

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
 - μ 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

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Tomography today

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

Example of a full study

Overview

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Biomedical imaging

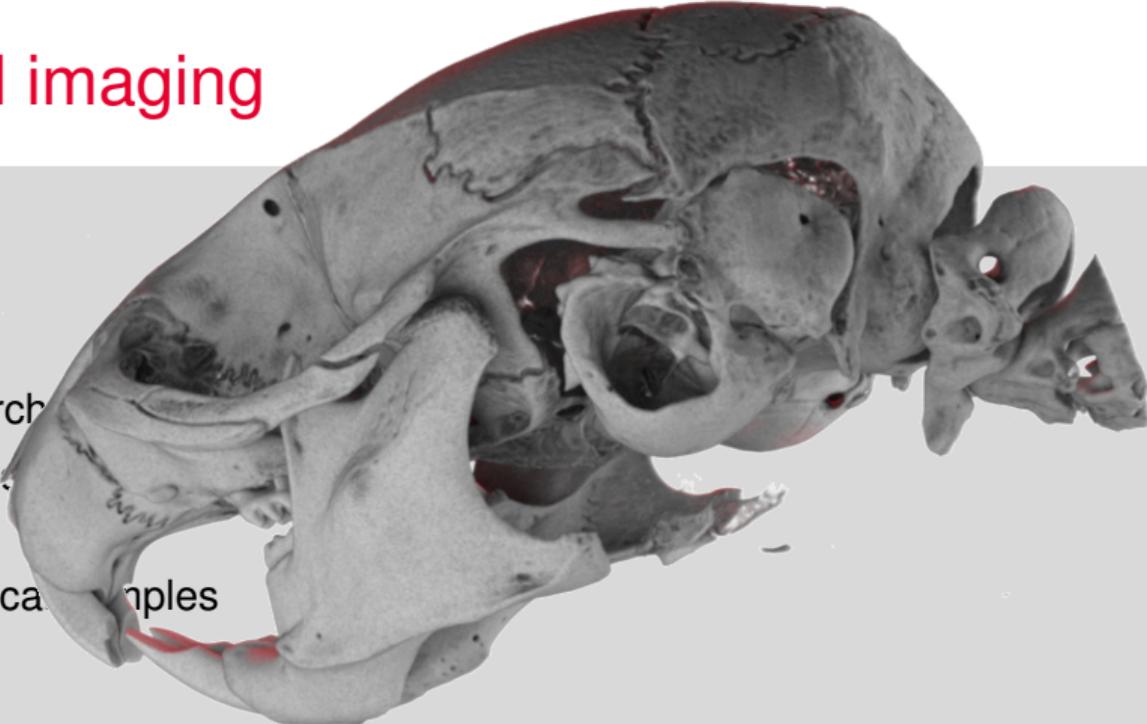
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



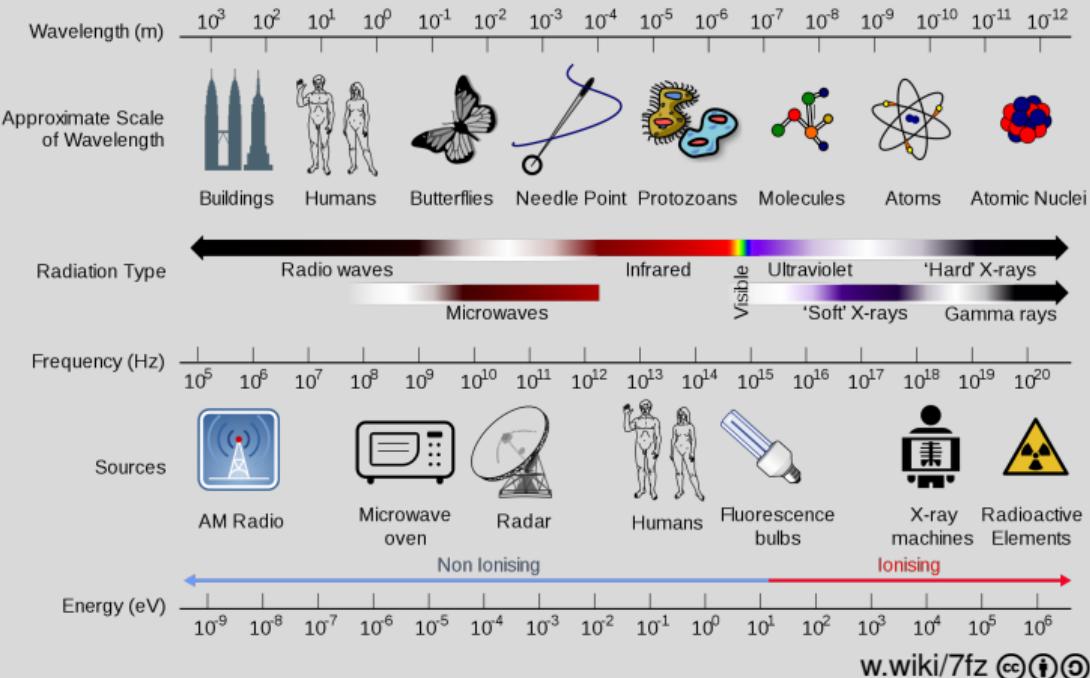
w.wiki/7g4 

Biomedical imaging

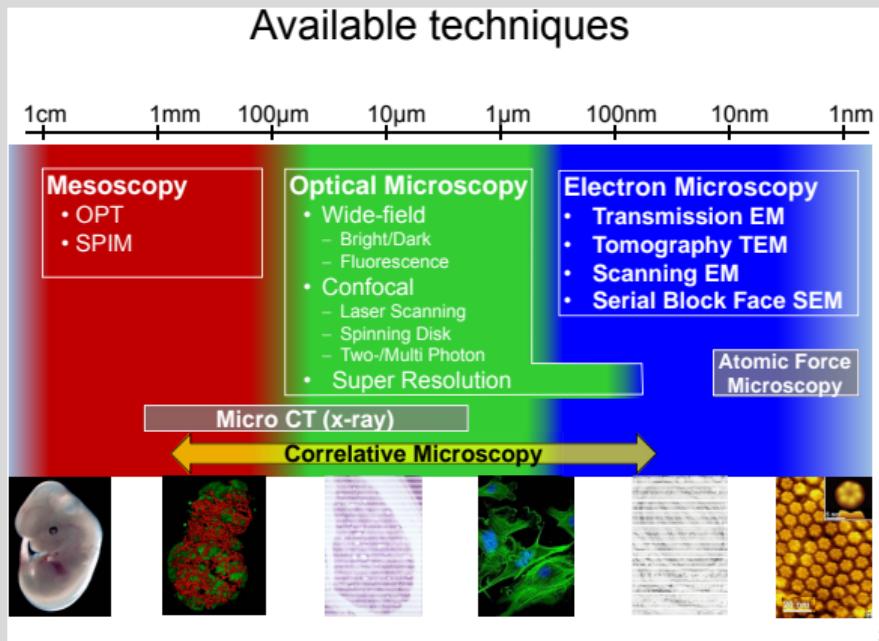
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Wavelength & Scale



Wavelength & Scale

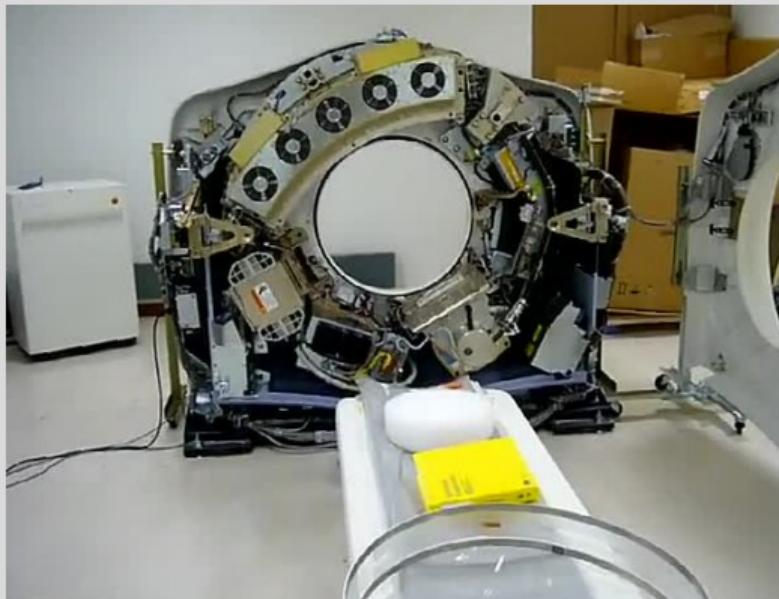


Yury Belyaev, MIC, slide from internal seminar presentation

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.

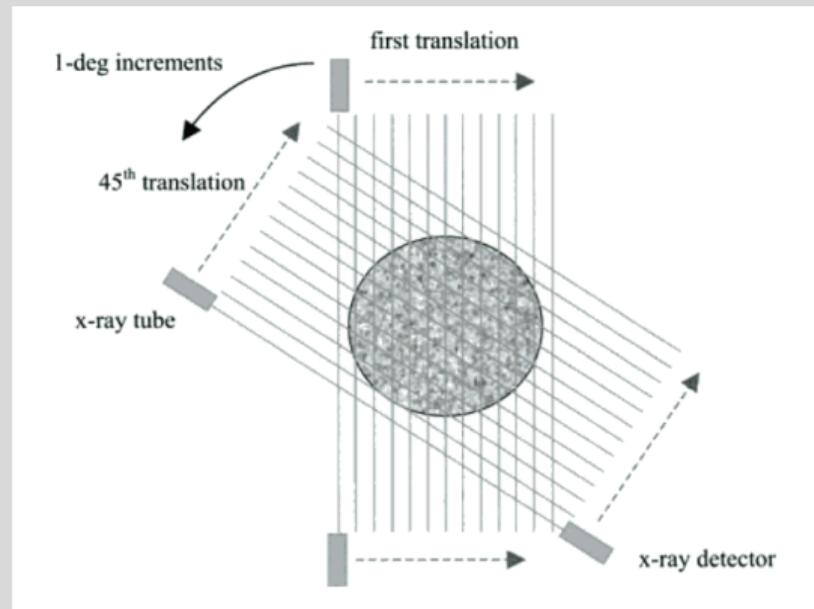
CT-Scanner



youtu.be/2CWpZKuy-NE

History

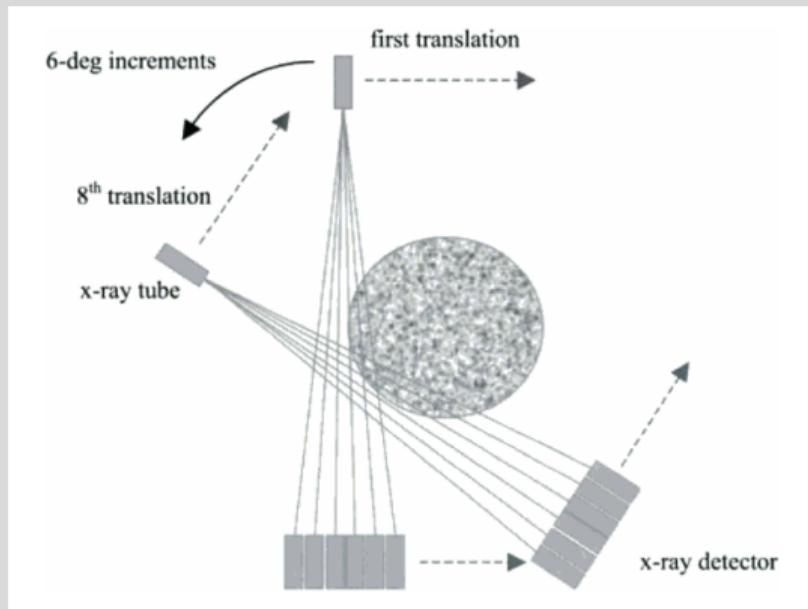
- Long history
 - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [3]
 - 1976: Hounsfield worked on first clinical scanner [4]
 - Nice overview by Hsieh [5]
- First, second and third generation of scanners



From [5], Figure 1.12

History

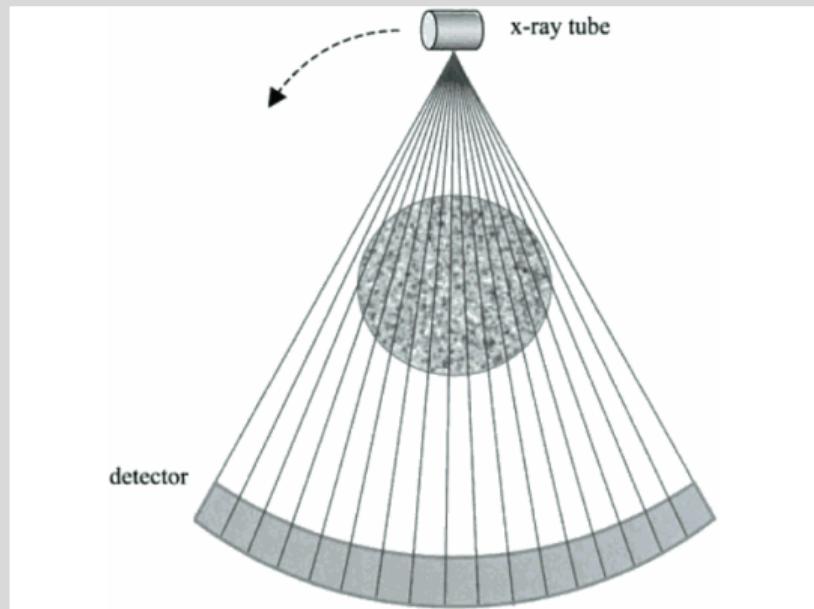
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From [5], Figure 1.13

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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 (τ) is strongly dependent on the atomic number Z of the absorbing material: $\tau \propto \frac{Z^4}{E^{3.5}}$
 - Compton scattering is one of the principle forms of photon interaction and is directly proportional to the (electron & physical) density of the material. It does *not* depend on the atomic number: $\lambda' - \lambda = \frac{h}{m_e c} (1 - \cos \theta)$
- Lowering x-ray energy increases contrast
- X-ray penetration decreases exponentially with sample thickness ([7, i. e. Beer-Lamberts law] $I(t) = I_0 e^{-\alpha z}$)

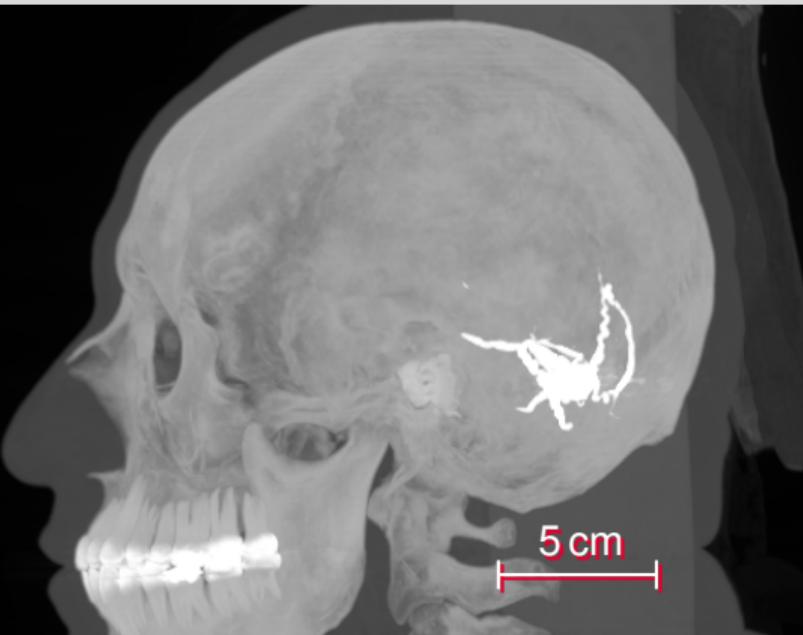


Composition of biological tissues

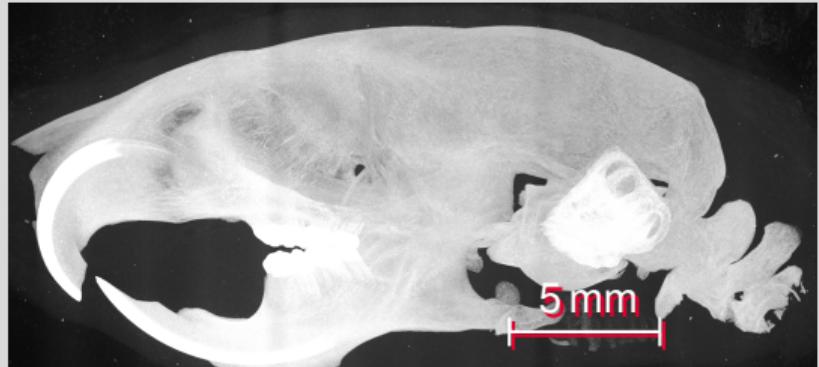
Tissue: content by mass percentage

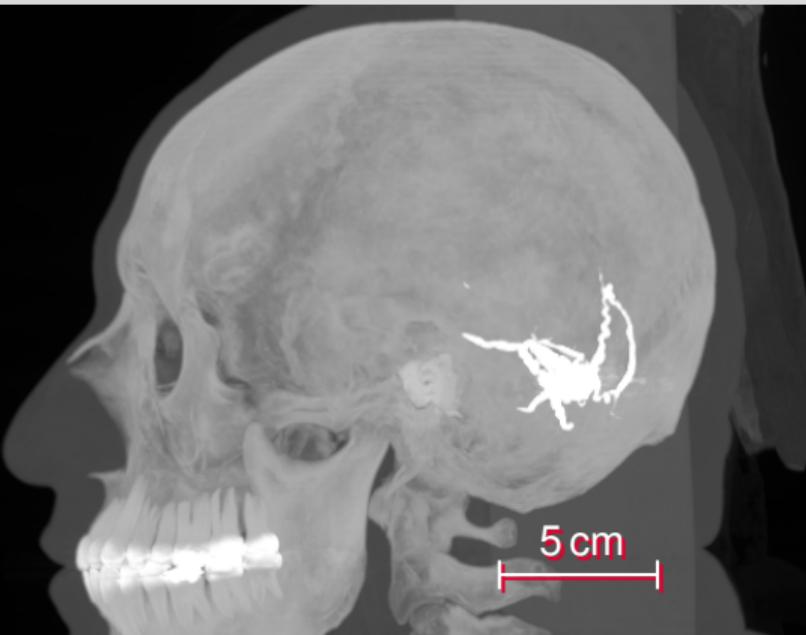
Element Atomic number	H 1	C 6	N 7	O 8	Na 11	P 15	S 16	Cl 17	K 19	Ca 20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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





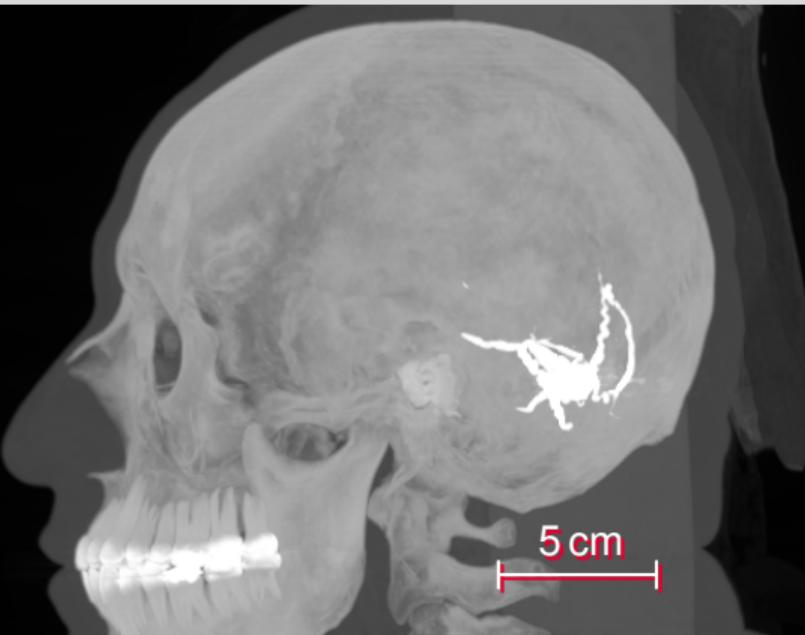
From [8], Subject C3L-02465



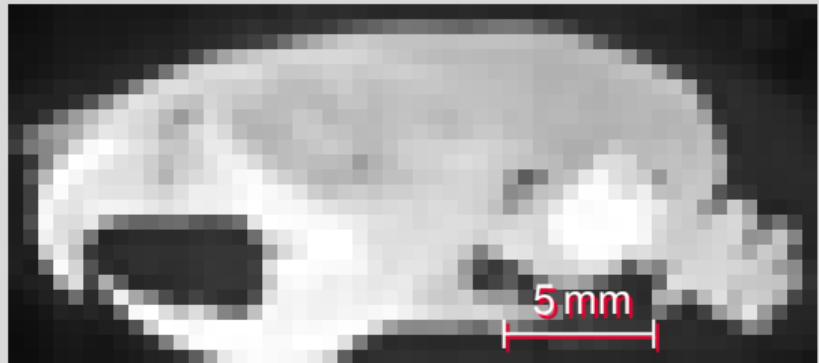


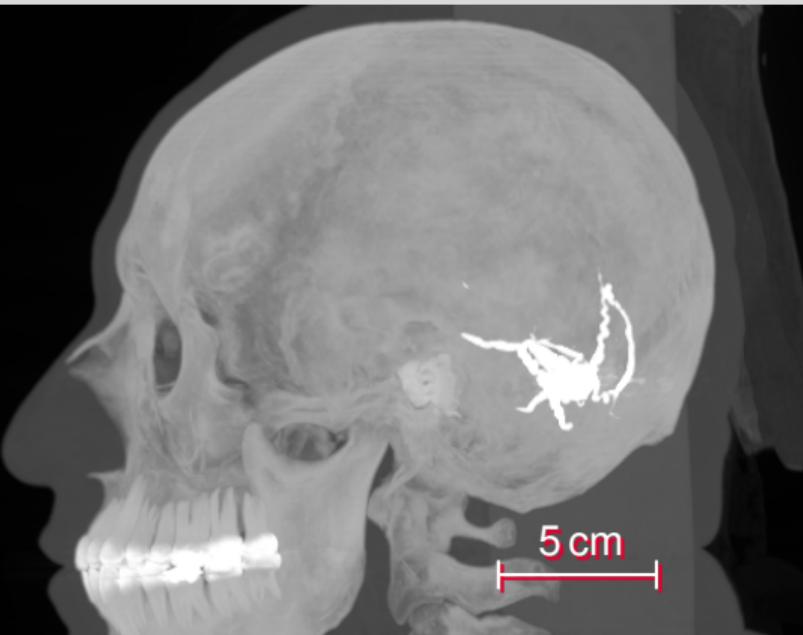
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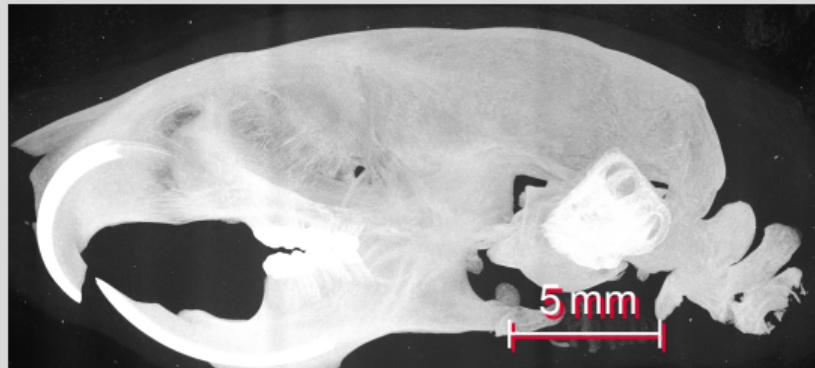


From [8], Subject C3L-02465





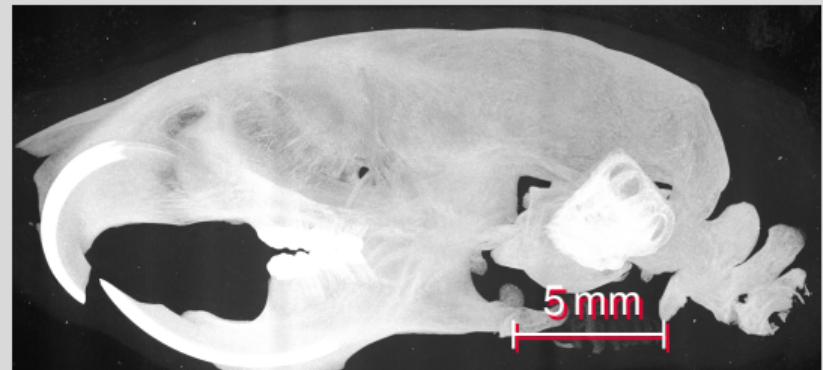
From [8], Subject C3L-02465



Why μ CT?



From [8], Subject C3L-02465



Machinery

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



flic.kr/p/D4rbom

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flic.kr/p/fpTrGu cc BY SA

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bruker.com/skyscan1272

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flic.kr/p/7Xhk2Y

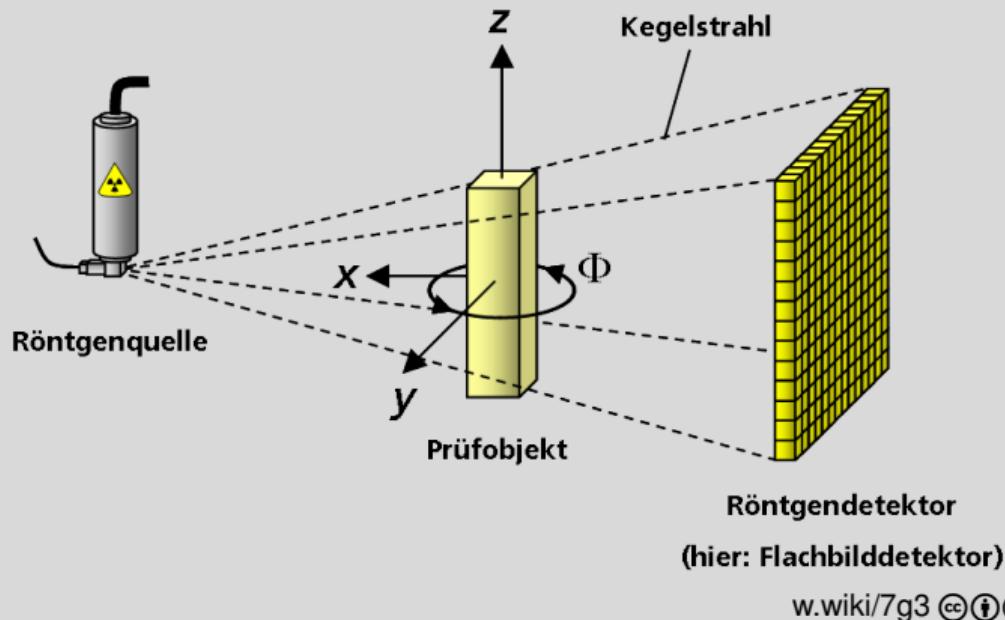
Machinery

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

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

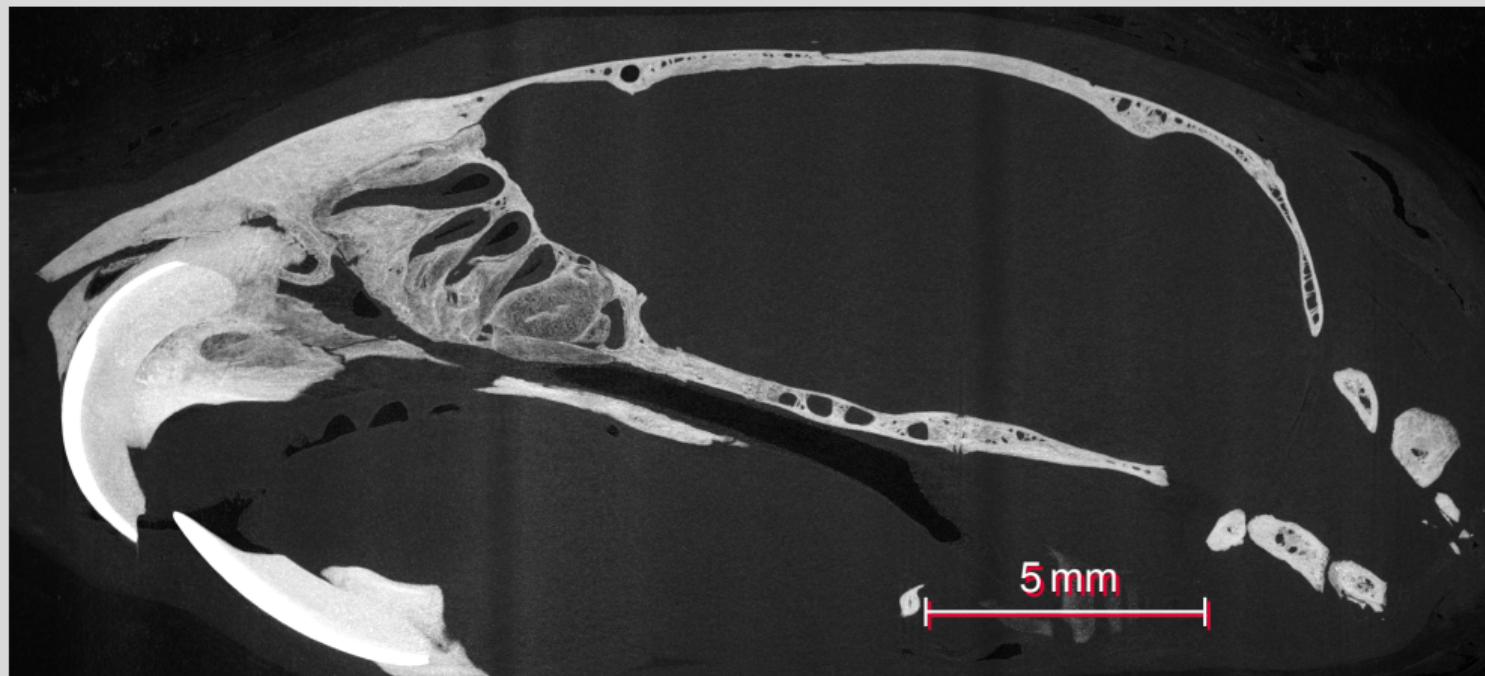
Machinery

What is happening?

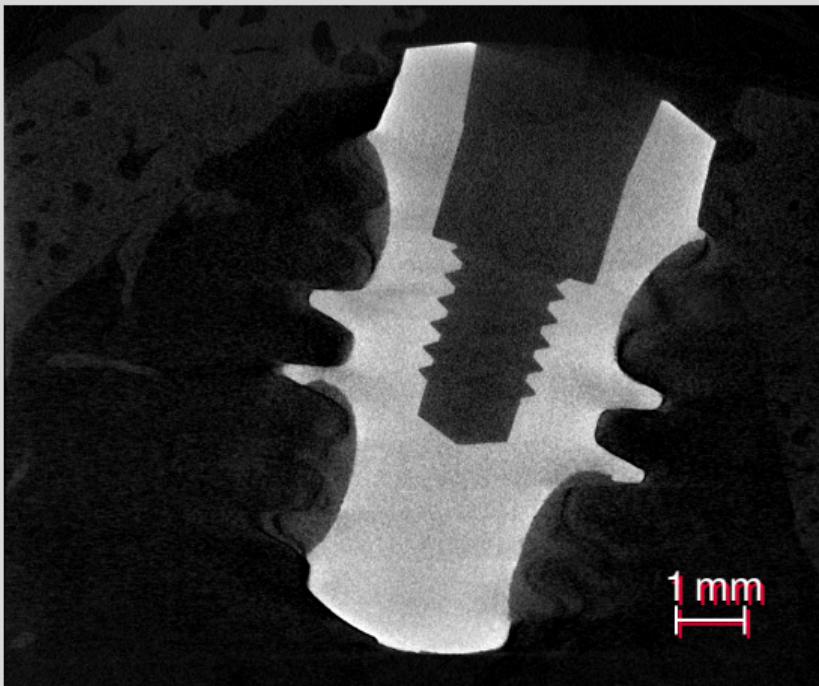


w.wiki/7g3

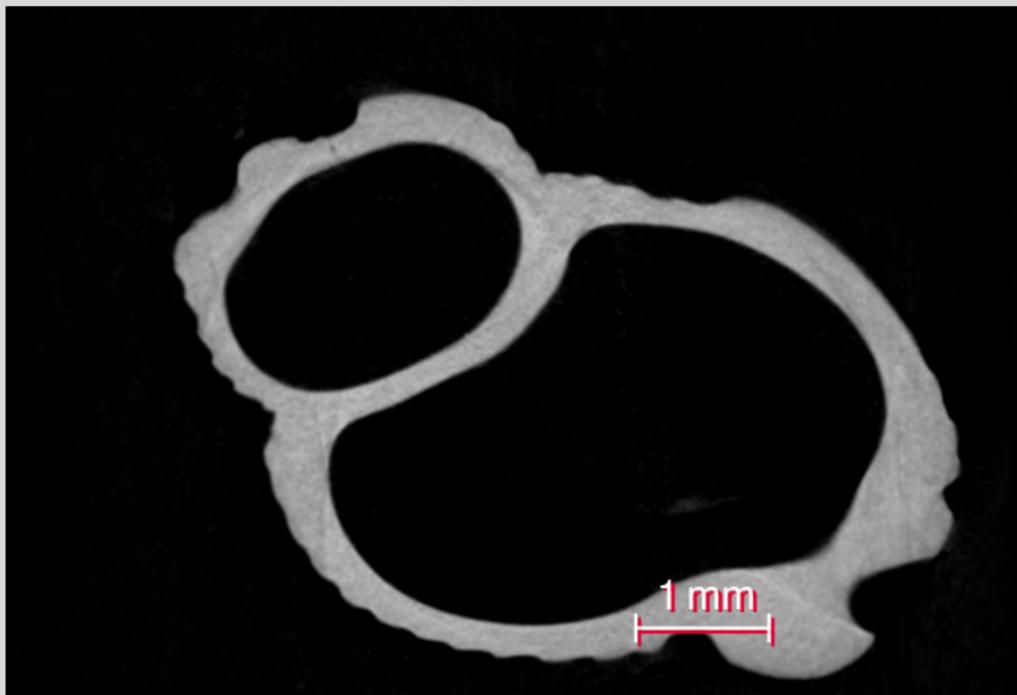
Examples



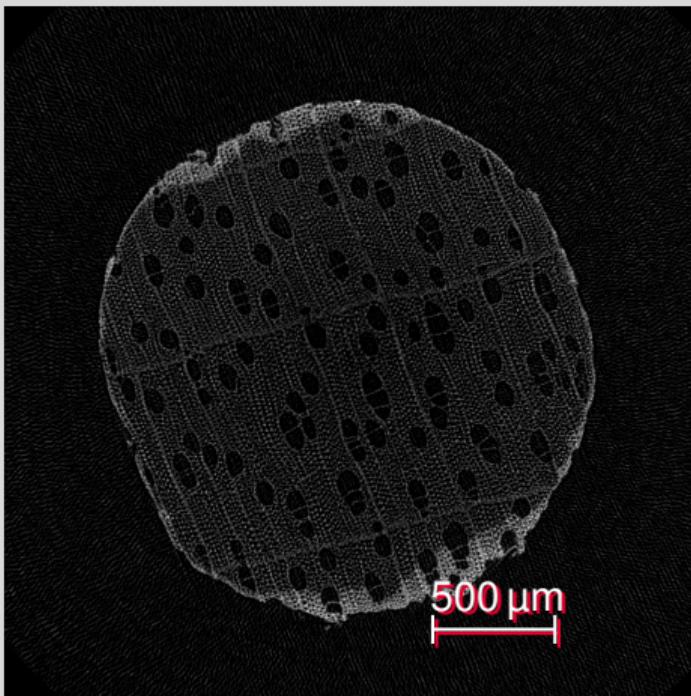
Examples



Examples



Examples



Examples

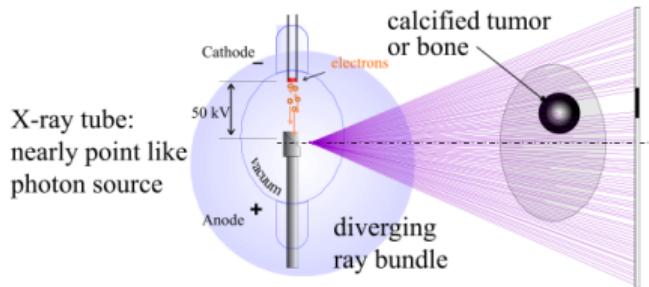


Preparation

- Study design
- Sample preparation

Projections

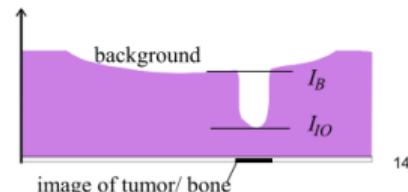
Image formation: shadow projection



X-ray tube:
nearly point like
photon source

Contrast is given by
absorption of intensity I
by object → negative

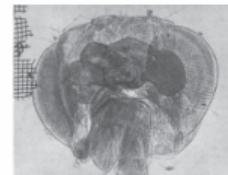
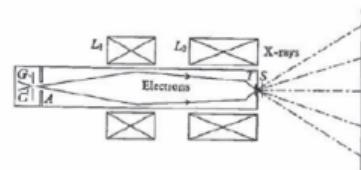
$$\text{Contrast} = \frac{I_{IO} - I_B}{I_B}$$



14

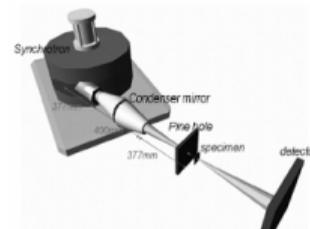
Image formation: shadow projection

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

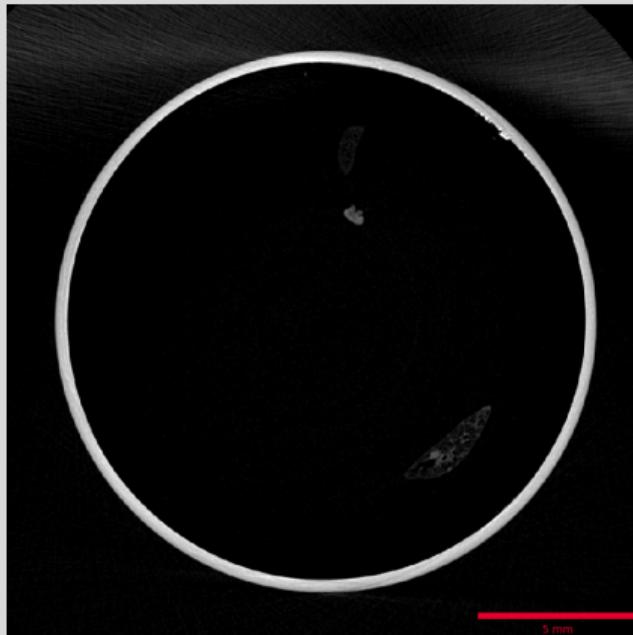
Projections



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

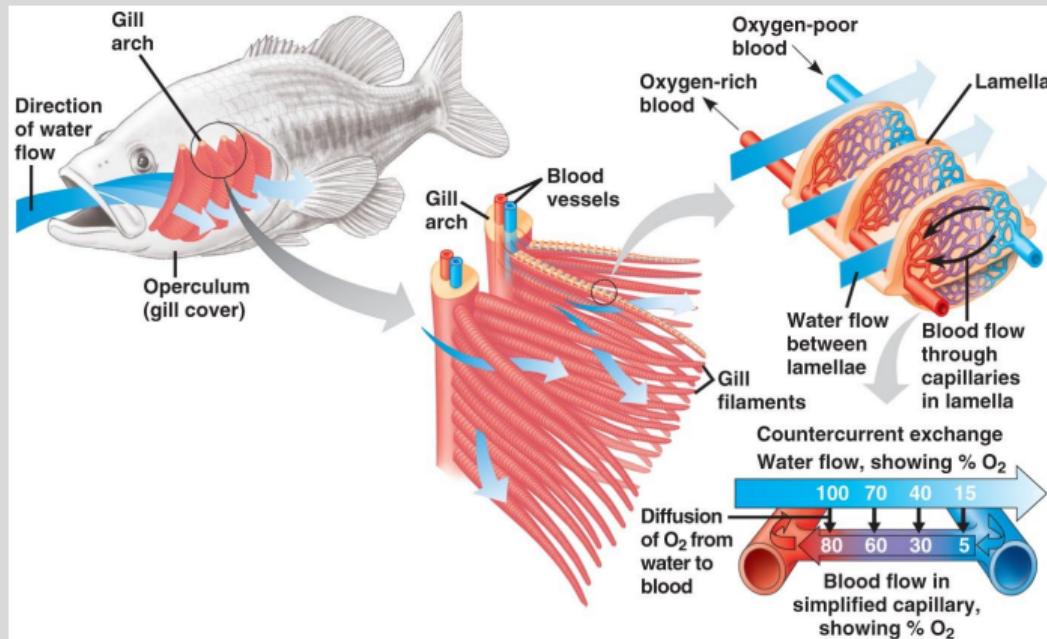
What to use?

- 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

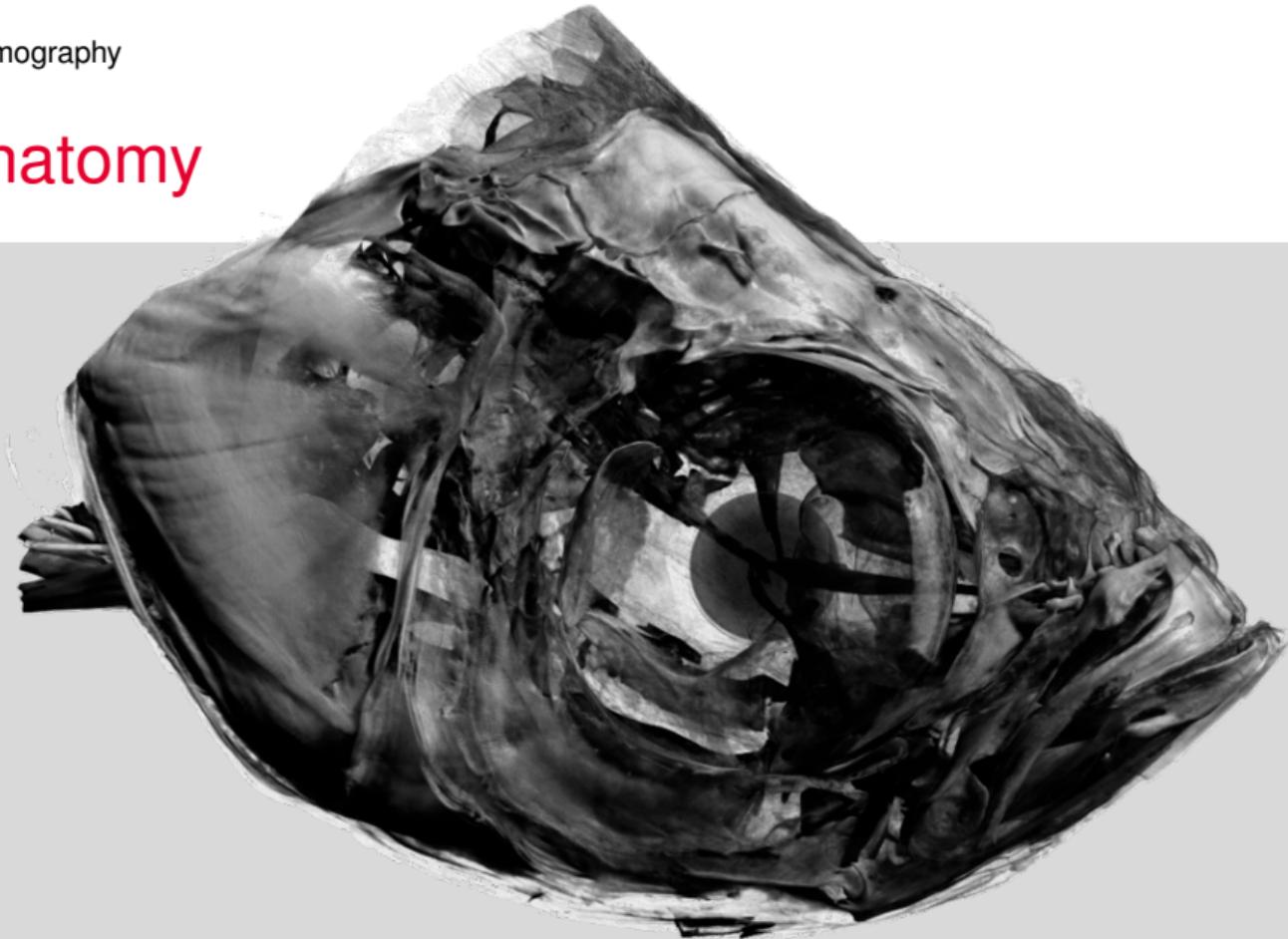
- Raw numbers instead of just pretty images
- Segmentation
- Characterization

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



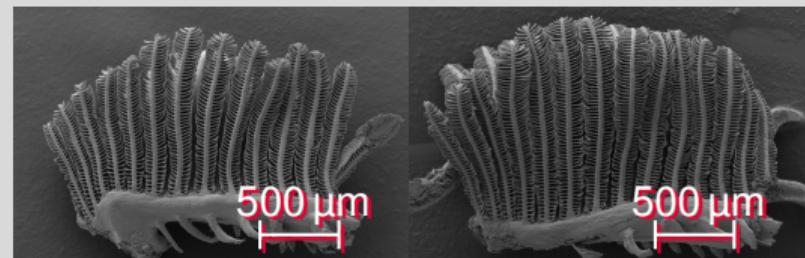
How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [12]
 - Endurance
- Morphology & Physiology
 - Body size & weight
 - O₂ consumption



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 - Gill structure



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- Critical point drying



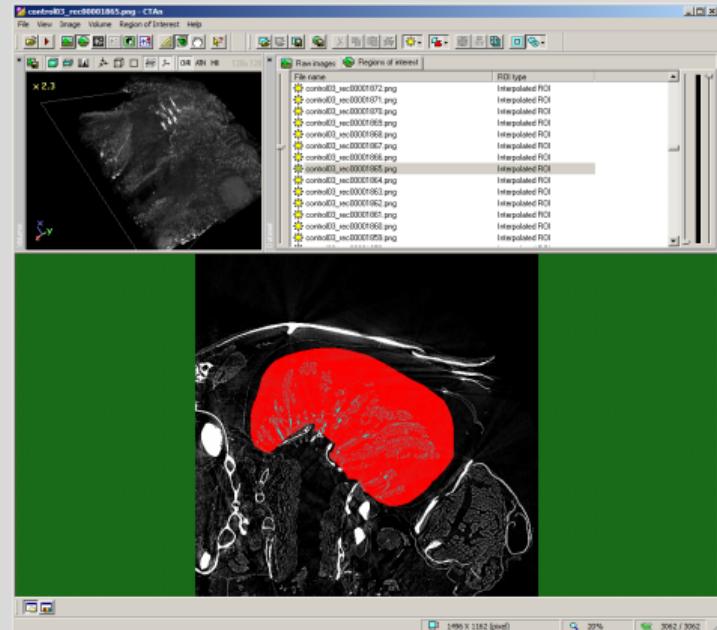
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- Scanning electron microscopy
 - Gill structure
- Critical point drying, µCT imaging

```
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?

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

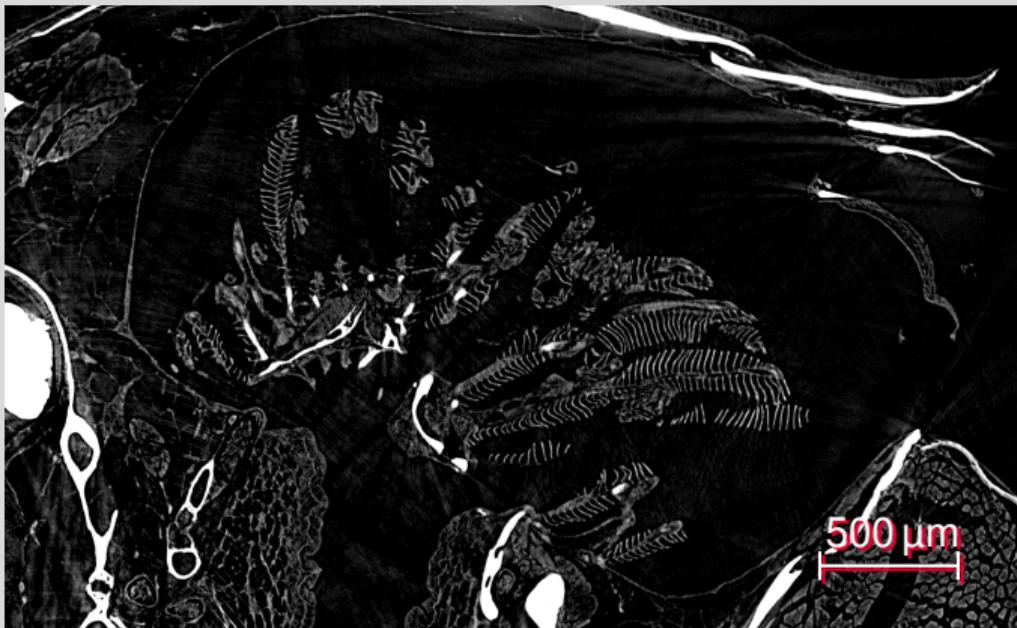


gph.is/2nqkple

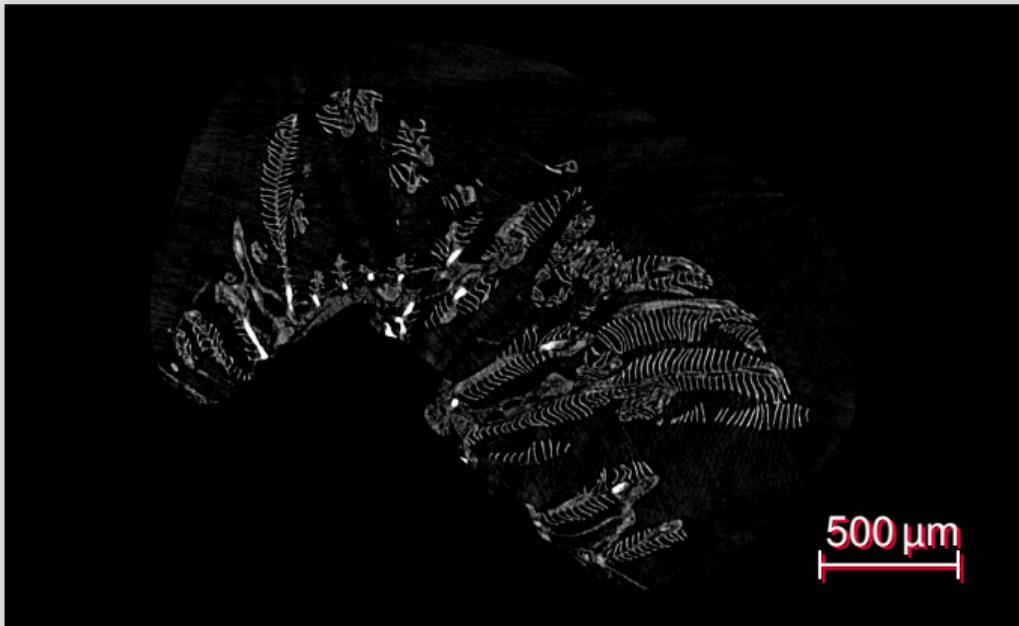
Gill volume



Gill volume



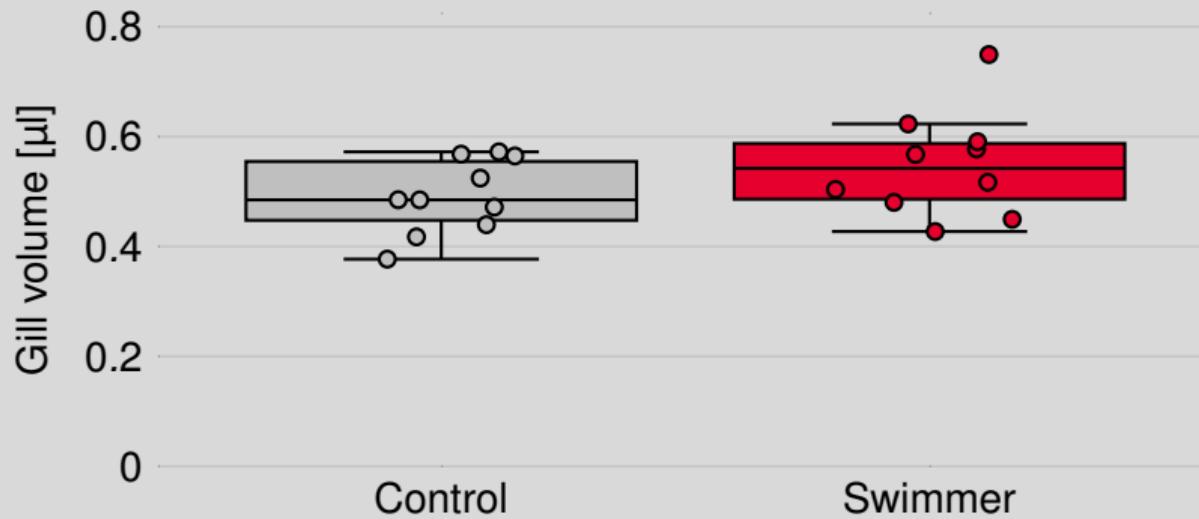
Gill volume



Gill volume



Gill volume



Gill complexity



Gill complexity

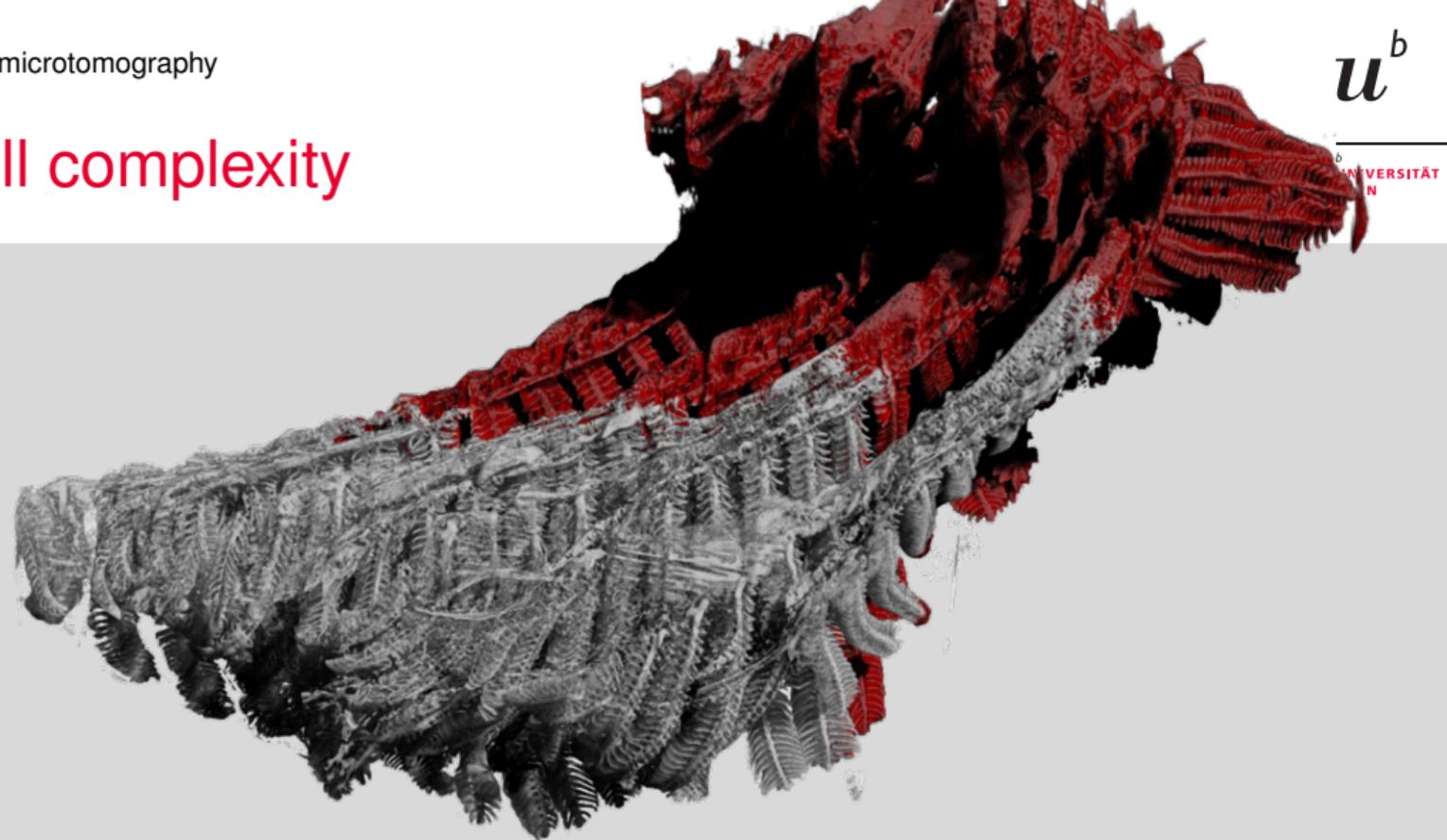


Gill complexity



X-ray microtomography

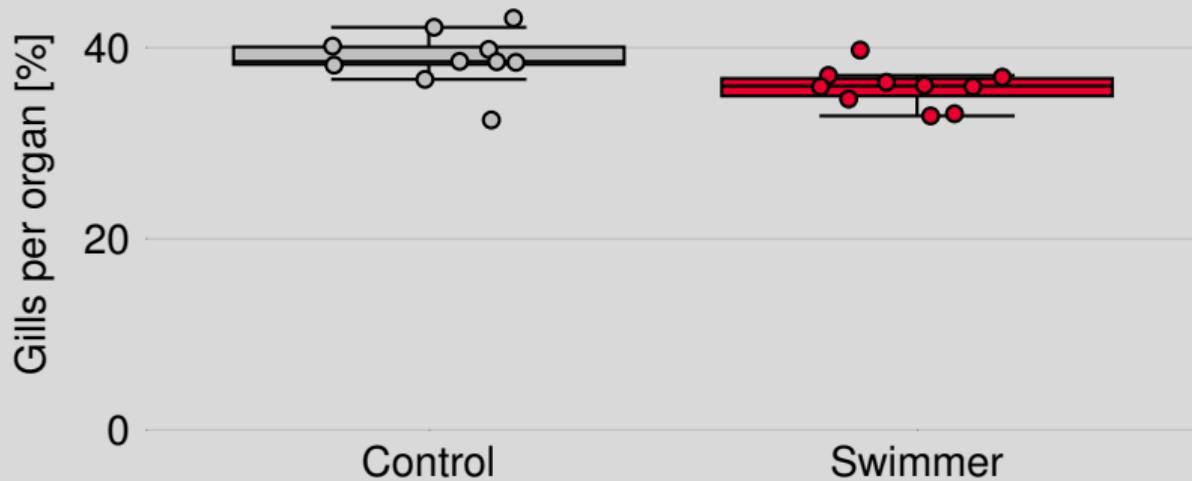
Gill complexity



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

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

References

- [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: [10.1371/journal.pone.0228333](https://doi.org/10.1371/journal.pone.0228333).
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- [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).