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X-ray microtomography

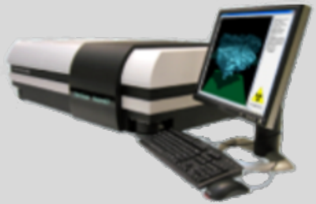
David Haberthür

December 3, 2021 | 9256-HS2021-0: Advanced Microscopy

Grüessech!

- David Haberthür
  - Physicist by trade
  - PhD in high resolution imaging of the lung, Institute of Anatomy, University of Bern, Switzerland
  - Post-Doc I: TOMCAT, Swiss Light Source, Paul Scherrer Institute, Switzerland
  - Post-Doc II: µCT group, Institute of Anatomy, University of Bern, Switzerland. Together with Ruslan Hlushchuk, Oleksiy-Zakhar Khoma and Tim Hoessly.

µCT-group



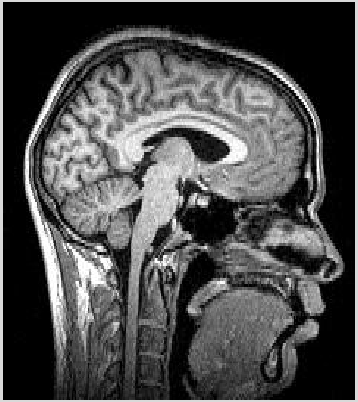
- microangioCT [1]
  - Angiogenesis: heart, musculature [2] and bones
  - Vasculature: (mouse) brain [3], (human) nerve scaffolds [4], (human) skin flaps [5] and tumors
- Zebrafish musculature and gills [6]
- (Lung) tumor detection and metastasis

brukersupport.com

Contents

Biomedical imaging

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



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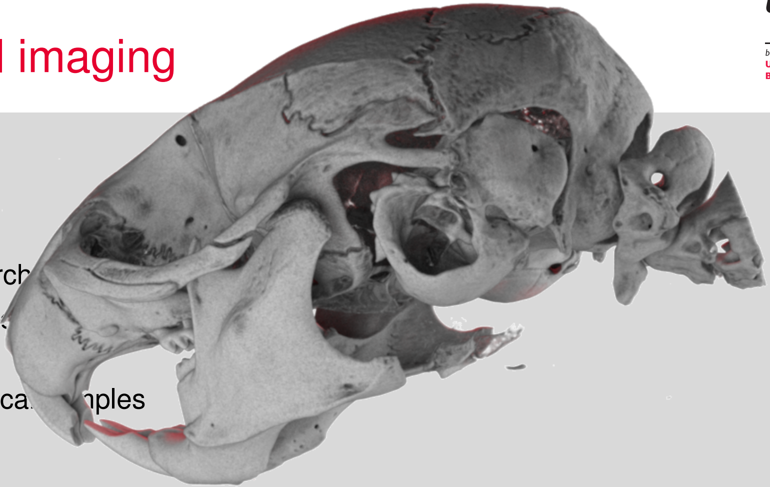
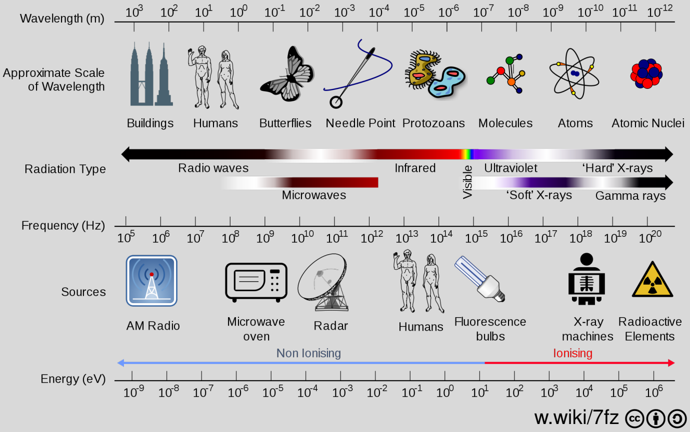
Biomedical imaging

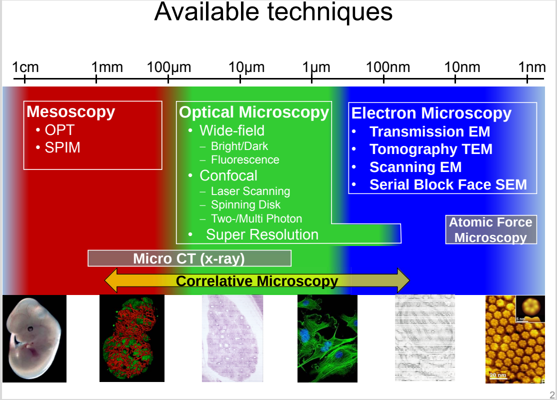


- Medical research
- Non-destructiv the samples
- (Small) Biologica mples

Wavelength & Scale



Wavelength & Scale



Yury Belyaev, MIC, slide from internal seminar presentation

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Imaging methods

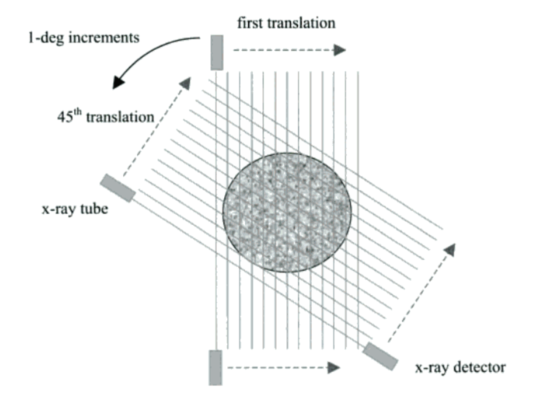
- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy: see lectures *Transmission Electron Microscopy* by Dimitri Vanhecke, *Scanning Electron Microscopy* by Michael Stoffel and *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

CT-Scanner

youtu.be/2CWpZKuy-NE

History

- Long history
  - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [10]
  - 1976: Hounsfield worked on first clinical scanner [11]
  - Nice overview by Hsieh [12]
- First, second and third generation of scanners



From [12], Figure 1.12

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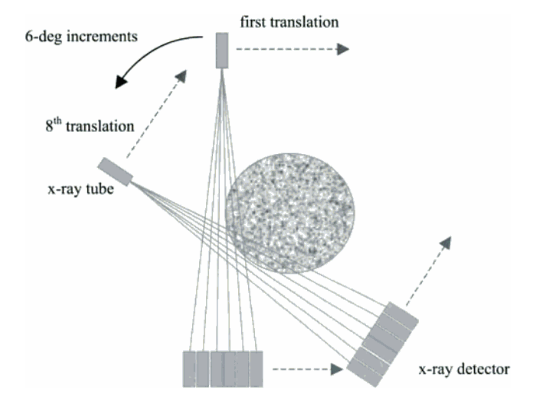

From [12], Figure 1.13

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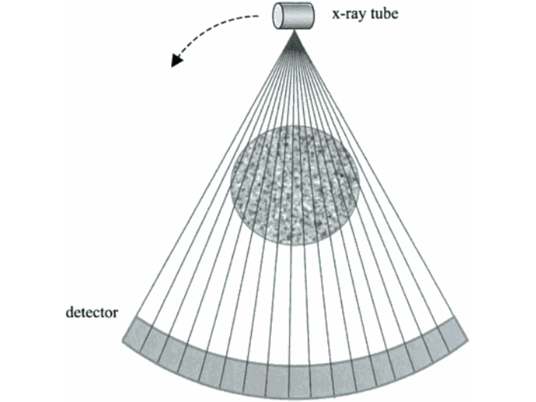


From [12], Figure 1.14

X-ray interaction

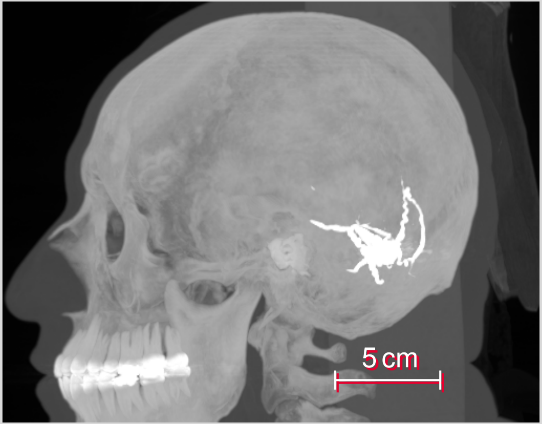
- "X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose." ([13])
  - Photoelectric absorption (τ) is strongly dependent on 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 ([14, i. e. Beer-Lamberts law] $I(t) = I_0 e^{-\alpha z}$

Composition of biological tissues

Tissue: content by mass percentage

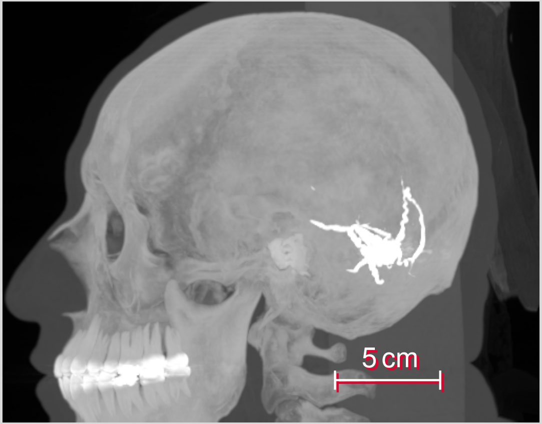
Element	H	C	N	O	Na	P	S	Cl	K	Ca
Atomic number	1	6	7	8	11	15	16	17	19	20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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

Why µCT?



From [15], Subject *C3L-02465*

Why µCT?



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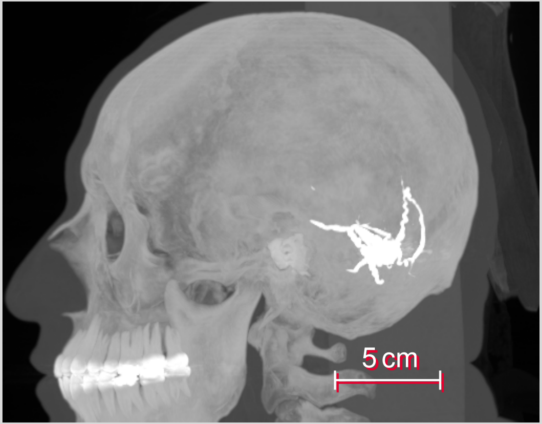

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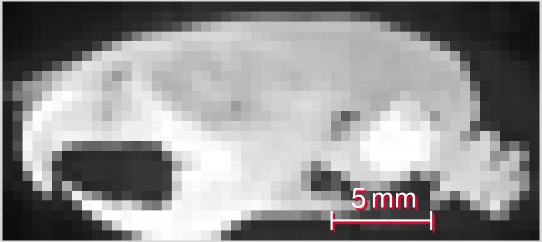

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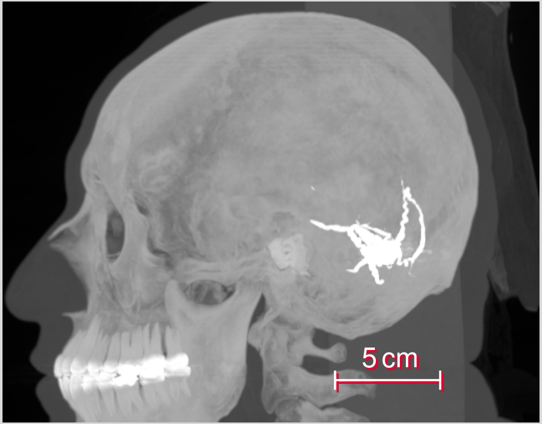

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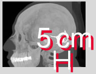


From [15], Subject *C3L-02465*

MIP

Volumetric representation

3D data can also be represented in 3D. Different strategies exist to show the depth e.g. projection of the brightest pixel along the viewing axis, surface representation etc.

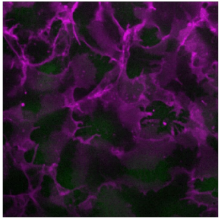
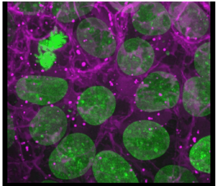


Image stack turned into transparent volume



Example dataset from scikit-imag.
Origin: Allen Institute for Cell Science

Fundamentals of Digital Image Processing by Guillaume Witz, Slide 20

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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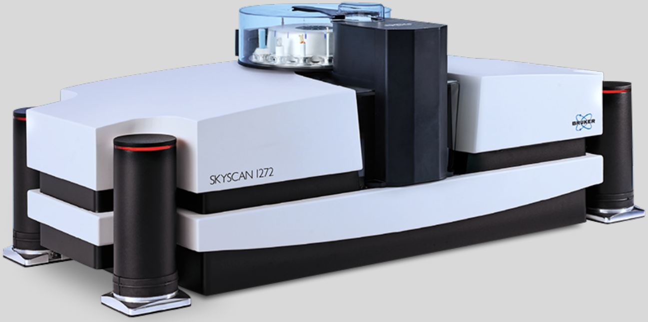

bruker.com/skyscan1272

Machinery

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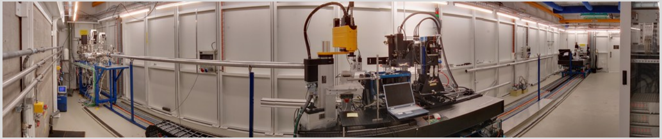

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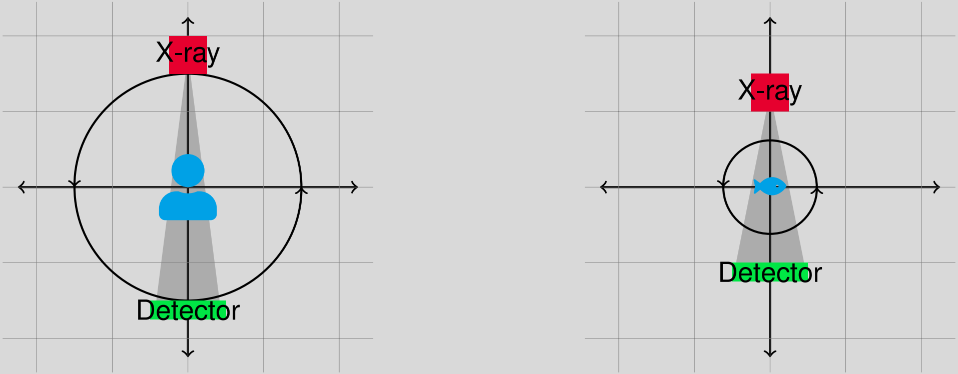
Machinery

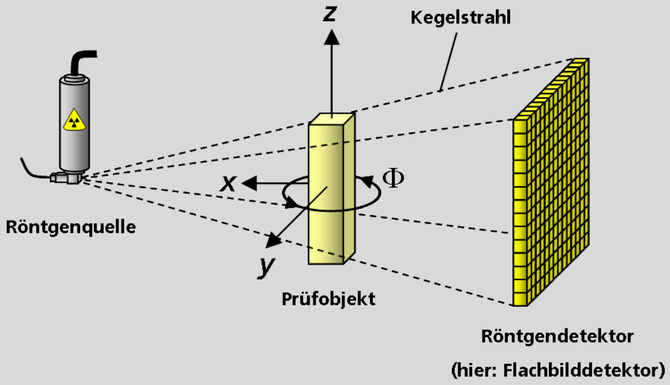
No matter what kind of machine, the basic principle is always the same

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

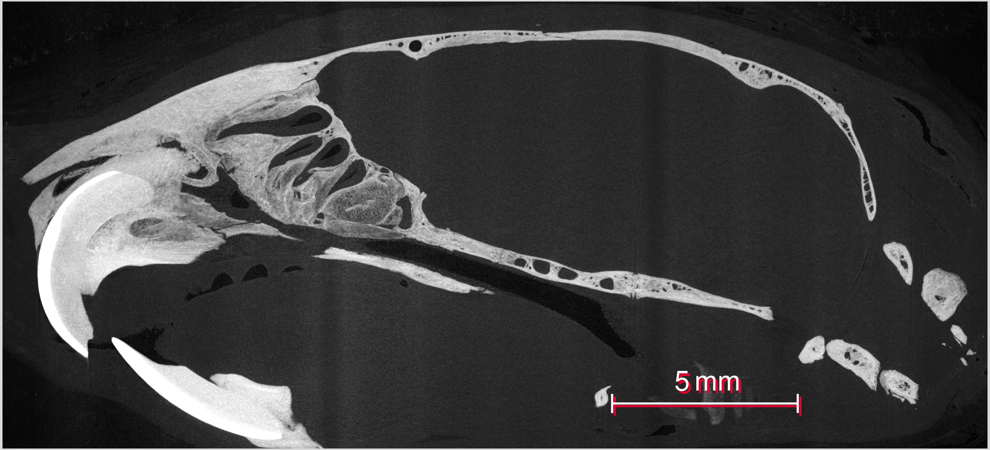
Machinery



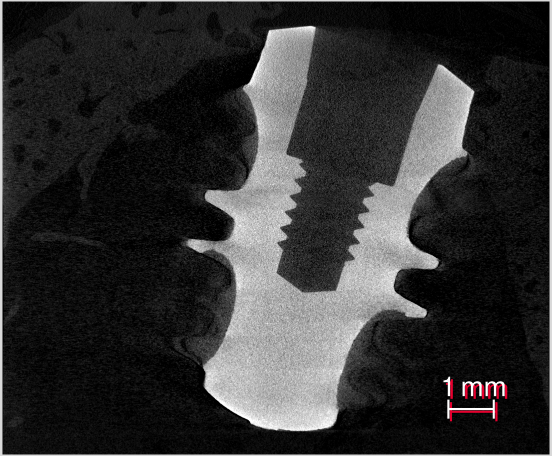
What is happening?



Examples



Examples



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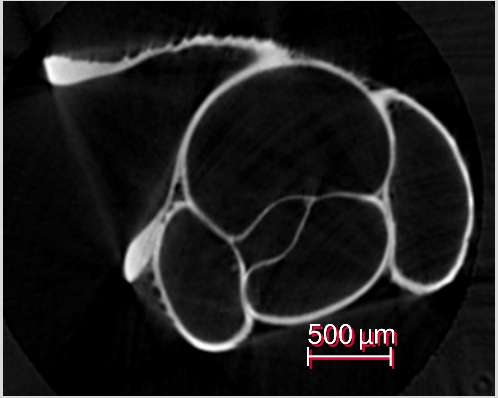
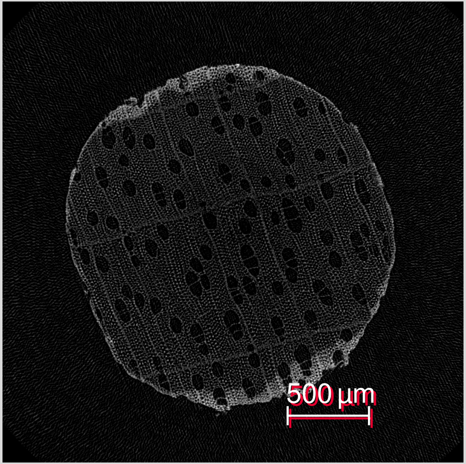
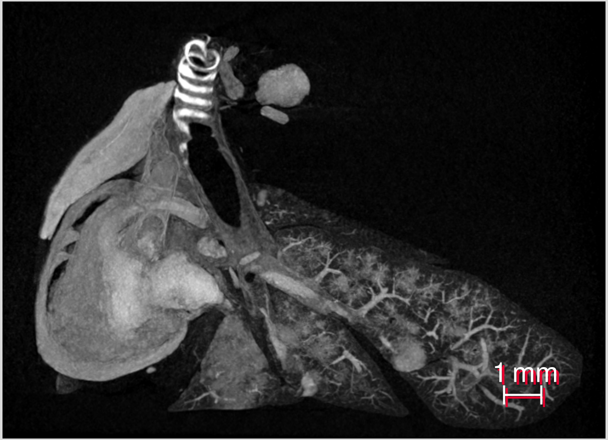


From [**Bochud2021**], *Diancta phoenīx*

Examples



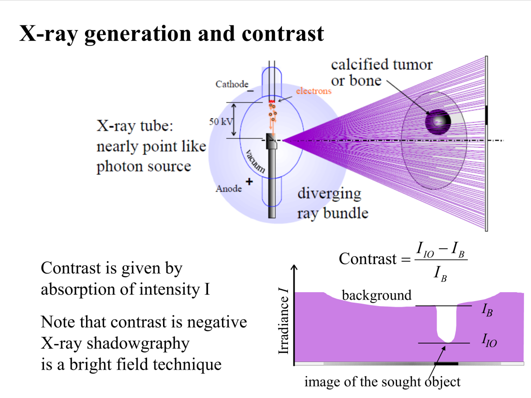
Examples



Preparation

- Study design
- Sample preparation

Projections



Laws of Physics for Microscopists by Martin Frenz, Slide 21

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 [16]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  - in Jupyter [17]
  - git
  - Script all your things!
  - Data repositories; i. e. sharing is caring!

Quantitative data

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

Internal morphology of human teeth

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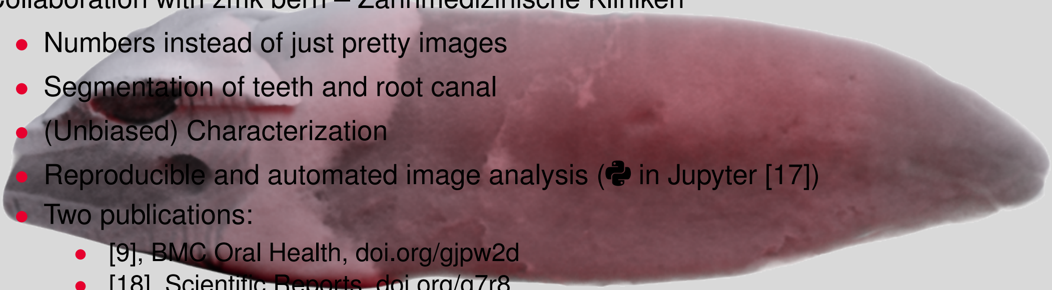
Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbers instead of just pretty images
- Segmentation of teeth and root canal
- (Unbiased) Characterization
- Reproducible and automated image analysis (in Jupyter [17])
- Two publications:
  - [9], BMC Oral Health, doi.org/gjpw2d
  - [18], Scientific Reports, doi.org/g7r8



Internal morphology of human teeth

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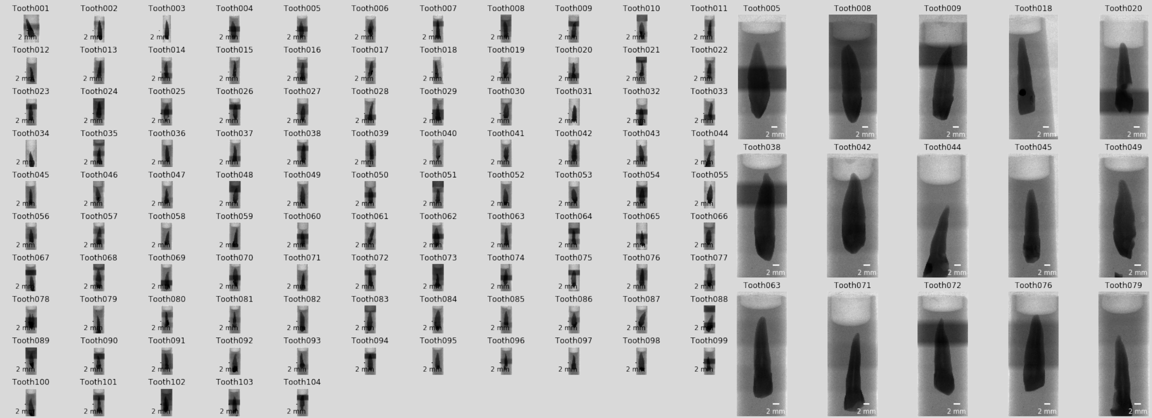
How?


```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
```

µCT imaging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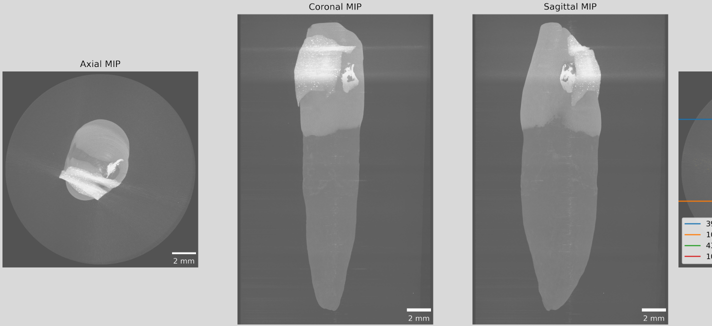
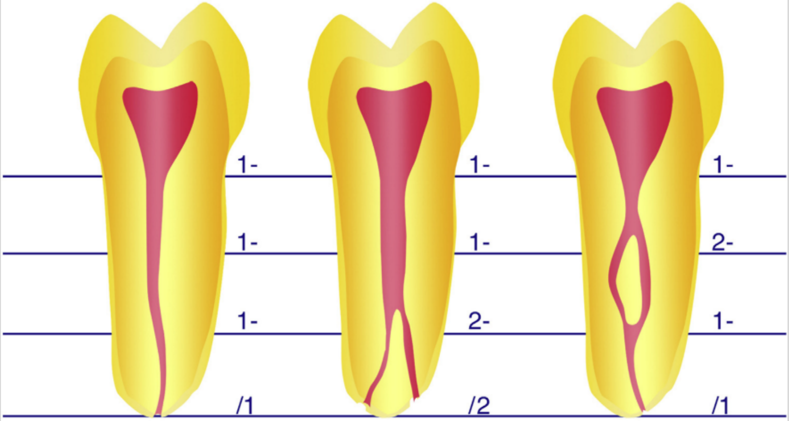
Tooth063 Tooth071 Tooth072 Tooth076 Tooth079
2 mm

Dataset cropping

- Full datasets: 326 GB
- Cropped datasets: 115 GB

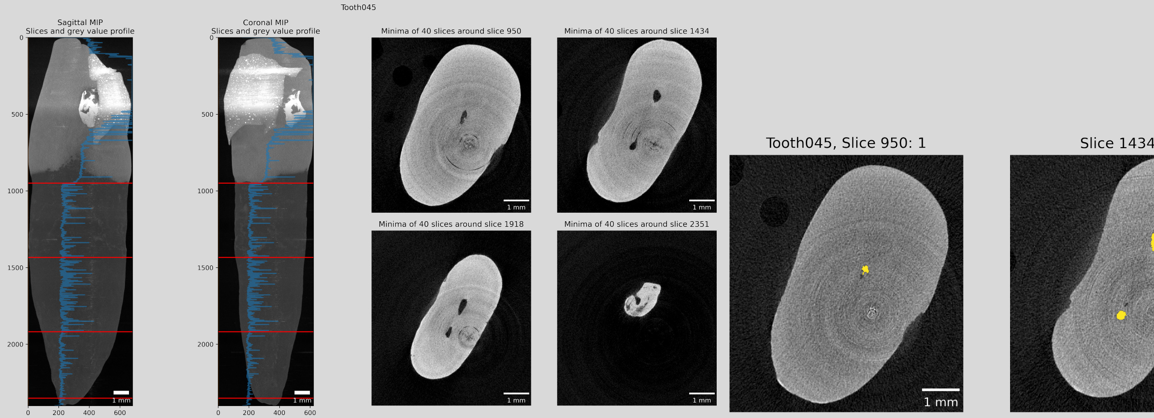


Tooth morphology



From [19], Fig. 2

Detection of enamel-dentin border



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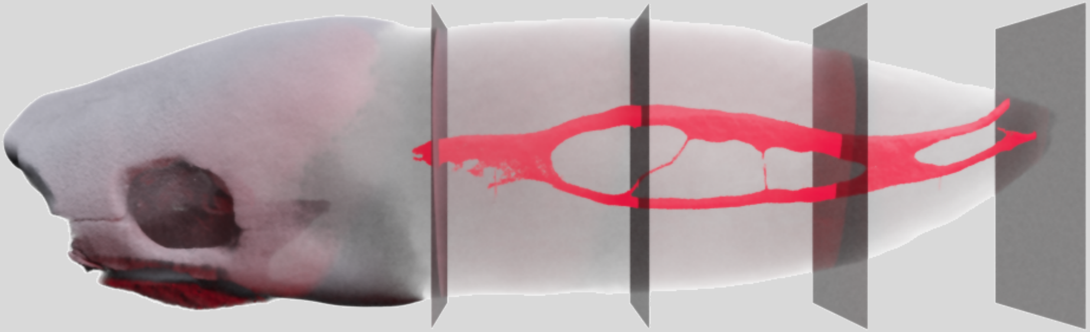
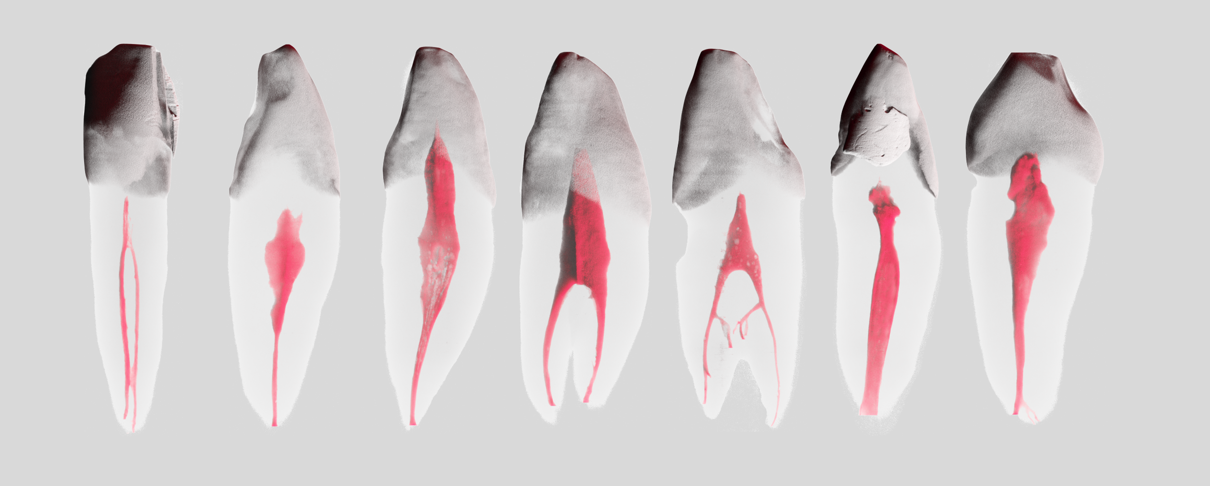
Outcome root canal configuration classification

Roots		RCC	#	%	Roots		RCC	#	%
Single (N=98)		1-1-1/1	73	74.5	Single (N=98)		1-1-1/1	73	74.5
		1-1-1/2	14	14.3			1-1-1/2	14	14.3
		1-1-1/3	1	1.0			1-1-1/3	1	1.0
		1-1-1/4	2	2.1			1-1-1/4	2	2.1
		1-1-2/1	1	1.0			1-1-2/1	1	1.0
		1-2-1/1	4	4.1			1-2-1/1	4	4.1
		1-2-1/2	1	1.0			1-2-1/2	1	1.0
		1-2-2/2	1	1.0			1-2-2/2	1	1.0
		2-3-1/1	1	1.0			2-3-1/1	1	1.0
Double (N=3)	Buccal	1-1-1/1	2	66.6	Double (N=3)	Buccal	1-1-1/1	2	66.6
		1-2-1/1	1	33.3			1-2-1/1	1	33.3
	Lingual	1-1-1/1	2	66.6		Lingual	1-1-1/1	2	66.6
		1-1-1/2	1	33.3			1-1-1/2	1	33.3

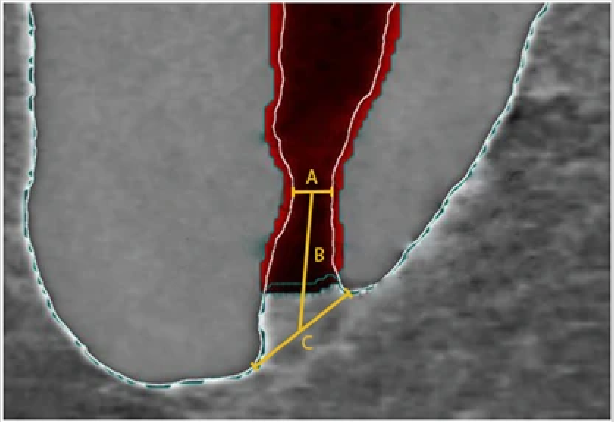
Extraction of root canal space



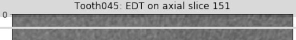
Outcome of root canal space extraction



Analysis of the physiological foramen geometry



From [20], Fig. 1



Outcome of foramen geometry analysis

	1 Foramen		2 Foramina		3 Foramina		4 Foramina	
	#	%	#	%	#	%	#	%
Oval	72	91.1	16	100.0	1	100.0	2	100.0
Round	6	7.6						
Irregular	1	1.3						
N	79		16		1		2	

Conclusion

- Efficient use of time, e. g. more teeth does not mean more (human) work
- Reproducible analysis with *free and open-source* software, usable by *anyone*
- Objective analysis, e. g. no operator bias

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 I

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

[2] Henry Nording et al. “The C5a/C5a receptor 1 axis controls tissue neovascularization through CXCL4 release from platelets”. DOI: 10.1038/s41467-021-23499-w.

[3] Ruslan Hlushchuk et al. “Innovative high-resolution microCT imaging of animal brain vasculature”. DOI: 10.1007/s00429-020-02158-8.

[4] Tsering Wüthrich et al. “Development of vascularized nerve scaffold using perfusion-decellularization and recellularization”. DOI: 10.1016/j.msec.2020.111311.

[5] Cédric Zubler et al. “The anatomical reliability of the superficial circumflex iliac artery perforator (SCIP) flap”. DOI: 10.1016/j.aanat.2020.151624.

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[8] Sebastian Halm et al. “Micro-CT imaging of Thiel-embalmed and iodine-stained human temporal bone for 3D modeling”. DOI: 10.1186/s40463-021-00522-0.

[9] David Haberthür et al. “Automated segmentation and description of the internal morphology of human permanent teeth by means of micro-CT”. DOI: 10.1186/s12903-021-01551-x.

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