

# X-ray microtomography

**David Haberthür**

December 20, 2019 | 9256-HS2019-0: Advanced Microscopy

# Hello!

- Office B311 | haberthuer@ana.unibe.ch
- Master in Physics
- PhD in high resolution imaging of the lung at the Institute of Anatomy
- Post-Doc
  - TOMCAT, Swiss Light Source, Paul Scherrer Institute
  - $\mu$ CT-group, Institute of Anatomy (Ruslan Hlushchuk, David Haberthür, Oleksiy-Zakhar Khoma, Fluri Wieland, Carlos Correa Shokiche)
- Biomedical research
  - microangioCT [1]: Tumor vasculature, angiogenesis in the heart, musculature and bones
  - Lung imaging: Tumor detection and classification
  - Cancer research: Melanoma
  - Physiology: Zebrafish musculature and gills [2]
  - SkyScan 1172 & 1272

# Contents

**Overview**

**Imaging**

**Tomography**

History

Interaction of x-rays with matter

Tomography today

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

**Example of a full study**

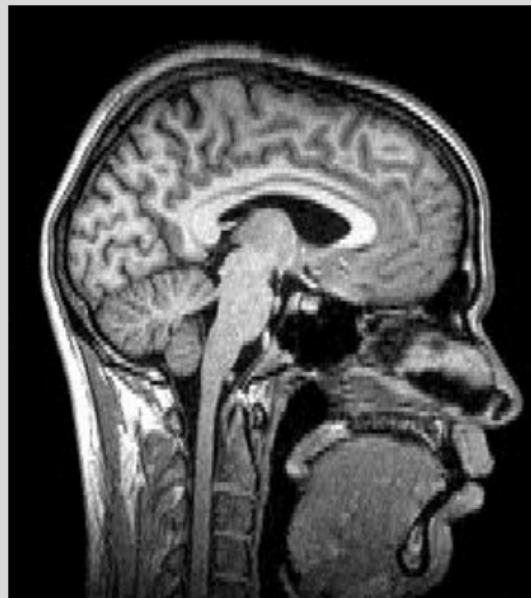
Overview

Setup

Image processing

# Biomedical imaging

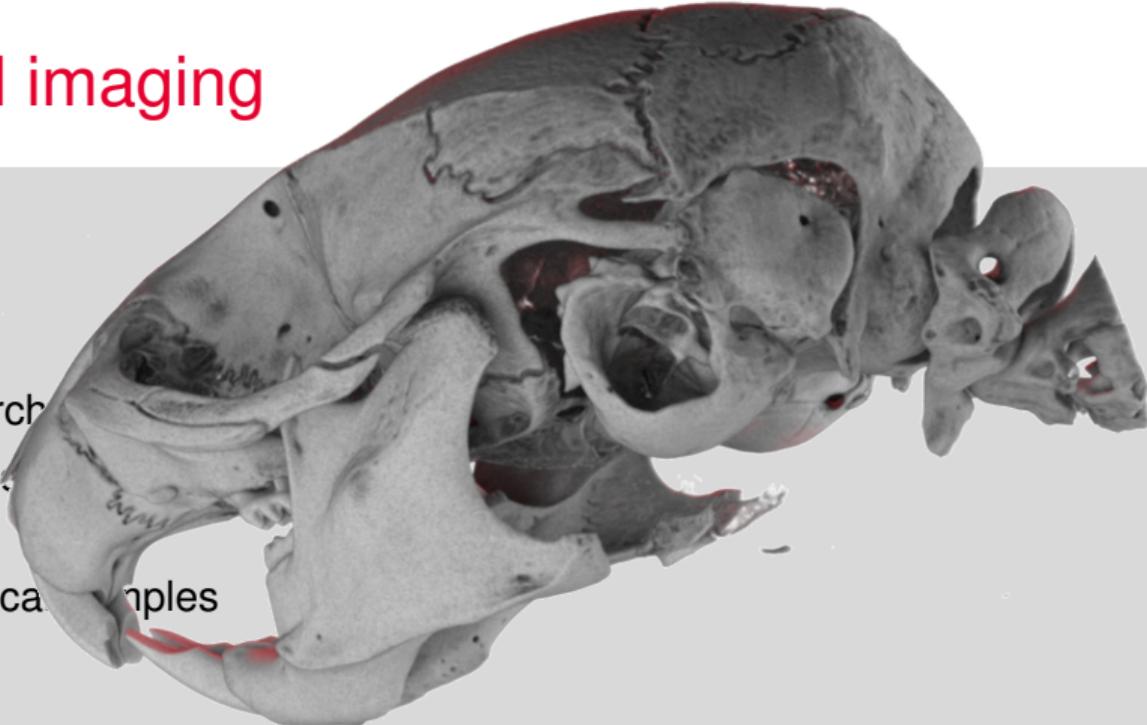
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



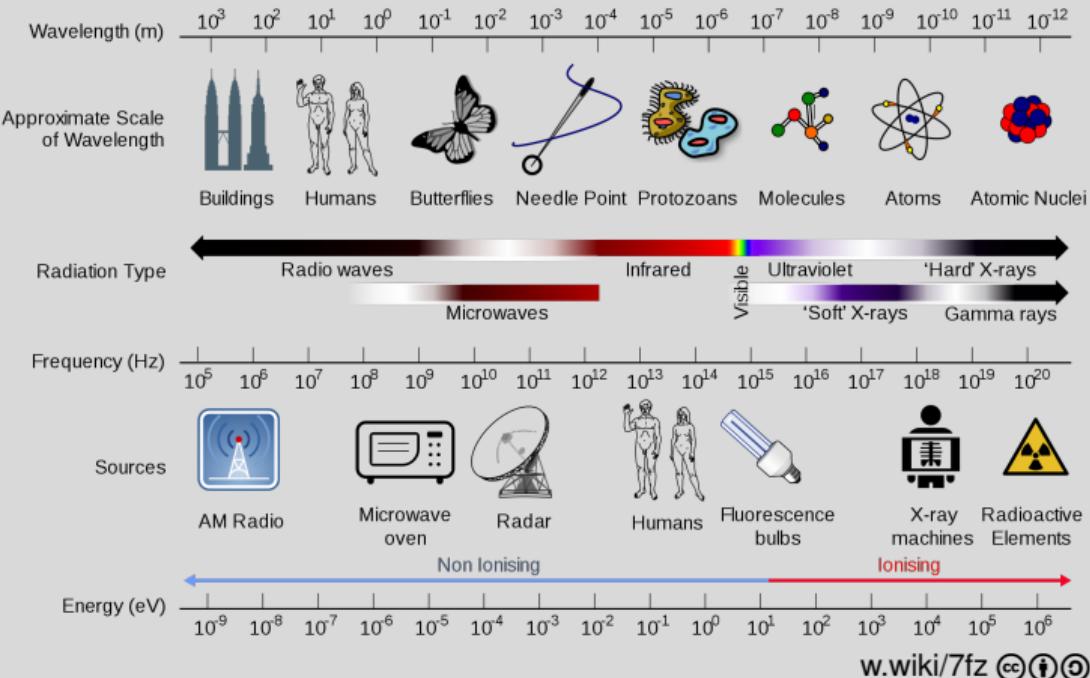
w.wiki/7g4 

# Biomedical imaging

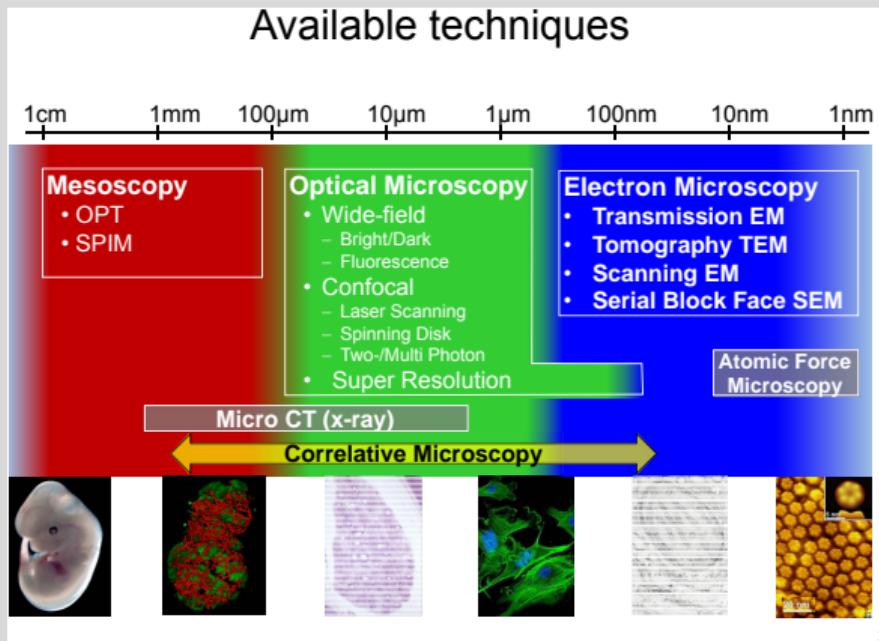
- Medical research
- Non-destructive analysis of the samples
- (Small) Biological samples



# Wavelength & Scale



# Wavelength & Scale

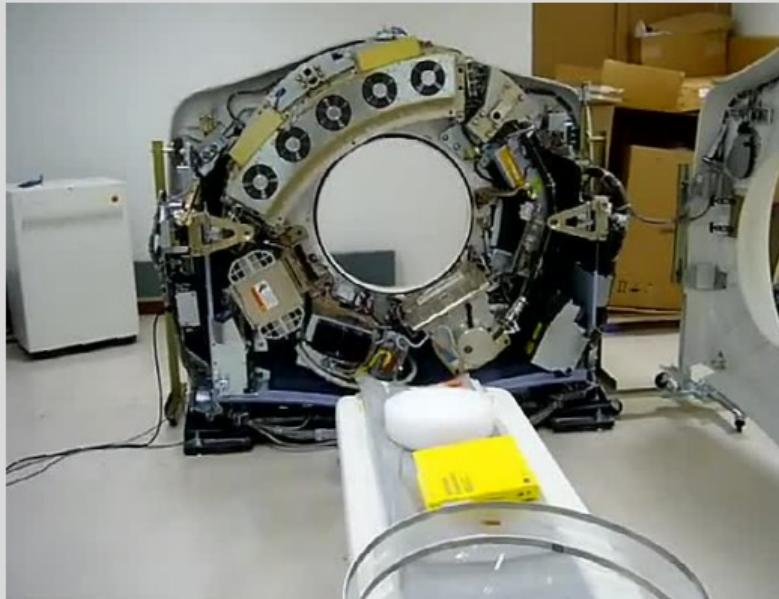


Yury Belyaev, MIC, slide from internal seminar presentation

# Imaging methods

- Light 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.

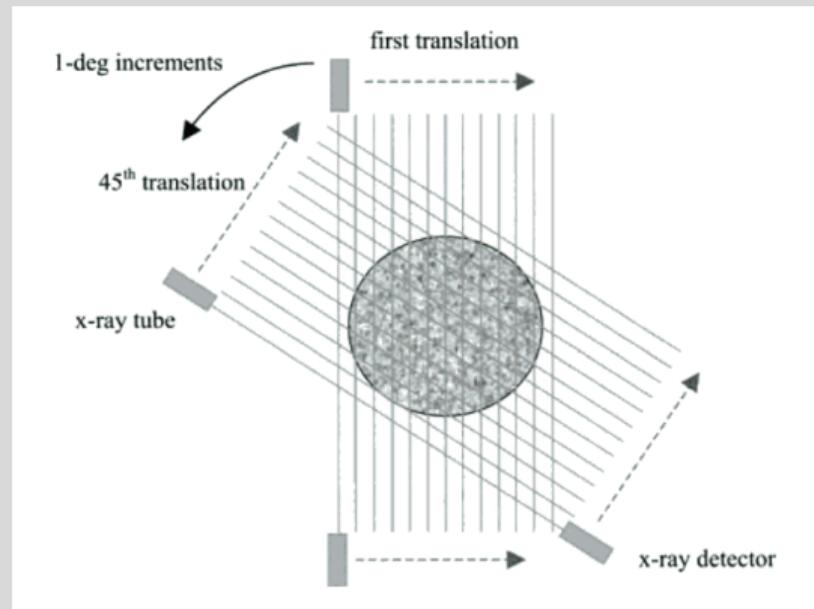
# CT-Scanner



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

# History

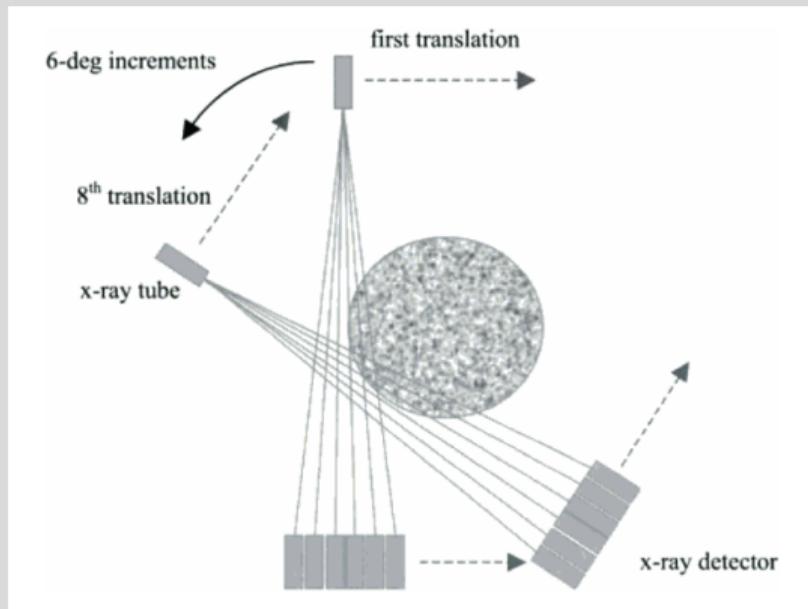
- Long history
  - 1963: Cormack used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector [3]
  - 1976: Hounsfield worked on first clinical scanner [4]
  - Nice overview by Hsieh [5]
- First, second and third generation of scanners



From [5], Figure 1.12

# History

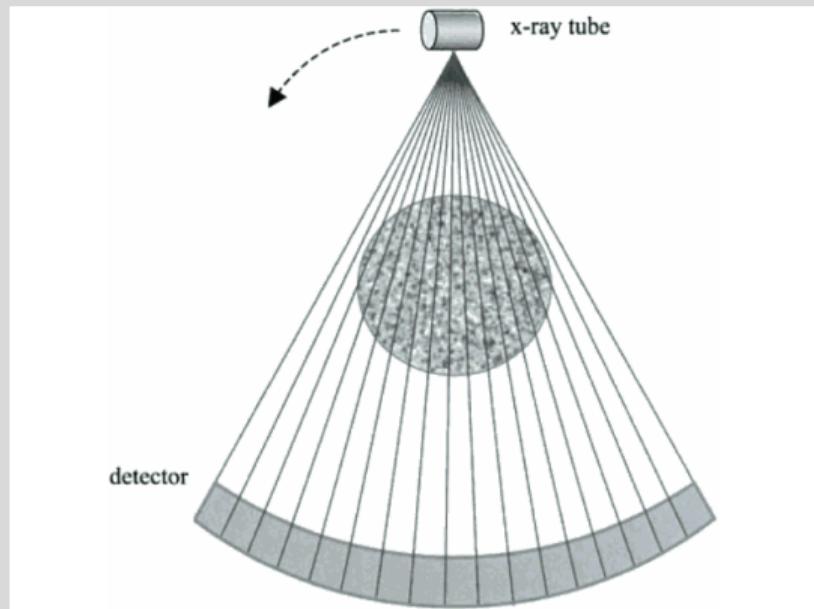
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From [5], Figure 1.13

# History

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From [5], Figure 1.14

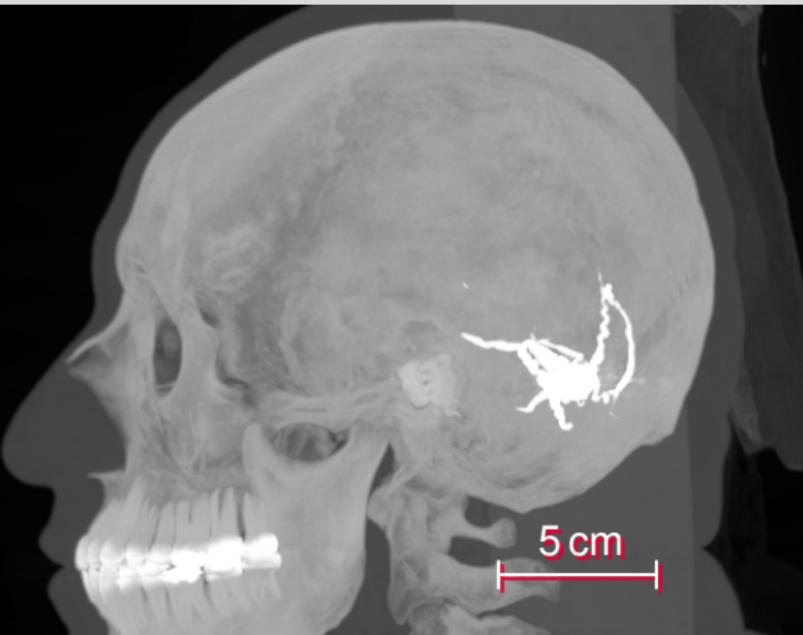
# X-ray interaction

- “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.” ([6])
  - 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 ([7, i. e. Beer-Lamberts law]  $I(t) = I_0 e^{-\alpha z}$ )

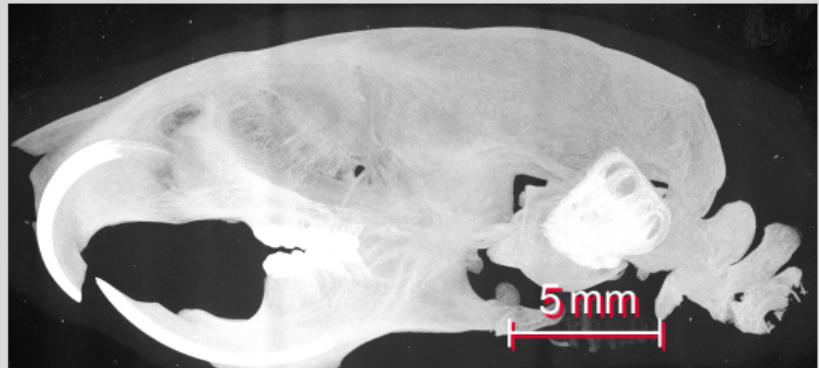
# Composition of biological tissues

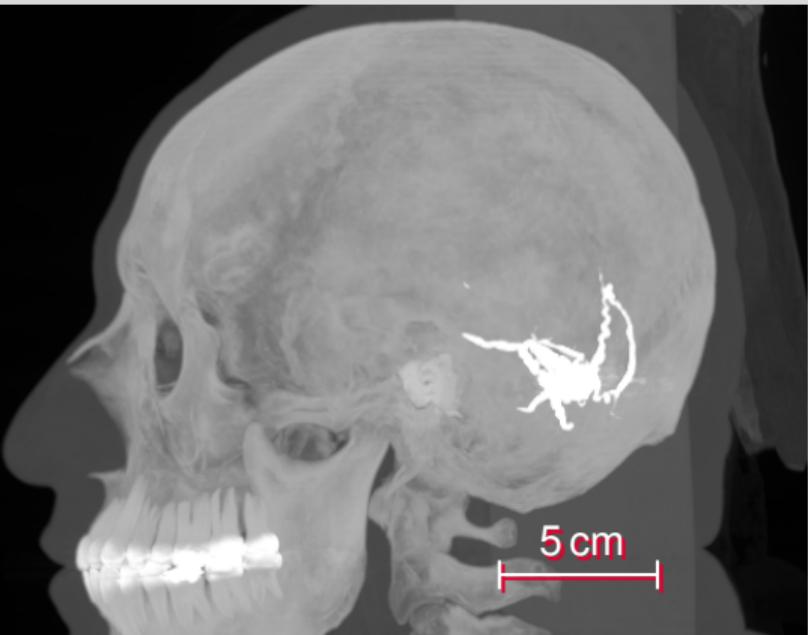
Tissue: content by mass percentage

Element Atomic number	H 1	C 6	N 7	O 8	Na 11	P 15	S 16	Cl 17	K 19	Ca 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



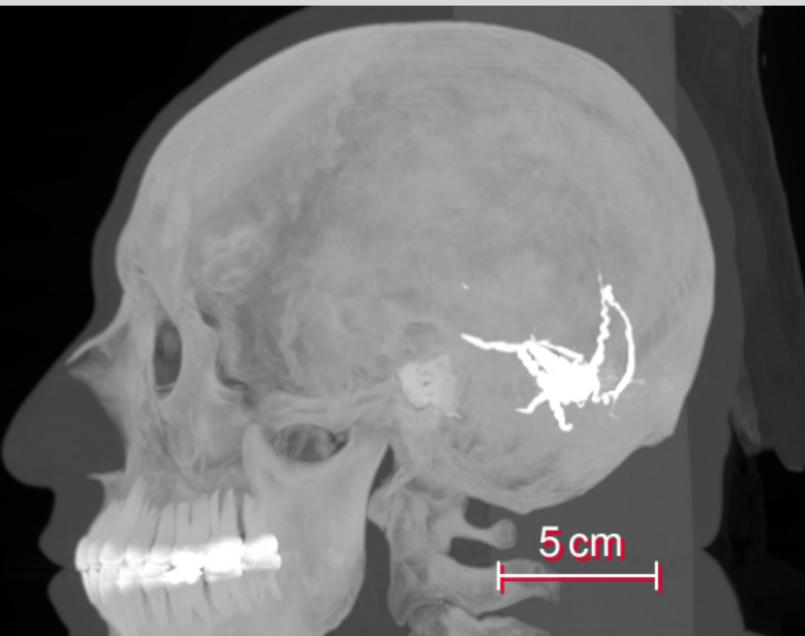
From [8], Subject C3L-02465



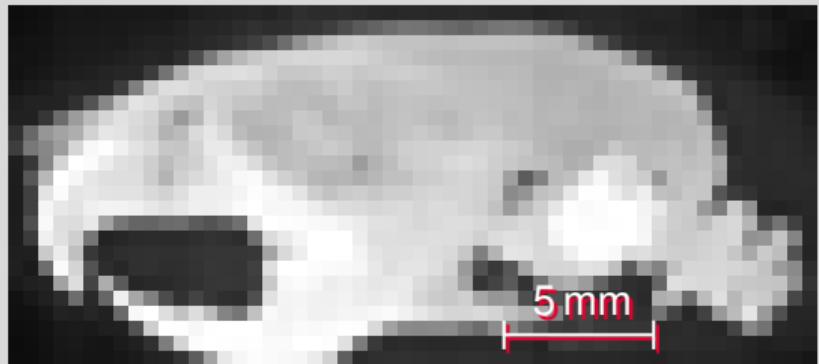


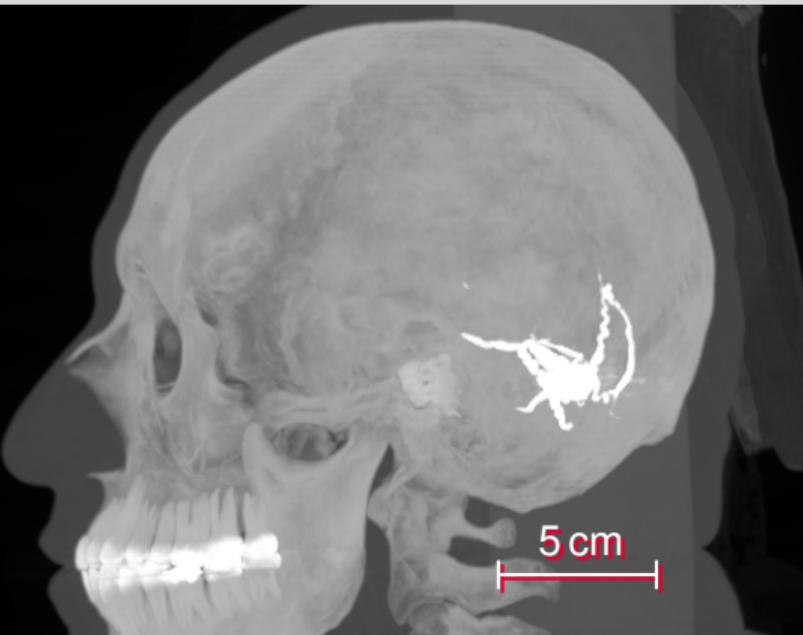
From [8], Subject C3L-02465



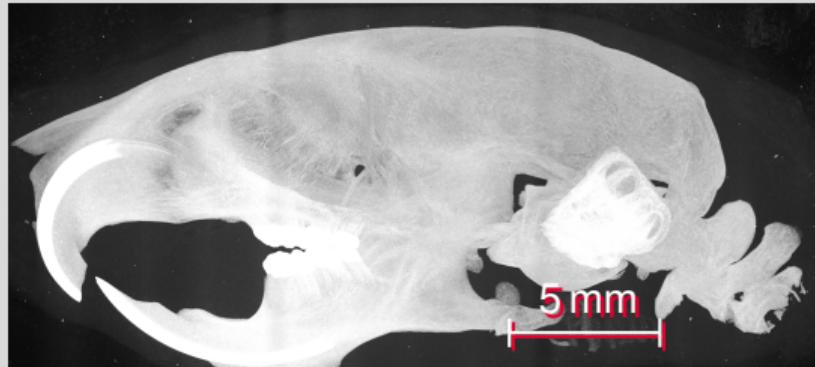


From [8], Subject C3L-02465





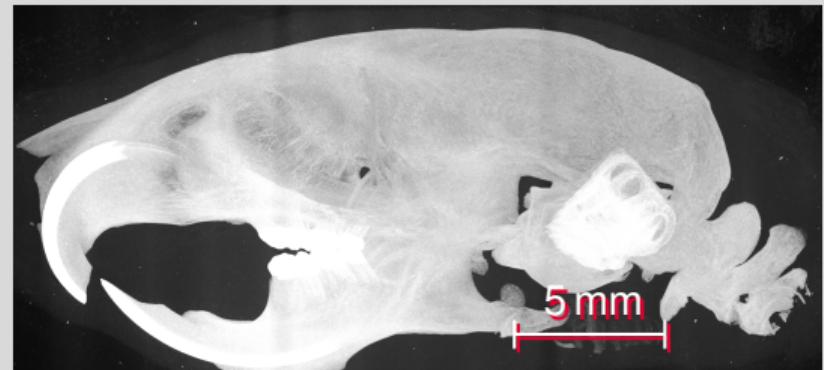
From [8], Subject C3L-02465



# Why $\mu$ CT?



From [8], Subject C3L-02465



## MIP

## Volumetric representation

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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.

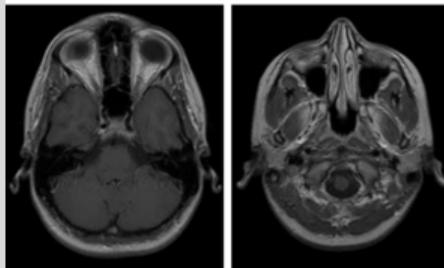
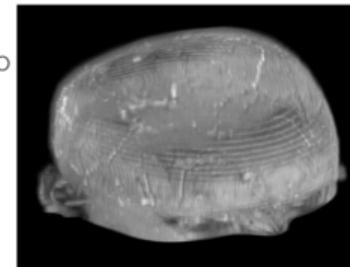


Image stack turned into  
transparent volume



# 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 cc BY SA

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

# Machinery

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flic.kr/p/7Xhk2Y © CC BY-NC-SA

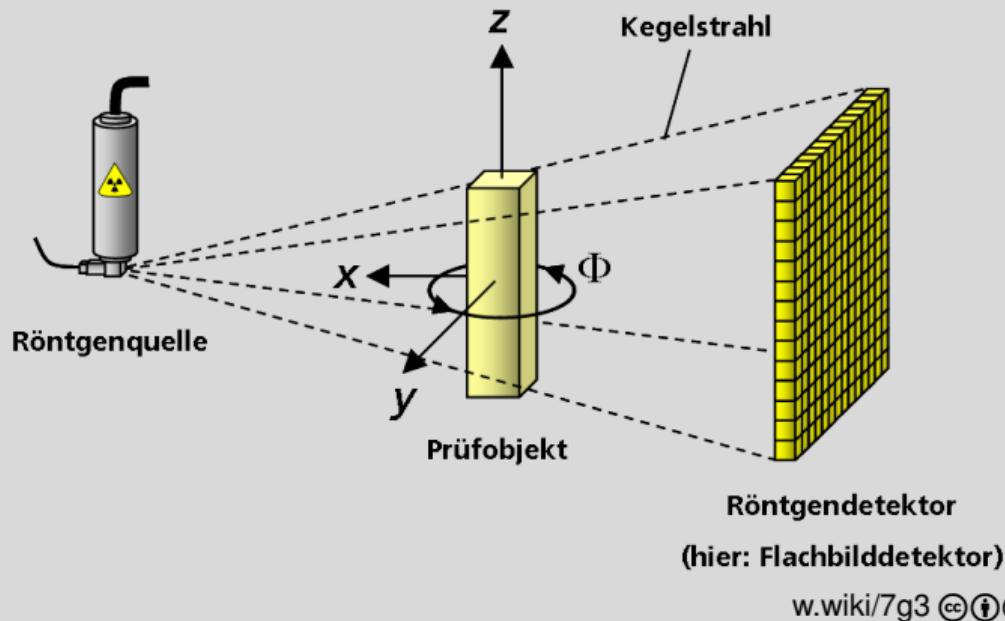
# Machinery

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

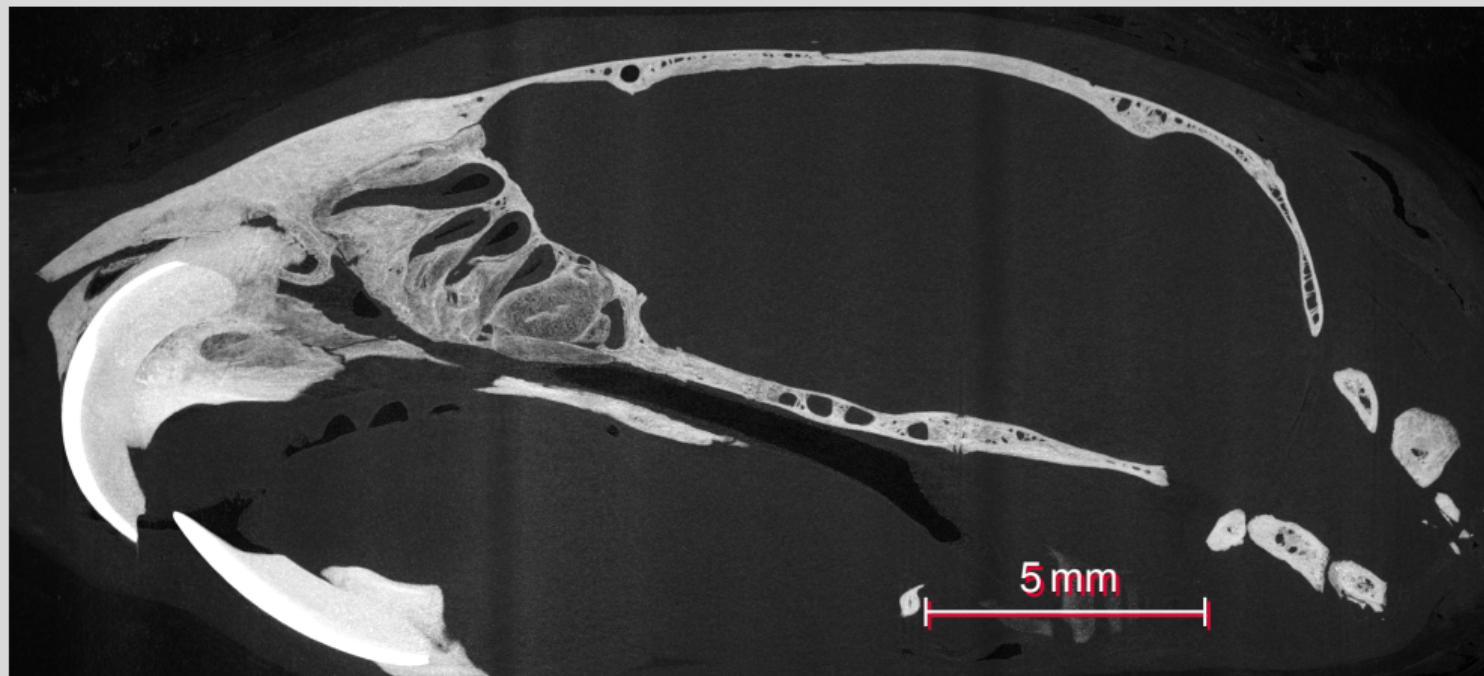
- an x-ray source
- a sample
- a detector

# Machinery

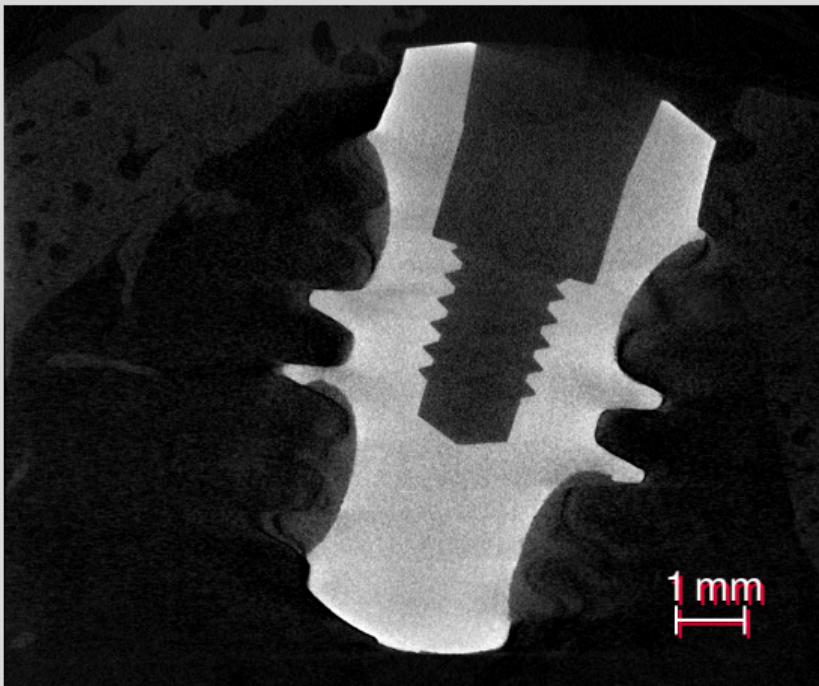
# What is happening?



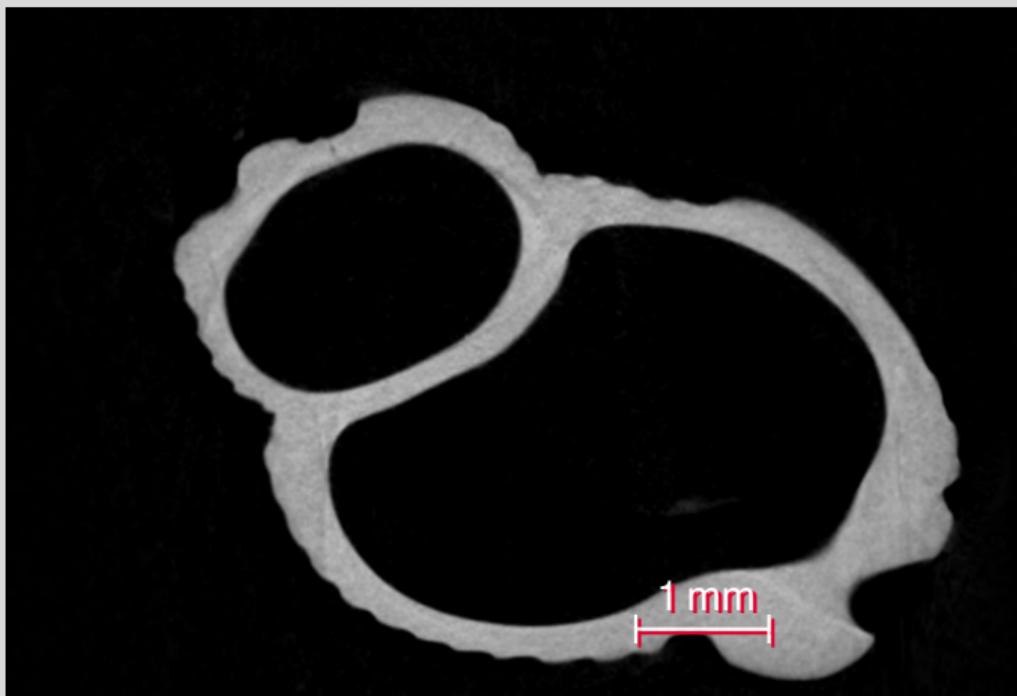
# Examples



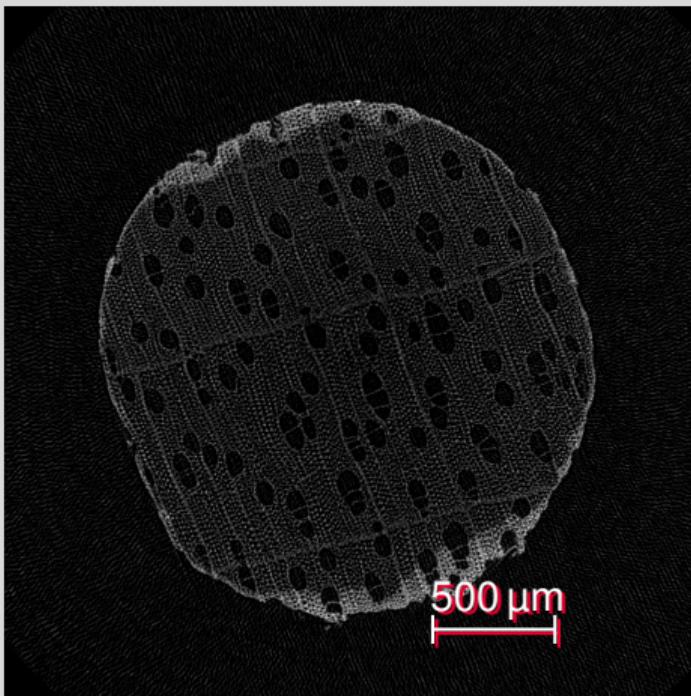
# Examples



## Examples



# Examples



# Examples



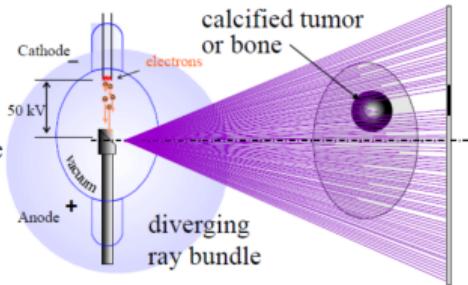
# Preparation

- Study design
- Sample preparation

# Projections

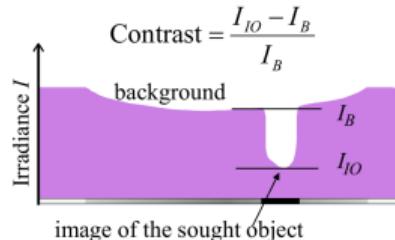
## X-ray generation and contrast

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



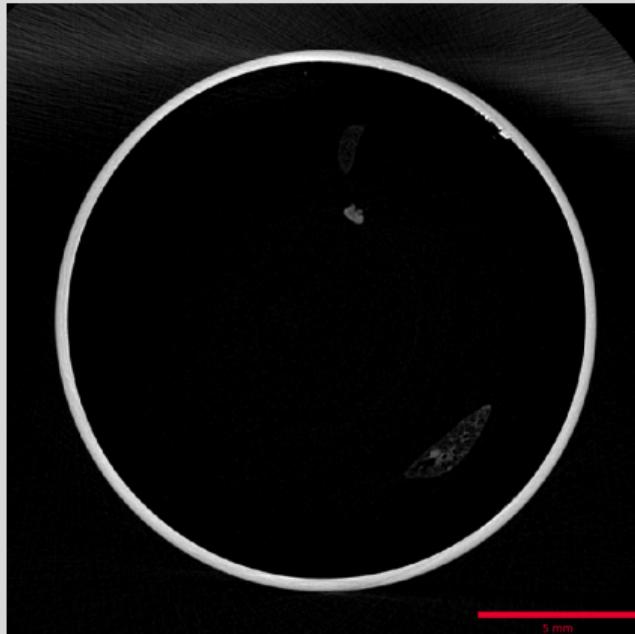
# Projections



# 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

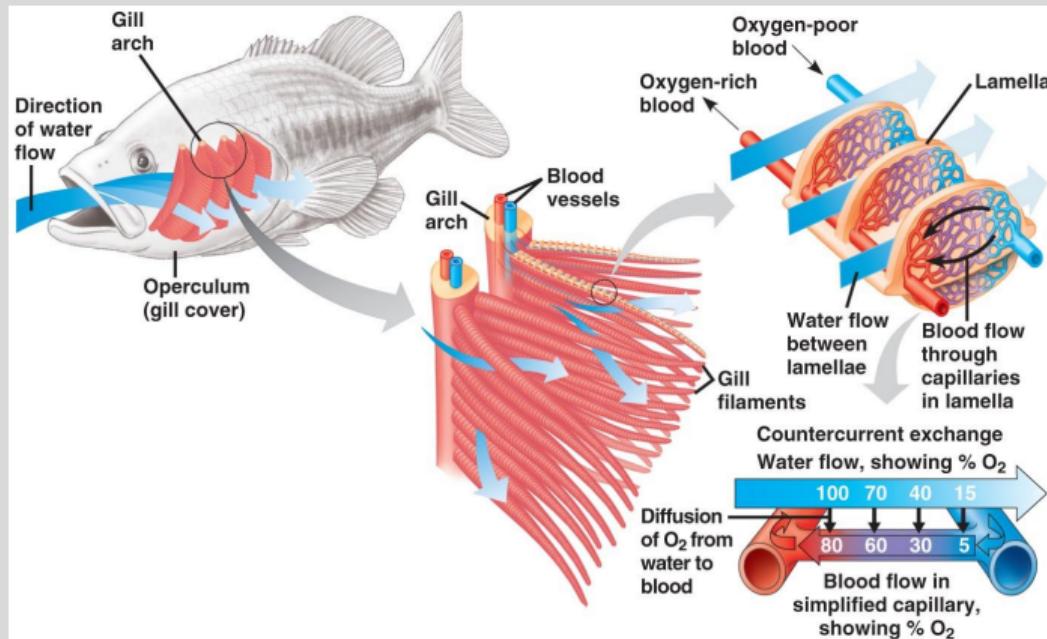
# What to use?

- ImageJ/Fiji [9]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  -  in Jupyter [10]
  - **git**
  - Script all your things!
  - Data repositories; sharing is caring!

# Quantitative data

- Raw numbers instead of just pretty images
- Segmentation
- Characterization

# An example: Do gills change with training?



Campbell Biology [11]

# Gill anatomy



X-ray microtomography

# Gill anatomy

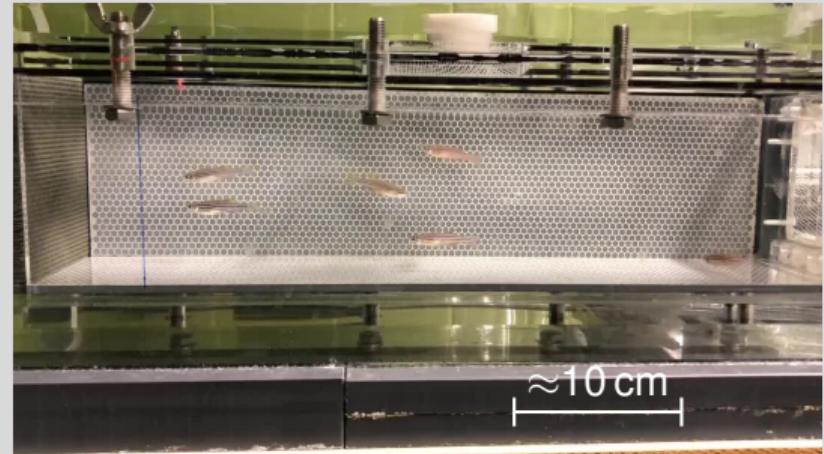


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# How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [12]
  - Endurance



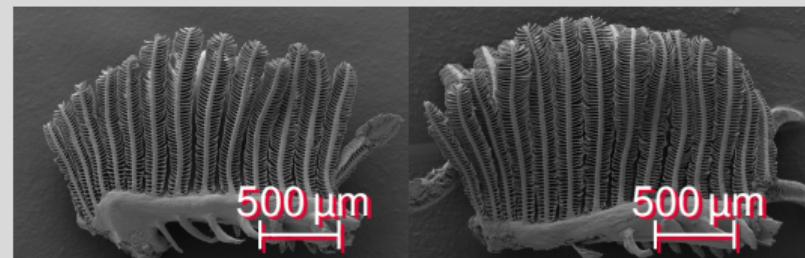
# How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [12]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption



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- Scanning electron microscopy
  - Gill structure



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  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying



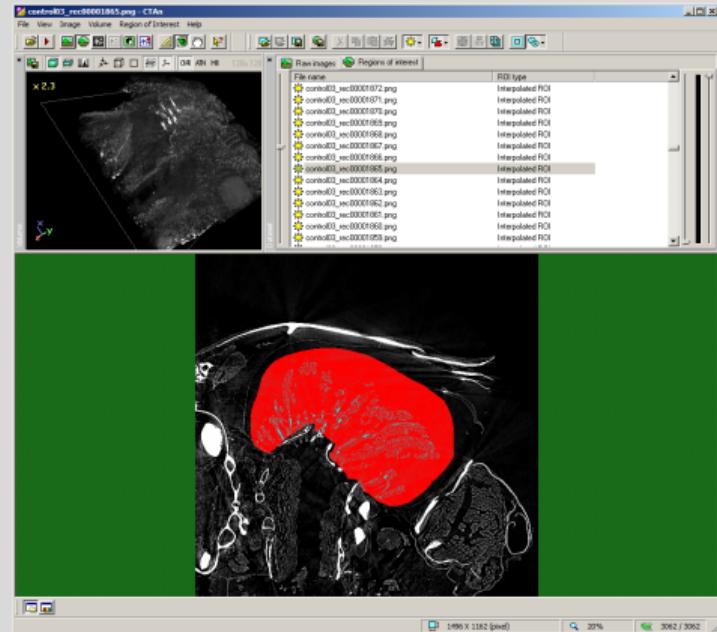
# How?

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  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging

```
Scanner=Skyscan1172
Instrument S/N=12001199
Hardware version=F
Software=Version 1. 5 (build 23)
Filename Prefix=Control05
Number of Files= 3979
Source Voltage (kV)= 49
Source Current (uA)= 167
Number of Rows= 2672
Number of Columns= 4000
Image Pixel Size (um)= 1.66
Object to Source (mm)=40.030
Camera to Source (mm)=212.399
Filter=No Filter
Exposure (ms)= 890
Rotation Step (deg)=0.050
Frame Averaging=ON (6)
Scan duration=08:55:28
Reconstruction Program=NRecon
Program Version=Version: 1.7.1.0
```

# How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [12]
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- Critical point drying, µCT imaging, delineation in CTAn



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- Scanning electron microscopy
  - Gill structure
- Critical point drying,  $\mu$ CT imaging, delineation in CTAn and analysis
  - Gill volume, structure and complexity

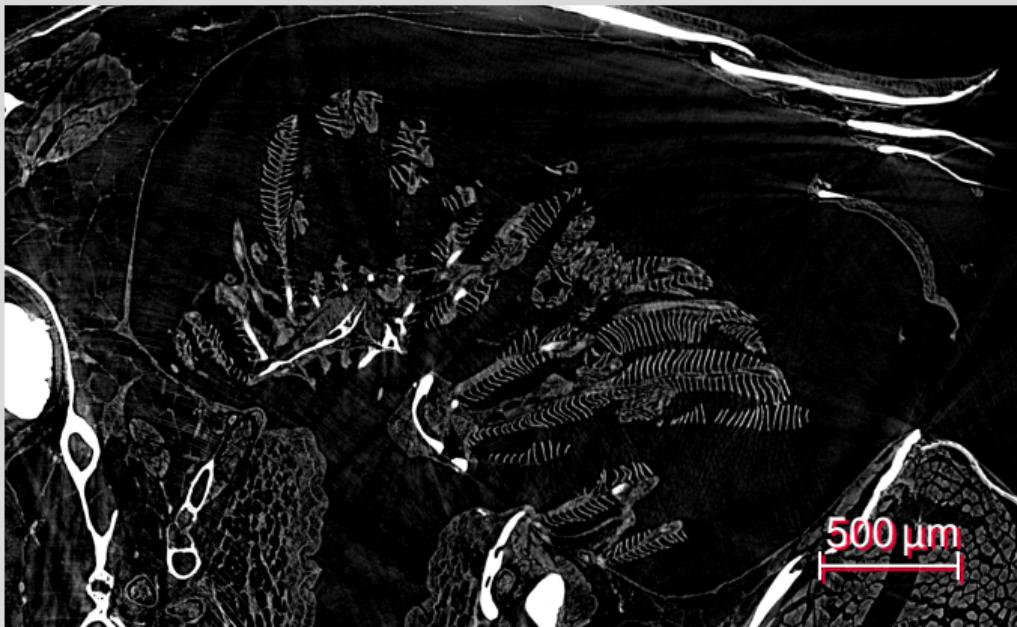


[gph.is/2nqkple](https://gph.is/2nqkple)

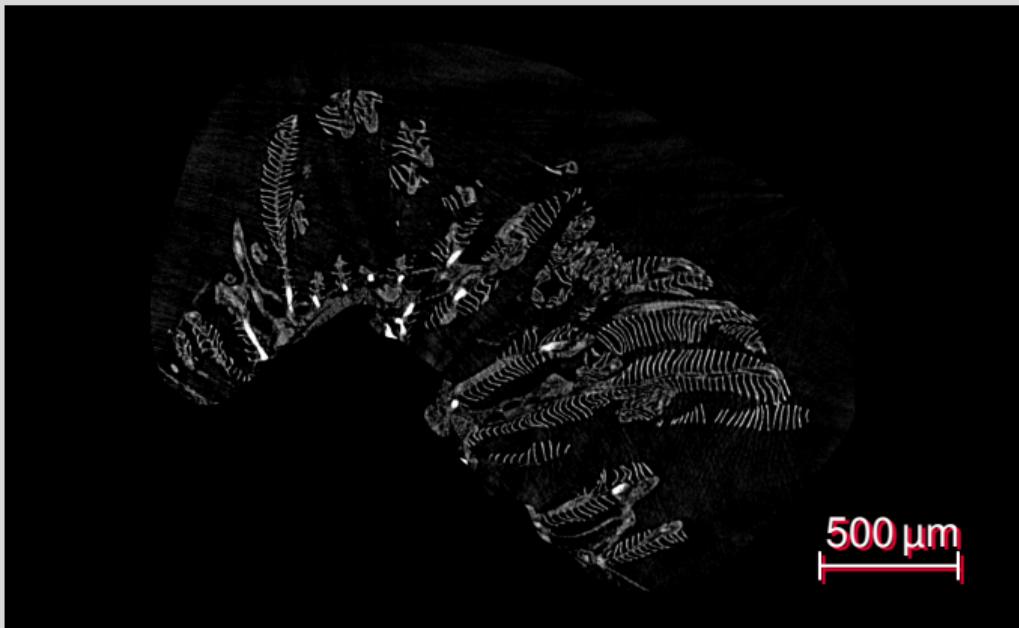
# Gill volume



# Gill volume



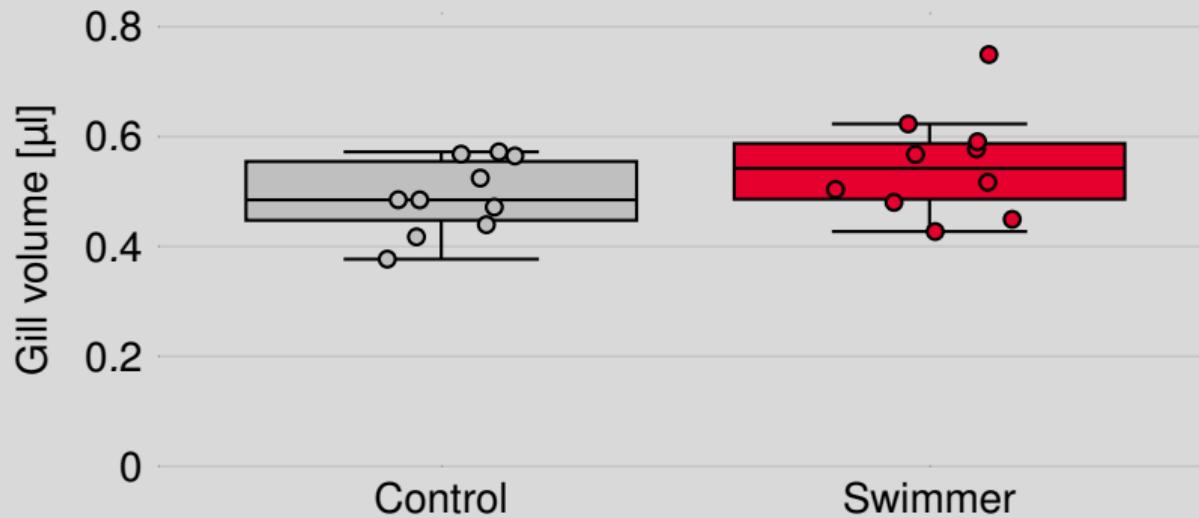
# Gill volume



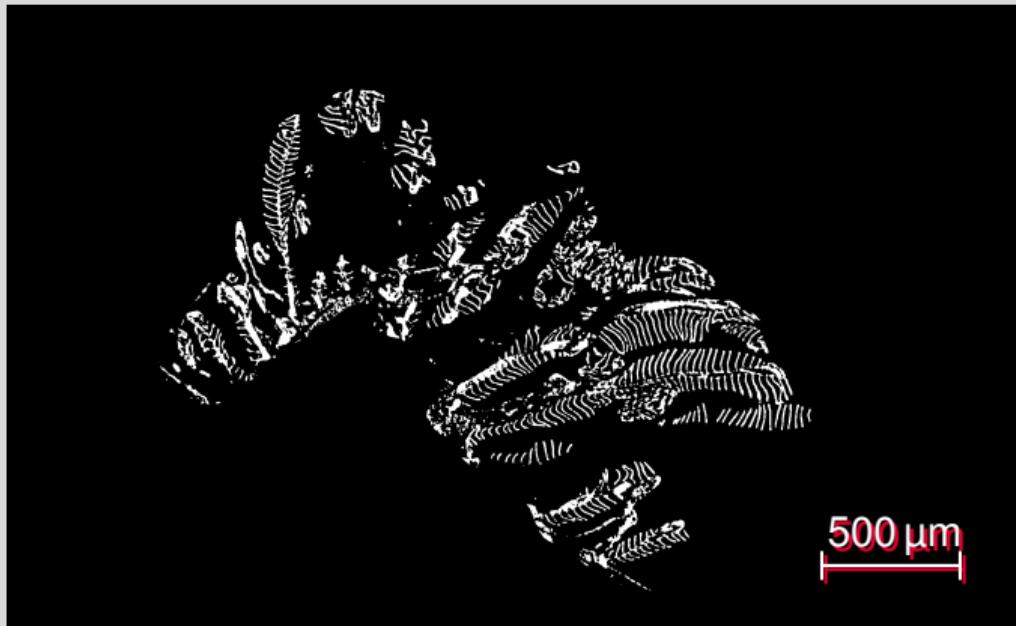
# Gill volume



# Gill volume



# Gill complexity



# Gill complexity

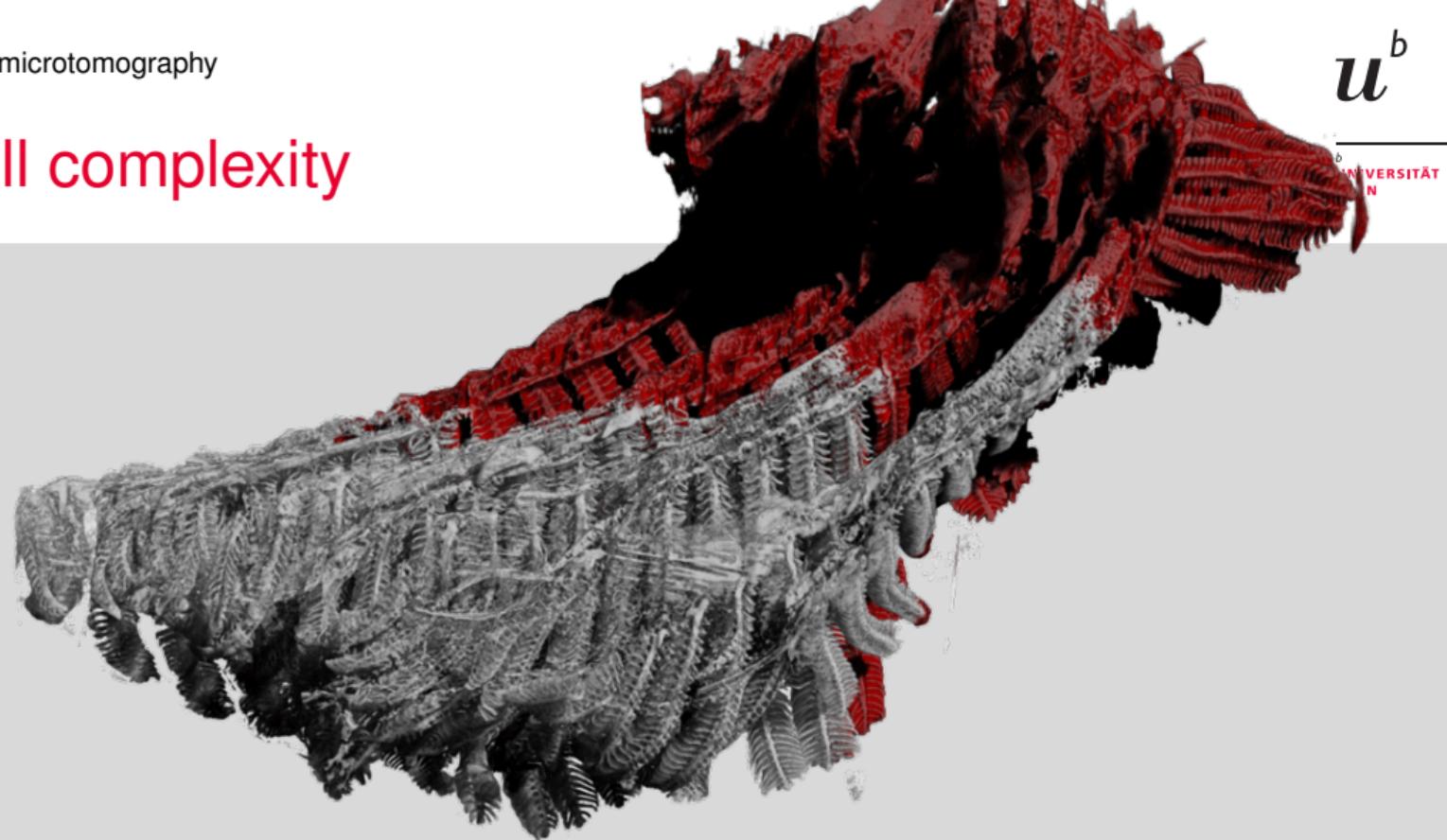


# Gill complexity



X-ray microtomography

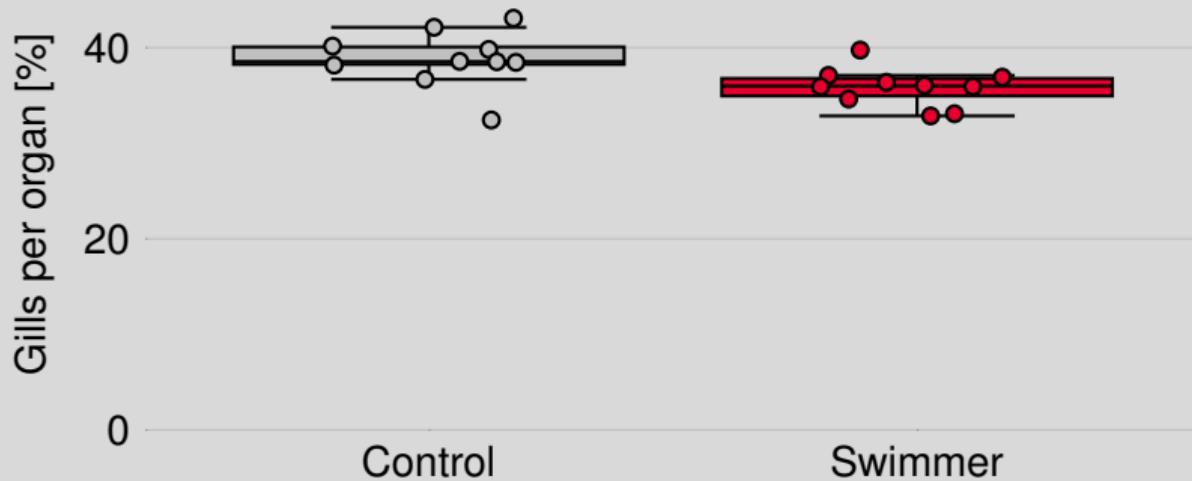
## Gill complexity



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# Gill complexity



# 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)

# References

- [1] Ruslan Hlushchuk et al. "Ex vivo microangioCT: Advances in microvascular imaging". DOI: [10.1016/j.vph.2018.09.003](https://doi.org/10.1016/j.vph.2018.09.003).
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- [11] Martha R. Taylor et al. *Campbell Biology: Concepts and Connections (9th Edition)*. ISBN: 9780134296012.
- [12] Arjan P. Palstra et al. "Establishing Zebrafish as a Novel Exercise Model: Swimming Economy, Swimming-Enhanced Growth and Muscle Growth Marker Gene Expression". DOI: [10.1371/journal.pone.0014483](https://doi.org/10.1371/journal.pone.0014483).