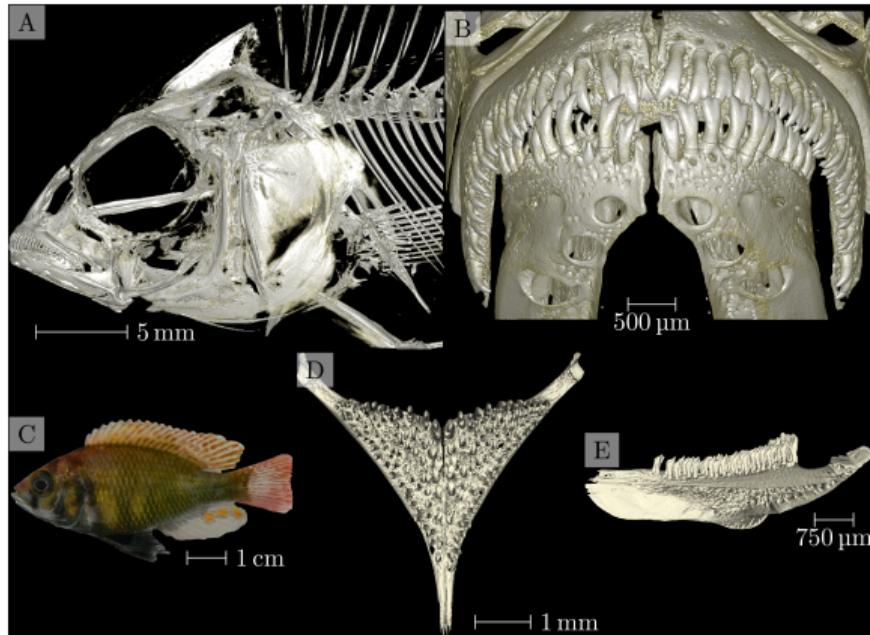


Etienne Berner NanoElectroCatalysis Group

*u*<sup>b</sup>

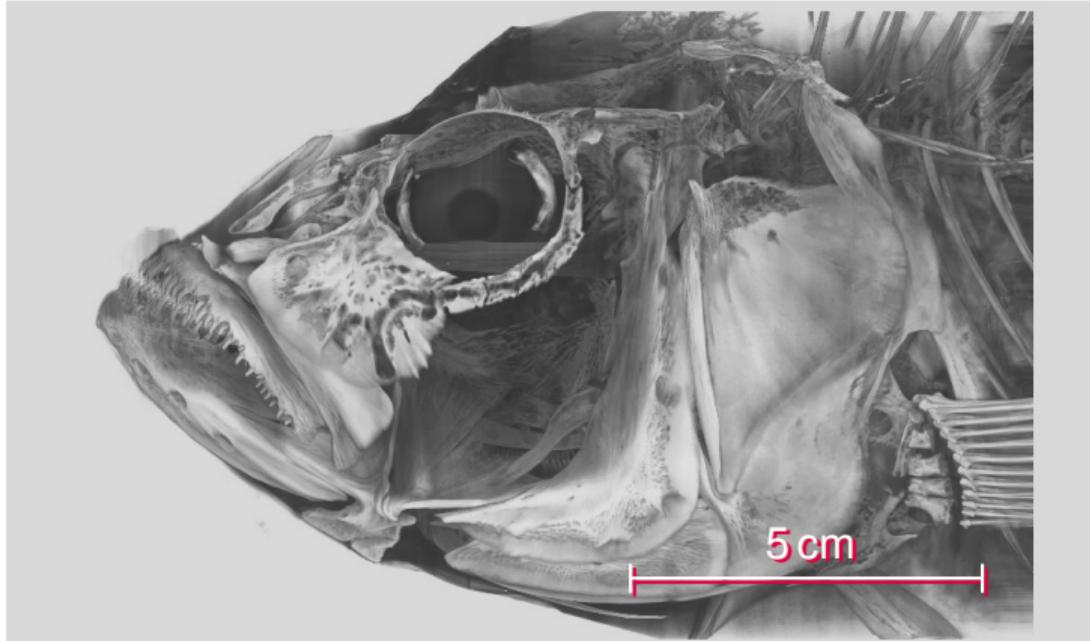
# Data wrangling by example: Cichlids

- 372 tomographic scans of 133 different Cichlids, from 6–18 cm **Haberthuer2023**
- 9.8 TB of projection images, 1.5 TB of reconstructions
- Reproducible and automated dataset wrangling, checking and image analysis ( in Jupyter) **Kluyver2016**



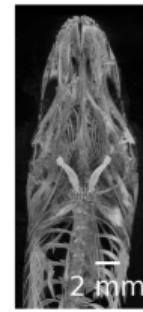
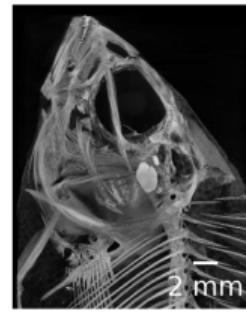
*u<sup>b</sup>*

# Cichlids



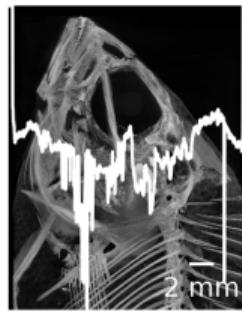
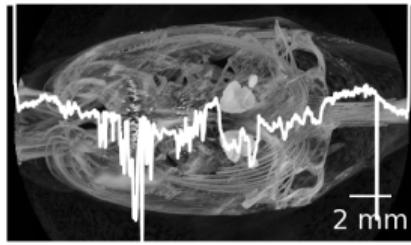
*u*<sup>b</sup>

# Data wrangling by example: Cichlids



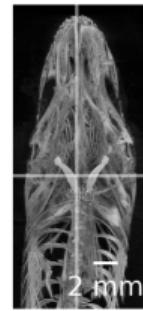
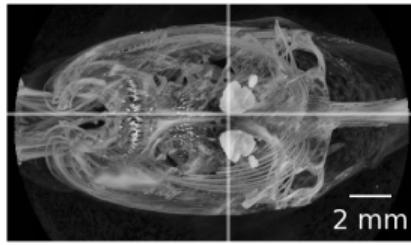
*u*<sup>b</sup>

# Data wrangling by example: Cichlids



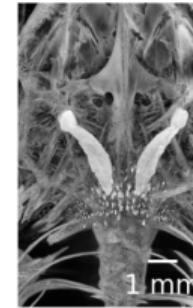
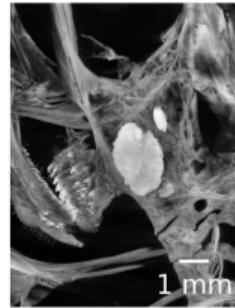
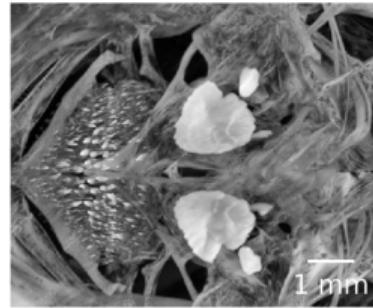
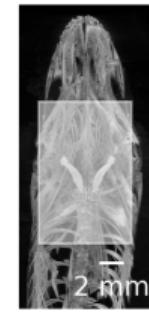
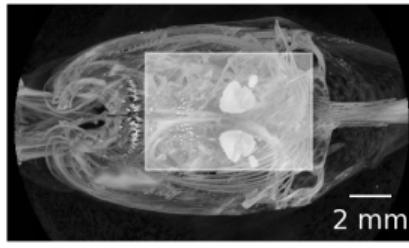
*u*<sup>b</sup>

# Data wrangling by example: Cichlids



*u<sup>b</sup>*

# Data wrangling by example: Cichlids



# *u<sup>b</sup>* 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<sup>1</sup> 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: [david.haberthuer@unibe.ch](mailto:david.haberthuer@unibe.ch)

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<sup>105</sup> September 25, 2024

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

*u*<sup>b</sup>

# References I