X-ray microtomography

David Haberthür

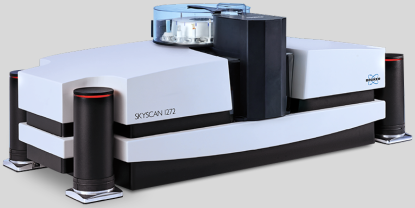
December 23, 2021 | 9256-HS2022-0: Advanced Microscopy

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 classification [7]
- Collaborations with museums [8] and scientist at our institute [9] to scan a wide range of specimens
- Automate *all* the things! [10]

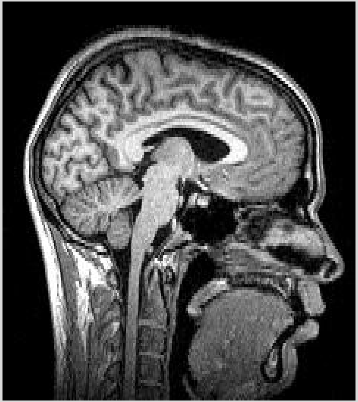


bruker.com/skyscan1272

Contents

Biomedical imaging

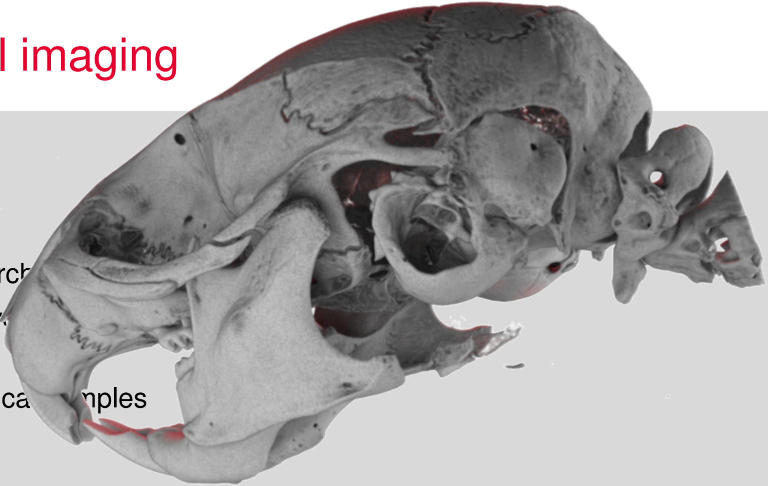
- Medical research
- Non-destructive insights into the samples
- (Small) Biological samples



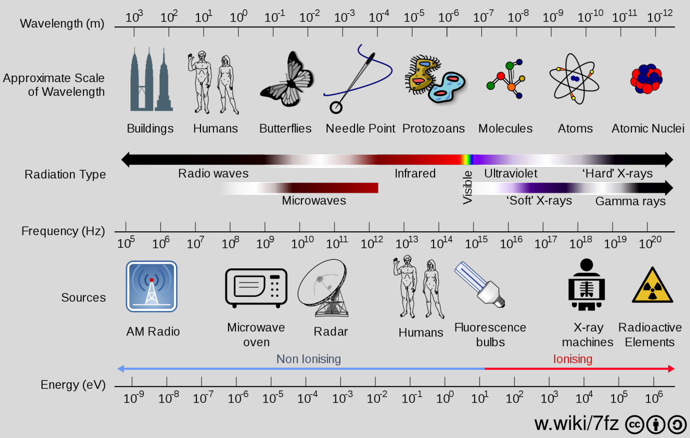
w.wiki/7g4

Biomedical imaging

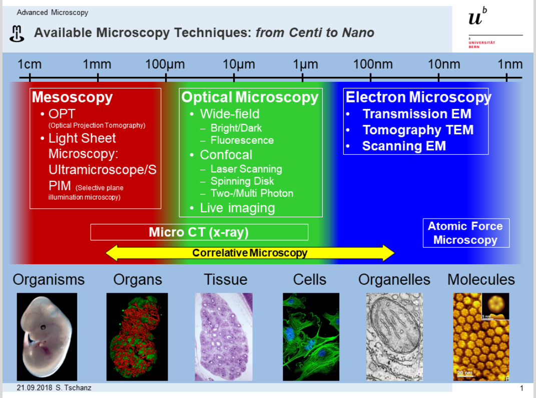
- Medical researc
- Non-destructiv
  the samples
- (Small) Biologica nples



Wavelength & Scale



Wavelength & Scale



Stefan Tschanz, with permission

Imaging methods

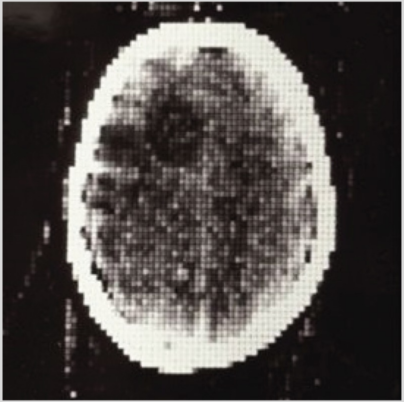
- Light (sheet) microscopy: see lecture of Nadia Mercader Huber
- X-ray imaging
- Electron microscopy: see lectures *Transmission Electron Microscopy* by Dimitri Vanhecke, *Scanning Electron Microscopy* by Michael Stoffel and *Cryoelectron Microscopy & Serial Block Face SEM* by Ioan Iacovache.

CT-Scanner

youtu.be/2CWpZKuy-NE

History

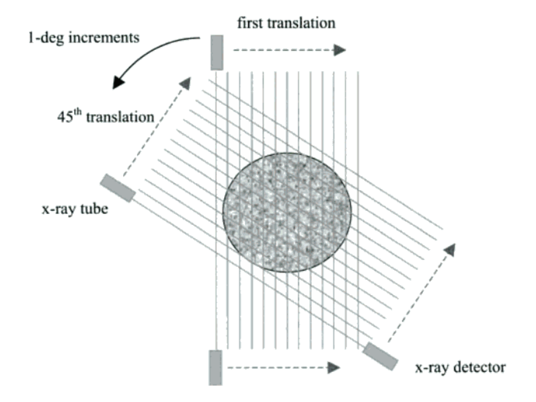
- Long history
  - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
  - 1976: Hounsfield worked on first clinical scanner [12]
  - Nice overview by Hsieh [13]



From [14], Figure 8.2

History

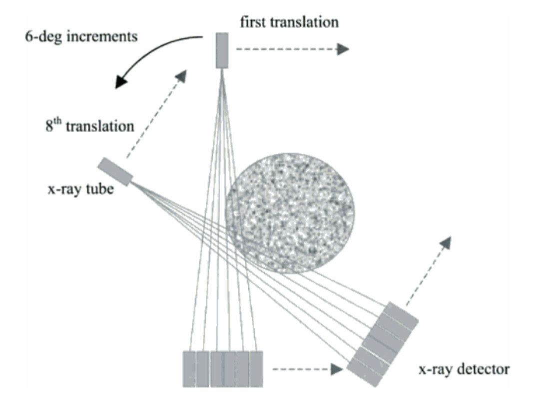
- Long history
  - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
  - 1976: Hounsfield worked on first clinical scanner [12]
  - Nice overview by Hsieh [13]
- CT scanner generations: First



From [13], Figure 1.12

History

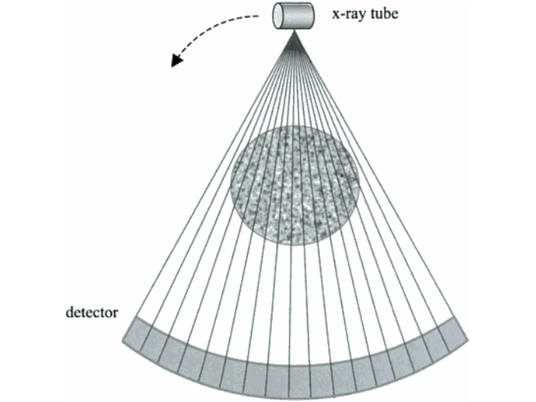
- Long history
  - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
  - 1976: Hounsfield worked on first clinical scanner [12]
  - Nice overview by Hsieh [13]
- CT scanner generations: First, second



From [13], Figure 1.13

History

- Long history
  - 1963: Cormack used a collimated ^{60}Co source and a Geiger counter as a detector [11]
  - 1976: Hounsfield worked on first clinical scanner [12]
  - Nice overview by Hsieh [13]
- CT scanner generations: First, second and third



From [13], Figure 1.14

X-ray interaction

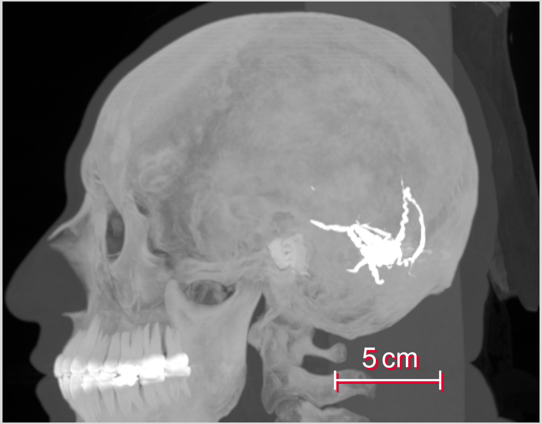
- "X-rays interact with tissue in 2 main ways: photoelectric effect and Compton scatter. To a first approximation, the photoelectric effect contributes to contrast while the Compton effect contributes to noise. Both contribute to dose." ([15])
  - Photoelectric absorption (τ) is strongly dependent on 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 ([16, i. e. Beer-Lamberts law] $I(t) = I_0 e^{-\alpha z}$

Composition of biological tissues

Tissue: content by mass percentage

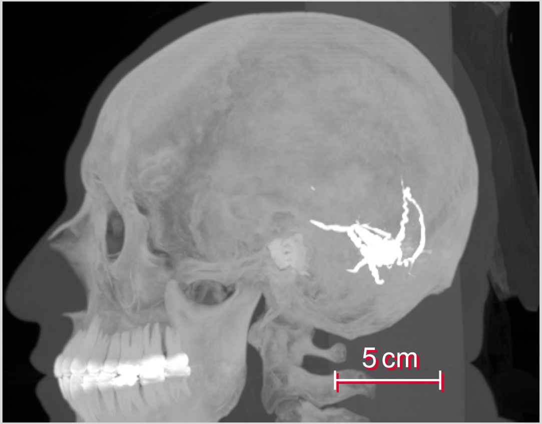
Element	H	C	N	O	Na	P	S	Cl	K	Ca
Atomic number	1	6	7	8	11	15	16	17	19	20
Fat	11.4	59.8	0.7	27.8	0.1		0.1	0.1		
Water	11.2			88.8						
Blood	10.2	11	3.3	74.5	0.1	0.1	0.2	0.3	0.2	
Liver	10.2	13.9	3	71.6	0.3	0.2	0.3	0.2	0.3	
Brain	10.7	14.5	2.2	71.2	0.2	0.4	0.2	0.3	0.3	
Bone	3.4	15.5	4.2	43.5	0.1	10.3	0.3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

Why µCT?



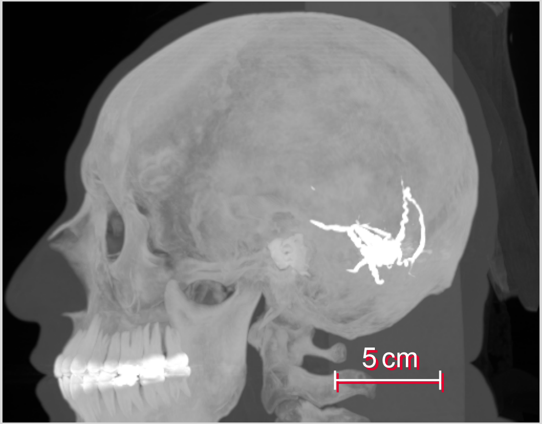
From [17], Subject *C3L-02465*

Why µCT?

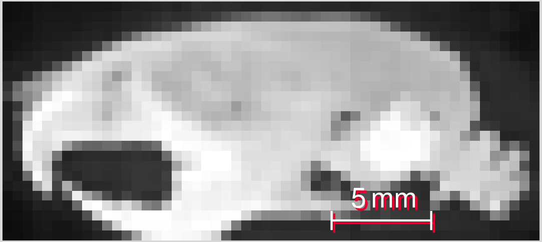


From [17], Subject *C3L-02465*

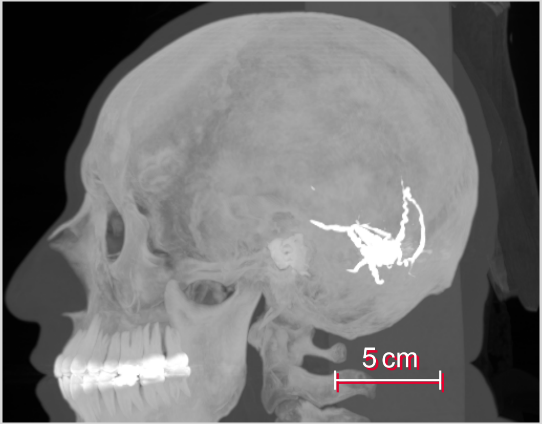
Why µCT?



From [17], Subject *C3L-02465*

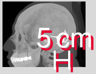


Why µCT?



From [17], Subject *C3L-02465*

Why µCT?



From [17], 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.

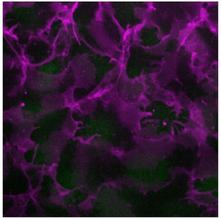
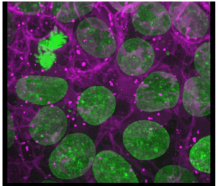


Image stack turned into transparent volume



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

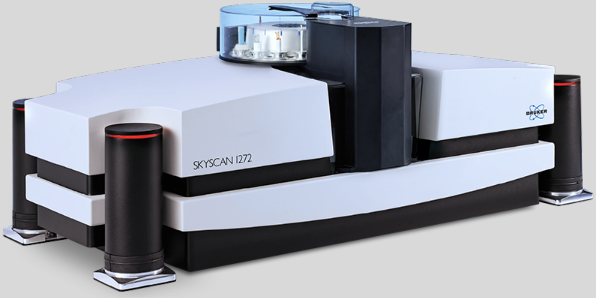
Machinery

- 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/fpTrGu

Machinery

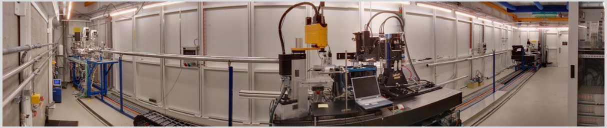
- Hospital CT
  - Voxel size around 0.5 mm
- Lab/Desktop CT
  - Voxel size around 7 µm (*in vivo*) or 0.5 µm (*ex vivo*)
- Synchrotron CT
  - Voxel size down to 160 nm



bruker.com/skyscan1272

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

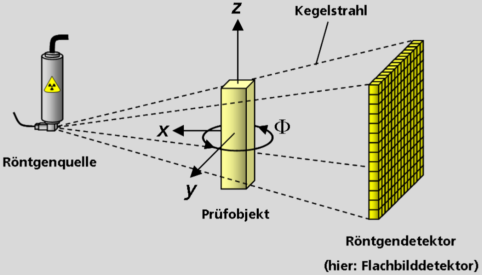


flic.kr/p/7Xhk2Y

What is happening?

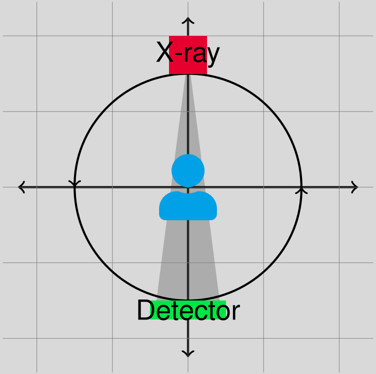
No matter what kind of machine, the basic principle is always the same

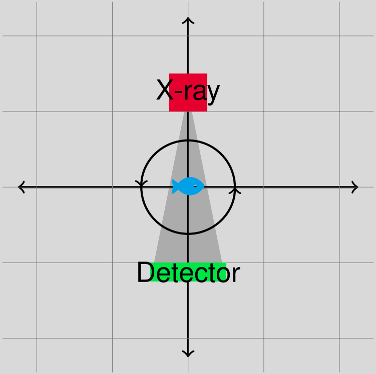
- an x-ray source
- a sample
- a detector



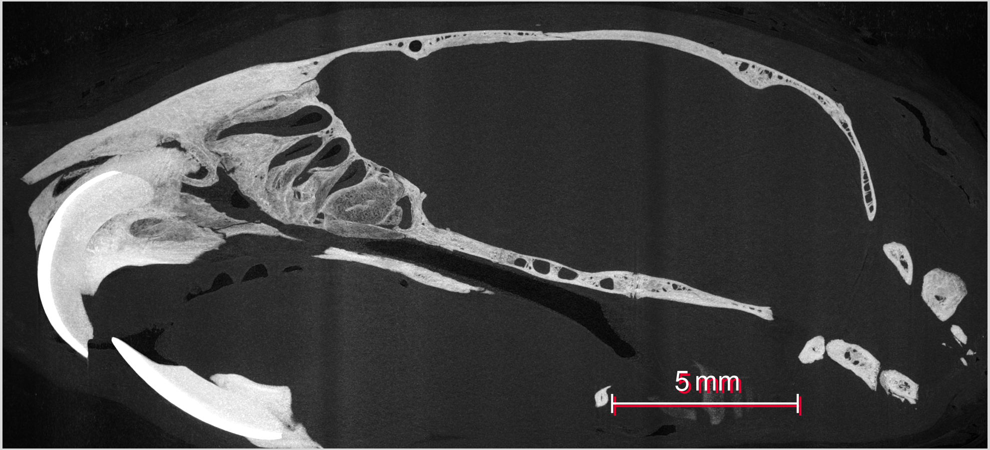
w.wiki/7g3

Machinery

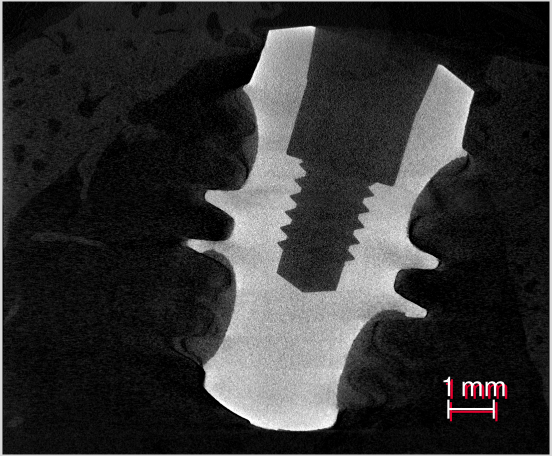




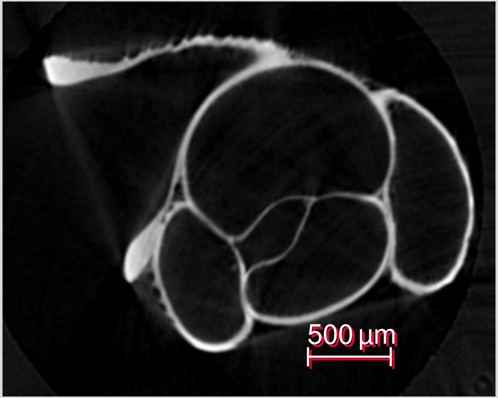
Examples



Examples

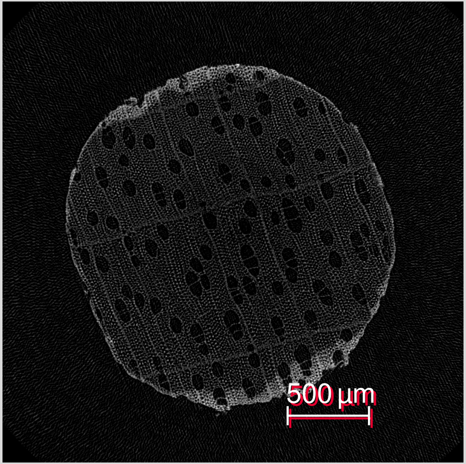


Examples

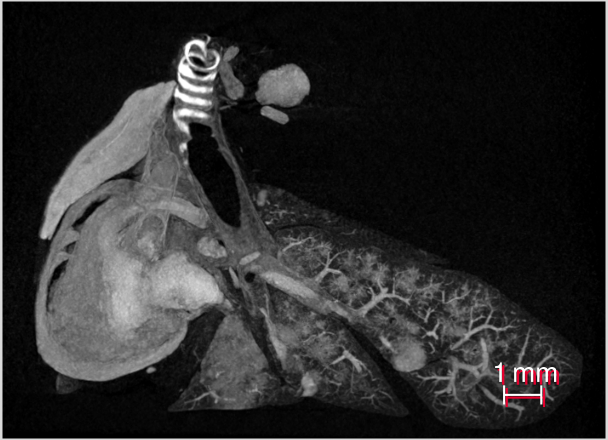


From [8], *Diancta phoenīx*

Examples



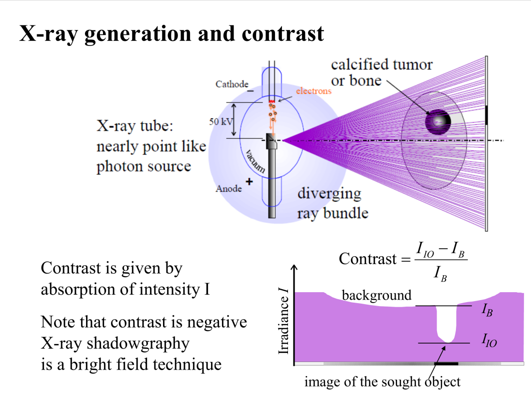
Examples



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 [18]
- Also see *Fundamentals of Digital Image Processing* by Guillaume Witz
- Reproducible research
  - in Jupyter [19]
  - git
  - Script all your things!
  - Data repositories; i. e. sharing is caring!

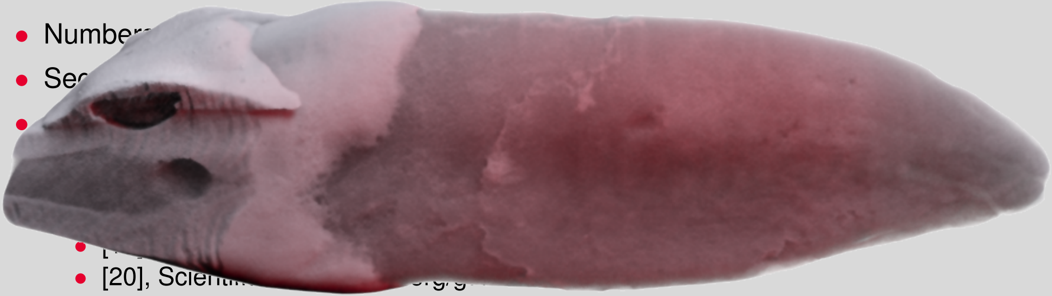
Quantitative data

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

Internal morphology of human teeth

Collaboration with zmk bern – Zahnmedizinische Kliniken

- Numbe
- Se
-



  - [20], Scien

Internal morphology of human teeth

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 [19])
- Two publications:
    - [10], BMC Oral Health, doi.org/gjpw2d
    - [20], Scientific Reports, doi.org/g7r8

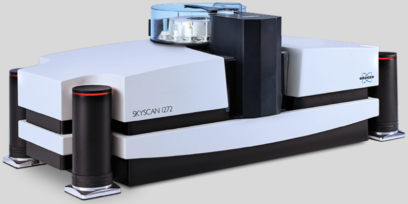
UNIVERSITÄT BERN

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
  - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
  - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!



bruker.com/skyscan1272

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
  - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!

```
Scanner=SkyScan1272
Instrument S/N=15G09089-B
Software Version=1.1.19
Filename Prefix=Tooth045~00
Number Of Files=  482
Number Of Rows= 1092
Number Of Columns= 1632
Source Voltage (kV)=  80
Source Current (uA)= 125
Image Pixel Size (um)=9.999986
Exposure (ms)=950
Rotation Step (deg)=0.400
Frame Averaging=ON (3)
Filter=Al 1mm
Study Date and Time=02 Jul 2020  08h:23m:34s
Scan duration=0h:39m:51s
```

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
  - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!

Sample changer on the SkyScan 1272

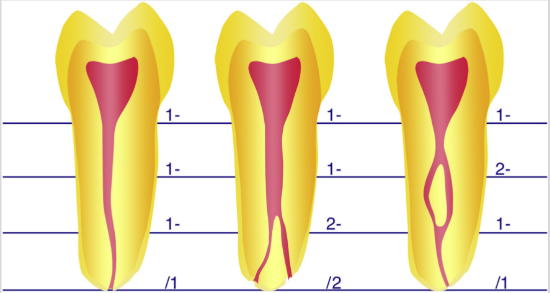
In total:

- 13 days of *continuous* µCT scanning
- 819 GB of raw data
  230 648 TIFF projections
- 326 GB data as input for analysis
  282 062 PNG reconstructions

UNIVERSITÄT BERN

How?

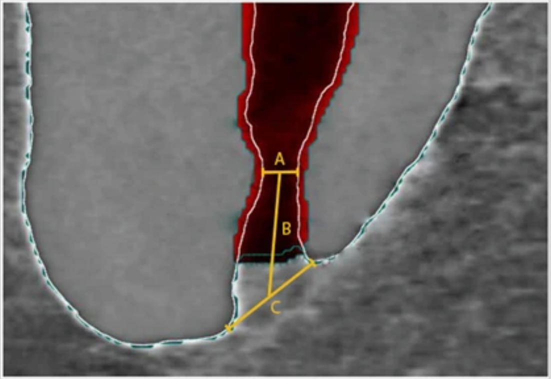
- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
  - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!



From [21], Fig. 2

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
  - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
  - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!



From [22], Fig. 1

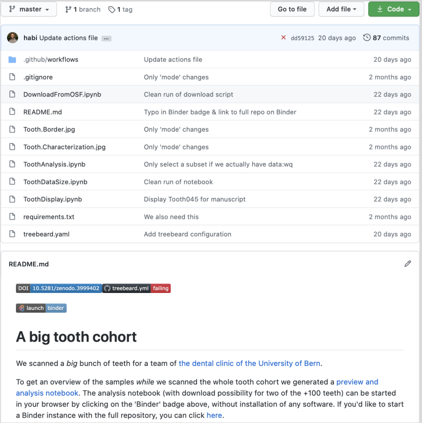
How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
    - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
    - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!

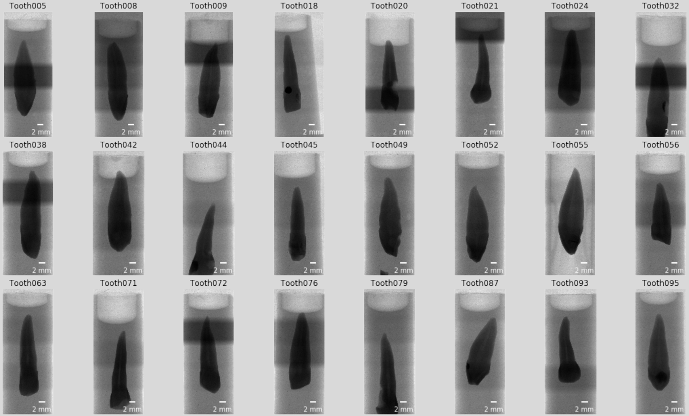
gph.is/2nqkple

How?

- 104 extracted human permanent mandibular canines
- µCT imaging
- Morphology
    - Root canal configuration (RCC), according to Briseño-Marroquín et al. [21]
    - Foramen geometry and size, according to Wolf et al. [22]
- *Reproducible* analysis [23], e. g. you can click a button to double-check or recalculate the results yourself!

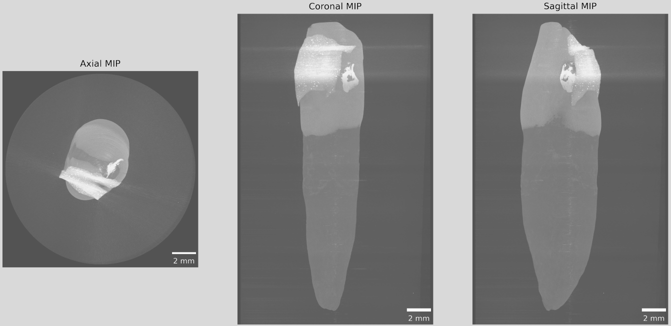


µCT imaging


Tooth005
Tooth008
Tooth009
Tooth018
Tooth020
Tooth021
Tooth024
Tooth032
Tooth038
Tooth042
Tooth044
Tooth045
Tooth049
Tooth052
Tooth055
Tooth056
Tooth063
Tooth071
Tooth072
Tooth076
Tooth079
Tooth087
Tooth093
Tooth095
2 mm

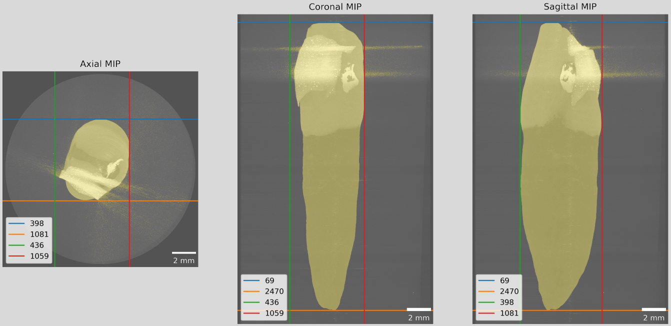
Dataset cropping

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

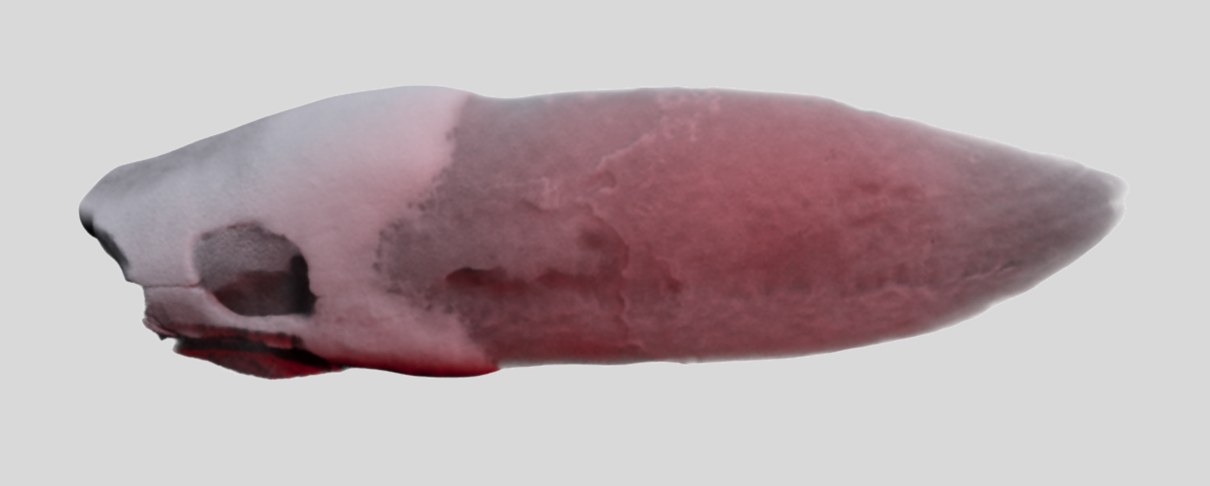


Dataset cropping

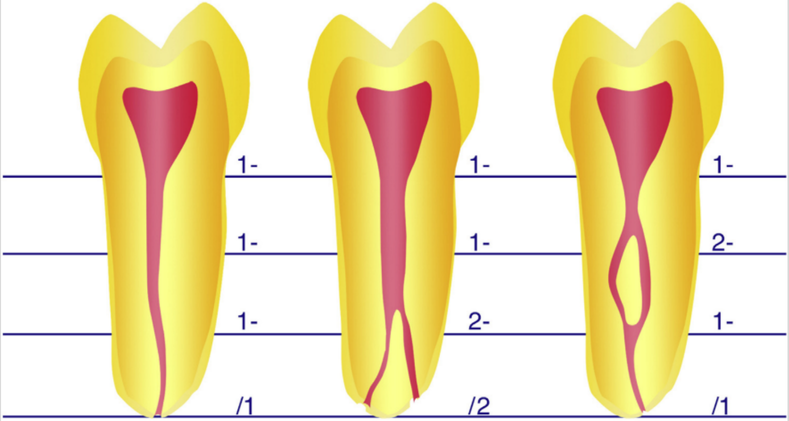
- Full datasets: 326 GB
- Cropped datasets: 115 GB



Tooth morphology



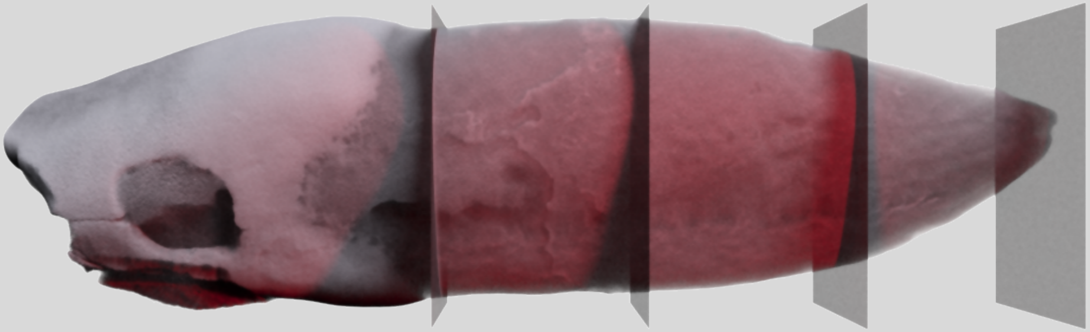
Tooth morphology



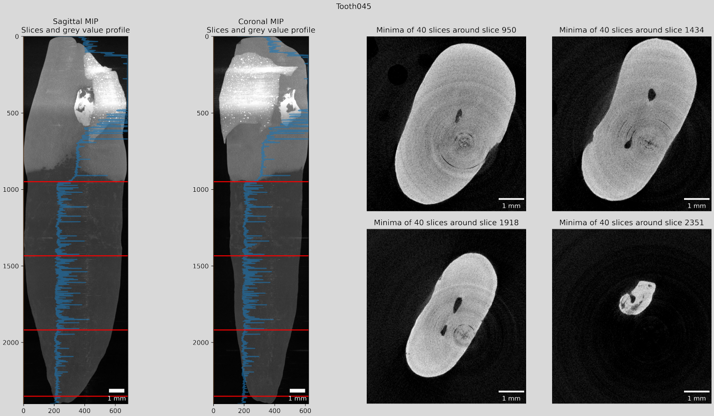
From [21], Fig. 2

Tooth morphology

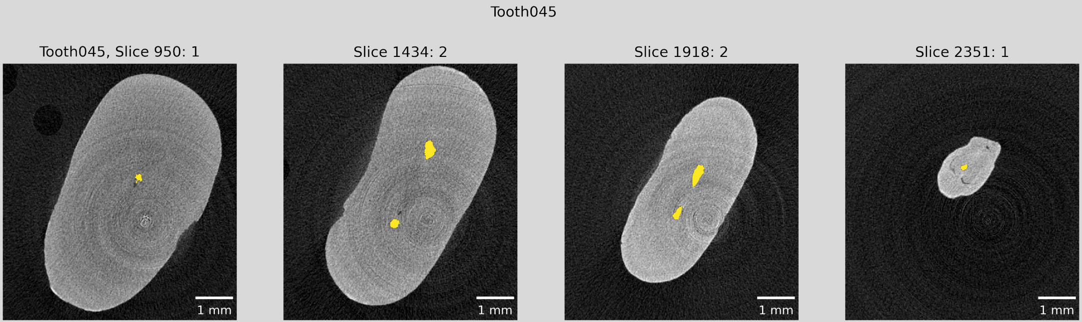
UNIVERSITÄT BERN



Detection of enamel-dentin border



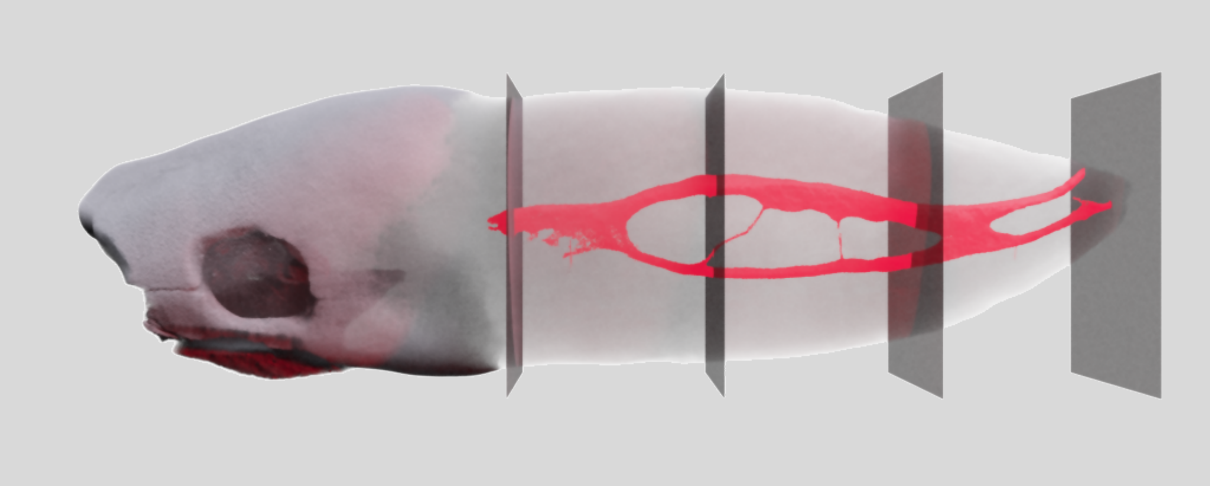
Detection of enamel-dentin border



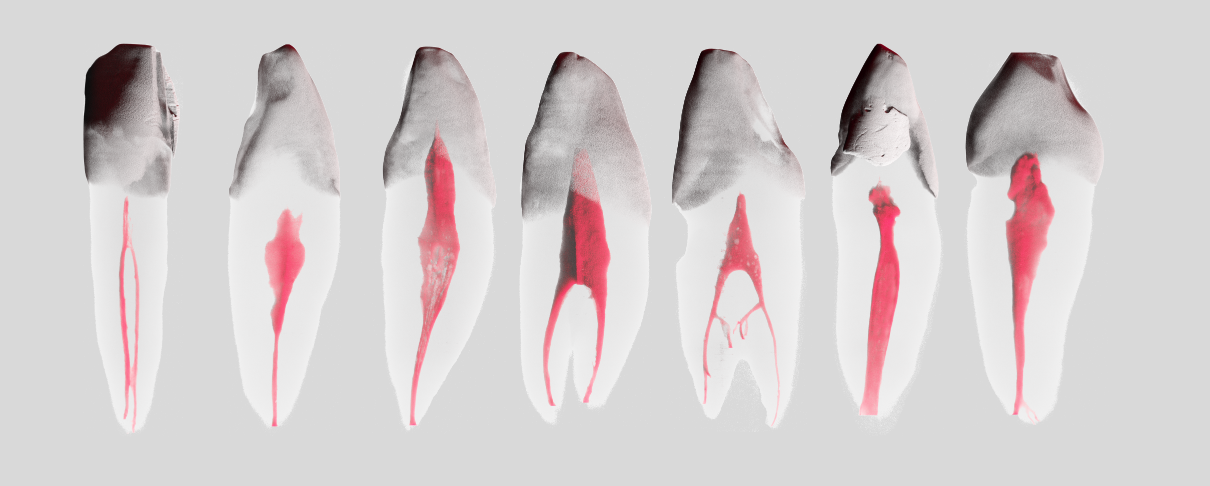
Outcome root canal configuration classification

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

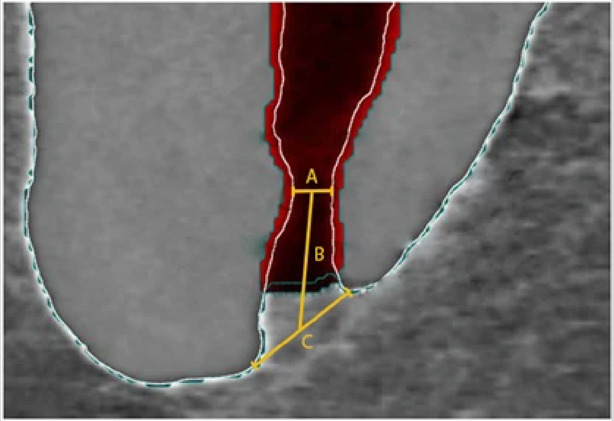
Extraction of root canal space



Outcome of root canal space extraction

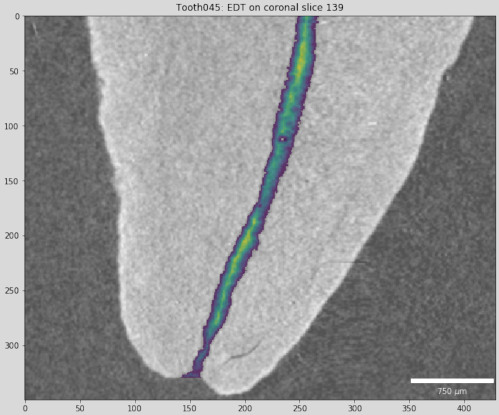


Analysis of the physiological foramen geometry



From [22], Fig. 1

Analysis of the physiological foramen geometry



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: david.haberthuer@unibe.ch

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

UNIVERSITÄT BERN

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.

[6] Matthias Messerli et al. “Adaptation mechanism of the adult zebrafish respiratory organ to endurance training”. DOI: 10.1371/journal.pone.0228333.

[7] Verdiana Trappetti et al. “Synchrotron Microbeam Radiotherapy for the treatment of lung carcinoma: a pre-clinical study”. DOI: 10.1016/j.ijrobp.2021.07.1717.

References II

[8] Estée Bochud et al. “A new Diancta species of the family Diplommatinidae (Cyclophoroidea) from Vanua Levu Island, Fiji”. DOI: 10.3897/zookeys.1073.73241.

[9] 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.

[10] 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.

[11] A. M. Cormack. “Representation of a Function by Its Line Integrals, with Some Radiological Applications”. DOI: 10.1063/1.1729798.

[12] Godfrey Newbold Hounsfield. “Historical notes on computerized axial tomography.”.

[13] J Hsieh. *Computed tomography: principles, design, artifacts, and recent advances*. Society of Photo Optical.

[14] Oliver Taubmann et al. “Computed Tomography”. DOI: 10.1007/978-3-319-96520-8_8.

[15] Mark Hammer. *X-Ray Physics: X-Ray Interaction with Matter, X-Ray Contrast, and Dose*.

[16] Wikipedia contributors. *Beer–Lambert law — Wikipedia, The Free Encyclopedia*.

References III

[17] Kenneth Clark et al. “The Cancer Imaging Archive (TCIA): Maintaining and Operating a Public Information Repository”. DOI: 10.1007/s10278-013-9622-7.

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

[19] Thomas Kluyver et al. “Jupyter Notebooks – a publishing format for reproducible computational workflows”. DOI: 10.3233/978-1-61499-649-1-87.

[20] Thomas Gerhard Wolf et al. “Internal morphology of 101 mandibular canines of a Swiss-German population by means of micro-CT: An ex vivo study”. DOI: 10.1038/s41598-021-00758-w.

[21] Benjamín Briseño-Marroquín et al. “Root Canal Morphology and Configuration of 179 Maxillary First Molars by Means of Micro–computed Tomography: An Ex Vivo Study”. DOI: 10.1016/j.joen.2015.09.007.

[22] Thomas Gerhard Wolf et al. “Three-dimensional analysis of the physiological foramen geometry of maxillary and mandibular molars by means of micro-CT”. DOI: 10.1038/ijos.2017.29.

[23] David Haberthür. “habi/zmk-tooth-cohort: Used for manuscript about method”. DOI: 10.5281/ZENODO.3999402.