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
  - µCT-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

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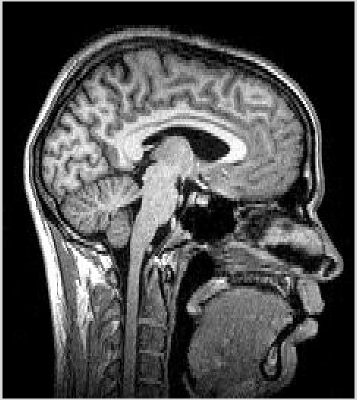
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



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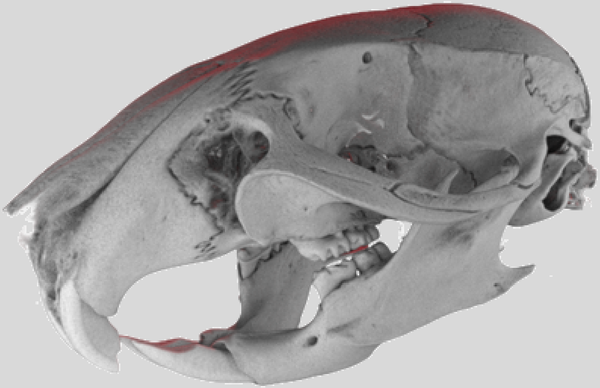
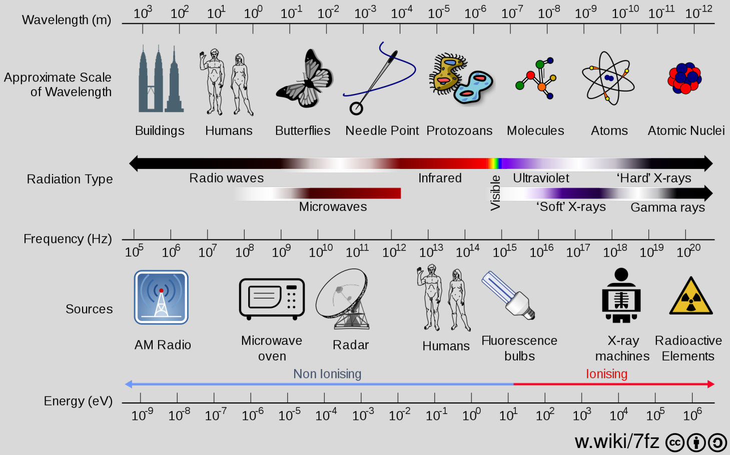
Biomedical imaging



Wavelength & Scale



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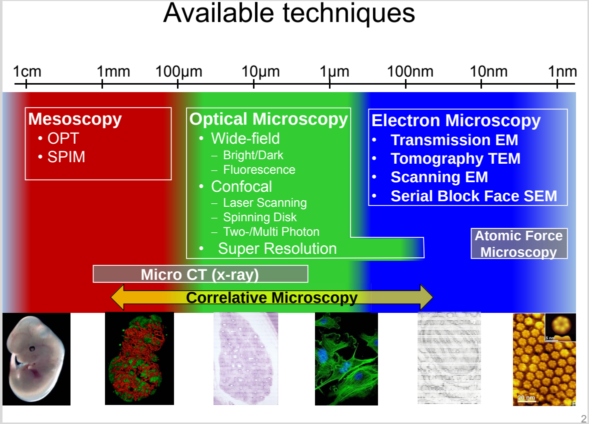


Yury Belyaev, MIC, slide from internal seminar presentation

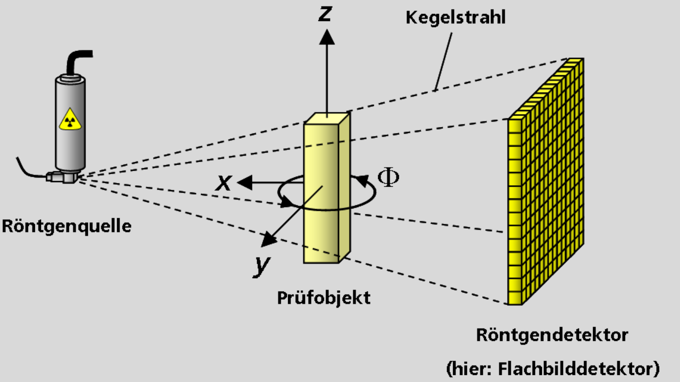
Imaging methods

- 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

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X-ray interaction

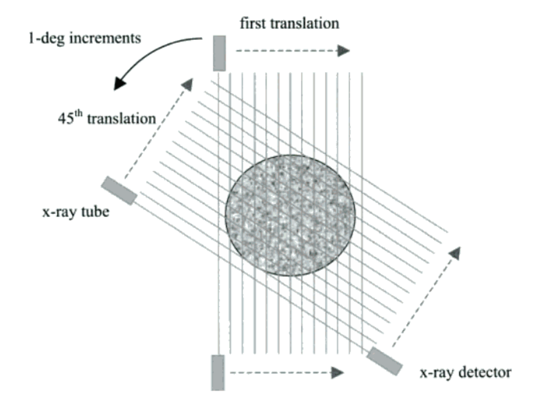
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([**xrayphysics**])
    - Photoelectric absorption (τ) is strongly 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

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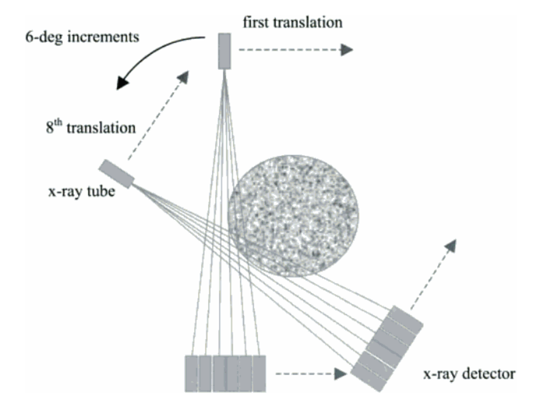

From [**Hsieh2003**], Figure 1.13

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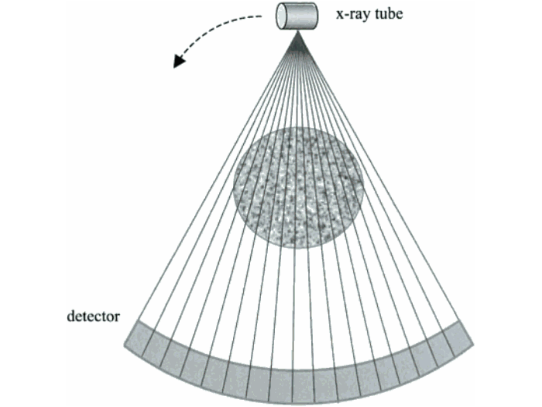
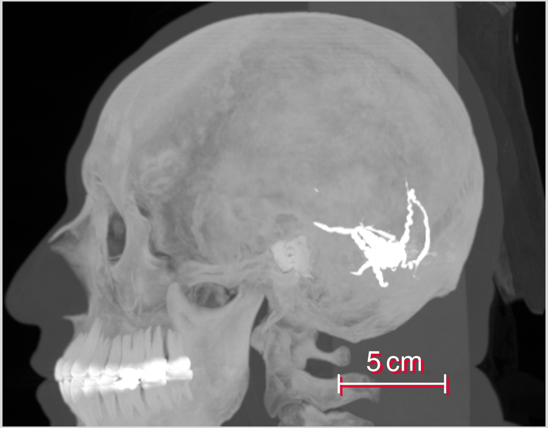


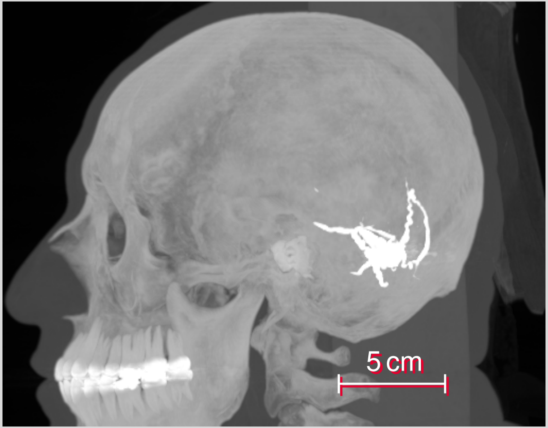
From [**Hsieh2003**], Figure 1.14

Why µCT?



From [**Clark2013**], Subject *C3L-02465*

Why µCT?



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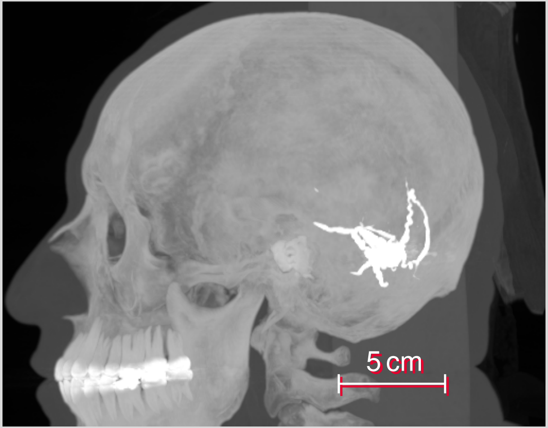

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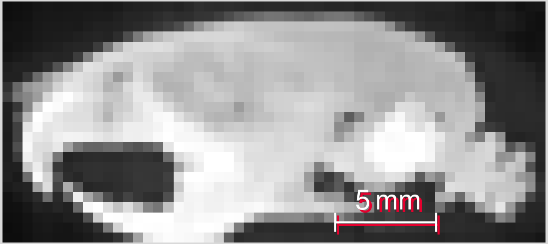

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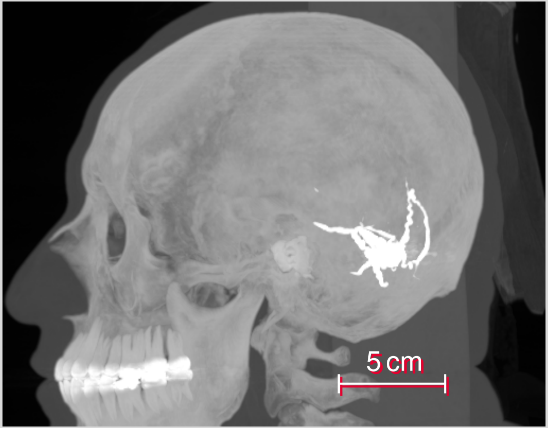

From [**Clark2013**], Subject *C3L-02465*

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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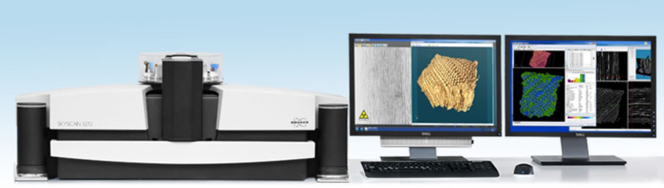

bruker.com/skyscan1272

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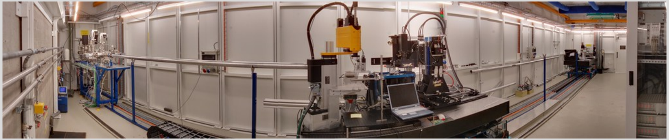

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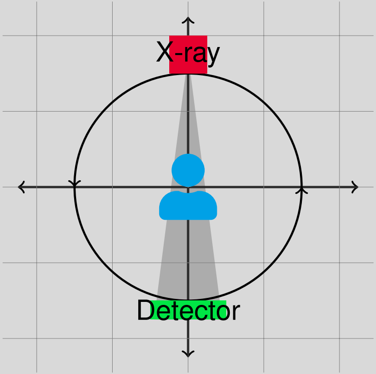
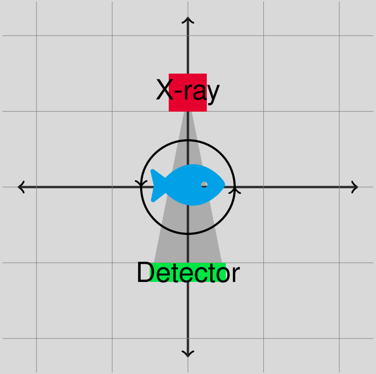
Machinery I

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

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

Machinery II





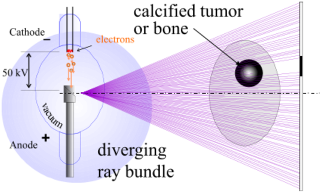
Preparation

- Study design
- Sample preparation

Projections

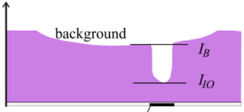
Image formation: shadow projection

X-ray tube:
nearly point like
photon source



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

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



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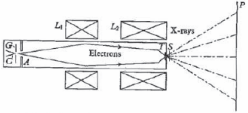
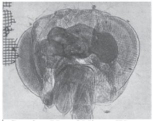
Laws of Physics for Microscopists by Michael Jaeger, Slide 14

Image formation: shadow projection

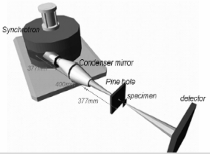
X-ray projection microscopy



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

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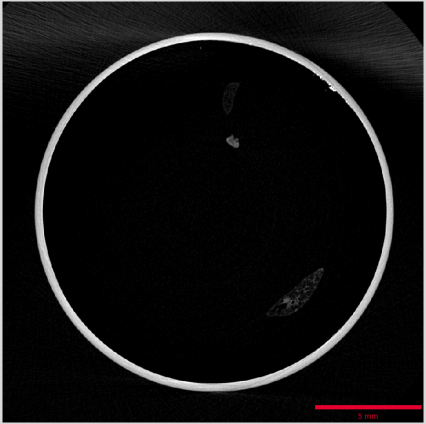
Laws of Physics for Microscopists by Michael Jaeger, Slide 15

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 the 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**.

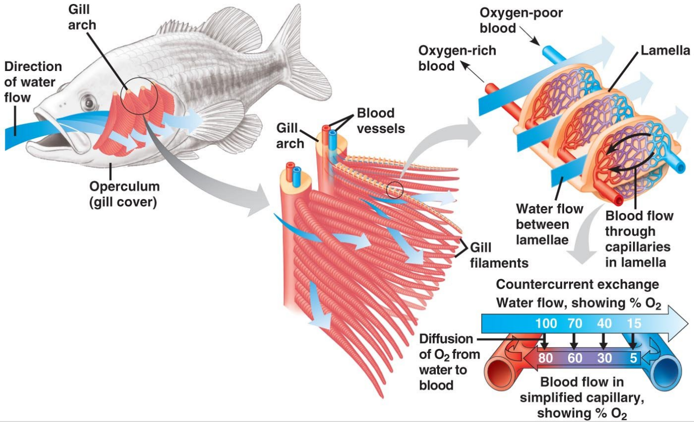
Quantitative data

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

Reproducible research

- **git**
- Jupyter
- Script all your things!
- Data repositories $\rightarrow$ Sharing is caring!

An example: Do gills change with training?



Campbell Biology [**Taylor2017**]

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

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    - Endurance
- Morphology & Physiology
    - Body size & weight
    - O_2 consumption

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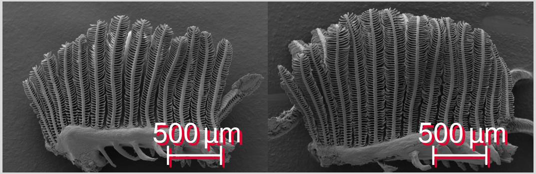
  - O_2 consumption
- Scanning electron microscopy
  - Gill structure



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  - 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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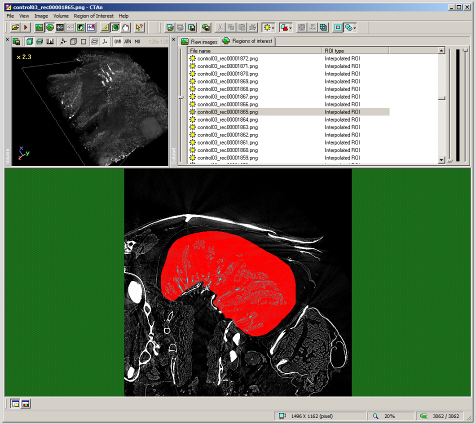
- Critical point drying, µCT imaging, delineation in CTAn



How?

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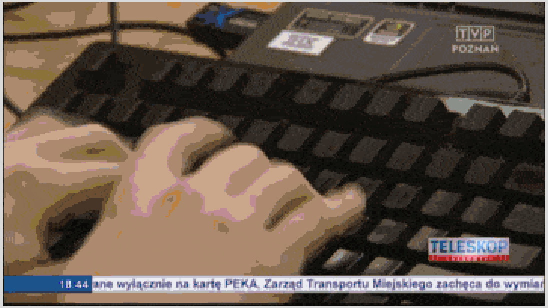
  - O_2 consumption
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging, delineation in CTAn and analysis
  - Gill volume, structure and complexity



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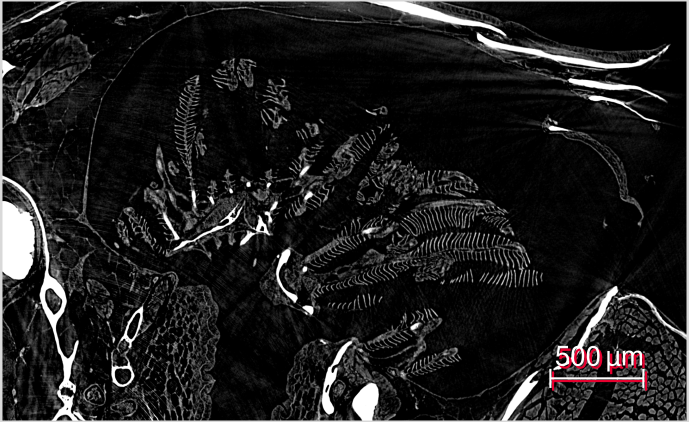
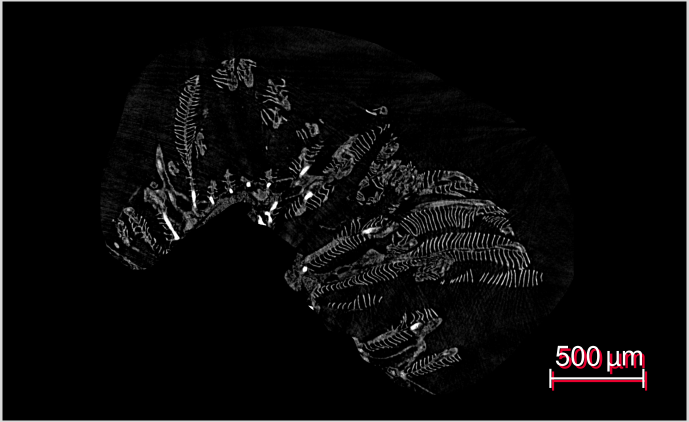
Gill volume

Gill volume



Gill volume



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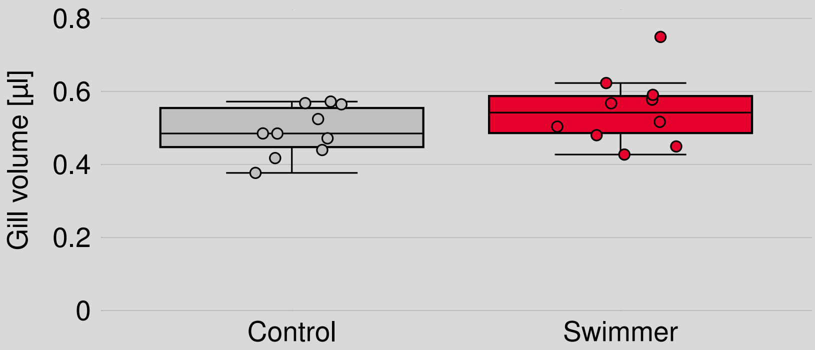

Gill complexity

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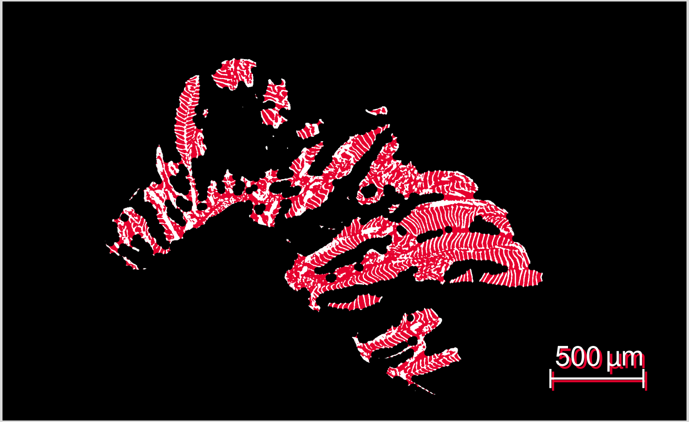
Gill complexity



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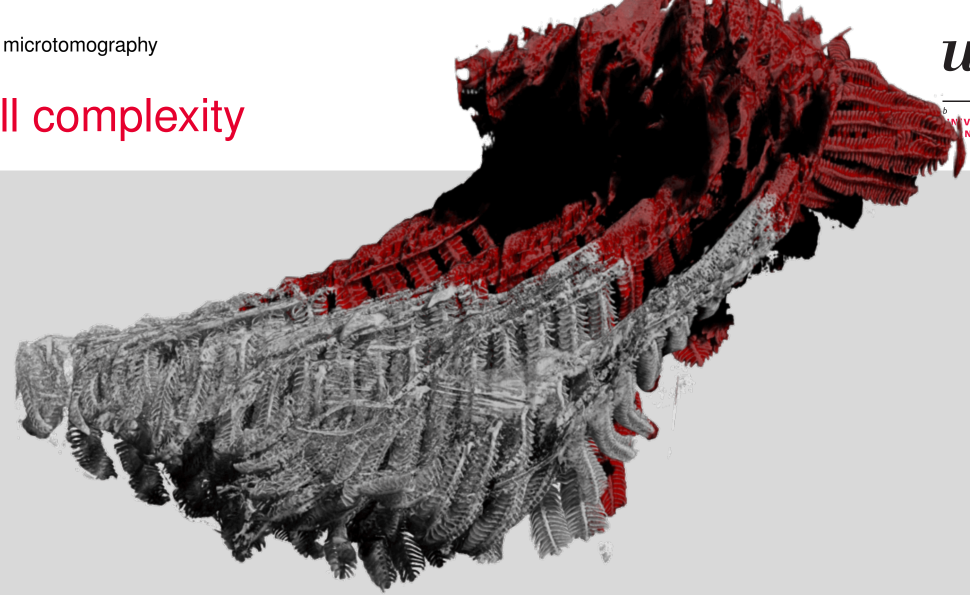
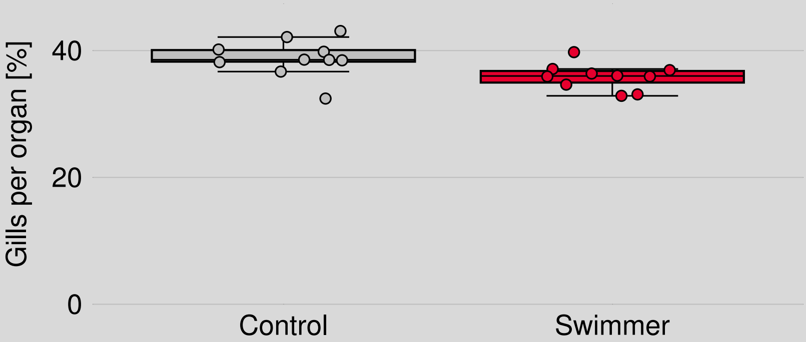


Gill complexity



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