PCMD MicroCT Imaging Core Learning Lunch Series

MicroCT 101:
How to get most out of your scans

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What is µCT?

- A non-destructive technique to produce 3D images of very high resolution using X-ray imaging and computed tomography

Recommended Reading:

Guidelines for assessment of bone microstructure in rodents using micro-computed tomography

Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R.

J Bone Miner Res. 2010 Jul;25(7):1468-86. doi: 10.1002/jbmr.141.

  - Disease progression
  - Drug treatments
- Input to micro finite element (µFE) models to estimate the mechanical properties of bone van Rietbergen+1998, Schulte+2011

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Available Scanners at PCMD µCT Imaging Core

- Specimen µCT
  - µCT 35
  - µCT 50
- *In Vivo* µCT
  - vivaCT 40
  - vivaCT 75
- Clinical µCT
  - XtremeCT II

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PCMD µCT Scanners

	µCT 35	µCT 50	vivaCT 40	vivaCT 75	XtremeCT II
Use	Specimen	Specimen	In Vivo Rodent	In Vivo Rodent	Clinical
X-Ray Source	30 - 70 kVp	30 - 90 kVp	30 - 70 kVp	30 - 70 kVp	68 kVp
Max Scan Size	37.9 x 120 mm (Ø x L)	50 x 120 mm (Ø x L)	38.9 x 145 mm (Ø x L)	79.9 x 145 mm (Ø x L)	140 x 200 mm (Ø x L)
Max Specimen Size	75.8 x 140 mm (Ø x L)	100 x 160 mm (Ø x L)	80 x 500 mm (Ø x L)	80 x 500 mm (Ø x L)	170 mm (Ø)
Best image voxel size	3.5 µm (Ø :7 mm)	1.5 µm (Ø: 3 mm)	10.5 µm (Ø :21.5 mm)	19 µm (Ø: 38.9 mm)	60 µm
Location	McKay Lab	VA Hospital	McKay Lab	VA Hospital	CHOP CTRC nutrition and growth lab

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Next generation *in vivo* µCT scanner

- Scanco vivaCT 80
  - Location: McKay Lab
  - *In vivo* µCT scanner
  - Best image voxel size:
    - 10.5 µm isotropic voxel size @ Ø 21.5 mm
  - Max Scan Size
    - 80 x 145 m (Ø x L)
- Capacity to scan rat vertebrae *in vivo* and large specimen (up to 80 mm in diameter)

Which Scanner Should I Use for My Study?

- vivaCT 40
  - Rodent study (rats and mice) study requiring in vivo scans (IACUC approval required)
  - When µCT 35 is fully occupied
- µCT 35
  - Small specimen scans (under diameter of 35 µm)
  - Mouse bone microstructure phenotyping must use µCT 35
- vivaCT 80
  - Rodent study (rats and mice) study requiring in vivo scans (IACUC approval required)
    - In vivo study of rat vertebrae and skull must use vivaCT 80 instead of vivaCT 40
  - Large specimen scans (above diameter of 40 µm)
- vivaCT 75 and µCT 50 (VA location)
  - Only accessible to investigator with VA affiliations
  - µCT 50: studies requiring high resolution characterization
  - vivaCT 75: large specimen scans (above diameter of 50 µm)
- Extreme CT II
  - Clinical studies (IRB approval required)
  - Large specimen scans (above diameter of 80 µm)
- For consistent results, please use the same model of scanner for all samples/animals from the same study

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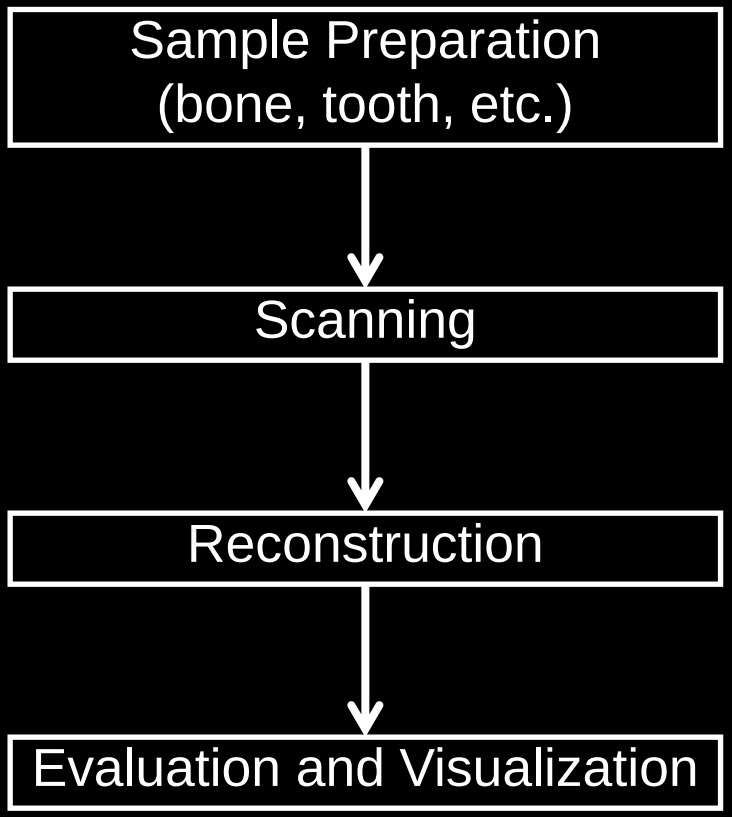
μCT Imaging



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Sample Preparation

- Specimens
  - Dry samples
    - Embedded in MMA √
    - Scanned in air (short scan time) for some soft tissue imaging √
  - Wet samples
    - Store in DI water √, saline √, PBS √, neutral buffer formalin √, ethonal √, etc.
    - Depend on the subsequent experiments (mechanical testing? Histology?)
- Image resolution
  - Purpose of the study
  - Dimension of the sample

Field of View (mm)	Projection /180°	Resolution (μm)
7.2	1000	3.5
12.3	1000	6
20.5	1000	10
30.7	1000	15
37.9	1000	18.5

- Sample holders
  - 7-mm (3.5μm), 11.5-mm (6μm), 20-mm (10μm), 30-mm (15μm), 37-mm (18.5μm) holders
  - 15mL tube (10μm) or 50mL tube (15μm)
- Position samples firmly in the holder (Very Important)
  - Use non-attenuating materials including foam, gauze, clay, paraffin, etc.

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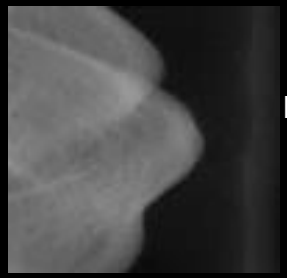
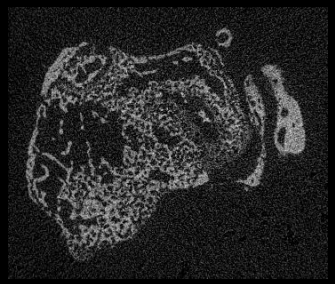
From Scan to Results



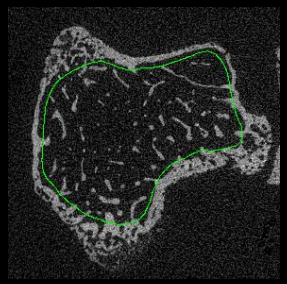
.RSQ
RAW sequence data

Reconstruction



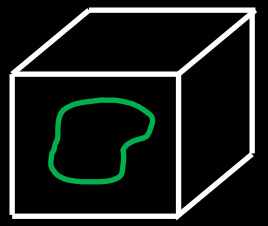
.ISQ
Image sequence data

Contouring



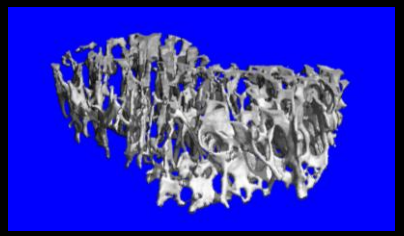
.GOBJ
Graphical object

3D evaluation



.AIM
White box
(gray scale)

Thresholding (Gauss)



_SEG.AIM
Segmented object
(binary file, black/white**)**

Calculating Morphometry

Final Results

- **Evaluation Sheet**
- **Trabecular Analysis**
  - **_TH.AIM**
  - **_SP.AIM**
  - **_TH.TXT**
  - **_SP.TXT**
- **Cortical Analysis**
  Midshaft Analysis
  _MOI_TXT

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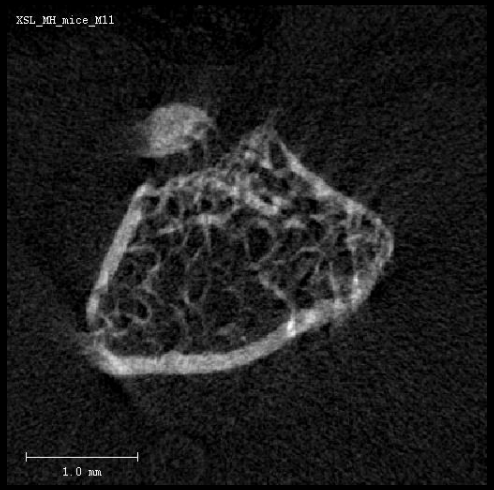
After Scanning

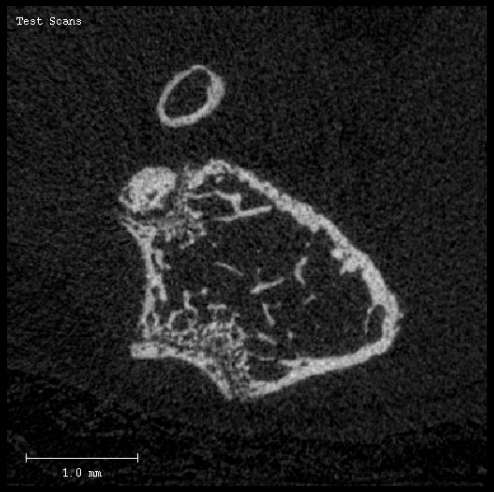
- Reconstruction
  - Automatic reconstruction in Scanco (our) systems

- Inspection of Images: Common Artifacts
  - Motion artifact
  - Metal artifact
  - Ring artifact

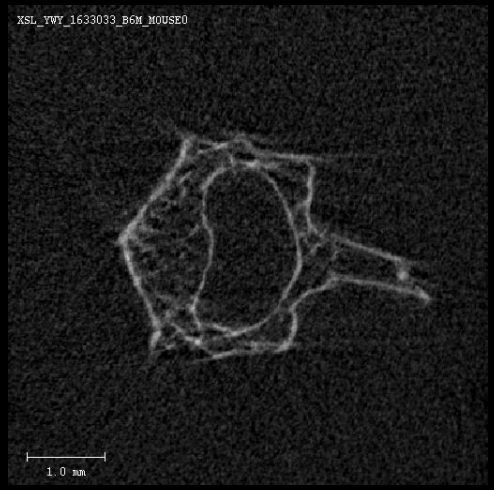
Motion Artifact

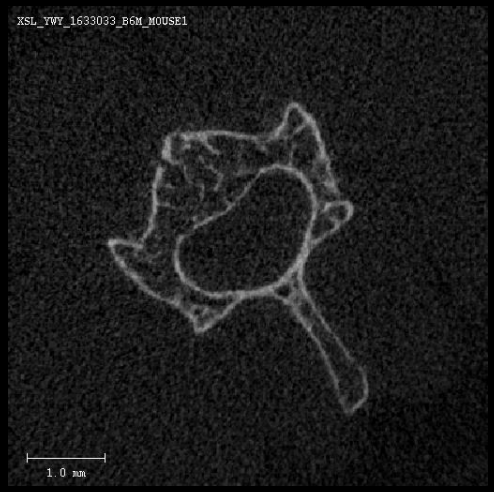
Distal Femur





L2 Vertebrae





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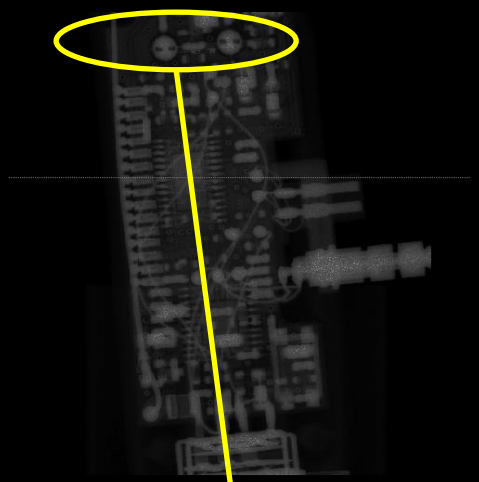
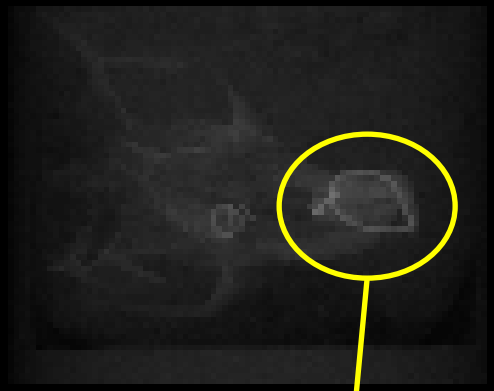
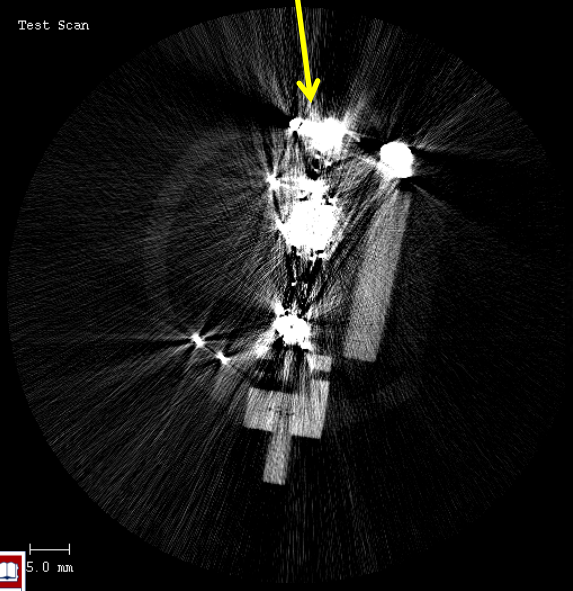
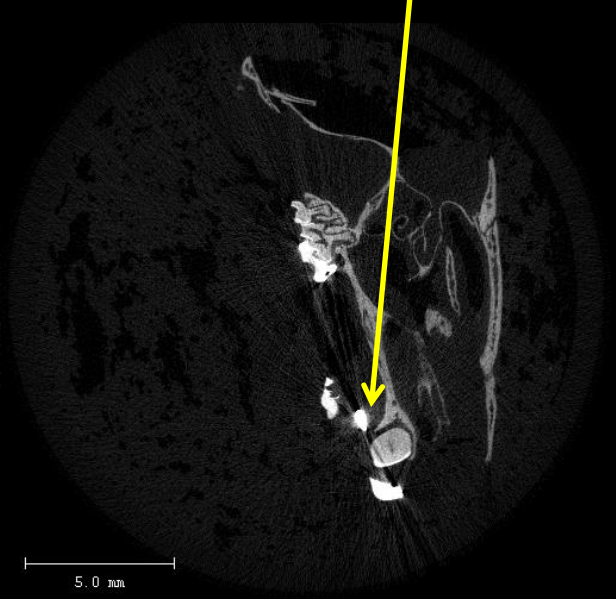
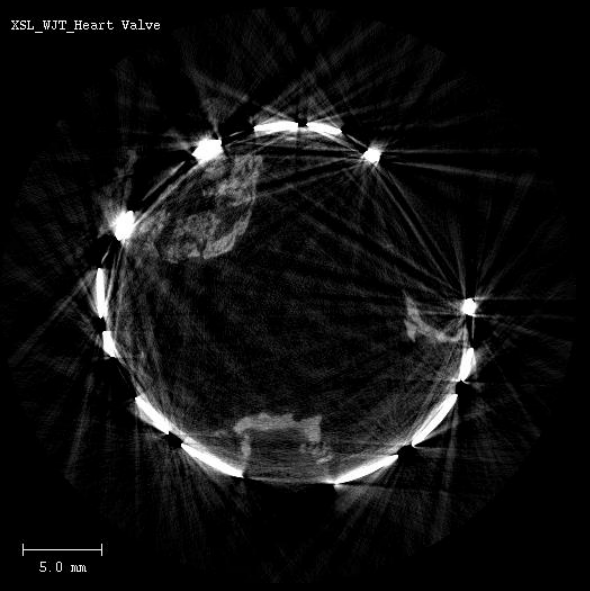
Metal Artifact



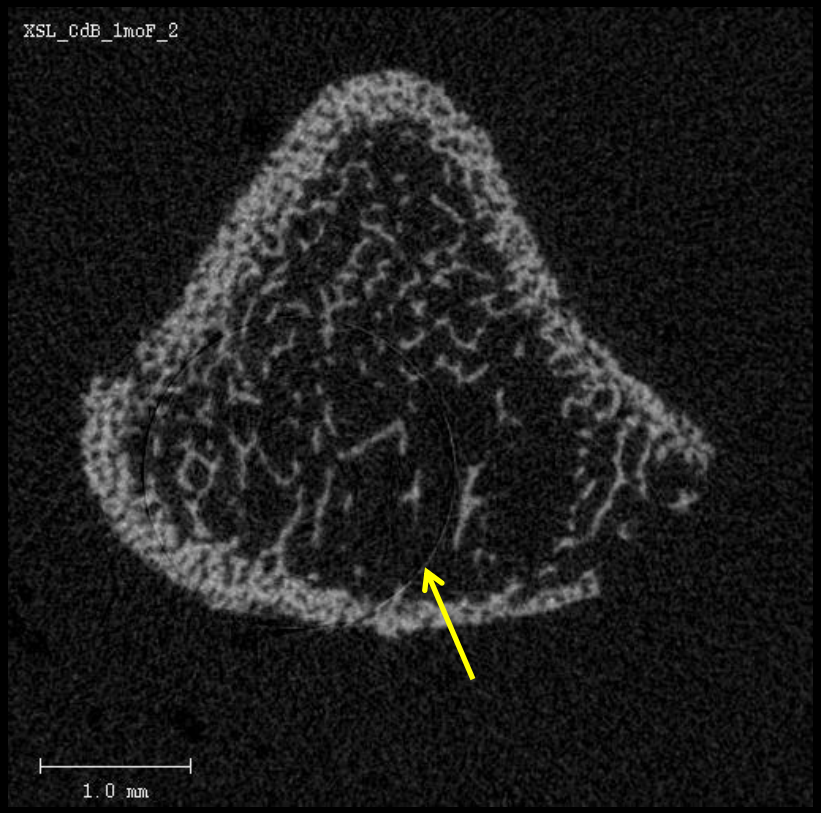


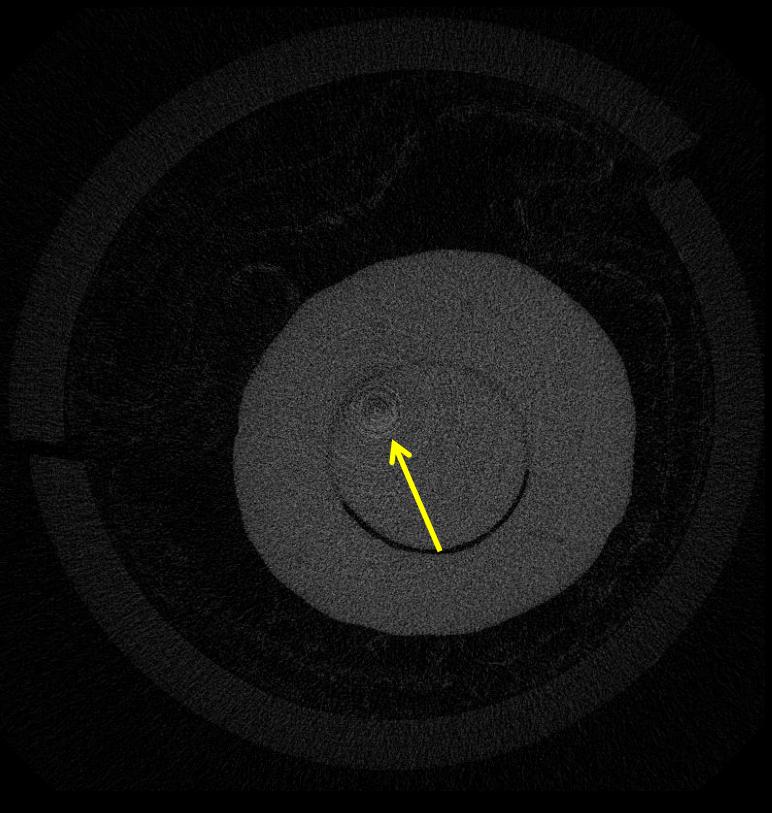






Ring Artifact





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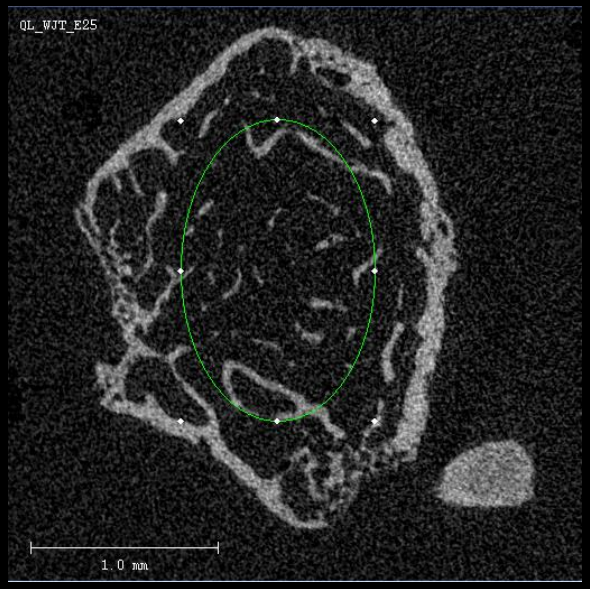
After Scanning

- Reconstruction
  - Automatic reconstruction in Scanco (our) system
- Inspection of Images: Common Artifacts
  - Motion artifact
  - Metal artifact
  - Ring artifact
- Image Processing
  - Contouring
  - Filtration
  - Segmentation

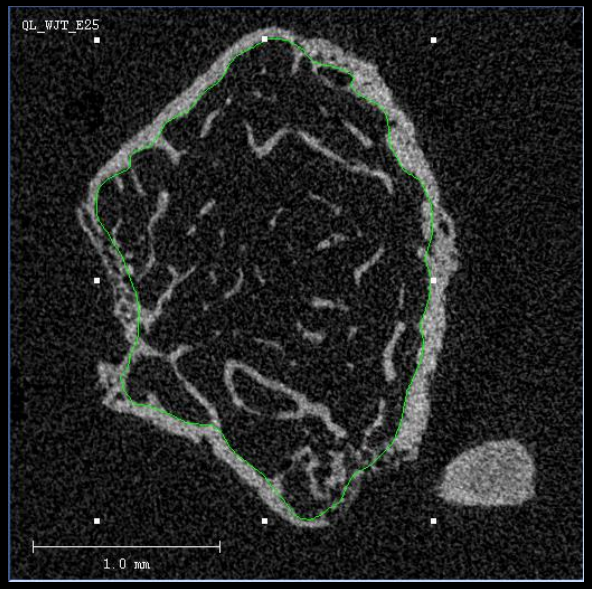
Contouring

- Choose Volume of Interest (VOI)

X



X



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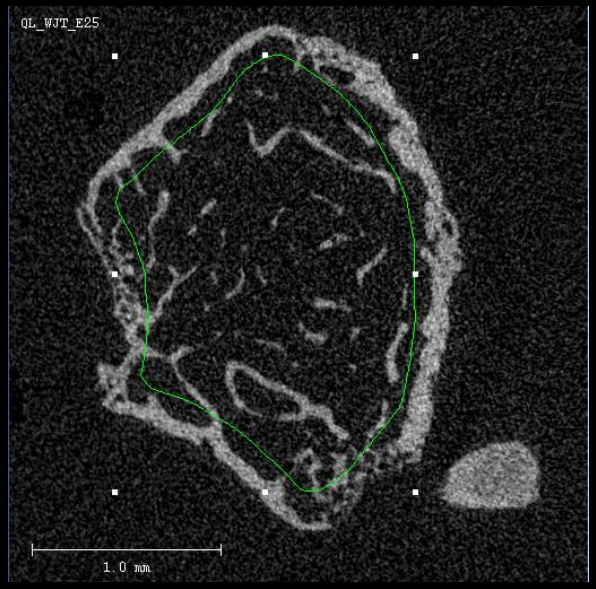
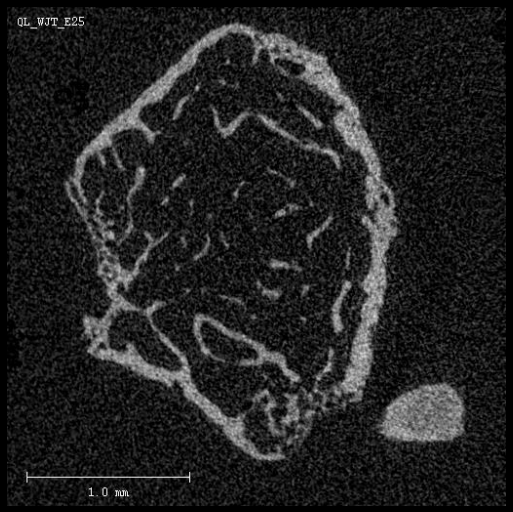
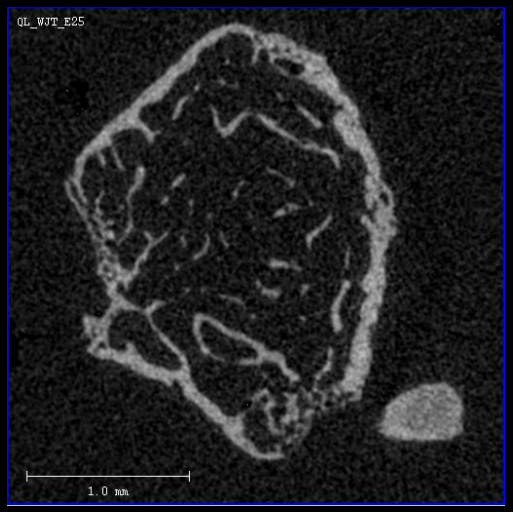


Filtration

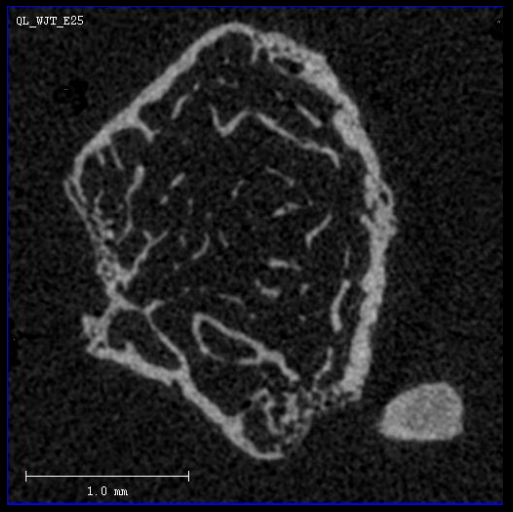
- Gaussian filter to suppress image noise
  - σ : Extent of the kernel or degree of smoothing
  - Support: the size of the discrete kernel (# of neighboring voxels that contributes to the weighted mean)



unfiltered



σ=1.2, support 2



σ=2, support 2

Recommended

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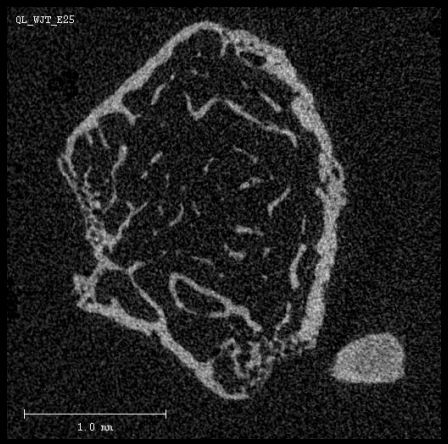
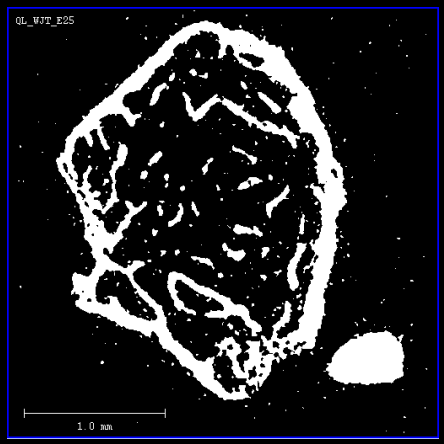
Segmentation

- Separate the mineralized and non-mineralized tissue
  - Low threshold: a value ~200-400; Cut off the bone marrow and other non-mineralized tissue
  - High threshold: a fixed value of 1000; Cut off the white noise
  - Recommended low threshold: trabecular bone ~330; cortical bone ~360

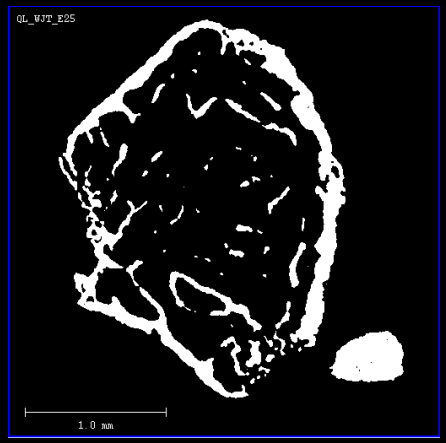
Grayscale



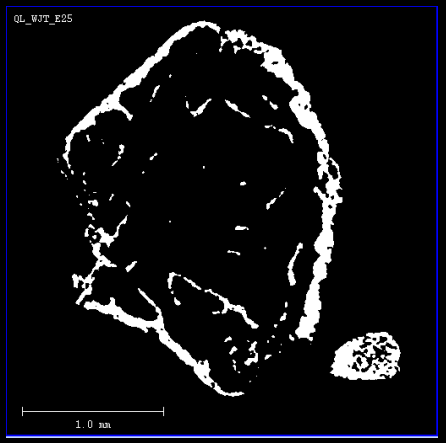
200-1000



Global Threshold
330-1000



450-1000



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After Scanning

- Reconstruction
  - Automatic reconstruction in Scanco (our) system
- Inspection of Images: Common Artifacts
  - Motion artifact
  - Metal artifact
  - Ring artifact
- Image Processing
  - Contouring
  - Filtration
  - Segmentation
- 3D Evaluation
  - Trabecular and Cortical bone density and microstructure

Trabecular Bone Density and Microstructure

	Variable	Unit
TV	Total volume	mm^3
BV	Bone volume	mm^3
BV/TV	Bone volume fraction	%
SMI	Structure model index: 0 for parallel plates, 3 for cylindrical rods	
Conn.D	Connectivity Density	$1/mm^3$
Tb.N	Trabecular number	1/mm
Tb.Th	Trabecular thickness	mm
Tb.Sp	Trabecular separation or spacing = marrow thickness	mm
BMD	Mean density values of everything within volume of interest	mg HA/cm^3
TMD	Mean density of segmented region, thus only of what was considered bone	mg HA/cm^3

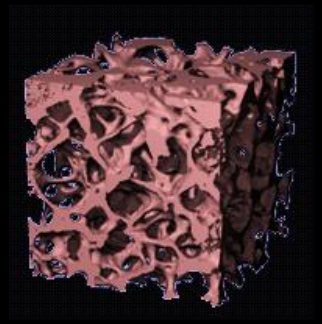
Trabecular Bone Density and Microstructure

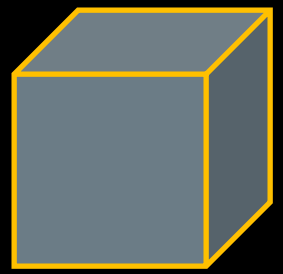
- Scale
  - Bone Volume Fraction (BV/TV)
  - Trabecular Number (Tb.N)
  - Trabecular Spacing (Tb.Sp)
  - Trabecular Thickness (Tb.Th) (Hildebrand & Ruegsegger, 1995)
- Topology
  - Connectivity (Conn.D) (Kinney and Labb, 1998; Kabel, *et al*. 1999)
  - Structural Model Index (SMI) (Wehrli *et al*., 2001; Hildebrand, *et al*. 1997)
- Orientation
  - Degree of Anisotropy (DA) (Cowin *et al*., 1986)
- Bone Density
  - Bone mineral density (BMD)
  - Tissue mineral density (TMD)

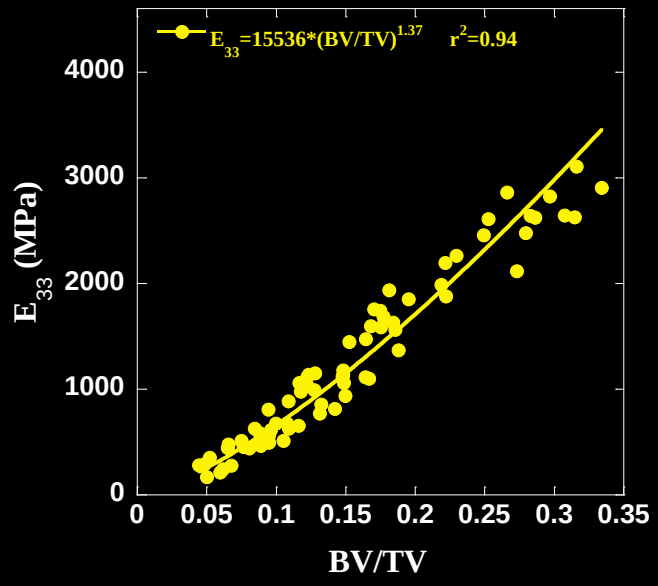
Bone Volume Fraction (BV/TV)

=

Bone Volume (BV) ÷ Total Volume (TV)







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Trabecular Bone Density and Microstructure

- Scale
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  - Trabecular Spacing (Tb.Sp)
  - Trabecular Thickness (Tb.Th) (Hildebrand & Ruegsegger, 1995)
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- Bone Density
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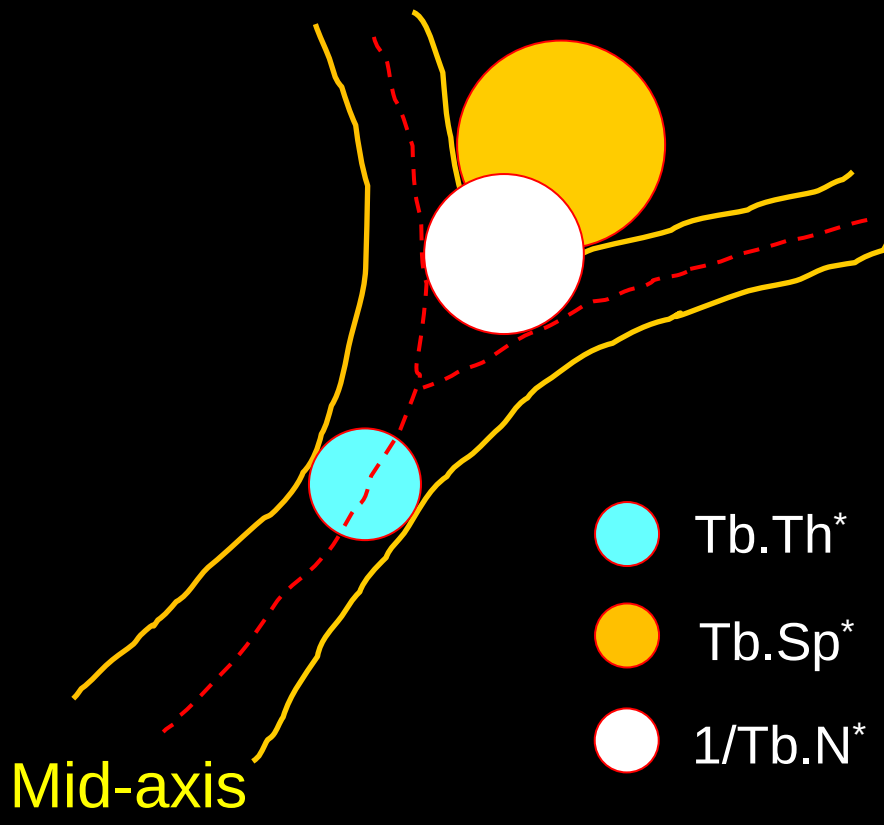
  - Tissue mineral density (TMD)



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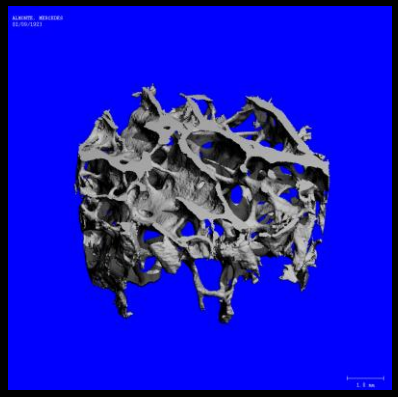
Trabecular Bone Density and Microstructure

- Topology
  - Connectivity density (Conn.D) (Kinney and Labb, 1998; Kabel, *et al*. 1999)
    - Indication of the integrity of trabecular bone network
    - Highly variable measurement outcome as it is sensitive to scan conditions, thresholding, etc
    - Limitation: plate perforation causes an increase in Conn.D
  - Structural Model Index (SMI) (Wehrli *et al*., 2001; Hildebrand, *et al*. 1997)
    - Indication of plate-likeness of trabecular bone network
    - Increase of SMI indicates a conversion of trabecular bone from plate-like to rod-like, which is associated with decreased bone strength and increased fracture risk
    - SMI can be negative in trabecular bone evaluation, indicating a highly plate-like structure
- Orientation
  - Degree of Anisotropy (DA) (Cowin *et al*., 1986)
    - Indication of how well trabeculae are aligned along the primary orientation within the network



A mix of plate- and rod-like structure

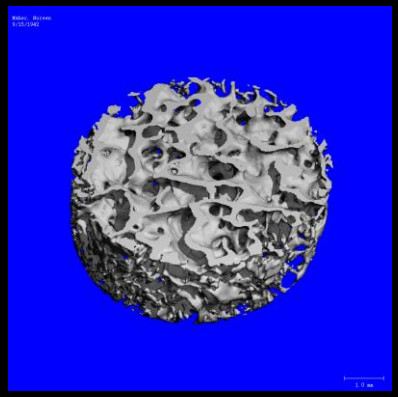


Plate-like structure

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Trabecular Bone Density and Microstructure

- Bone Density
  - Bone mineral density (BMD)
    - Total bone mineral content divided by the total volume (BMC/TV)
    - Mean density values of everything within the total volume
    - Rat tibia: ~300-400 mgHA/cm^3
    - B6 WT Mice tibia: ~100-200 mgHA/cm^3
    - B6 WT Mice femur: ~150-250 mgHA/cm^3
  - Tissue mineral density (TMD)
    - Total bone mineral content divided by the bone volume (BMC/BV)
    - Mean density values of everything within the bone volume
    - Cortical bone: ~1000-1200 mgHA/cm^3
    - Trabecular bone: ~800-1000 mgHA/cm^3

Cortical Bone Morphometry

	Variable	Unit
Ct.Area	Cortical Area	mm^2
Ct.Th	Cortical thickness	mm
Ct.Po	Cortical Porosity	%
pMOI	Polar moment of inertia	mm^4
Ps.Pm	Periosteal perimeter	mm
Es.Pm	Endocorical perimeter	mm
BMD	Mean density values of everything within volume of interest	mg HA/cm^3
TMD	Mean density of segmented region, thus only of what was considered bone	mg HA/cm^3

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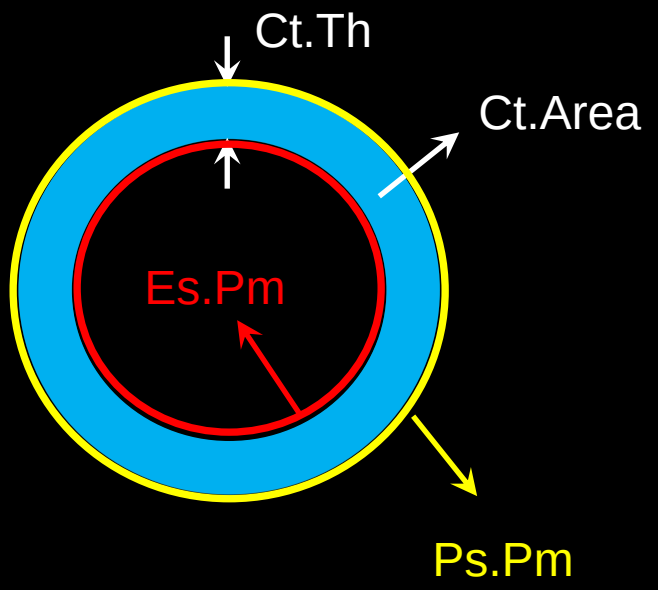
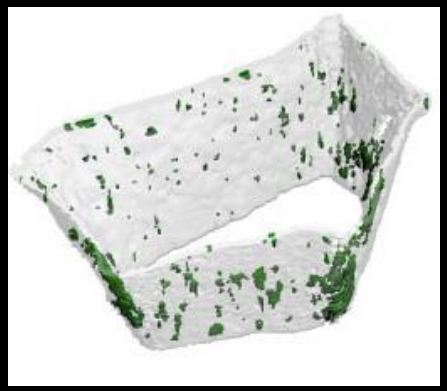
Cortical Bone Morphometry

- Cortical thickness (Ct.Th)
  - Thickness of the cortex
- Cortical area (Ct.Area)
  - Mean surface area of the cortex
  - Bone volume divided by the bone length
- Periosteal perimeter (Ps.Pm)
- Endosteal perimeter (Es.Pm)
- Cortical porosity (Ct.Po)
  - Percent of void volume over bone volume
  - ~1-10%





Burghardt *et al.* J Clin Endocrinol Metab 2010

Post-Processing

- Visualization – 3D Rendering
- Export your scans
  - DICOM format
  - TIFF format
- Software (free) for post-processing
  - Scanco Image Process Language
  - ImageJ – BoneJ (Analysis)
  - MicroView (Analysis)
  - CTAn (Bruker) (Analysis)
  - OsiriX (Segmentation, Visualization, Analysis)
  - ITK-SNAP (Segmentation, Visualization)
  - Slicer (Segmentation, Registration)
- Export for 3D printing
  - STL format

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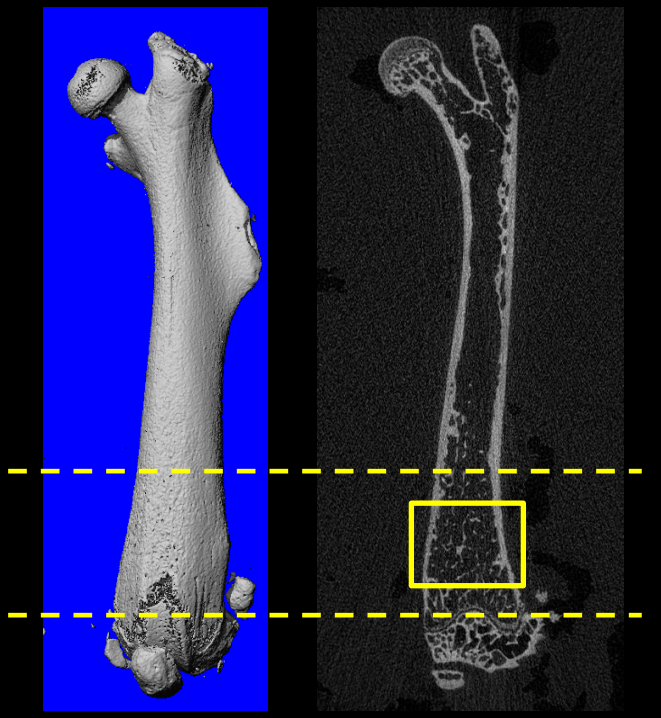
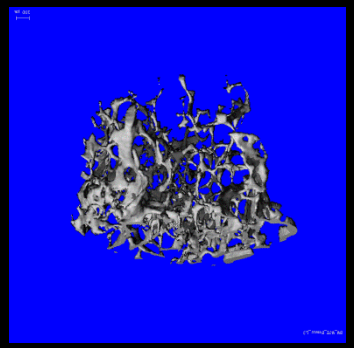
Phenotyping

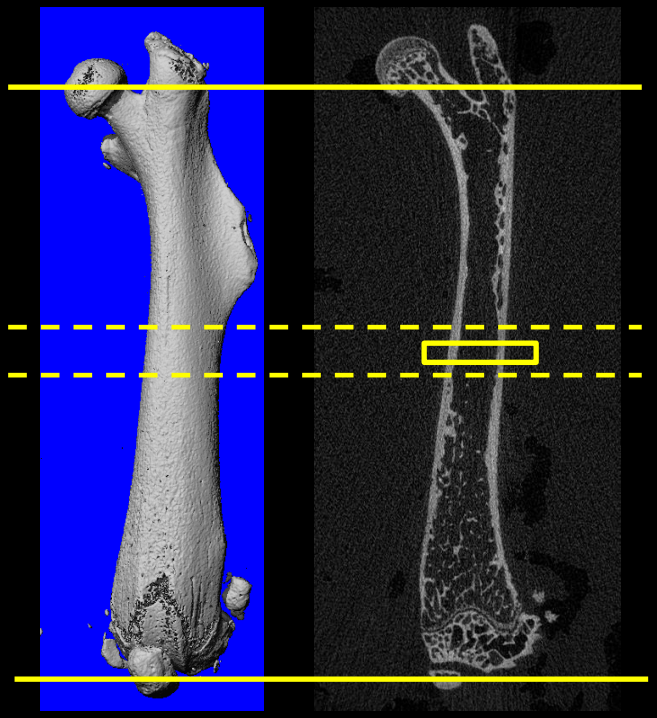
- Mouse trabecular bone (long bone)
  - Distal Femur, proximal tibia
  - 6 µm isotropic voxel size
  - Scan Region
    - 1-2 stacks, ~210-420 slices
  - Average scan time: 24-48 mins
  - Analysis region
    - 100-200 slices, 0.5-1mm from the growth plate
  - Outcome measures: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn.D, BMD, TMD

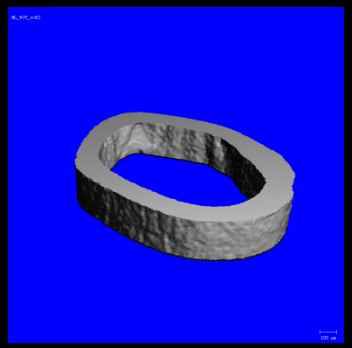




Phenotyping

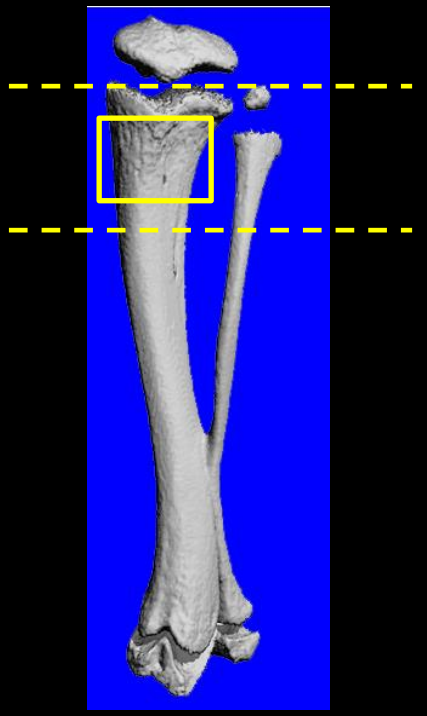
- Mouse cortical bone (long bone)
  - Midshaft of tibia or femur
  - 6-10 µm isotropic voxel size
  - Scan Region
    - 1 stacks, ~210 slices
  - Average scan time: 24 mins
  - Analysis region
    - Middle 50 slices
  - Outcome measures: Ct.Area, Ct.Th, pMOI, Ct.Po, TMD

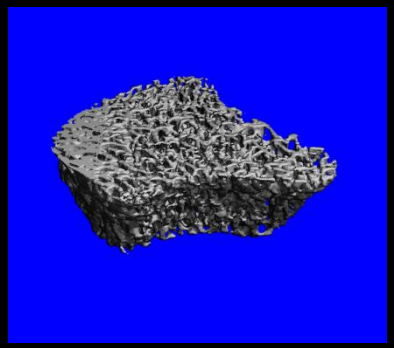




Phenotyping

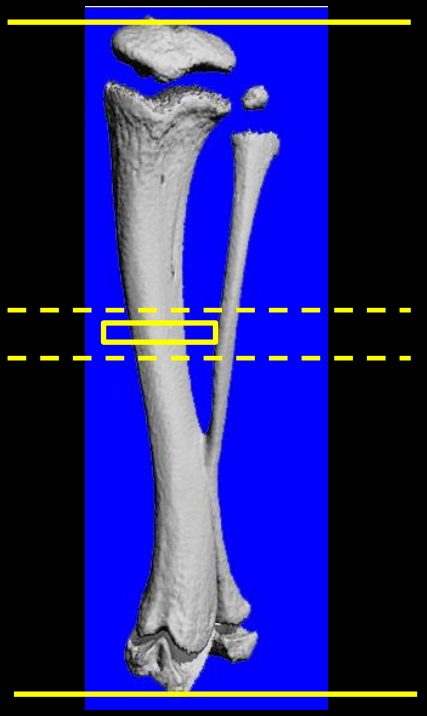
- Rat trabecular bone (long bone)
  - Proximal tibia
  - 6-10 µm isotropic voxel size
  - Scan Region
    - 2 stacks, ~420 slices
  - Average scan time: 24-48 mins
  - Analysis region
    - ~200 slices, 1-2mm distal to the growth plate
  - Outcome measures: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn.D, TMD

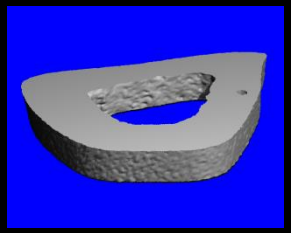




Phenotyping

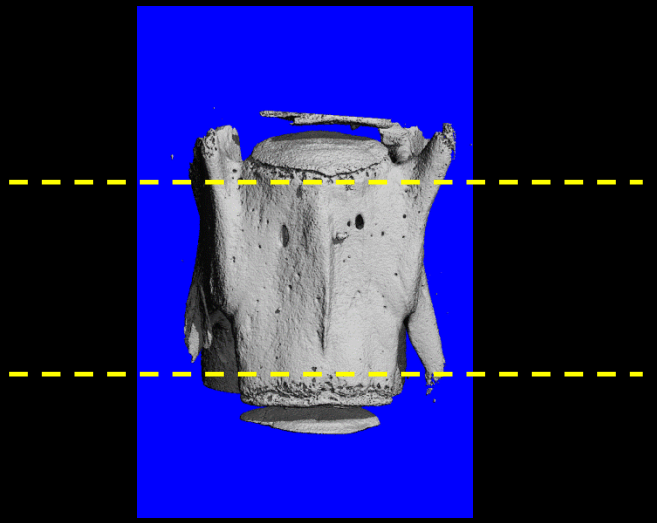
- Rat cortical bone (long bone)
  - Midshaft of tibia or femur
  - 6-10 μm isotropic voxel size
  - Scan Region
    - 1 stacks, ~210 slices
  - Average scan time: 24 mins
  - Analysis region
    - Middle 50 slices
  - Outcome measures: Ct.Area, Ct.Th, pMOI, Ct.Po, TMD

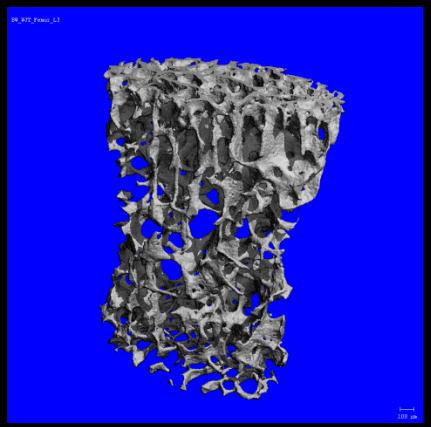




Phenotyping

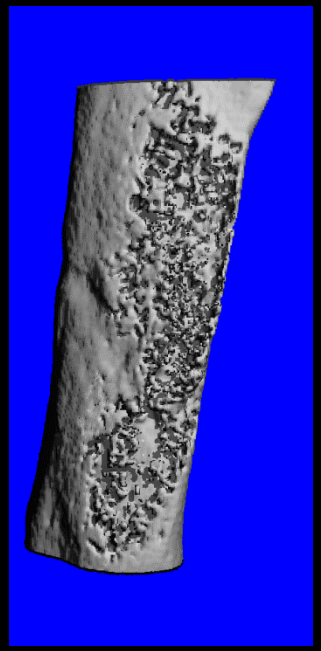
- Mouse/Rat vertebral trabecular bone
  - Lumbar vertebra L1-L4
  - 6 µm (mouse) / 6-10 µm (rat) isotropic voxel size
  - Scan Region
    - Between end plates
  - Average scan time: 48-72 mins
  - Analysis region
    - Between two end plates
    - Middle 150-200 slices
  - Outcome measures: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn.D, BMD, TMD

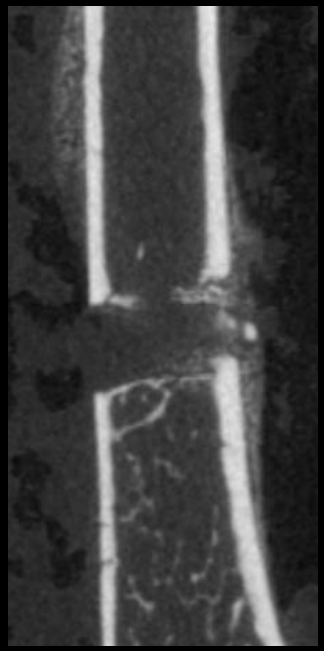


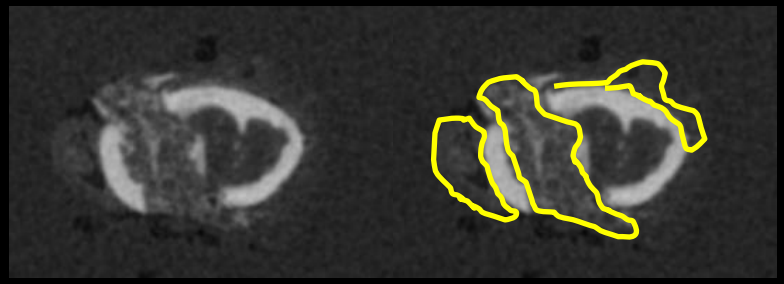


Fracture Healing

- 6-10 µm isotropic voxel size
- Scan region
  - Whole bone
- Average scan time
  - Scan vertically: 1-2 hours
  - Scan horizontally: 15-30 mins
- Analysis region
  - whole healing region excluding cortical and trabecular bone
- Outcome measures
  - BV: Callus Volume
  - Avg callus area = Callus Volume/ Total Length

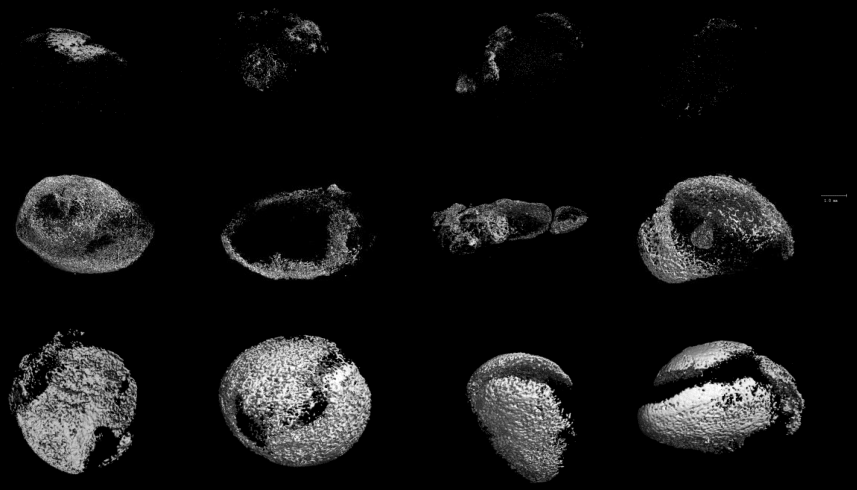






Scaffolds

- PLA, PGA, PCL, etc.
  - 6 -10 µm isotropic voxel size
  - Scan Region:
    - Whole scaffold
  - Average scan time:
    - 0.5-2 hours
  - Analysis Region:
    - Whole scaffold
  - Outcome measure:
    - Total mineralized tissue content (BV * TMD)
    - Total mineralized tissue volume (BV)

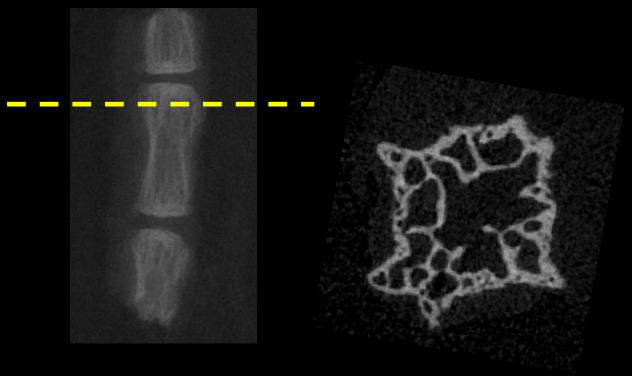


Courtesy of Dr. Masahiro and Dr. Kenta Uchibe

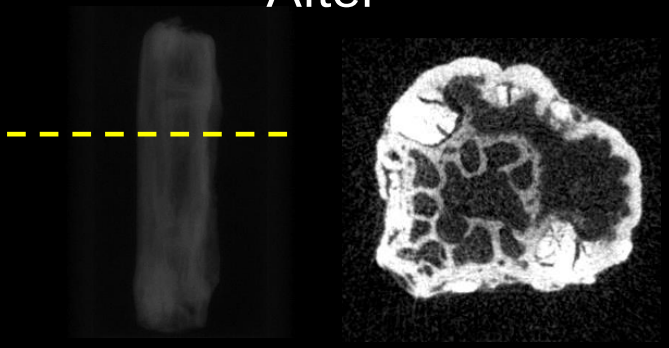
Soft Tissue µCT Imaging

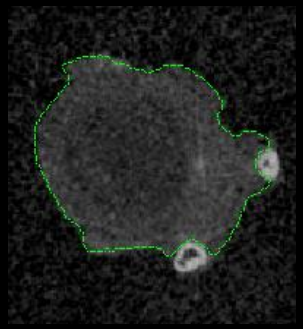
- Contrast-Enhanced µCT Imaging
  - Mouse Intervertebral disc
  - 18.5µm or lower voxel size
  - Spine segment scanned before and after Lugols staining
  - Scan Region:
    - Whole segment
  - Average scan time:
    - 0.5-2 hours
  - Outcome measures
    - Disc thickness (Tb.Th)

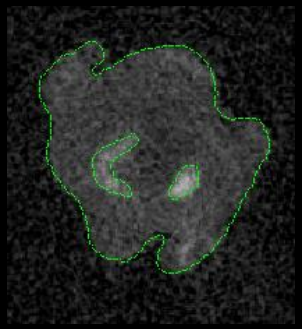
Before

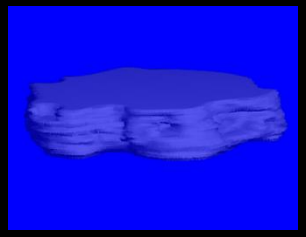


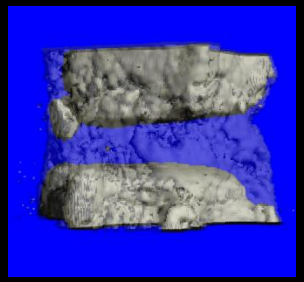
After











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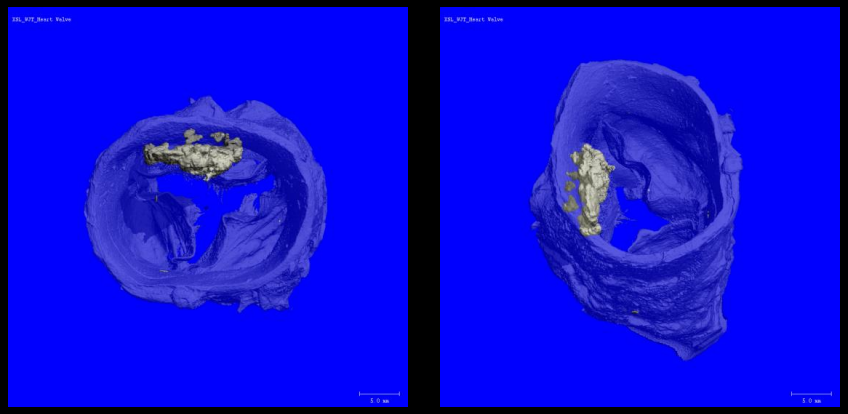
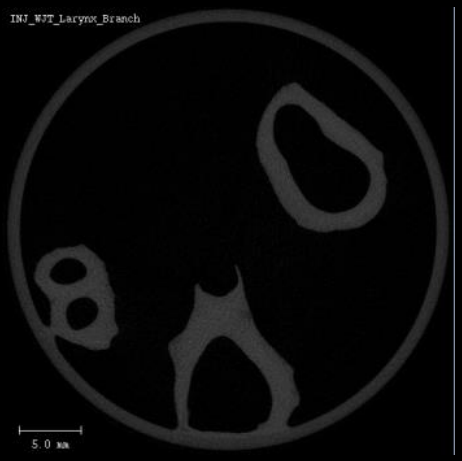
Soft Tissue µCT Imaging

- Soft tissue scanned in air
  - Heart Valve
  - Larynx Branch



Heart Valve



Larynx Branch

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Questions?

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