

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 [**Hlushchuk2018**]: Tumor vasculature, angiogenesis in the heart, musculature and bones
 - Cancer research: Melanoma
 - Lung imaging: Tumor detection and classification
 - Physiology: Zebrafish musculature and gills [**Messerli2019**]
 - SkyScan 1172 & 1272

Contents

Biomedical imaging

Imaging

Tomography

X-ray production

Interaction of x-rays with matter

History

A scan, from start to finish

Example

Imaging performance

Image processing

Image display

Biomedical imaging

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

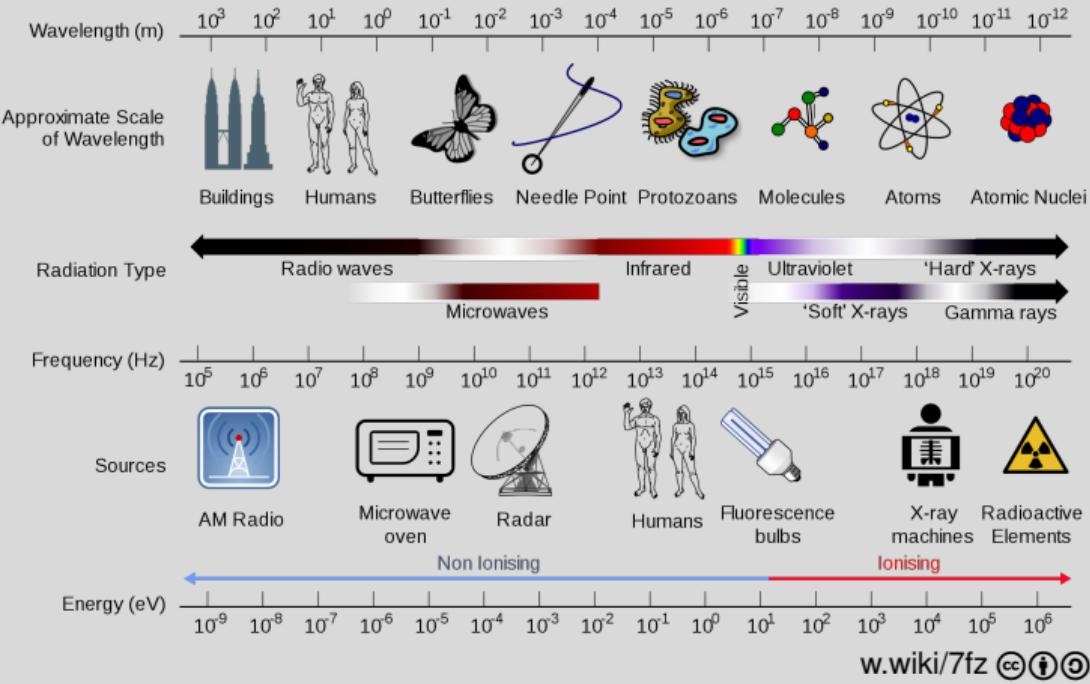


w.wiki/7g4 CC BY NC SA

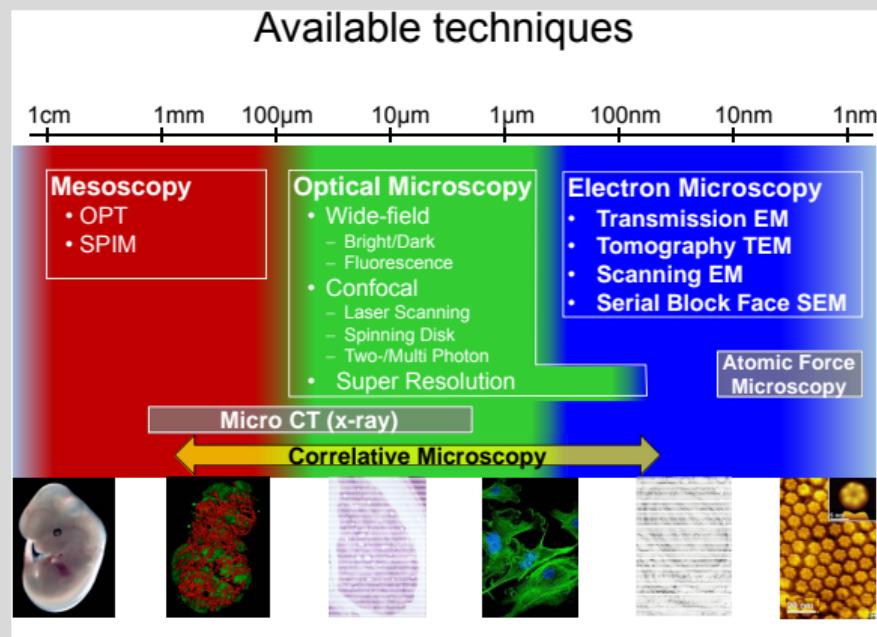
Biomedical imaging



Wavelength & Scale



Wavelength & Scale



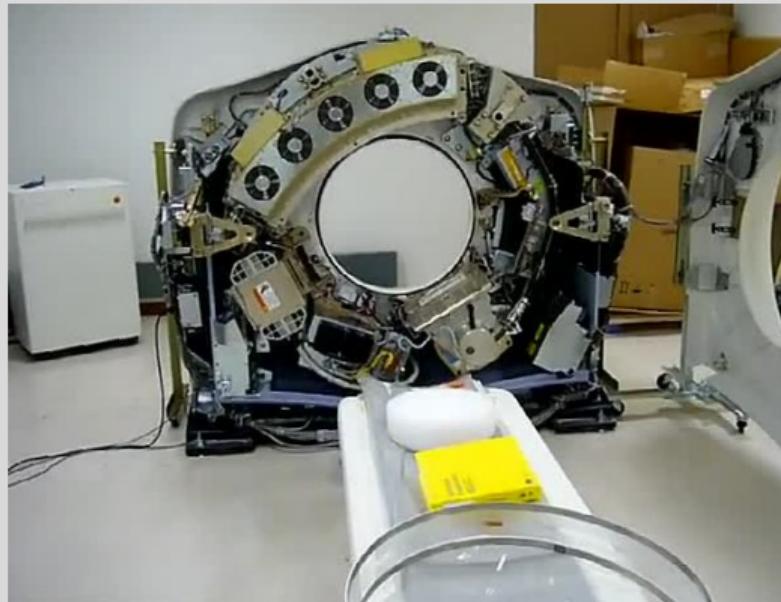
2

Yury Belyaev, MIC, slide from internal seminar presentation

Imaging methods

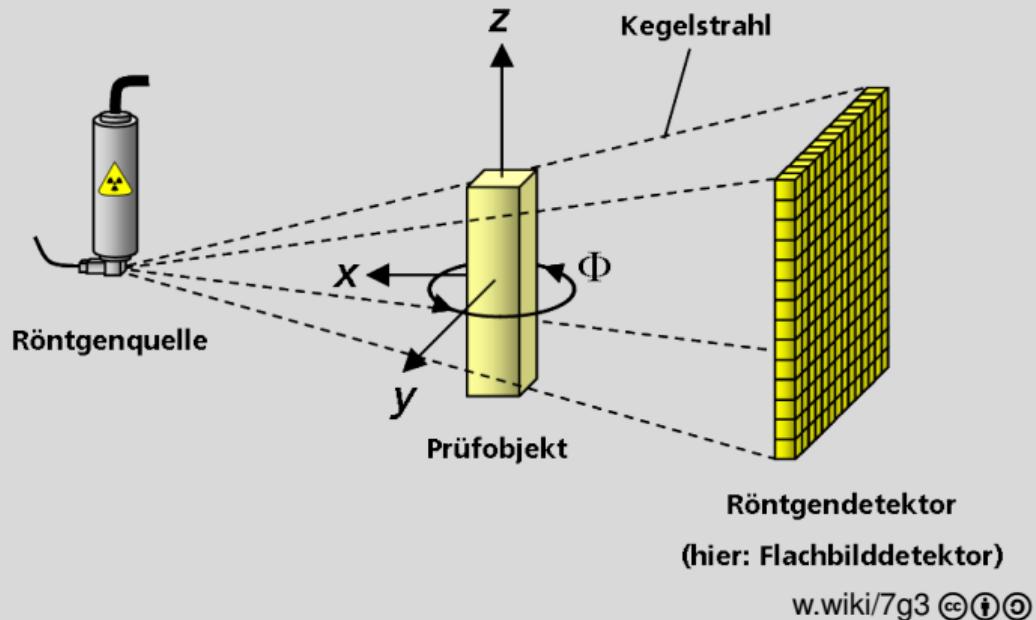
- Light microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy: see lectures *Transmission Electron Microscopy* by Dimitri Vanhecke, *Scanning Electron Microscopy* by Michael Stoffel and *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

CT-Scanner



youtu.be/2CWpZKuy-NE

What is happening?



X-ray generation

- How are x-rays generated
- Why do we need them

X-ray interaction

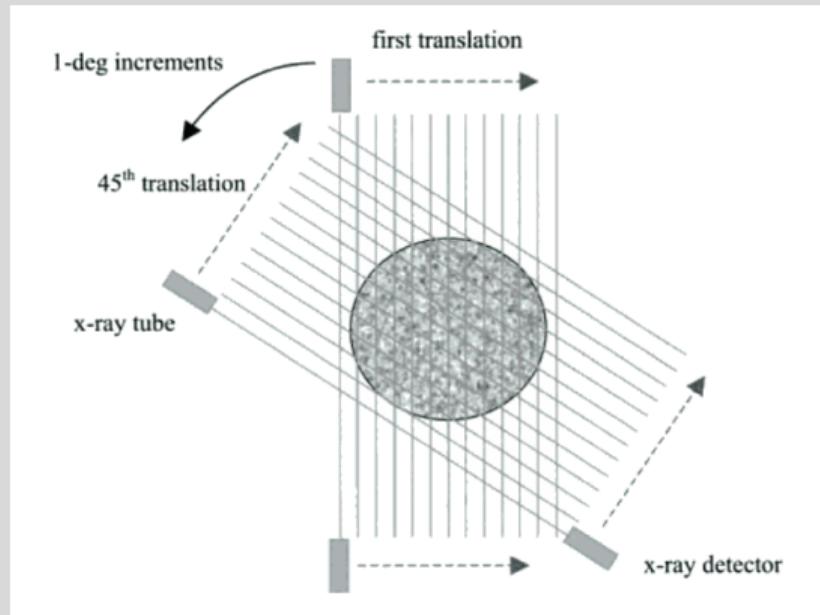
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” (**[xrayphysics]**)
 - Photoelectric absorption (τ) is strongly 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 (**[wiki:beer-lambert]**
 $I(t) = I_0 e^{-\alpha z}$)

Composition of biological tissues

| 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 |

History

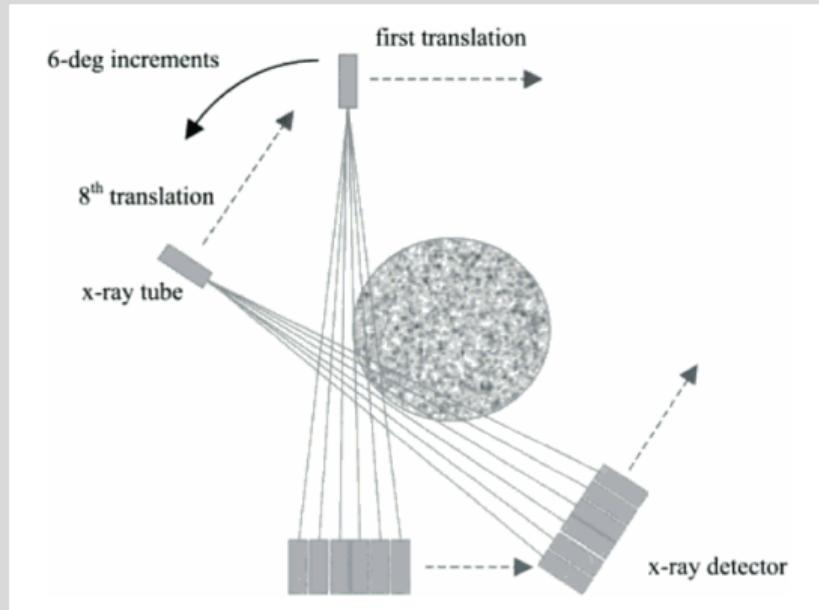
- Extensive history is found in [Cormack1963, Hsieh2003]
- First, second and third generation of scanners



From [Hsieh2003], Figure 1.12

History

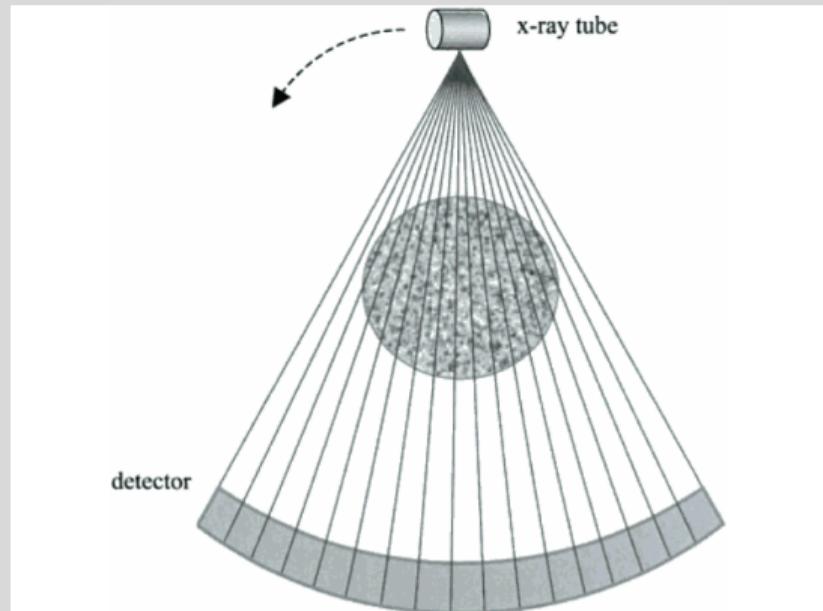
- Extensive history is found in [Cormack1963, Hsieh2003]
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From [Hsieh2003], Figure 1.13

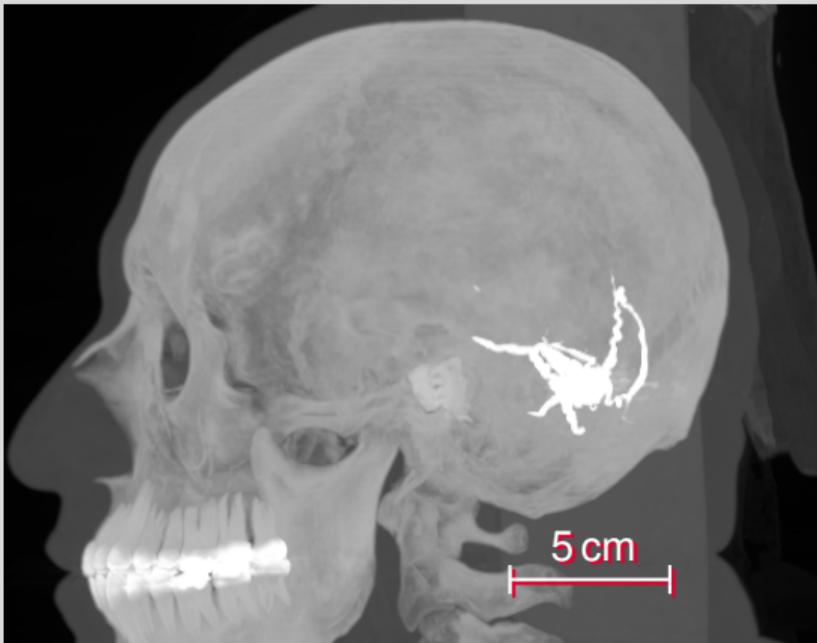
History

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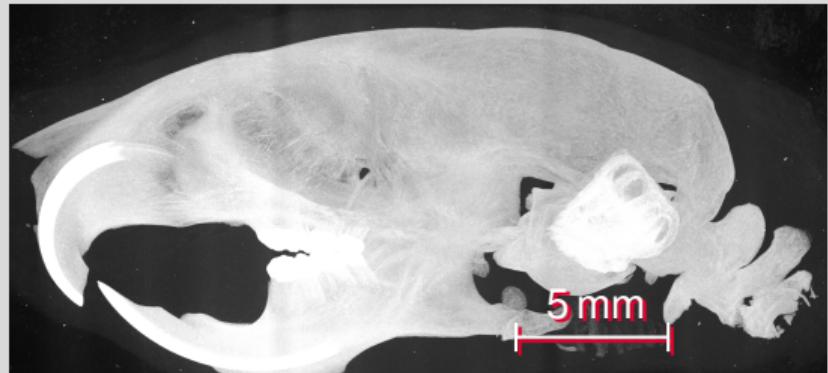


From [**Hsieh2003**], Figure 1.14

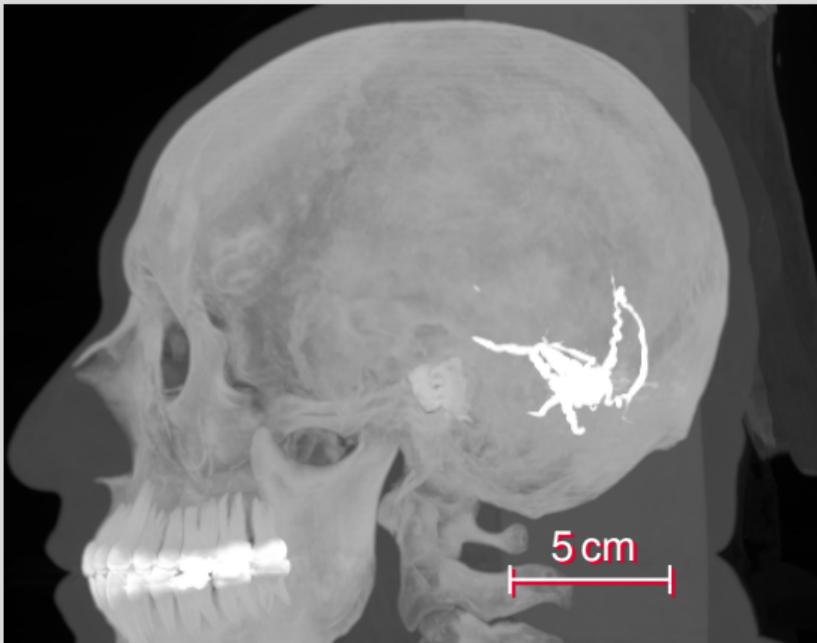
Why μ CT?



From [Clark2013], Subject C3L-02465



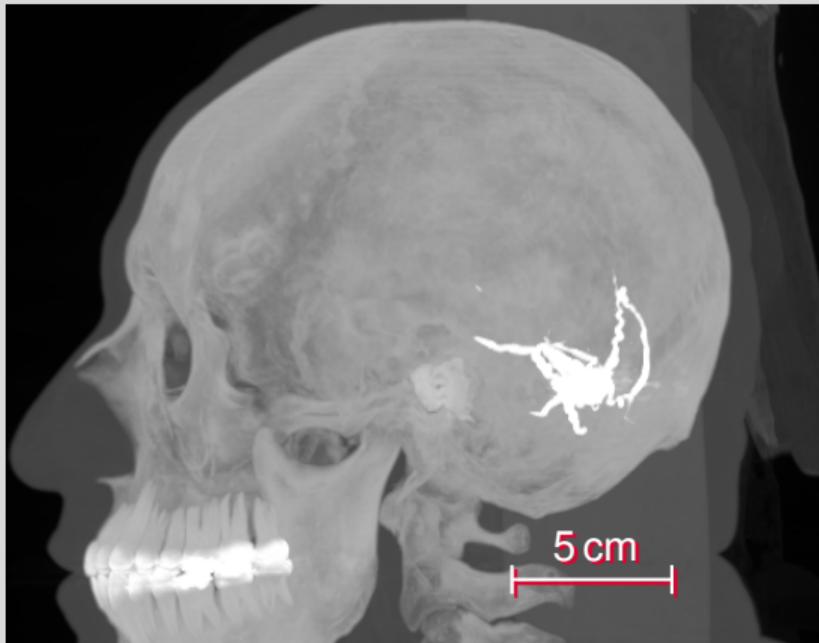
Why μ CT?



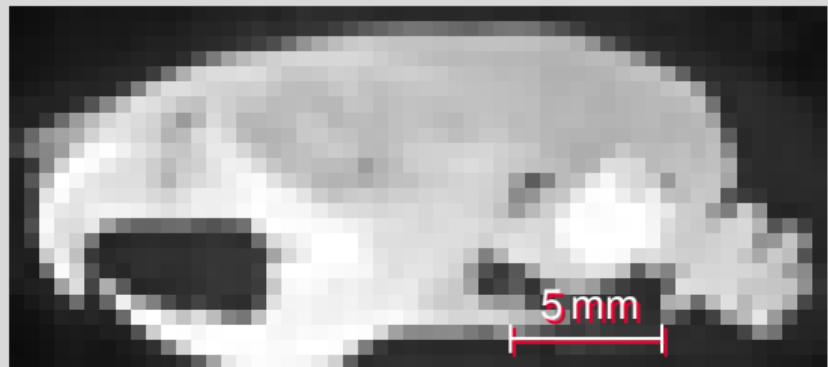
From [Clark2013], Subject C3L-02465



Why μ CT?



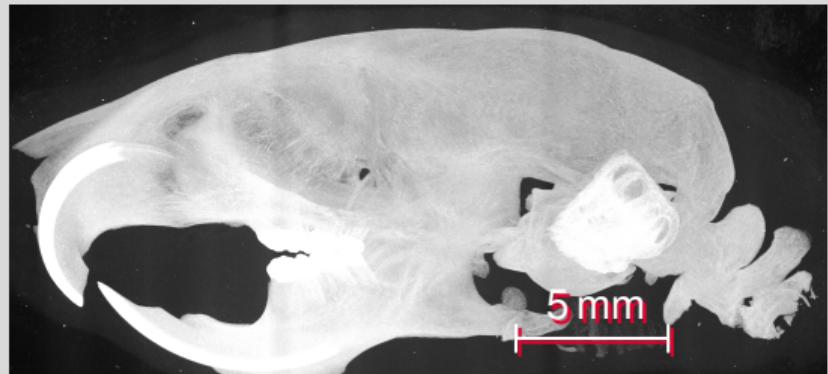
From [Clark2013], Subject C3L-02465



Why μ CT?



From [Clark2013], 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

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bruker.com/skyscan1272

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flic.kr/p/7Xhk2Y

Machinery I

Independent on the machine, technically they are all a simple combination of

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

Machinery II

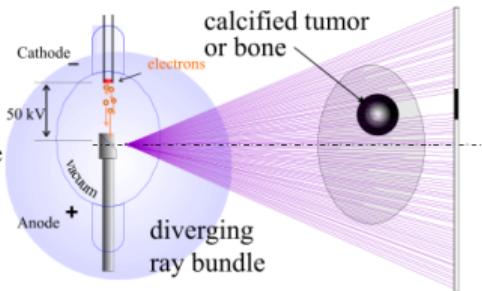
Preparation

- Study design
- Sample preparation

Projections

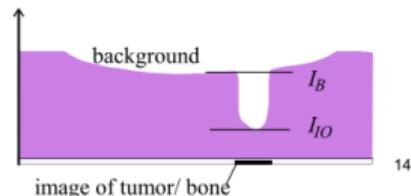
Image formation: shadow projection

X-ray tube:
nearly point like
photon source



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

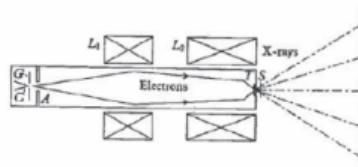
$$\text{Contrast} = \frac{I_{IO} - I_B}{I_B}$$



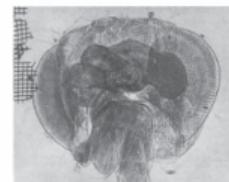
Laws of Physics for Microscopists by Michael Jaeger, Slide 14 Laws of Physics for Microscopists by Michael Jaeger, Slide 15

Image formation: shadow projection

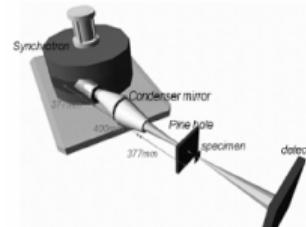
X-ray projection microscopy



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

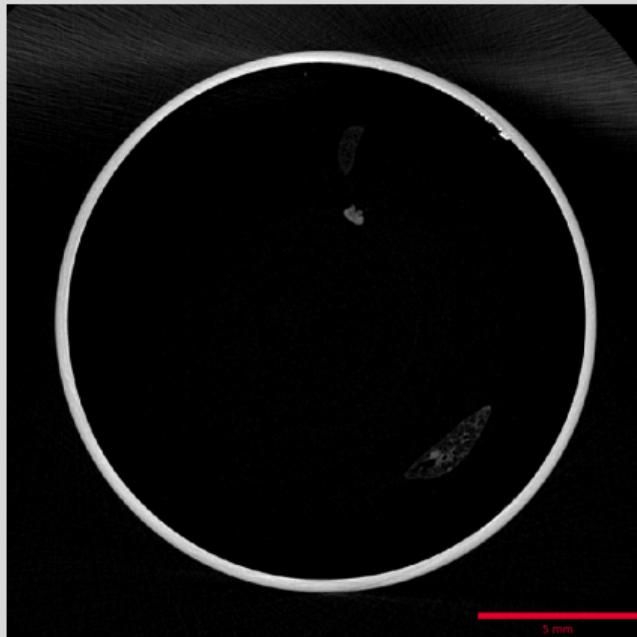
Projections



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 [1]
-  in Jupyter [**Kluyver2016**]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz

[1] Schindelin2012.

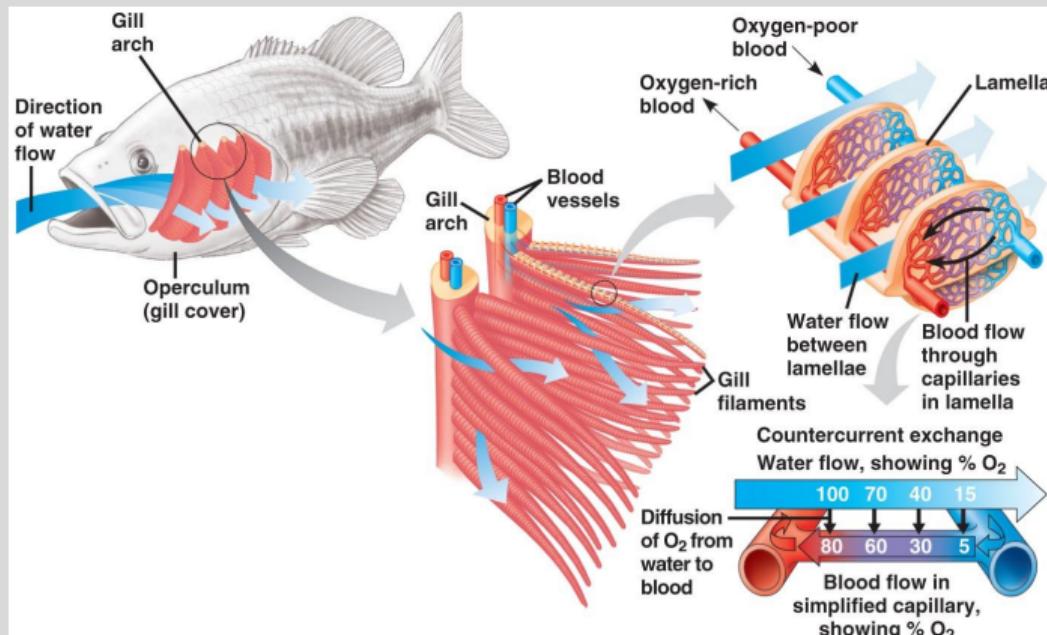
Quantitative data

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

Reproducible research

- git
- Jupyter
- Script all your things!
- Data repositories → Sharing is caring!

An example: Do gills change with training?



Campbell Biology [Taylor2017]

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 [**Palstra2010**]
 - Endurance



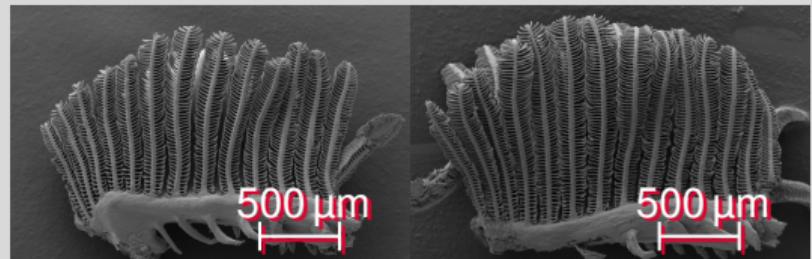
How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [**Palstra2010**]
 - Endurance
- Morphology & Physiology
 - Body size & weight
 - O₂ consumption



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 - Gill structure



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- Critical point drying



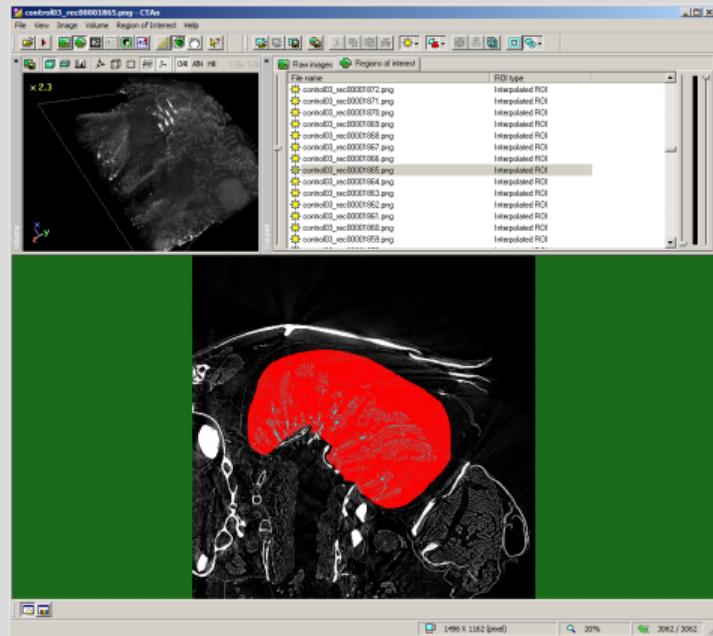
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- Critical point drying, µCT imaging

```
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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- Critical point drying, μ CT imaging, delineation in CTAn



How?

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

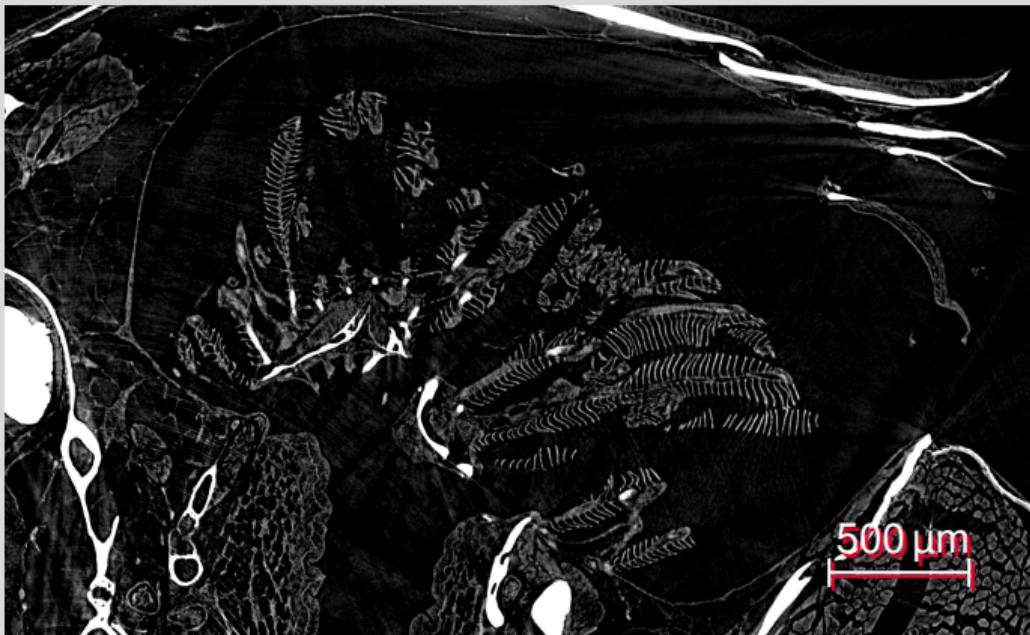


gph.is/2nqkple

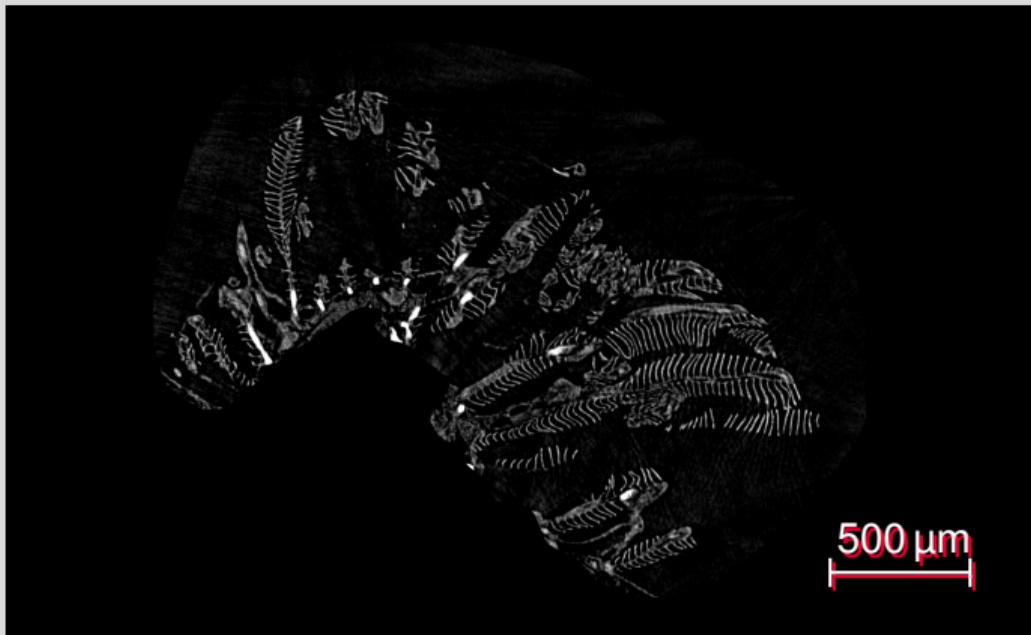
Gill volume



Gill volume



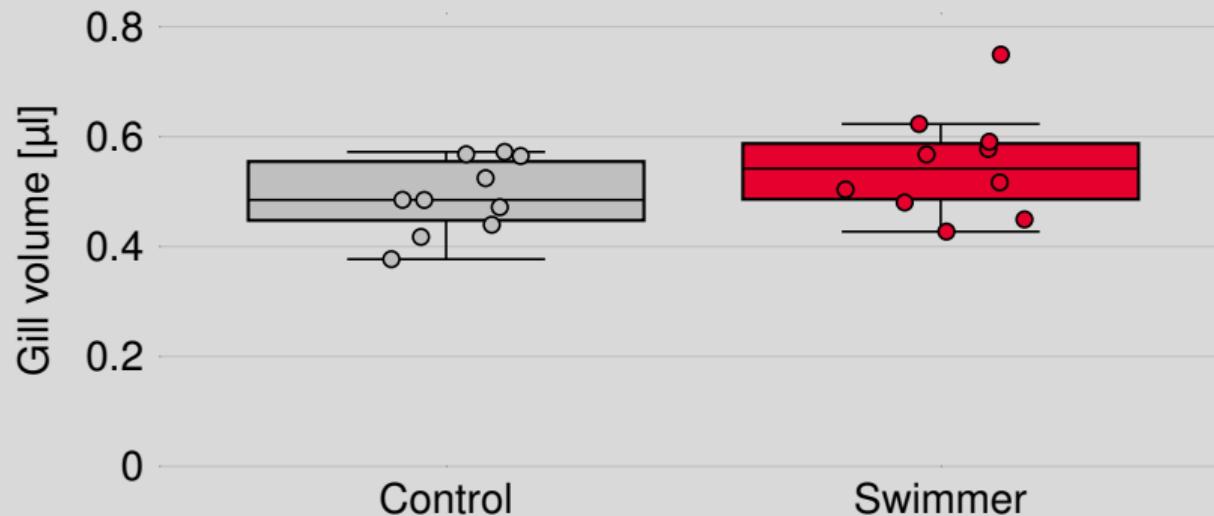
Gill volume



Gill volume



Gill volume



Gill complexity



Gill complexity

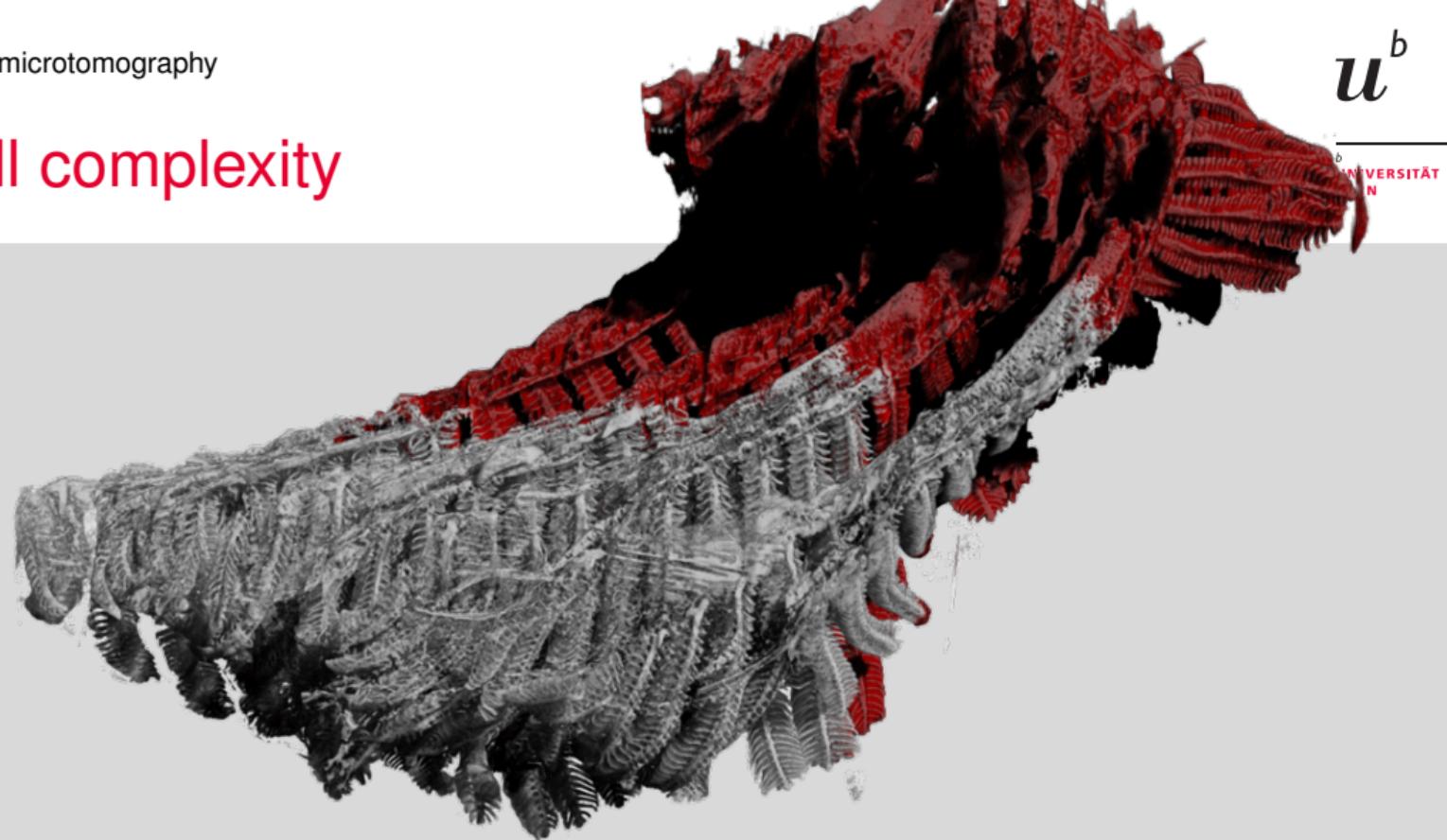


Gill complexity



X-ray microtomography

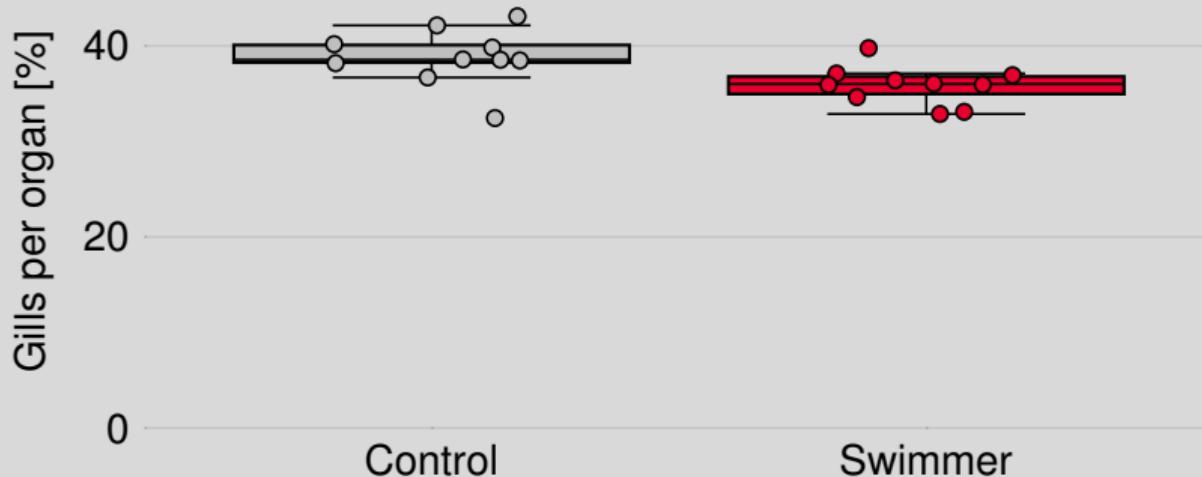
Gill complexity



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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 [2] to a (handout) PDF which you can access here: git.io/JeQxO
- Spotted an error?
 - File an issue: git.io/fjpPb
 - Submit a pull request: git.io/fjpPN
 - Send me an email: haberthuer@ana.unibe.ch

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

References