

# X-ray microtomography

David Haberthür

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X-ray microtomography

## 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 [**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

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X-ray microtomography

## 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

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## Biomedical imaging

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

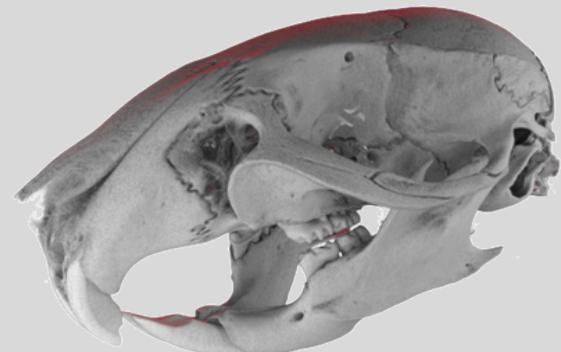


w.wiki/7g4

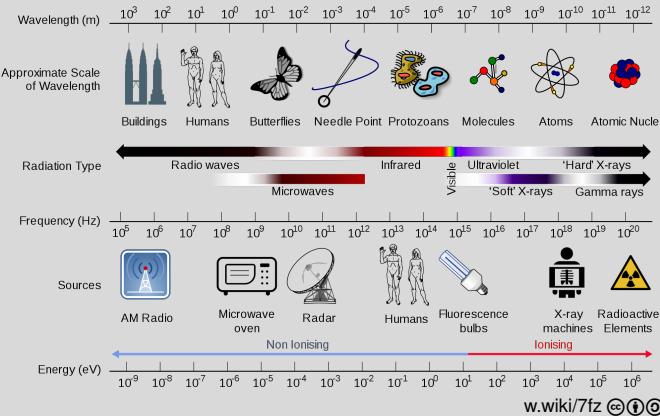
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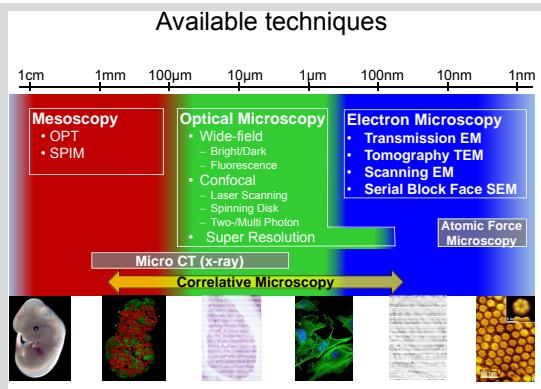
## Biomedical imaging

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## Wavelength & Scale



## Wavelength & Scale

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## Imaging methods

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- 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 *Cryo-electron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

## CT-Scanner



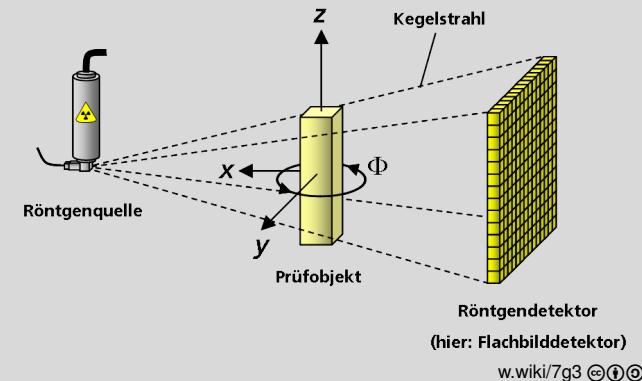
[youtu.be/2CWpZKuy-NE](https://youtu.be/2CWpZKuy-NE)

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## What is happening?



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## X-ray generation

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

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## 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 ([\[wiki:beer-lambert\]](#))  
 $I(t) = I_0 e^{-\alpha z}$

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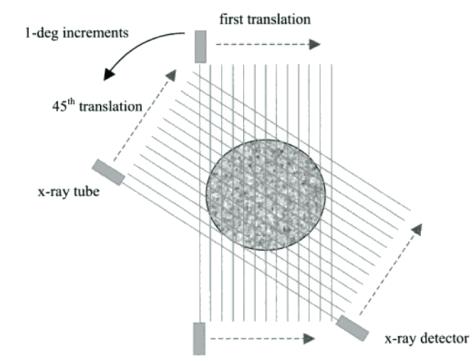
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## Composition of biological tissues

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	0.2
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	0.3
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	0.3
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3			22.5

## History

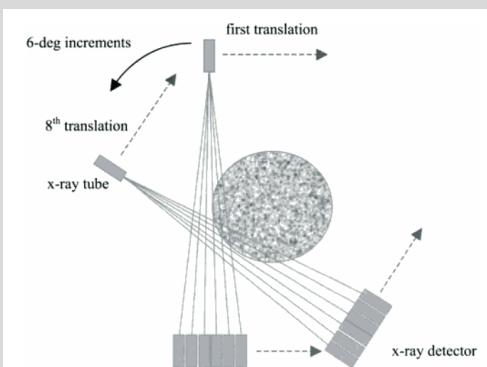
- Extensive history is found in [Cormack1963, Hsieh2003]
- First, second and third generation of scanners



From [Hsieh2003], Figure 1.12

## History

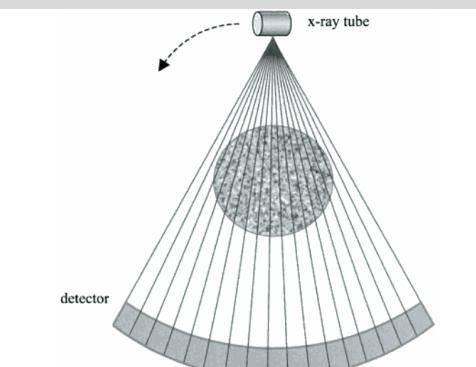
- Extensive history is found in [Cormack1963, Hsieh2003]
- First, second and third generation of scanners



From [Hsieh2003], Figure 1.13

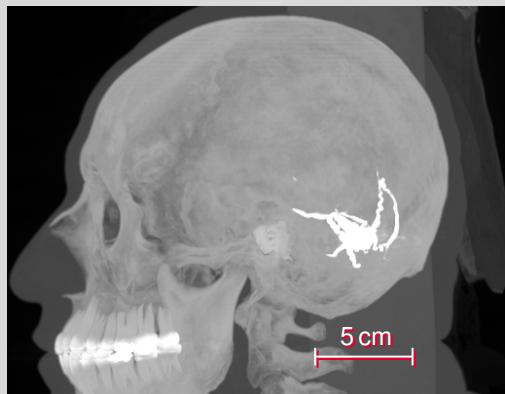
## History

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



From [Hsieh2003], Figure 1.14

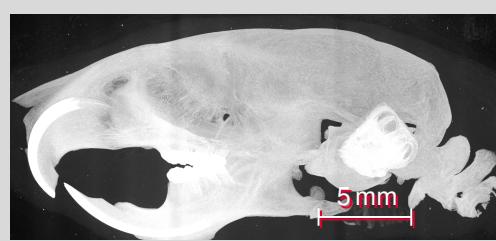
## Why µCT?



From [Clark2013], Subject C3L-02465

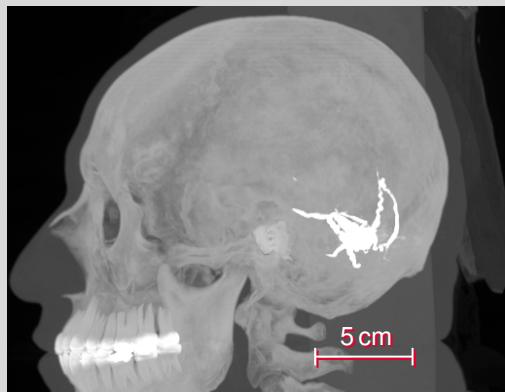
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## Why µCT?



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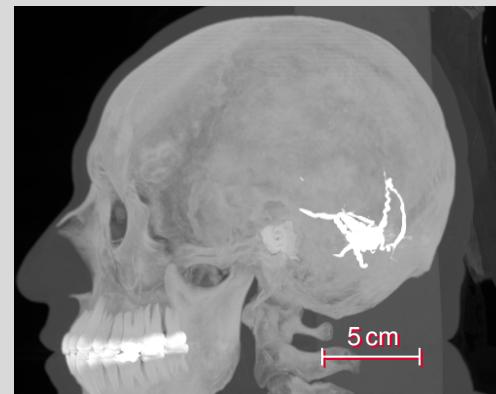
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## Why µCT?



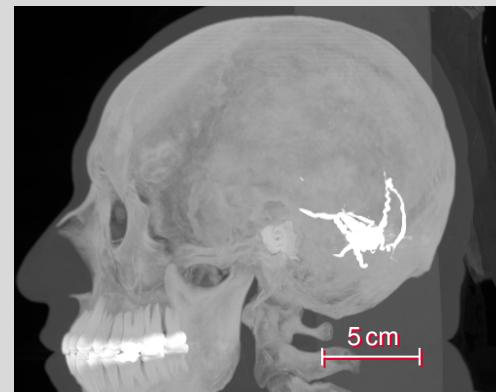
From [Clark2013], Subject C3L-02465

5 cm

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## Why µCT?



From [Clark2013], Subject C3L-02465

5 cm

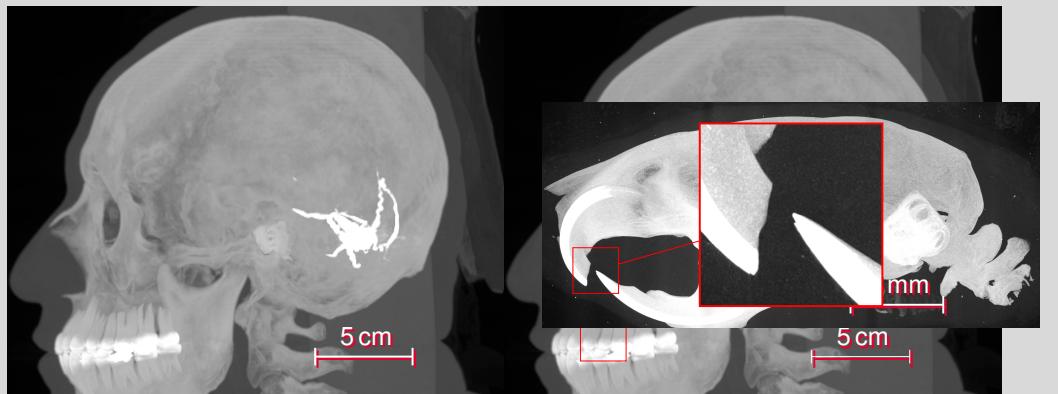
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## Why µCT?

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## 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 ☺①④③

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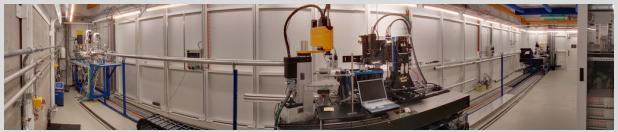


bruker.com/skyscan1272

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## Machinery

- Hospital CT
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- Synchrotron CT
  - Voxel size down to 160 nm



flic.kr/p/7Xhk2Y

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## Machinery I

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

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

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## Machinery II

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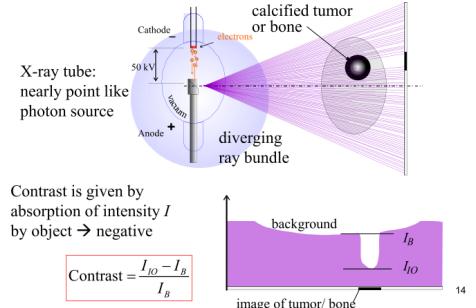
## Preparation

- Study design
- Sample preparation

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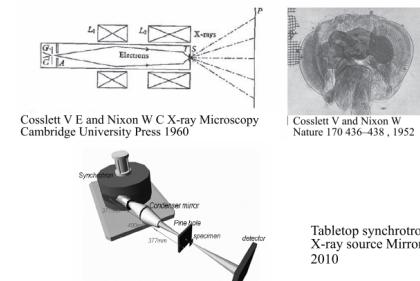
## Projections

### Image formation: shadow projection



### Image formation: shadow projection

#### X-ray projection microscopy



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

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## Projections

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## 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

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## Reconstructions

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## 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

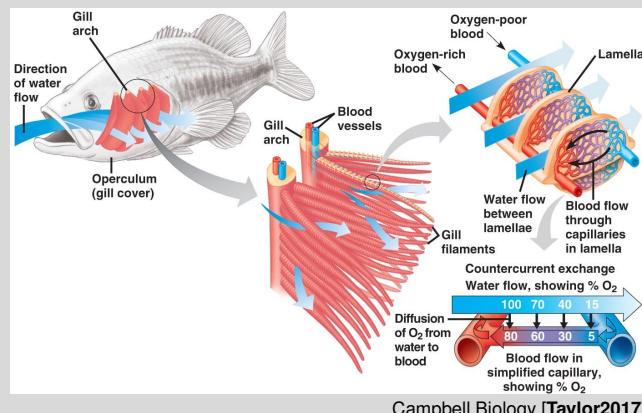
## Quantitative data

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

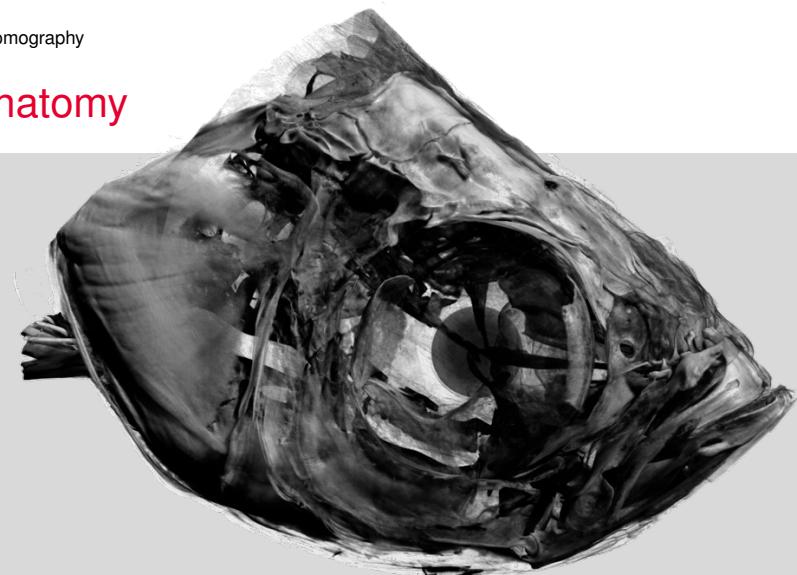
## Reproducible research

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

## An example: Do gills change with training?



## Gill anatomy

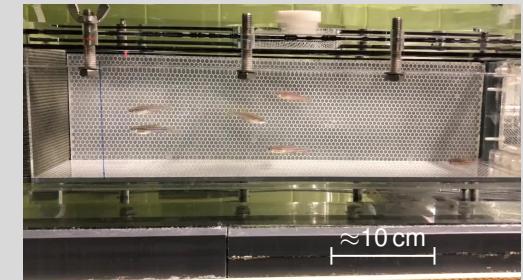


## Gill anatomy

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## How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [Palstra2010]
  - Endurance



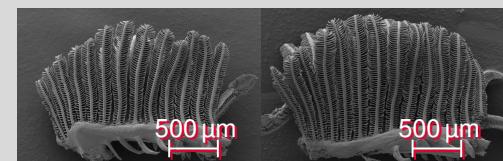
## How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [Palstra2010]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption

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## How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [Palstra2010]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure



## How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [Palstra2010]
  - Endurance
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  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying



## How?

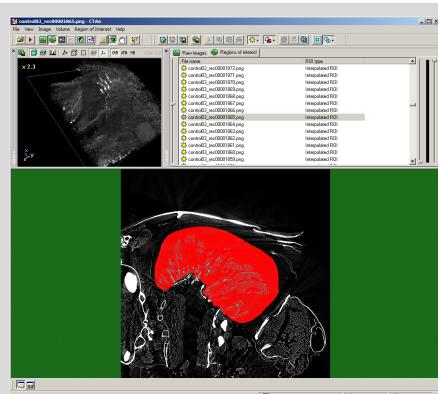
- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [Palstra2010]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging

```

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 [Palstra2010]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging, delineation in CTAn



## How?

- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [Palstra2010]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O<sub>2</sub> consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging, delineation in CTAn and analysis
  - Gill volume, structure and complexity



X-ray microtomography

## Gill volume

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X-ray microtomography

## Gill volume

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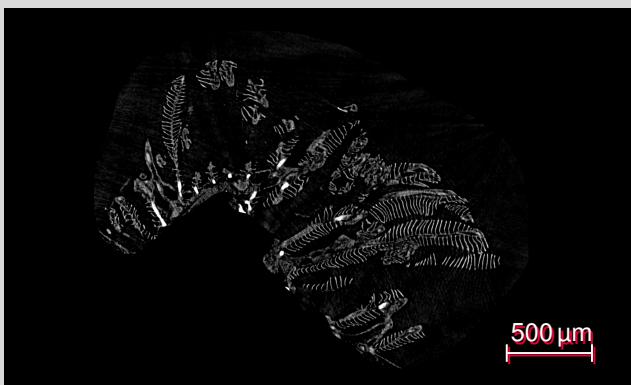
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X-ray microtomography

## Gill volume

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X-ray microtomography

## Gill volume

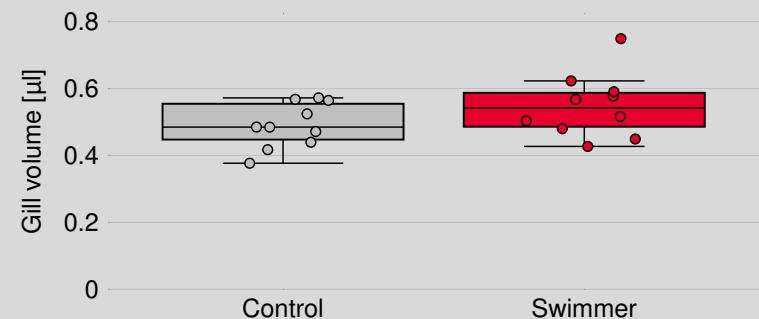
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## Gill volume



## Gill complexity

500  $\mu\text{m}$ 

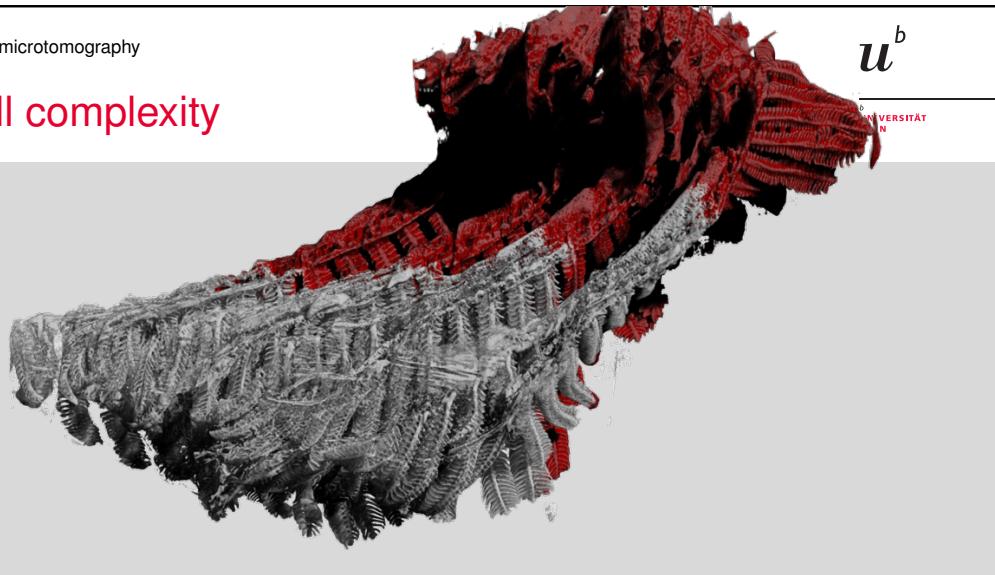
## Gill complexity

500  $\mu\text{m}$ 

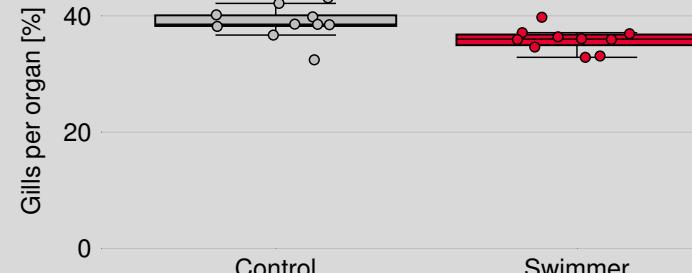
## Gill complexity

500  $\mu\text{m}$

## Gill complexity



## Gill complexity



## Colophon

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- This BEAMER presentation was crafted in  $\text{\LaTeX}$  with the (slightly adapted) template from *Corporate Design und Vorlagen* of the University of Bern.
  - Full source code: [git.io/fjpP7](https://git.io/fjpP7)
  - The  $\text{\LaTeX}$  code is automatically compiled with a GitHub action [2] to a PDF which you can access here: [git.io/JeMjP](https://git.io/JeMjP)
  - Spotted an error?  
Then please file an issue ([git.io/fjpPb](https://git.io/fjpPb)) or (even better) submit a pull request ([git.io/fjpPN](https://git.io/fjpPN)).

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

## References

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