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X-ray microtomography

David Haberthür
December 20, 2019 | 9256-HS2019-0: Advanced Microscopy

Hello!

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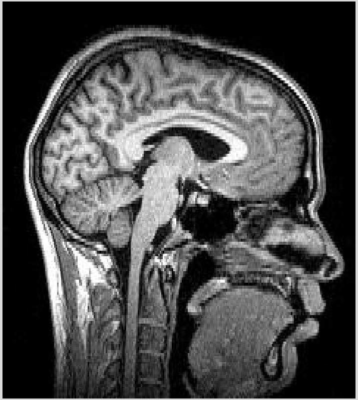
- Office B311 | haberthuer@ana.unibe.ch
- Master in Physics
- PhD in high resolution imaging of the lung at the Institute of Anatomy
- Post-Doc
  - TOMCAT, Swiss Light Source, Paul Scherrer Institute
  - µCT-group, Institute of Anatomy (Ruslan Hlushchuk, David Haberthür, Oleksiy-Zakhar Khoma)
- Biomedical 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 & 2214

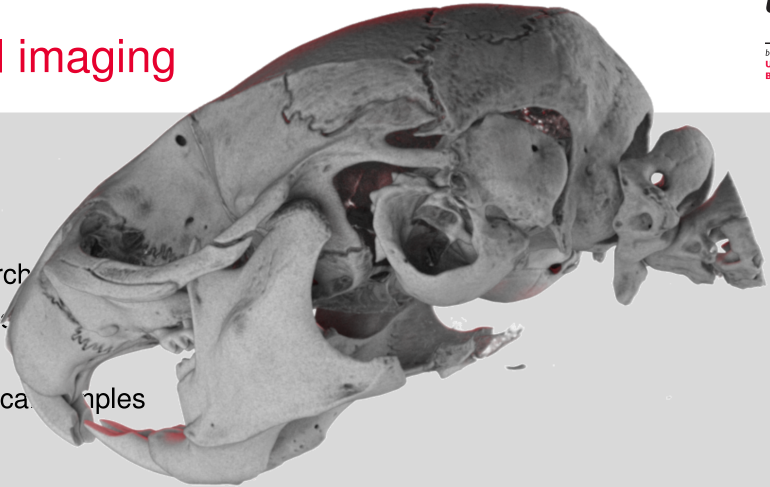
Contents

Biomedical imaging

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

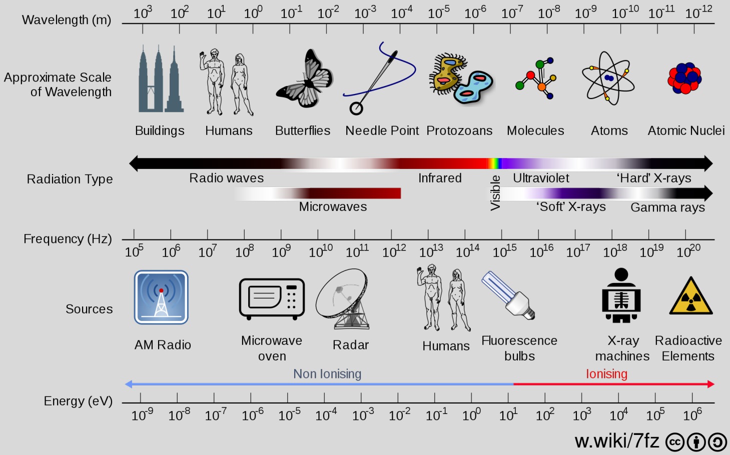


Biomedical imaging



- Medical research
- Non-destructiv
  the samples
- (Small) Biologica nples

Wavelength & Scale



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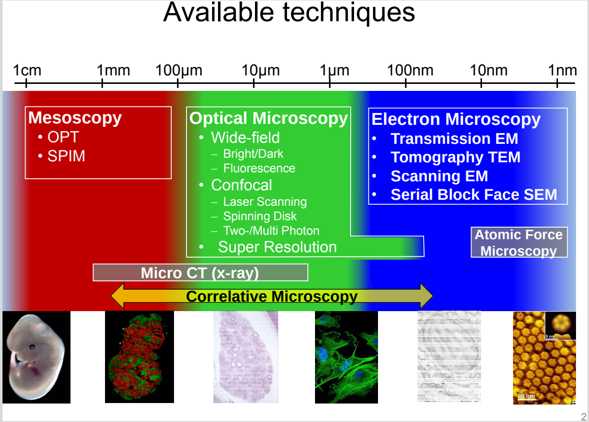

Yury Belyaev, MIC, slide from internal seminar presentation

Imaging methods

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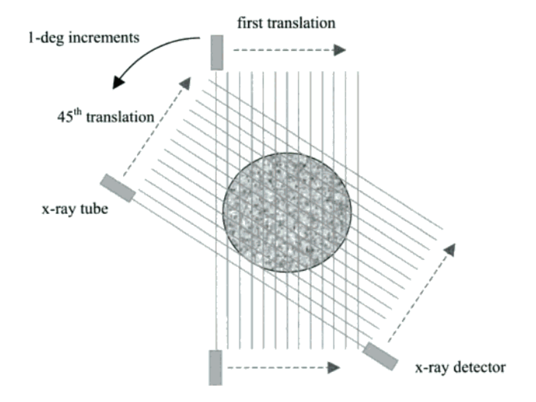
- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy: see lectures *Transmission Electron Microscopy* by Dimitri Vanhecke, *Scanning Electron Microscopy* by Michael Stoffel and *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

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

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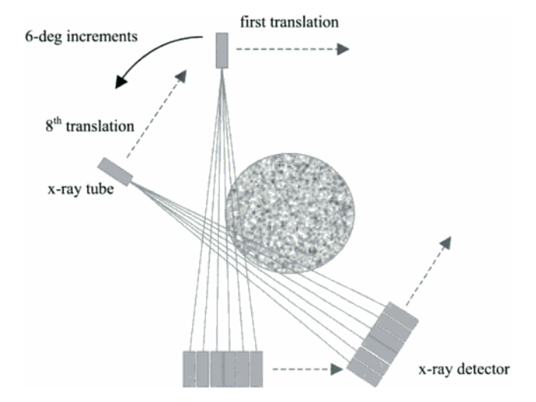

From [5], Figure 1.13

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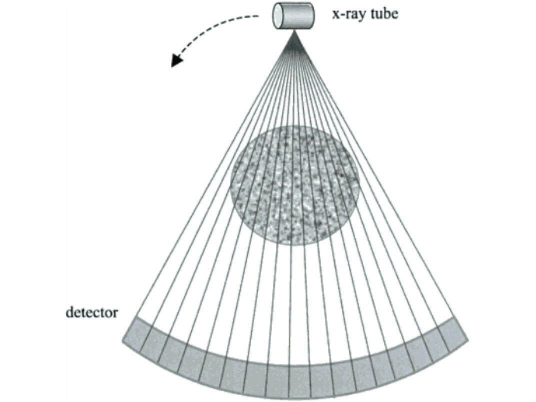


From [5], Figure 1.14

X-ray interaction

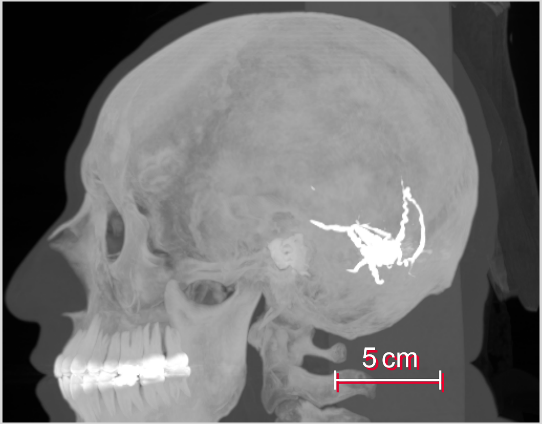
- “X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose.” ([6])
  - Photoelectric absorption (τ) is strongly dependent on 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

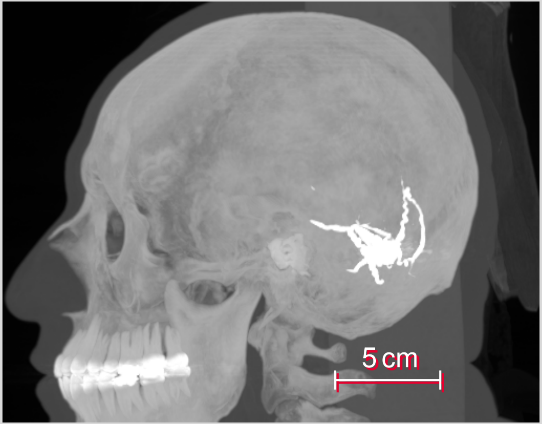
Element	H	C	N	O	Na	P	S	Cl	K	Ca
Atomic number	1	6	7	8	11	15	16	17	19	20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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

Why µCT?



From [8], Subject *C3L-02465*

Why µCT?



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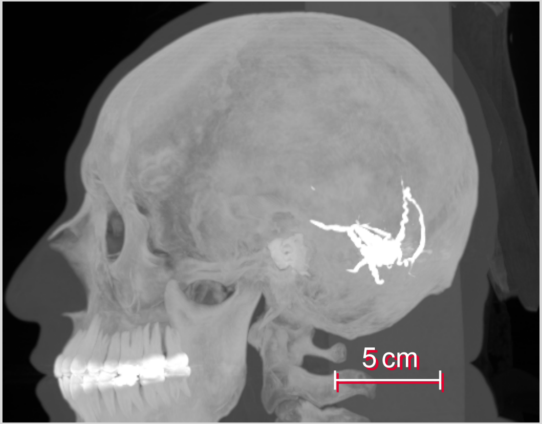

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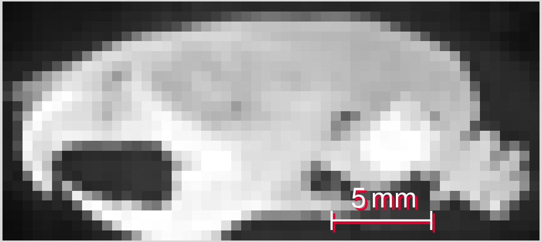

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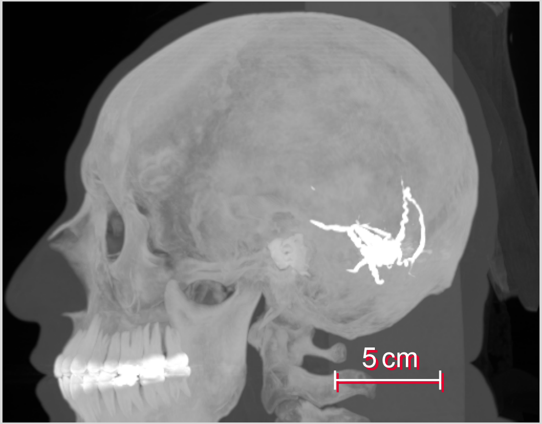

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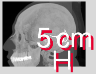

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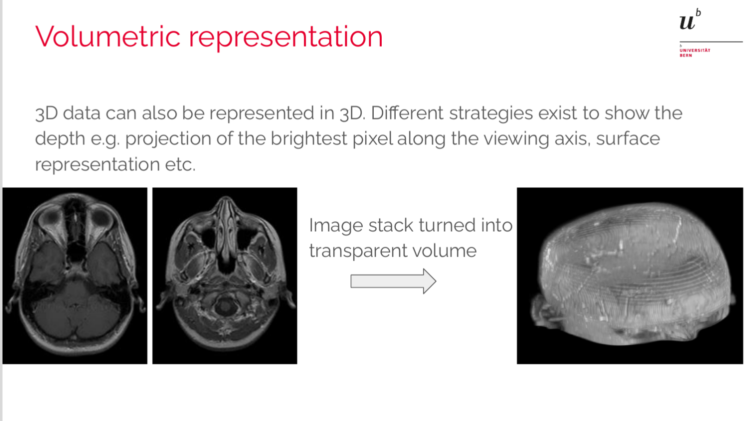
MIP



Fundamentals of Digital Image Processing by Guillaume Witz, Slide 21

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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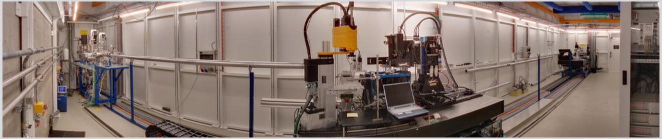

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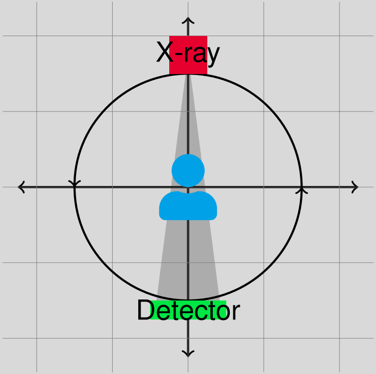
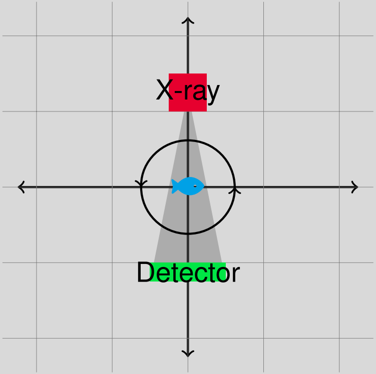
Machinery

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

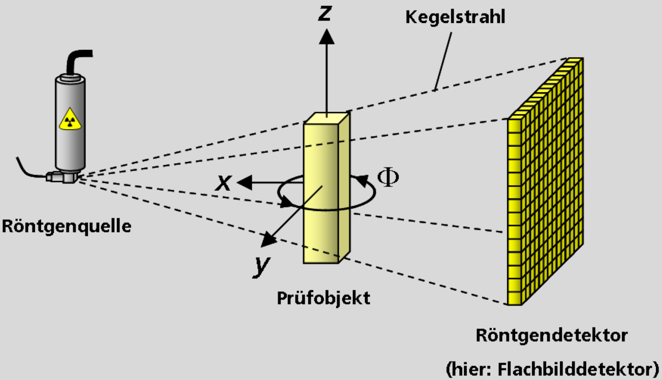
- an x-ray source
- a sample
- a detector

Machinery



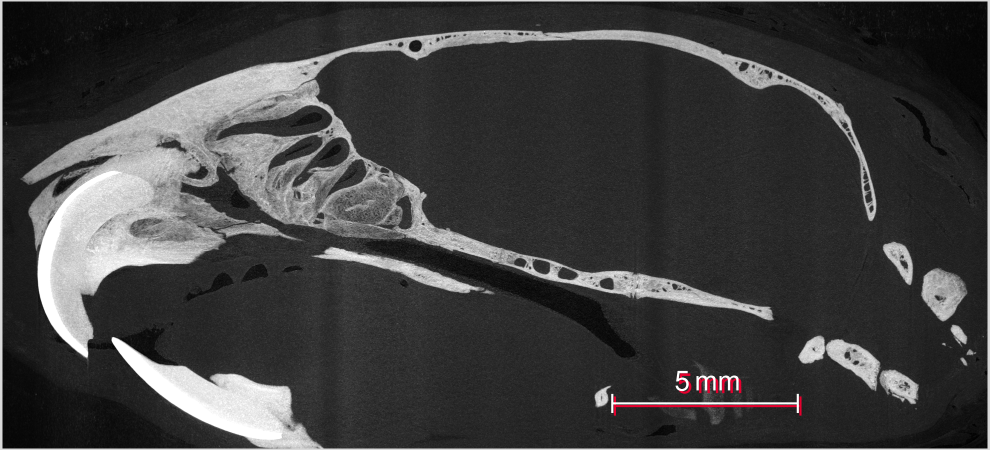


What is happening?

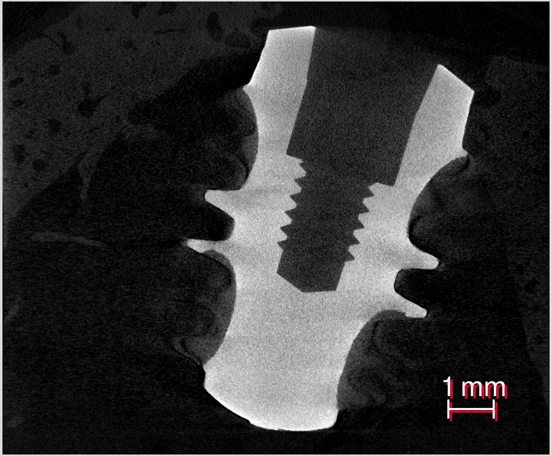


w.wiki/7g3

Examples



Examples



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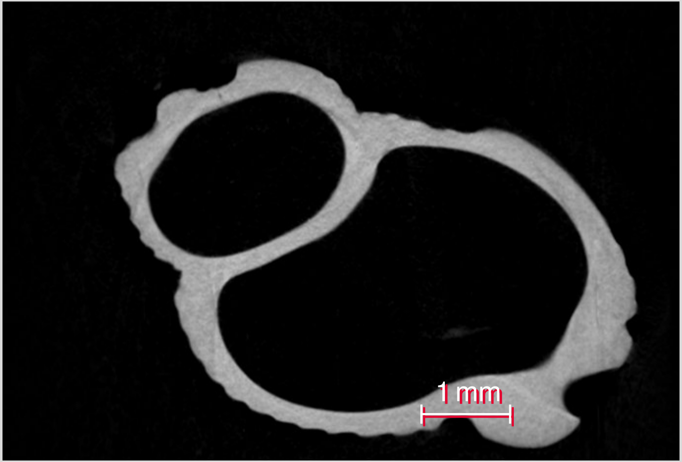

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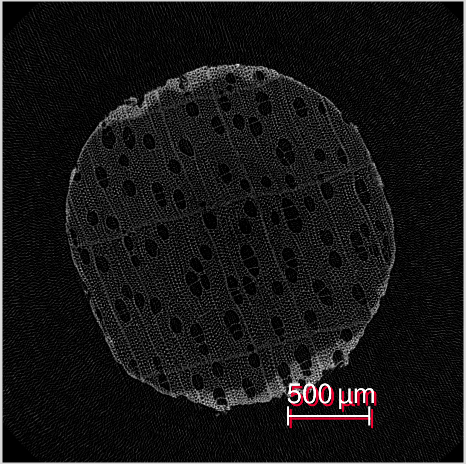

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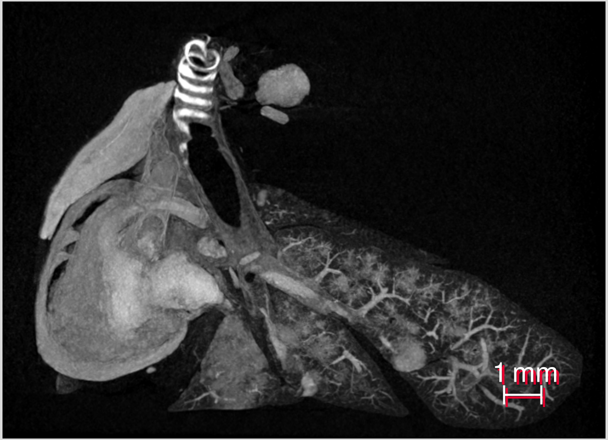

Preparation

- Study design
- Sample preparation

Projections

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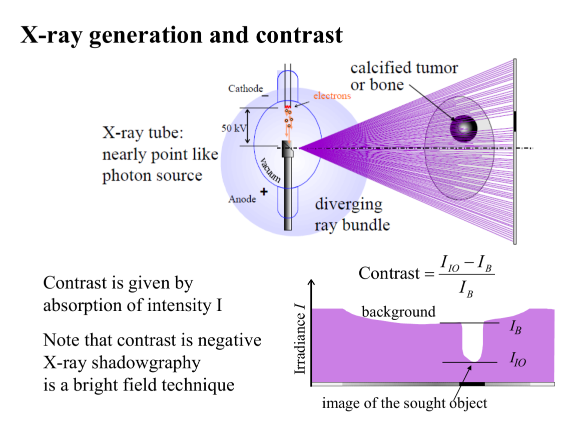


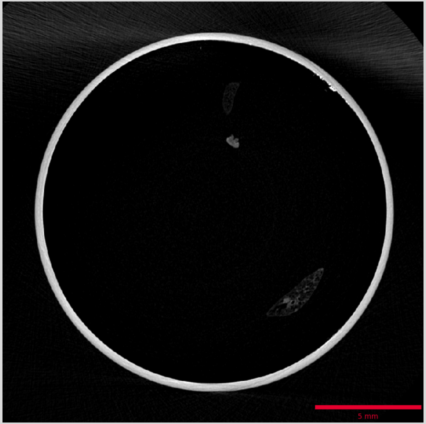
Laws of Physics for Microscopists by Martin Frenz, Slide 15

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

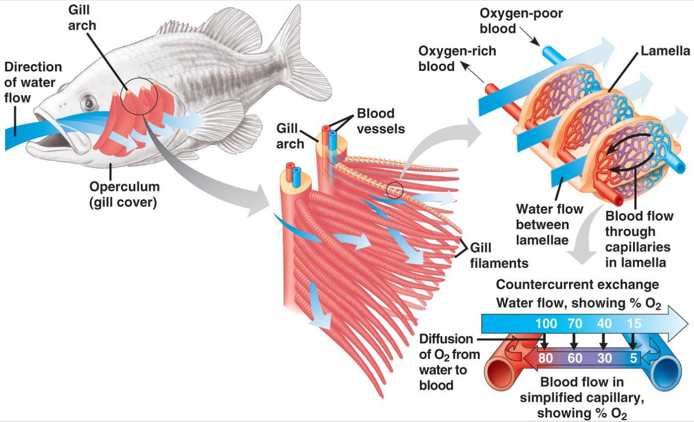
What to use?

- ImageJ/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

- Pretty images are nice, but we need quantitative numbers
- Segmentation
- Characterization

An example: Do gills change with training?



Campbell Biology [11]

Gill anatomy

Gill anatomy

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How?

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

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- Training for 5 w, 5 d/w, 6 h/d, at 66 % of critical speed [12]
  - Endurance
- Morphology & Physiology
  - Body size & weight
  - O_2 consumption

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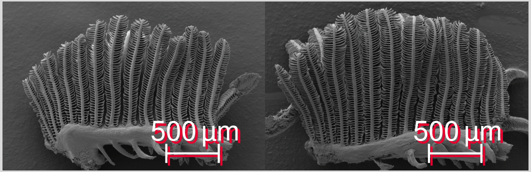
    - Body size & weight
    - O_2 consumption
- Scanning electron microscopy
    - Gill structure



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- Critical point drying

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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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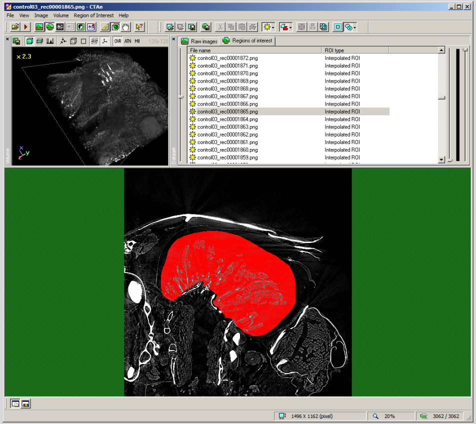

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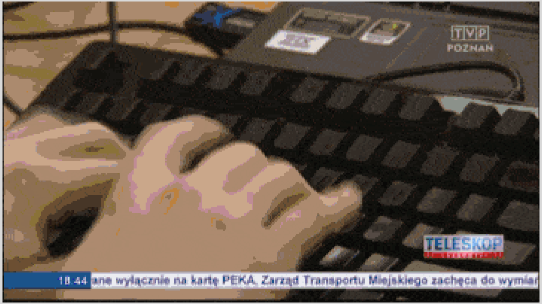
- Scanning electron microscopy
  - Gill structure
- Critical point drying, µCT imaging, delineation in CTAn and analysis
  - Gill volume, structure and complexity



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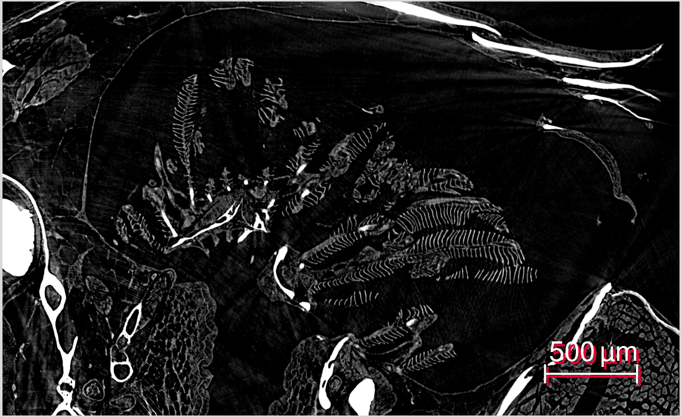
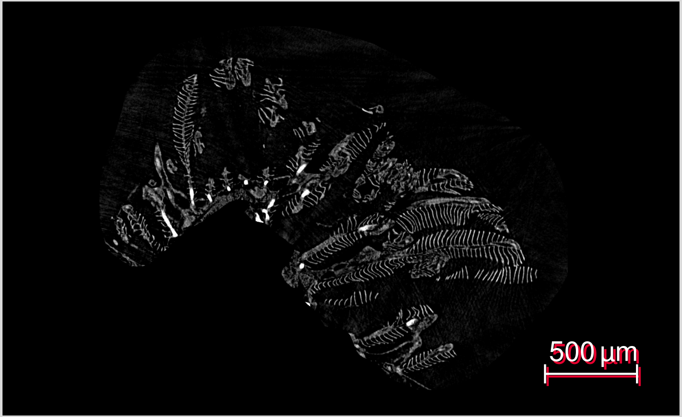
Gill volume

Gill volume



Gill volume



Gill volume

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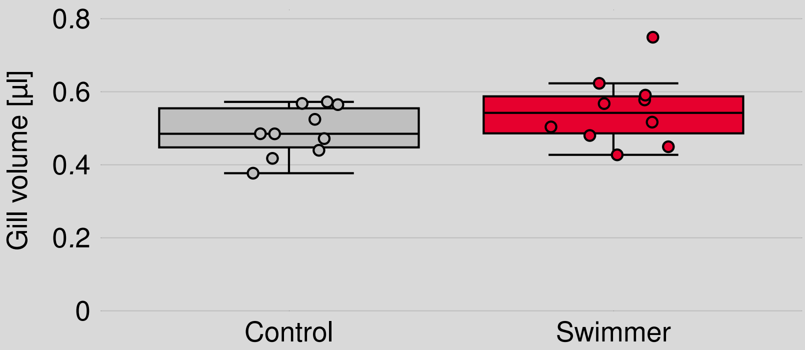

Gill complexity

Gill complexity

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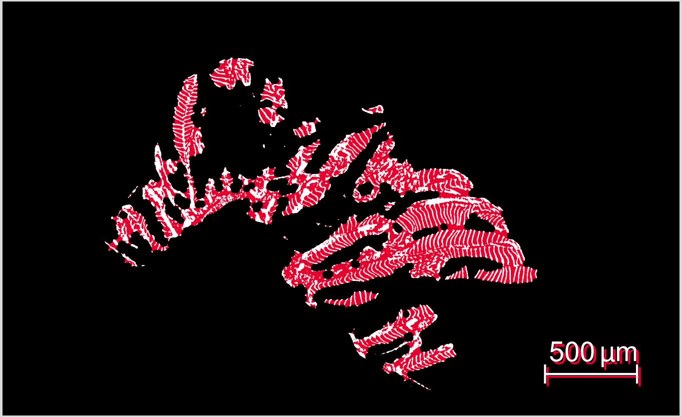
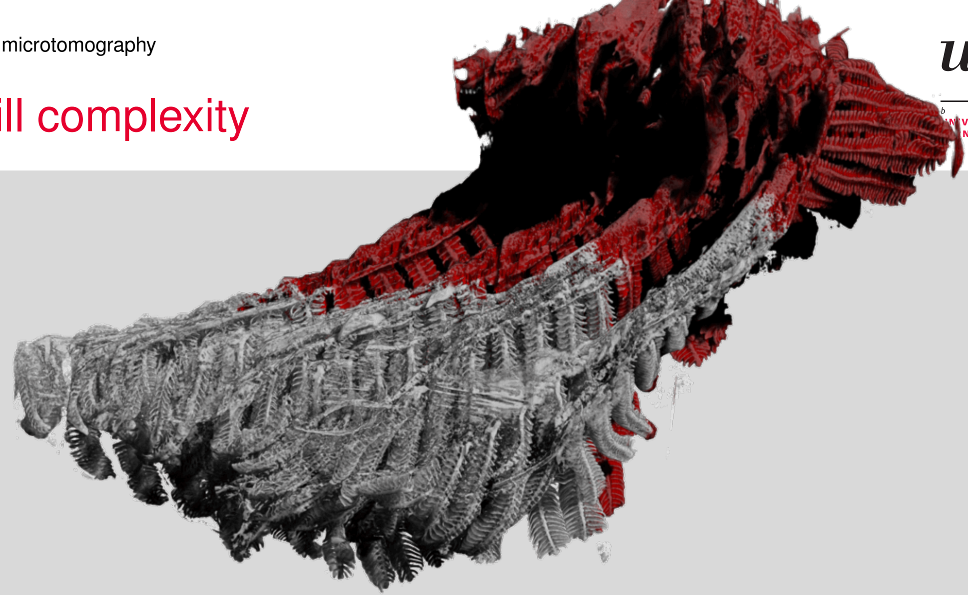
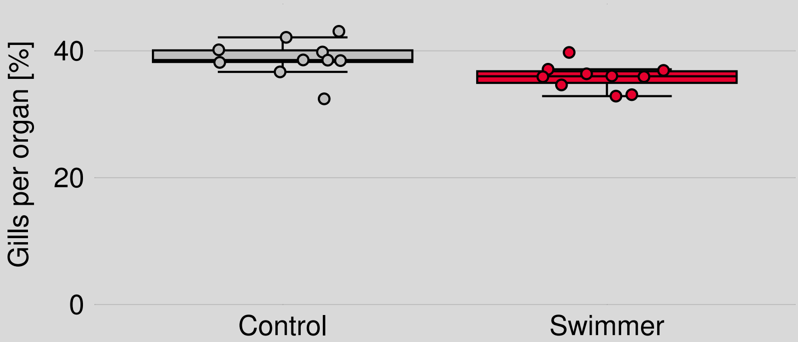


Gill complexity



Gill complexity



Thanks!

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- 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

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References

[1] Ruslan Hlushchuk et al. “Ex vivo microangioCT: Advances in microvascular imaging”. DOI: 10.1016/j.vph.2018.09.003.

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[9] Johannes Schindelin et al. “Fiji: an open-source platform for biological-image analysis”. DOI: 10.1038/nmeth.2019.

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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.