

# 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

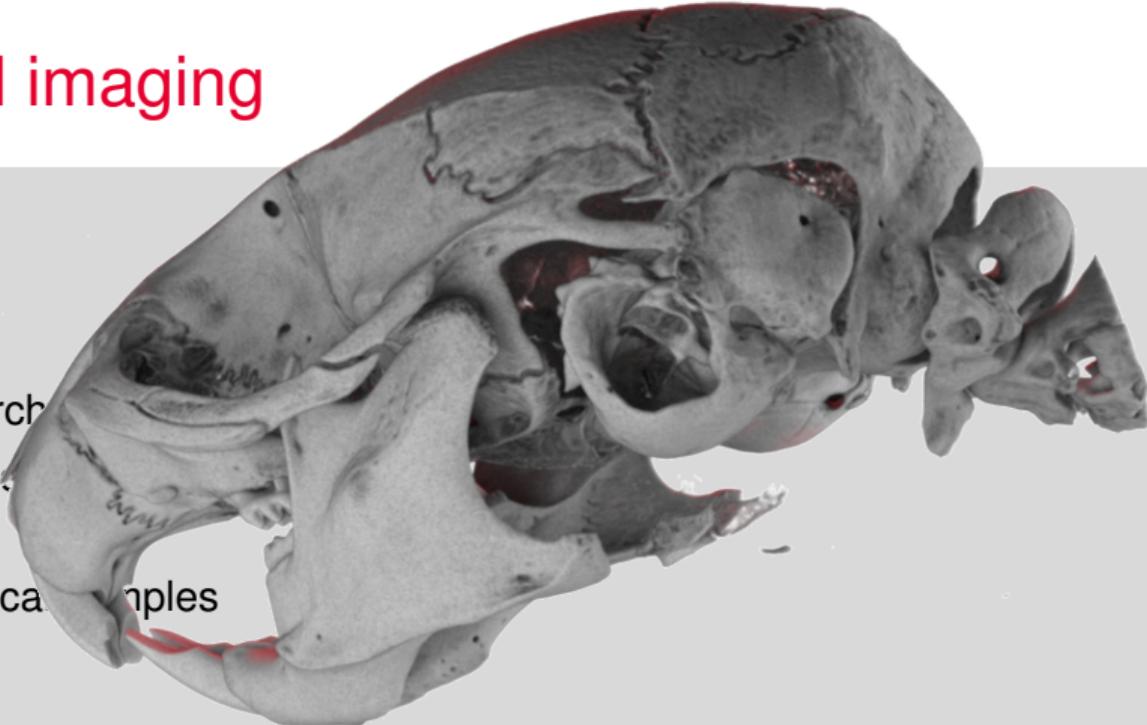
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



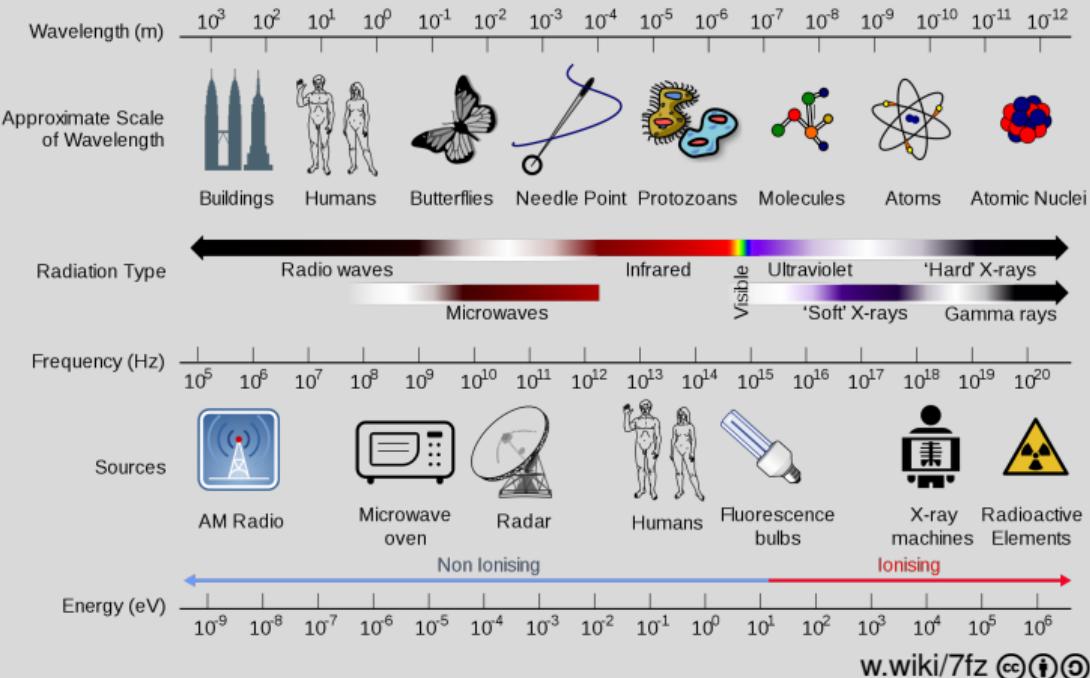
w.wiki/7g4 

# Biomedical imaging

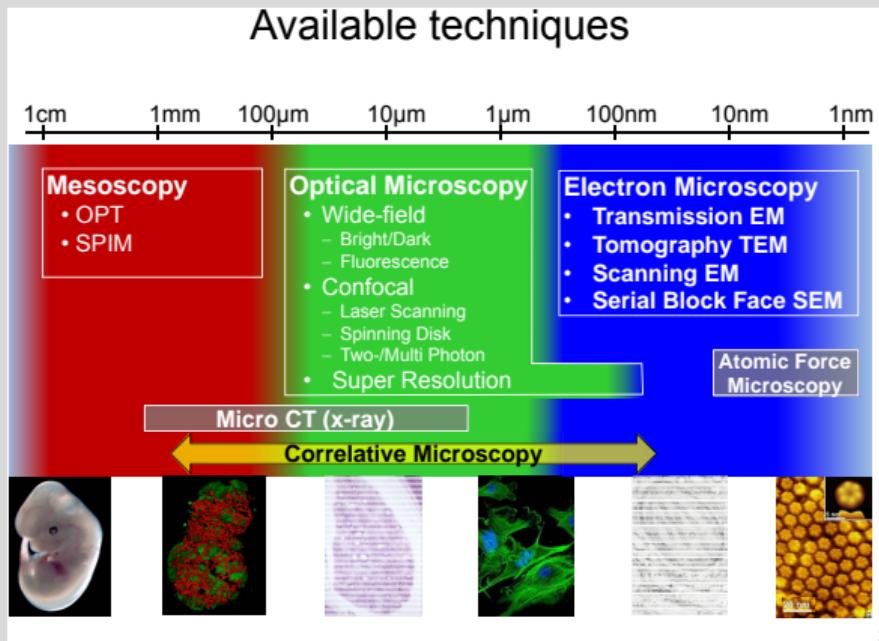
- Medical research
- Non-destructive analysis of the samples
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# Wavelength & Scale



# Wavelength & Scale

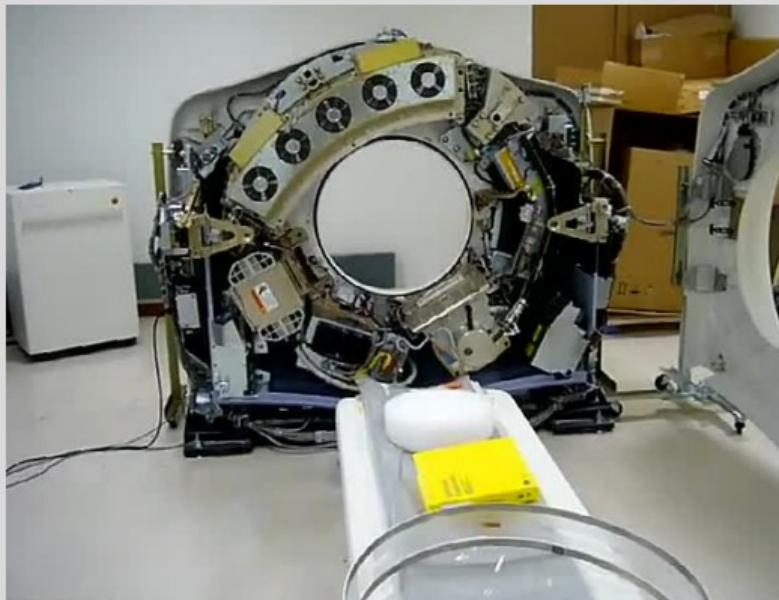


Yury Belyaev, MIC, slide from internal seminar presentation

# 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.

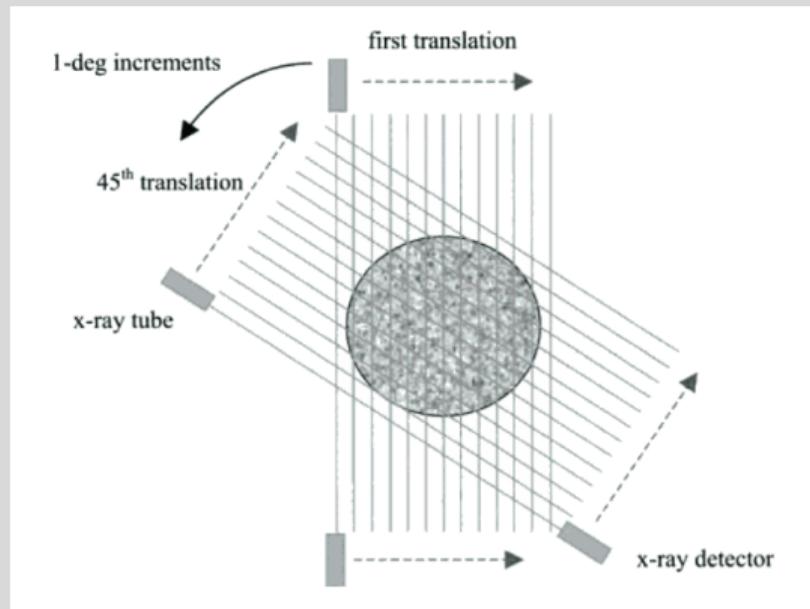
# CT-Scanner



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

# History

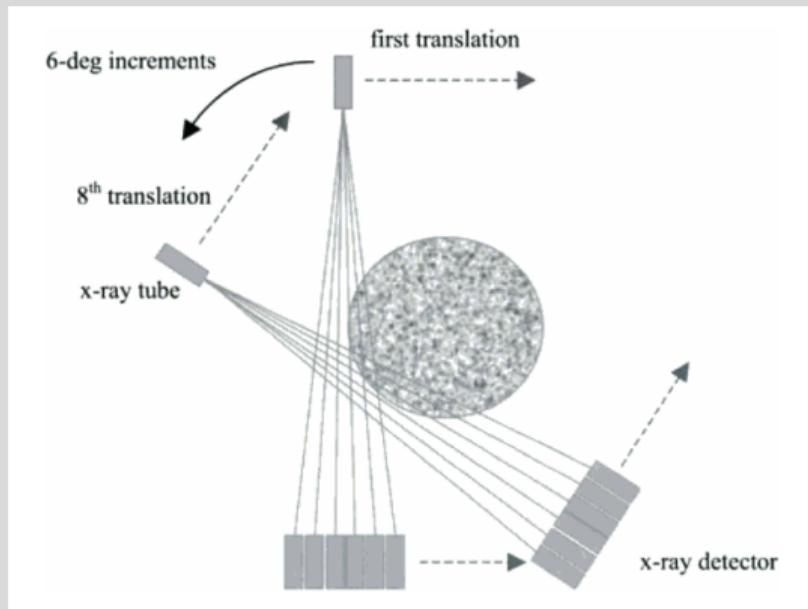
- Long history
  - 1963: Cormack used a collimated  $^{60}\text{Co}$  source and a Geiger counter as a detector [3]
  - 1976: Hounsfield worked on first clinical scanner [4]
  - Nice overview by Hsieh [5]
- First, second and third generation of scanners



From [5], Figure 1.12

# History

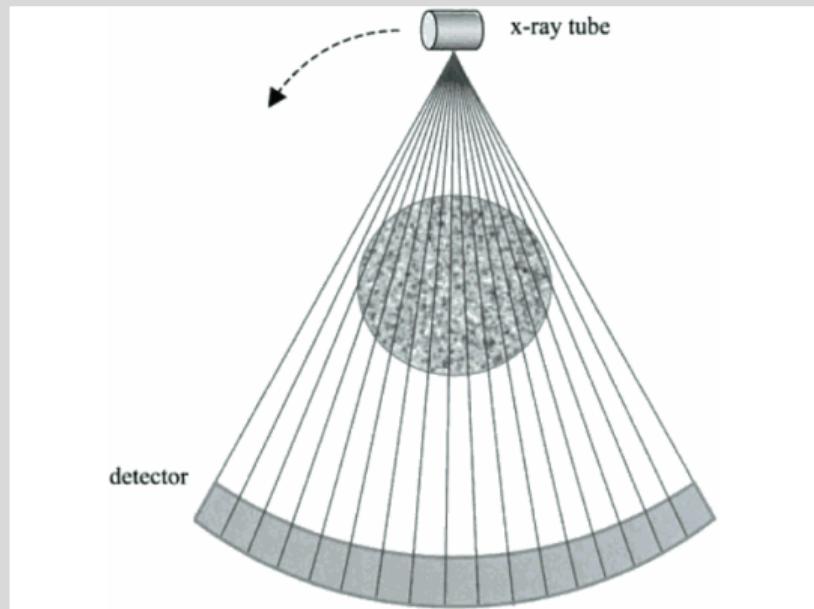
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From [5], Figure 1.13

# 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]  $I(t) = I_0 e^{-\alpha z}$

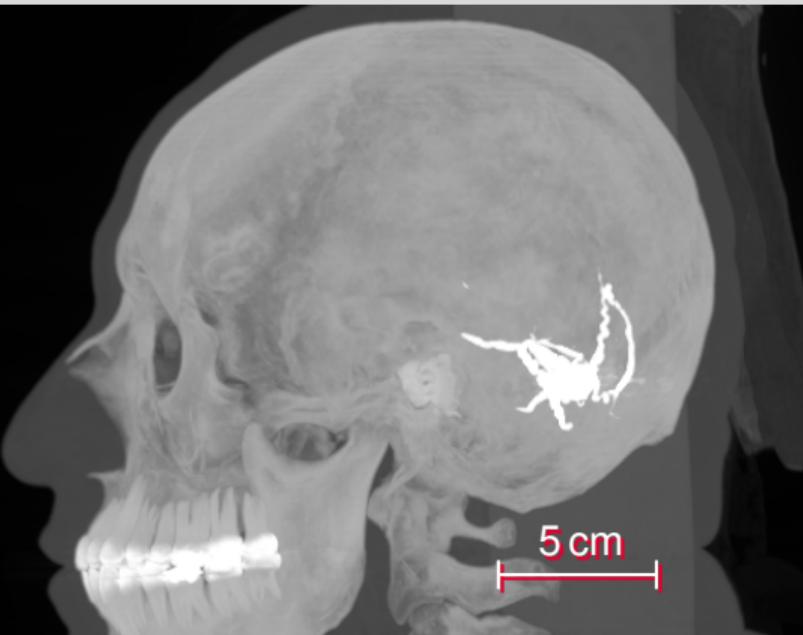


# Composition of biological tissues

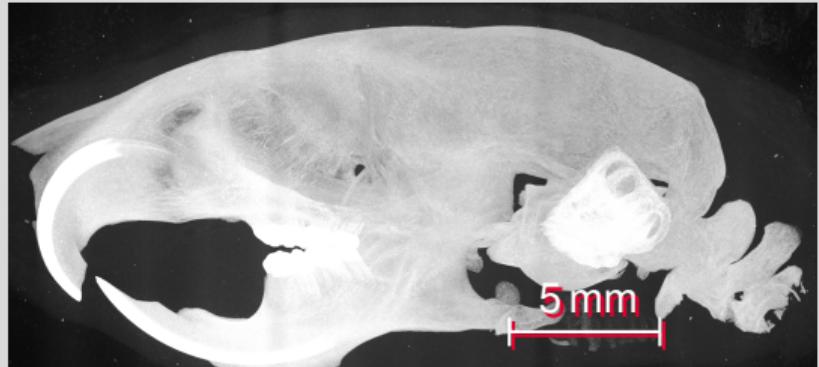
Tissue: content by mass percentage

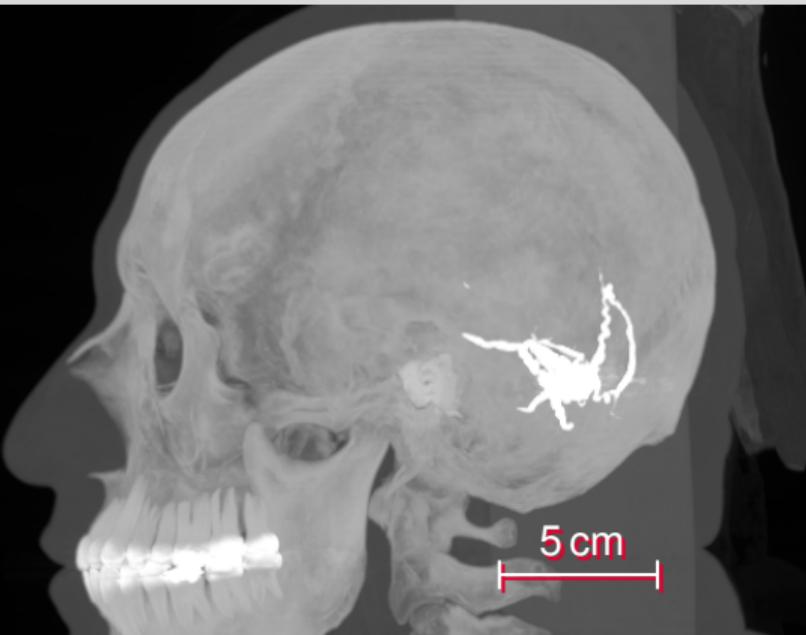
Element Atomic number	H 1	C 6	N 7	O 8	Na 11	P 15	S 16	Cl 17	K 19	Ca 20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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





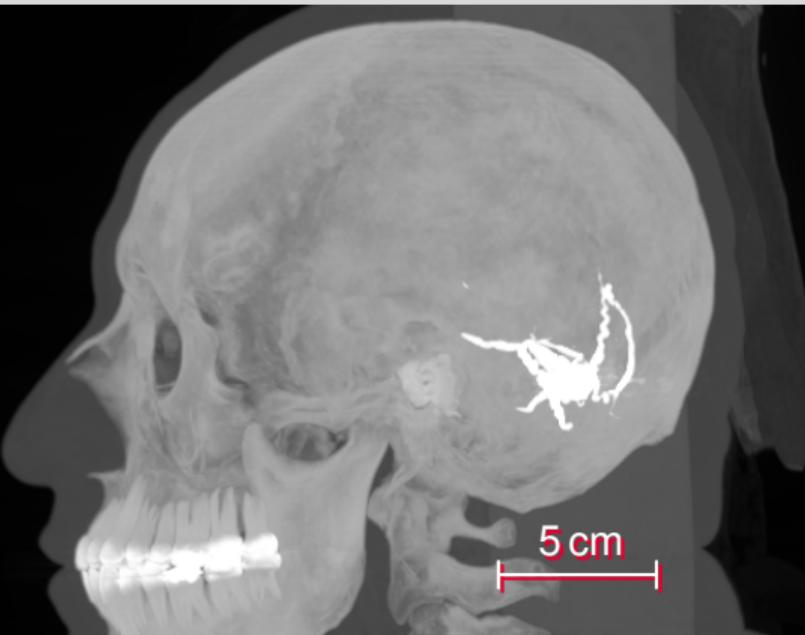
From [8], Subject C3L-02465



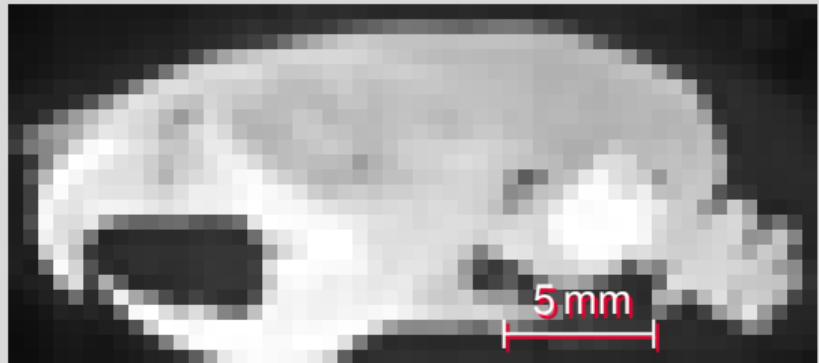


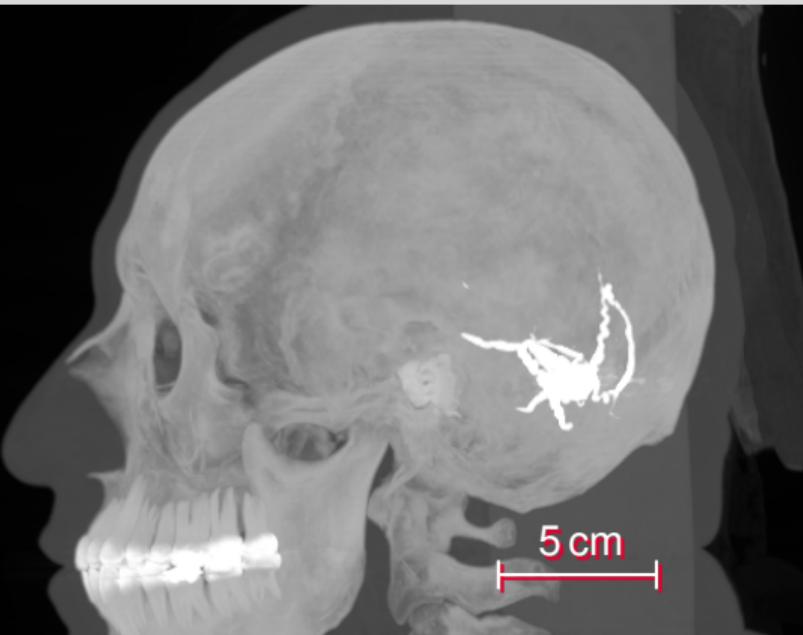
From [8], Subject C3L-02465



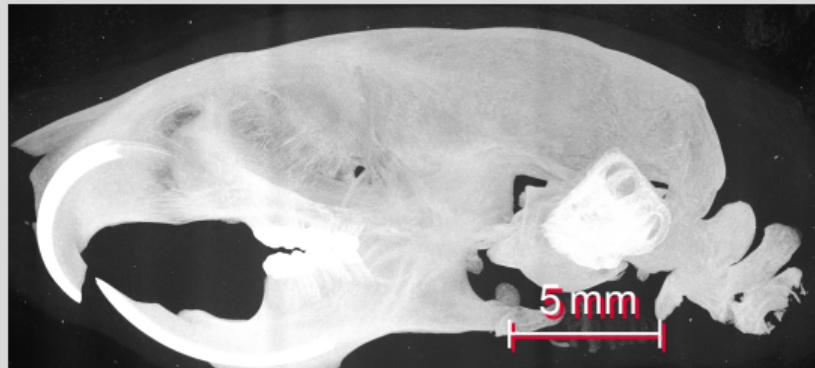


From [8], Subject C3L-02465





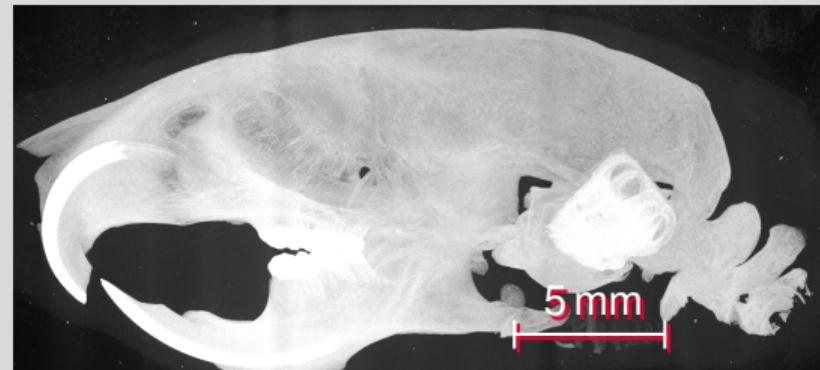
From [8], Subject C3L-02465



# Why $\mu$ CT?



From [8], Subject C3L-02465



# 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)

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flic.kr/p/7Xhk2Y © CC BY-NC-SA

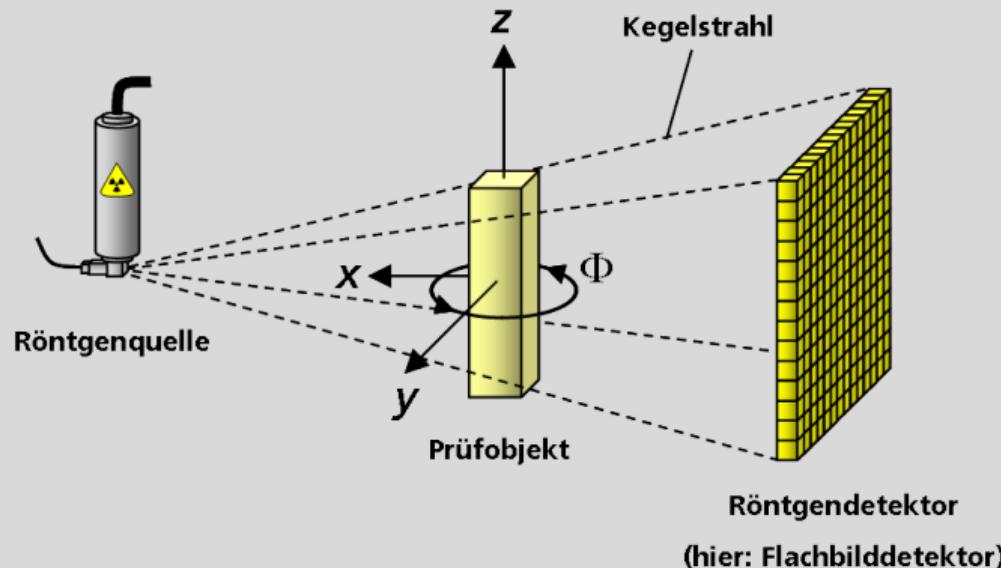
# Machinery

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

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

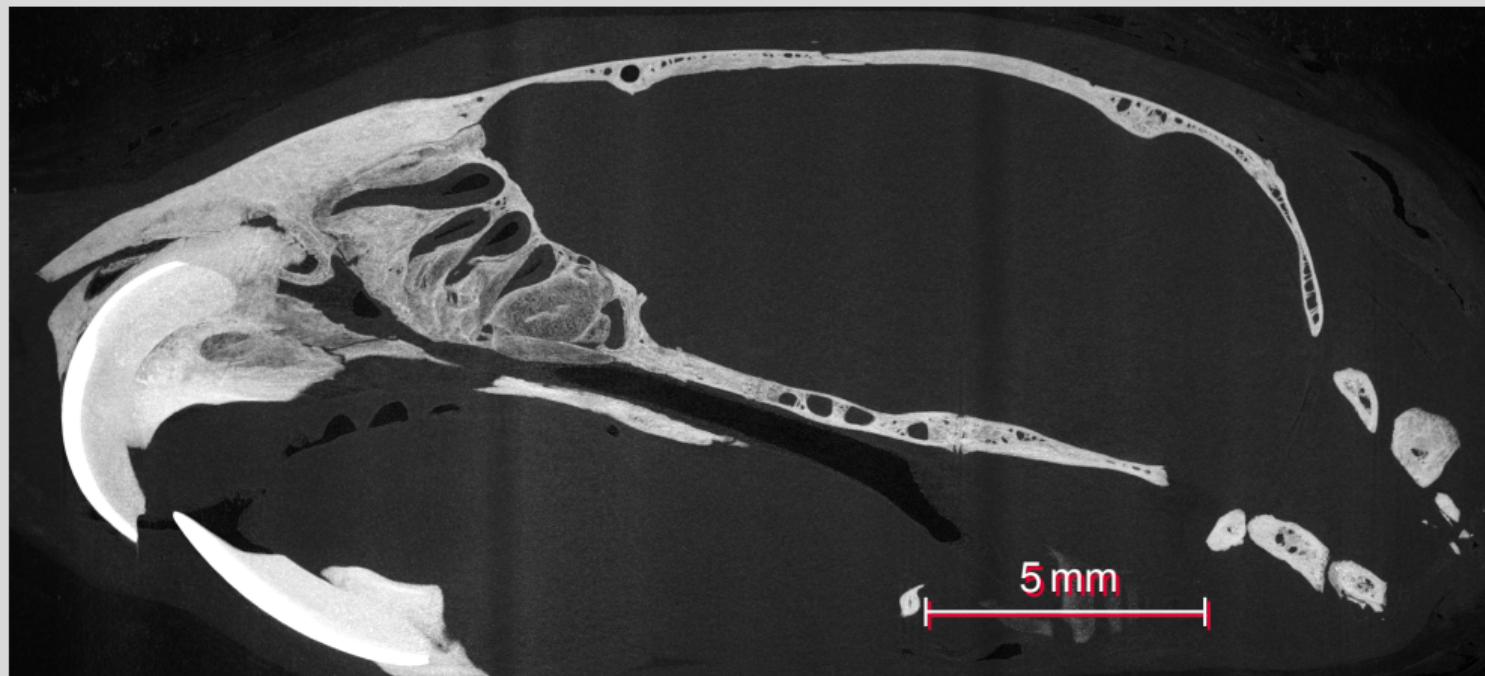
# Machinery

# What is happening?

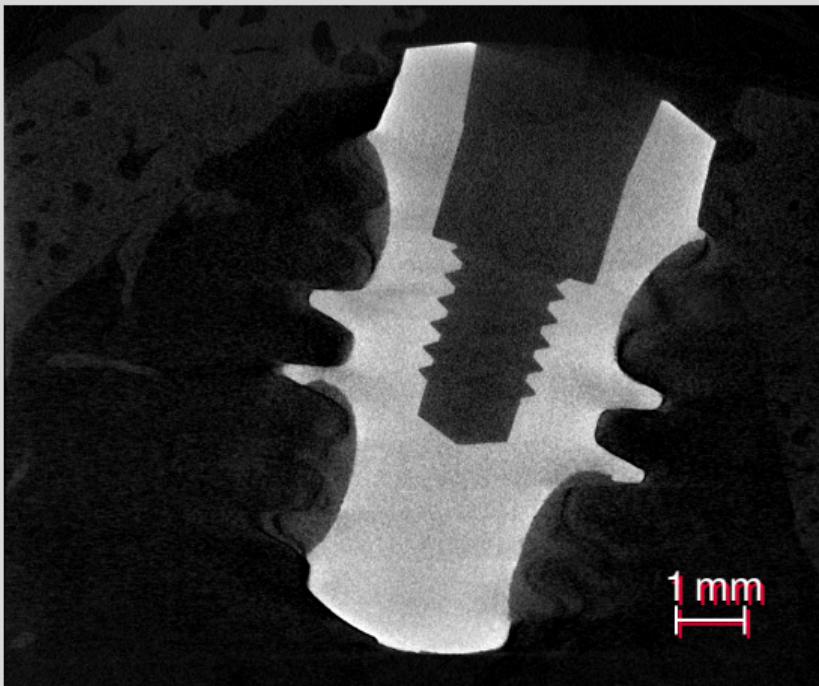


w.wiki/7g3

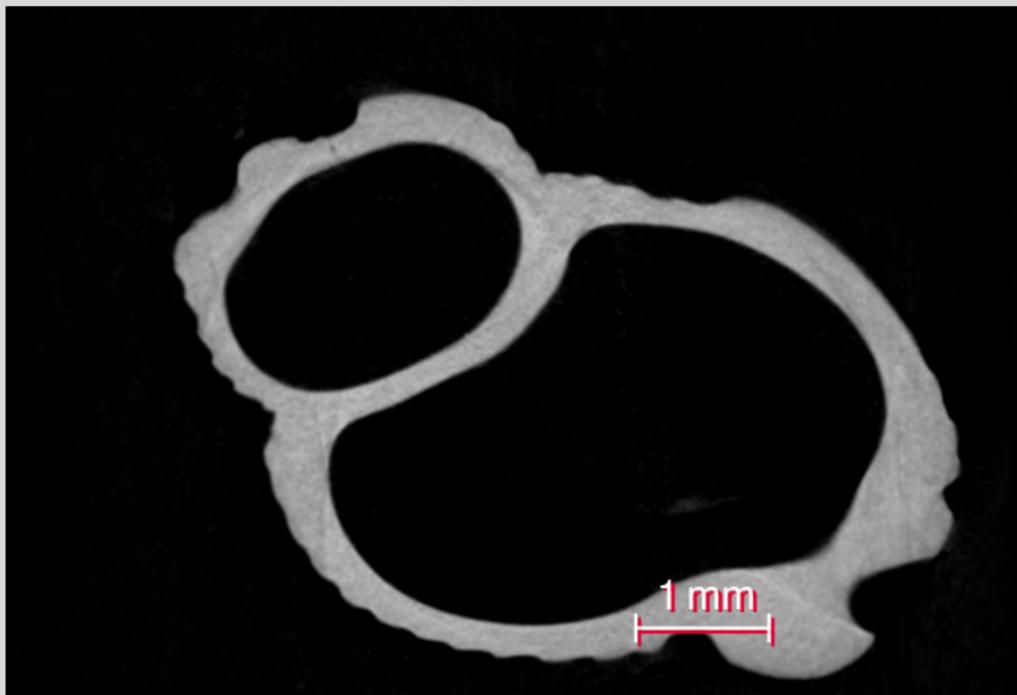
# Examples



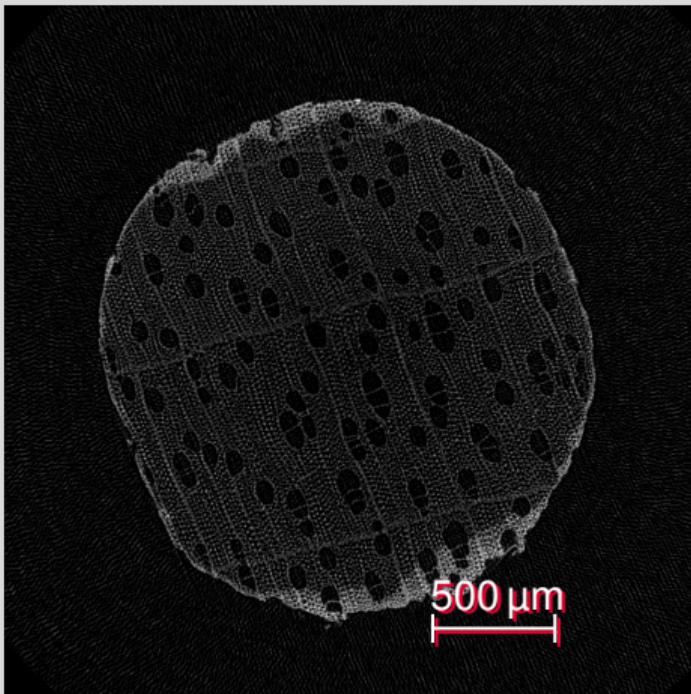
# Examples



## Examples



# Examples



# Examples

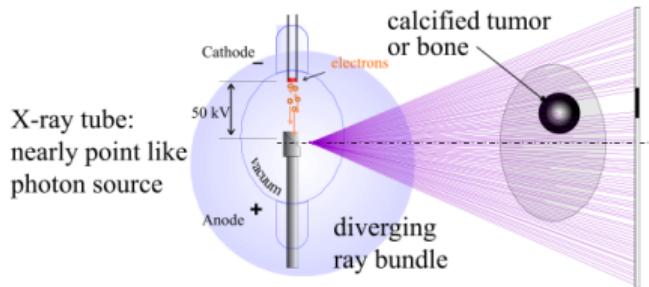


# Preparation

- Study design
- Sample preparation

# Projections

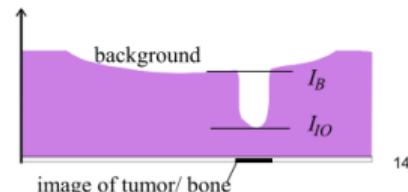
## Image formation: shadow projection



X-ray tube:  
nearly point like  
photon source

Contrast is given by  
absorption of intensity  $I$   
by object  $\rightarrow$  negative

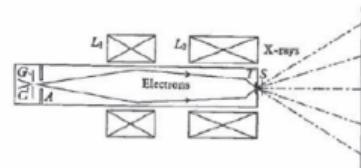
$$\text{Contrast} = \frac{I_{IO} - I_B}{I_B}$$



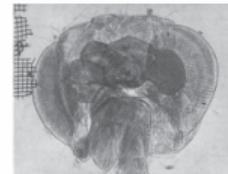
14

## Image formation: shadow projection

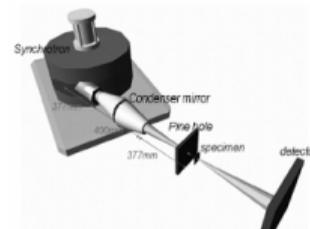
### X-ray projection microscopy



Cosslett V E and Nixon W C X-ray Microscopy  
Cambridge University Press 1960



Cosslett V and Nixon W  
Nature 170 436–438 , 1952



Tabletop synchrotron  
X-ray source Mirrorcle  
2010

15

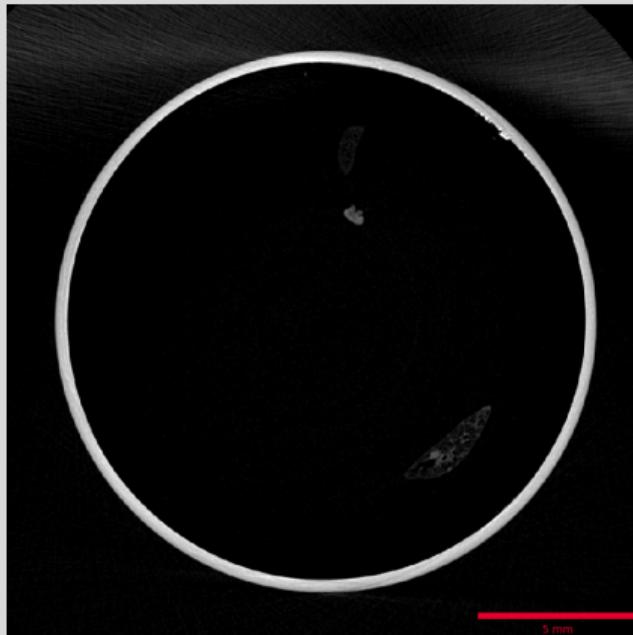
# Projections



# 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

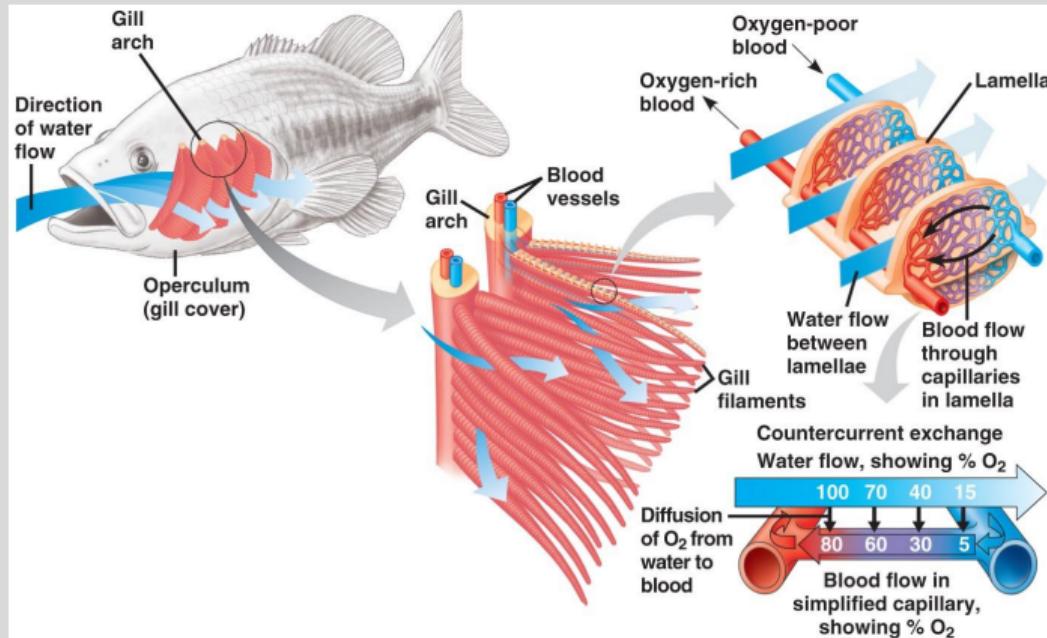
# What to use?

- 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

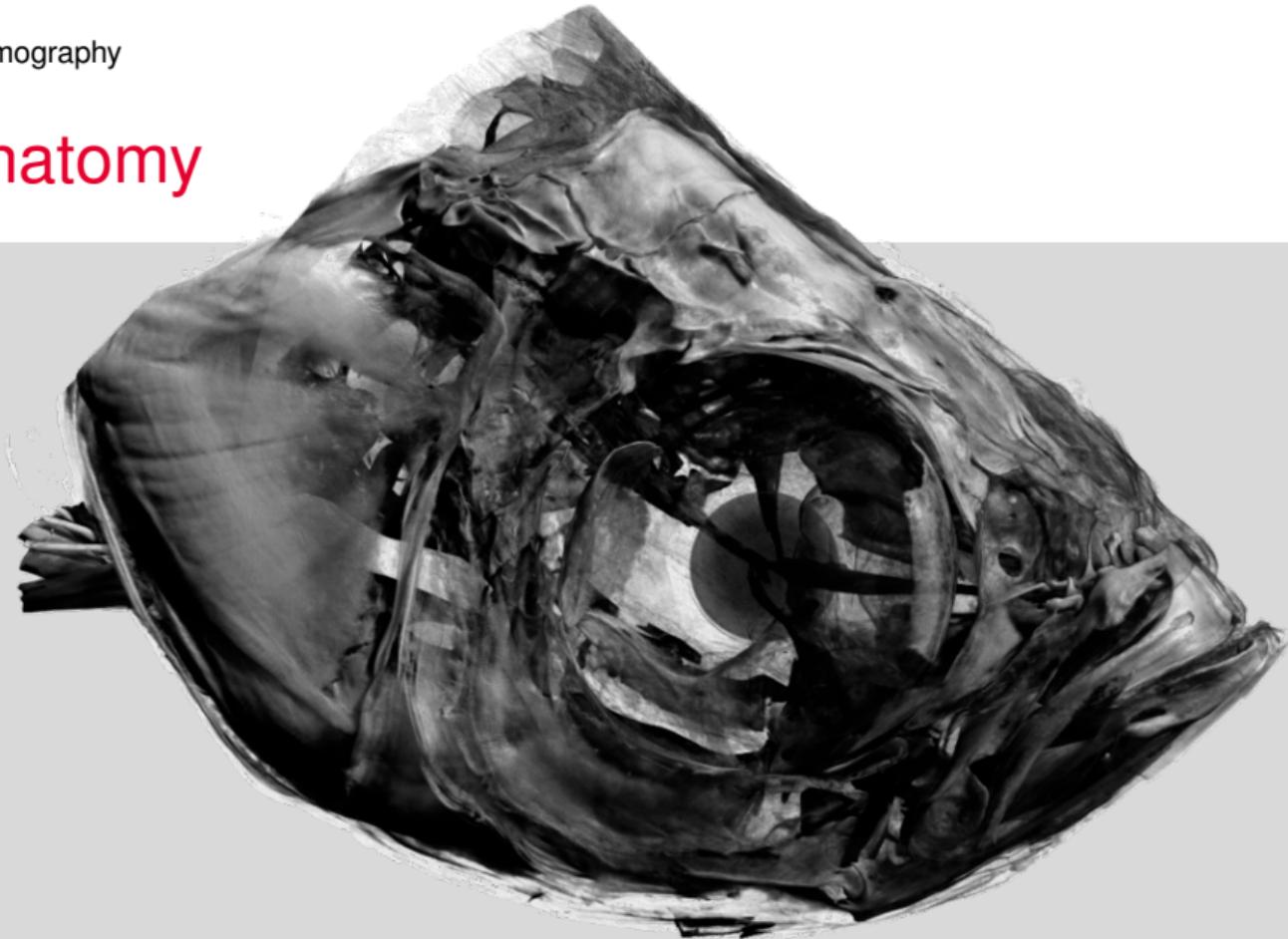
- Raw numbers instead of just pretty images
- Segmentation
- Characterization

# An example: Do gills change with training?



Campbell Biology [11]

# Gill anatomy



X-ray microtomography

# Gill anatomy

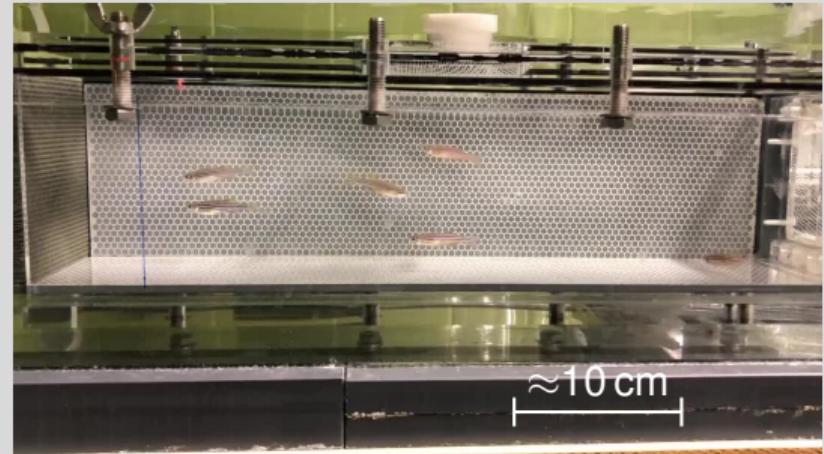


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# How?

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



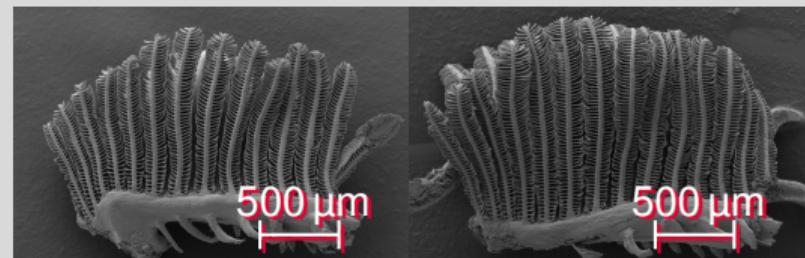
# How?

- 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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- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [12]
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  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure



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- Scanning electron microscopy
  - Gill structure
- Critical point drying



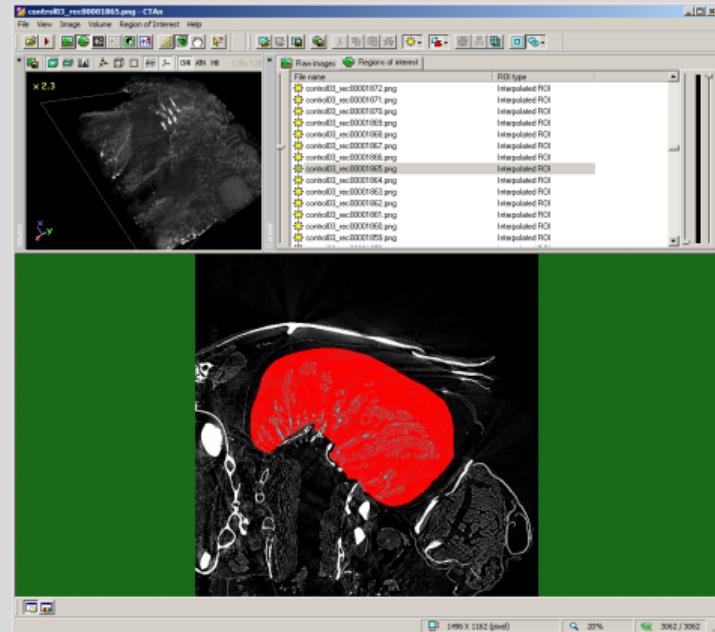
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  - Endurance
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  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging

```
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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  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying,  $\mu$ CT imaging, delineation in CTAn and analysis
  - Gill volume, structure and complexity

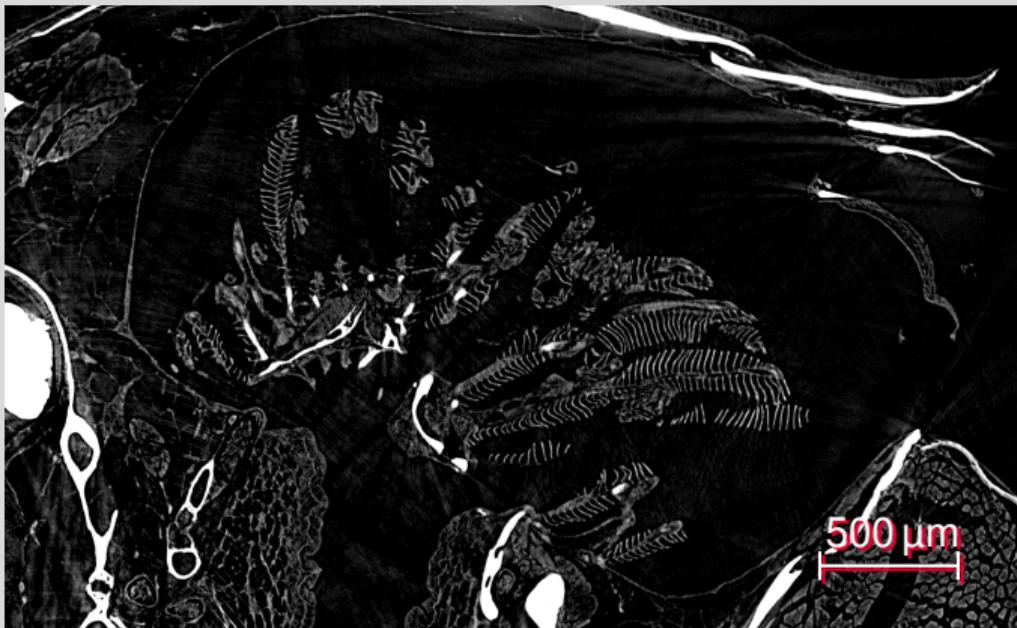


[gph.is/2nqkple](https://gph.is/2nqkple)

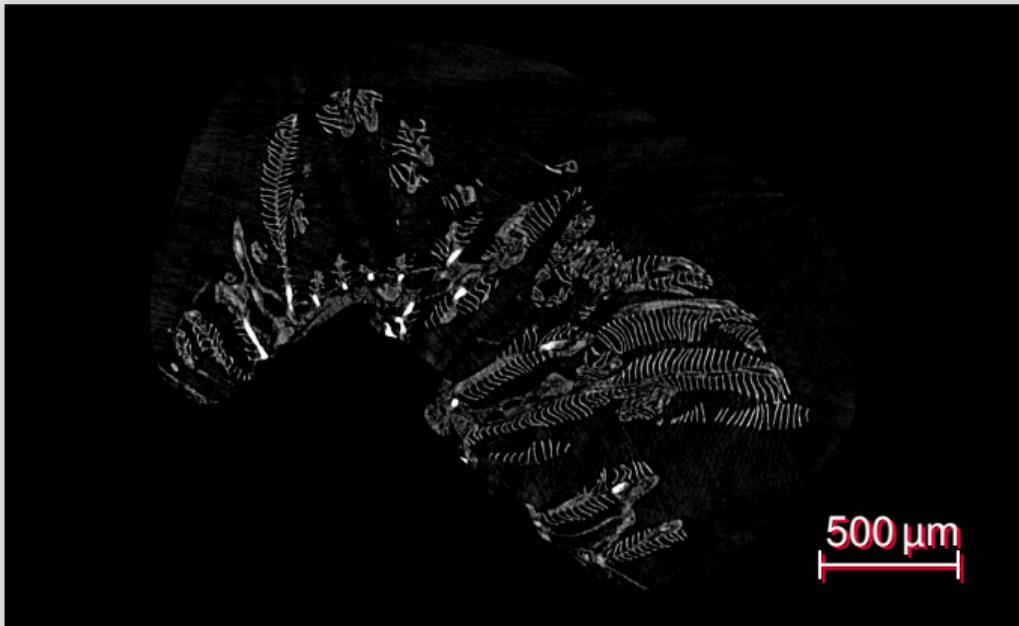
# Gill volume



# Gill volume



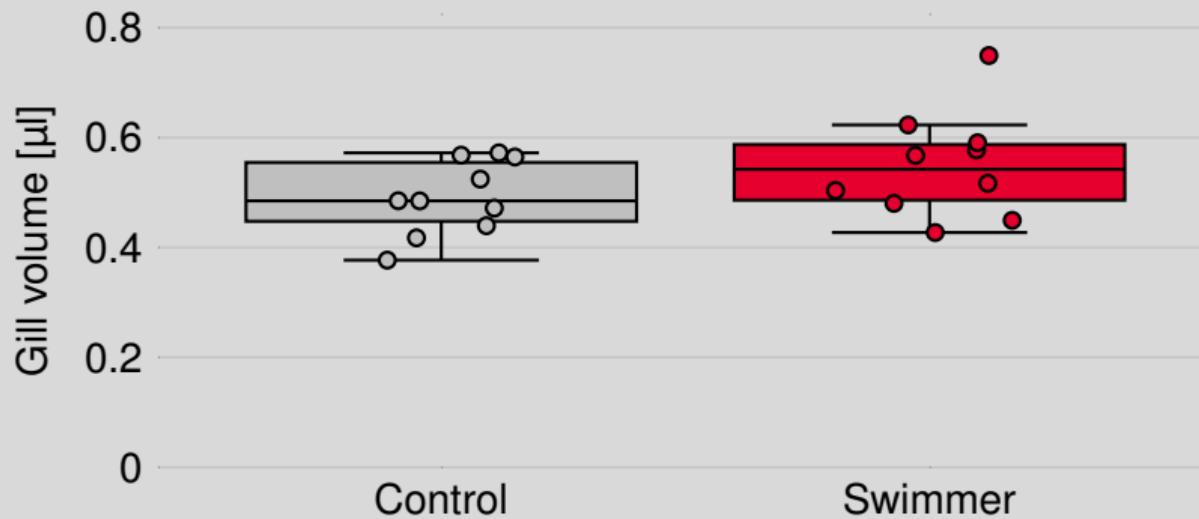
# Gill volume



# Gill volume



# Gill volume



# Gill complexity



# Gill complexity

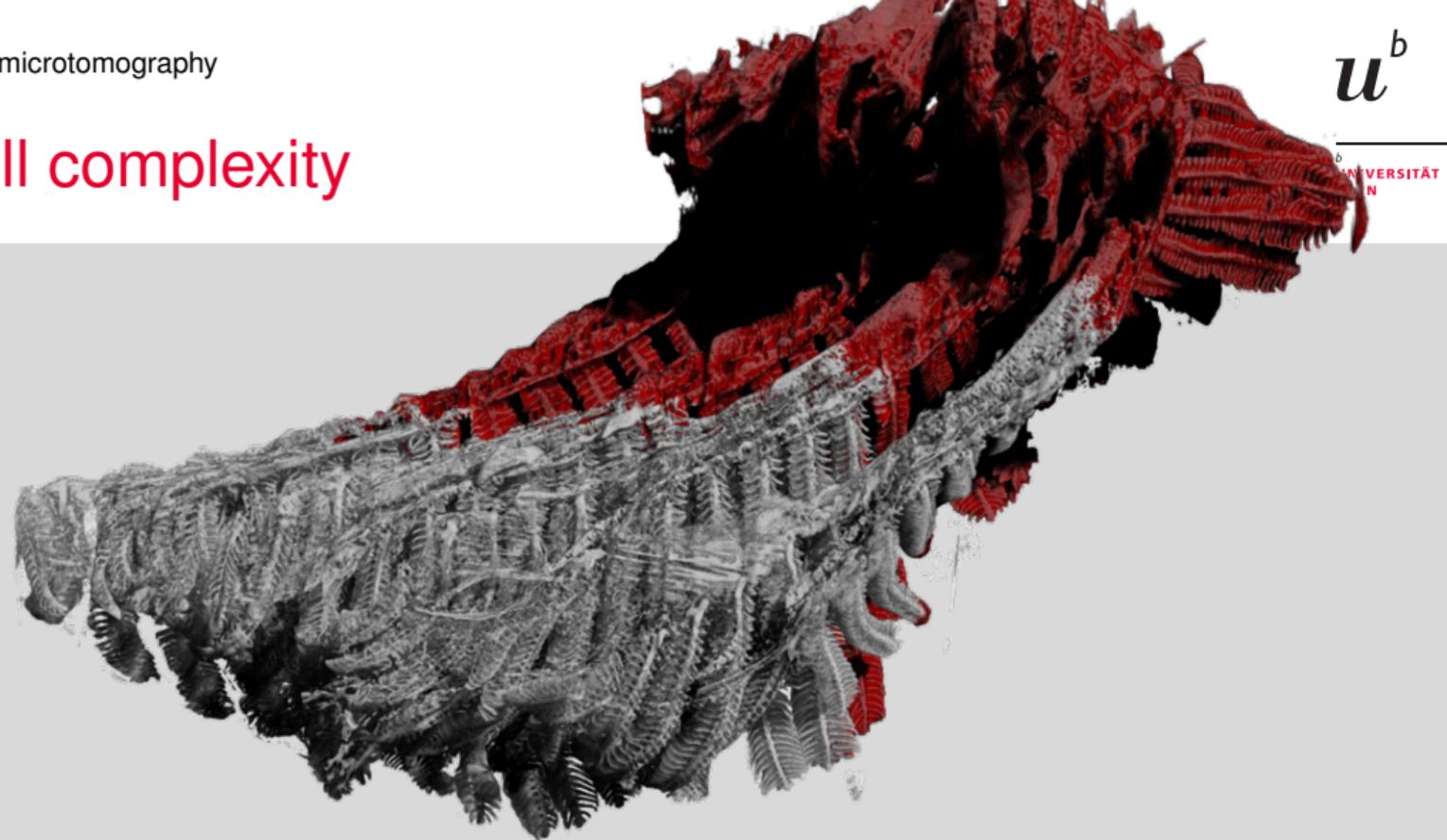


# Gill complexity



X-ray microtomography

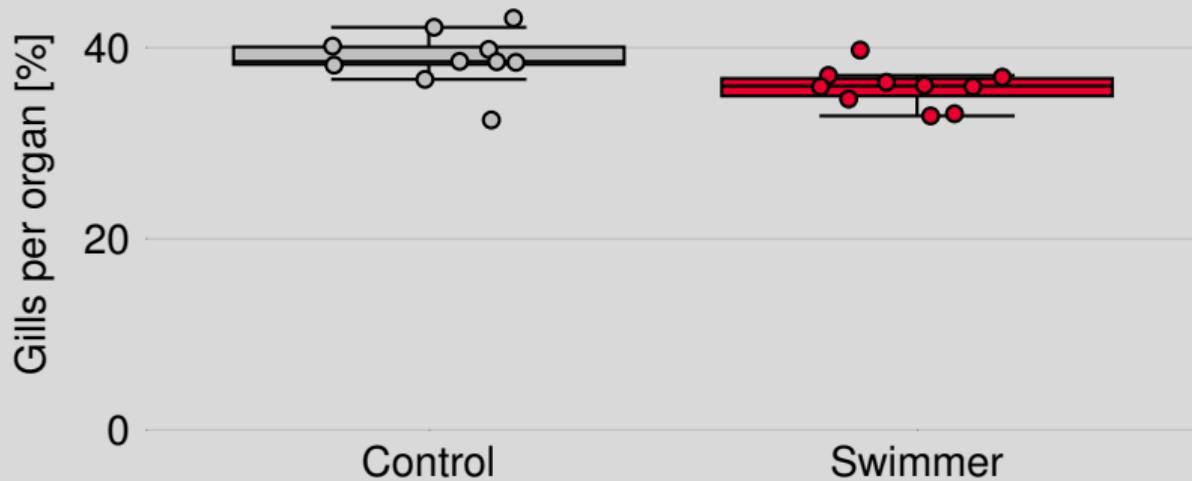
## Gill complexity



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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).
- [2] Matthias Messerli et al. "Adaptation mechanism of the adult zebrafish respiratory organ to endurance training". DOI: [10.1101/744300](https://doi.org/10.1101/744300).
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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).