*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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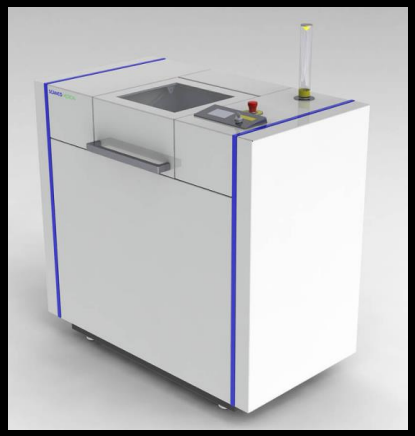
# Available Scanners at PCMD µCT Imaging Core

- Specimen µCT
  - µCT 35
  - µCT 50
  - µCT 45 *new!*
- *In Vivo* µCT
  - vivaCT 40
  - vivaCT 75
  - vivaCT 80 *new!*
- Clinical µCT
  - XtremeCT II





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# PCMD *ex vivo* µCT Scanners

| | µCT 35 | µCT 45 | µCT 50 |
|---|---|---|---|
| Use | Specimen | Specimen | Specimen |
| X-Ray Source | 30 - 70 kVp | 20 - 90 kVp | 30 - 90 kVp |
| Max Scan Size | 37.9 x 120 mm (Ø x L) | 50 x 120 mm (Ø x L) | 50 x 120 mm (Ø x L) |
| Max Specimen Size | 75.8 x 140 mm (Ø x L) | 90 x 120 mm (Ø x L) | 100 x 160 mm (Ø x L) |
| Best image voxel size | 3.5 µm (Ø :7 mm) | 2.5 µm (Ø: 5 mm) | 1.5 µm (Ø: 3 mm) |
| Location | McKay Lab | McKay Lab | VA Hospital |

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# PCMD *in vivo* µCT Scanners

| | vivaCT 40 | vivaCT 80 | vivaCT 75 | XtremeCT II |
|---|---|---|---|---|
| Use | In Vivo Rodent | In Vivo Rodent | In Vivo Rodent | Clinical |
| X-Ray Source | 30 - 70 kVp | 30 - 70 kVp | 30 - 70 kVp | 68 kVp |
| Max Scan Size | 38.9 x 145 mm (Ø x L) | 80 x 145 mm (Ø x L) | 79.9 x 145 mm (Ø x L) | 140 x 200 mm (Ø x L) |
| Max Specimen Size | 80 x 500 mm (Ø x L) | 90 x 500 mm (Ø x L) | 80 x 500 mm (Ø x L) | 170 mm (Ø) |
| Best image voxel size | 10.5 µm (Ø :21.5 mm) | 10.4 µm (Ø :21.5 mm) | 19 µm (Ø: 38.9 mm) | 60 µm |
| Location | McKay Lab | McKay Lab | VA Hospital | CHOP CTRC nutrition and growth lab |

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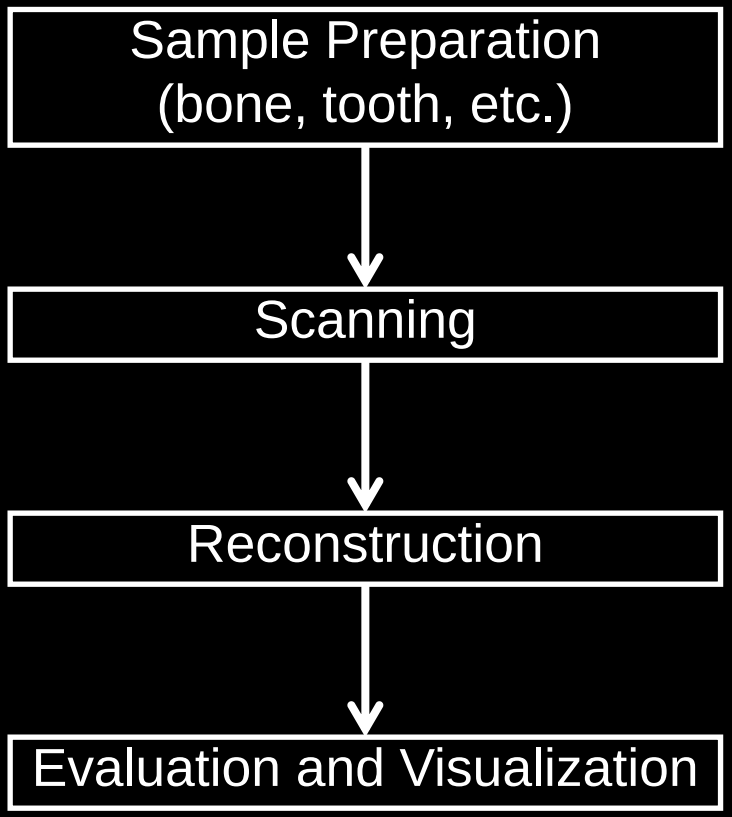
# Which Scanner Should I Use for My Study?

- µCT 35 and µCT 45
  - Small specimen scans (under diameter of 35 µm)
  - Mouse bone microstructure phenotyping must use µCT 35/45
- vivaCT 40
  - Rodent study (rats and mice) requiring in vivo scans (IACUC approval required)
  - When µCT 35/45 is fully occupied
- vivaCT 80
  - Rodent study (rats and mice) 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 50 µm)
- vivaCT 75 and µCT 50 (VA location)
  - Only accessible to investigators 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

# μCT Imaging

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# Sample Preparation

- Specimens
  - Dry samples
    - Embedded in MMA √
    - Scanned in air (short scan time) for special purposes of soft tissue imaging √
  - Wet samples
    - Stored in DI water √, saline √, PBS √, neutral buffer formalin √, ethonol √, 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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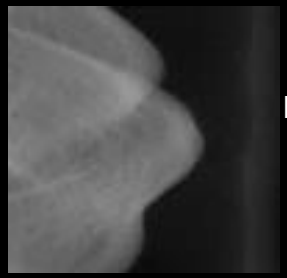
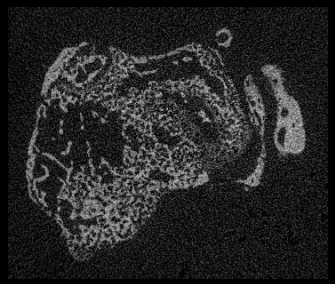
# From Scan to Results



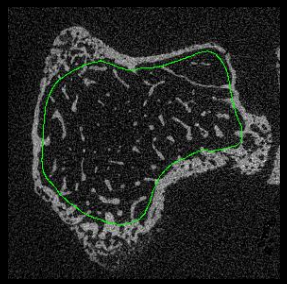
**.RSQ**
RAW sequence data

**Reconstruction**



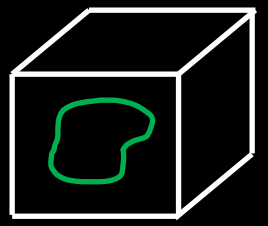
**.ISQ**
Image sequence data

**Contouring**



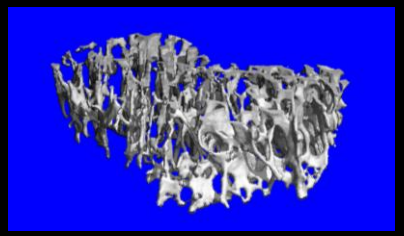
**.GOBJ**
Graphical object

**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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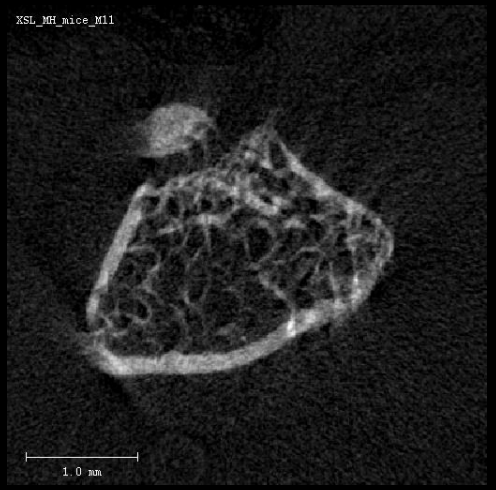
# After Scanning

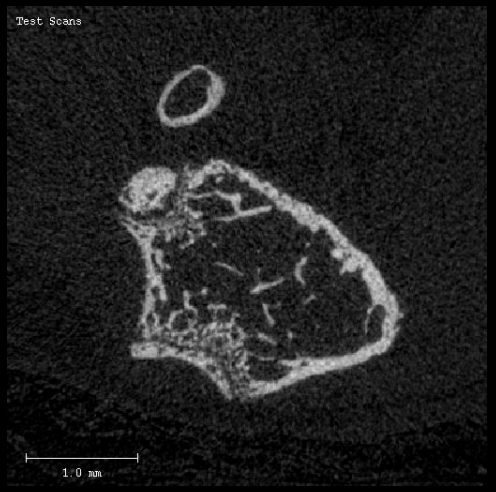
- Reconstruction
  - Automatic reconstruction in Scanco (our) systems

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

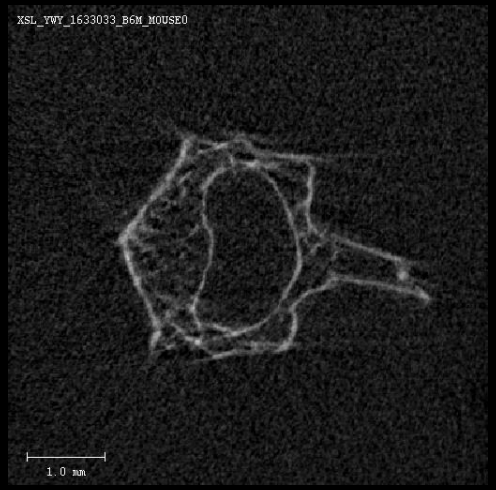
# Motion Artifact

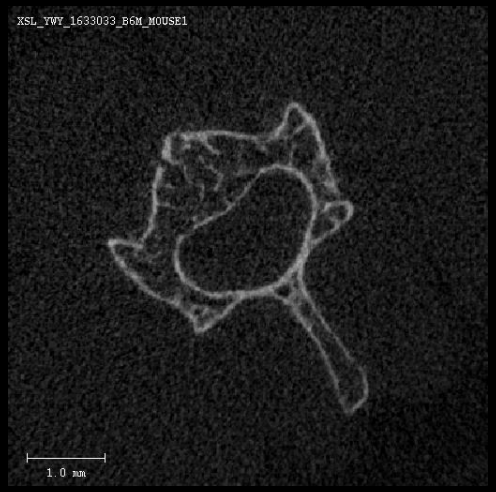
**Distal Femur**




**L2 Vertebrae**




Sometimes re-scans required

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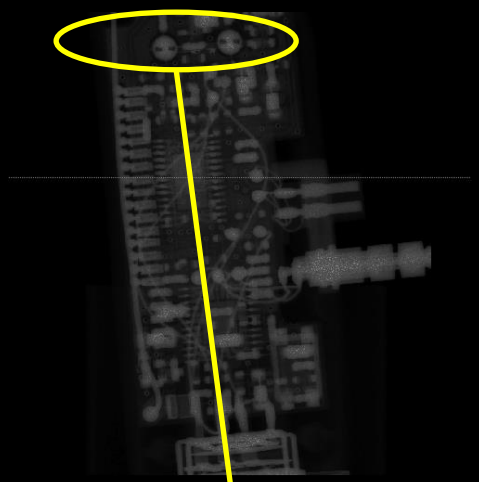
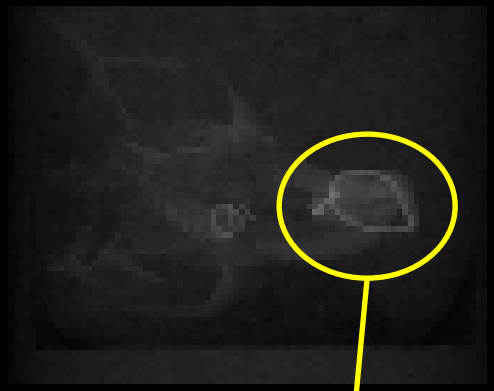
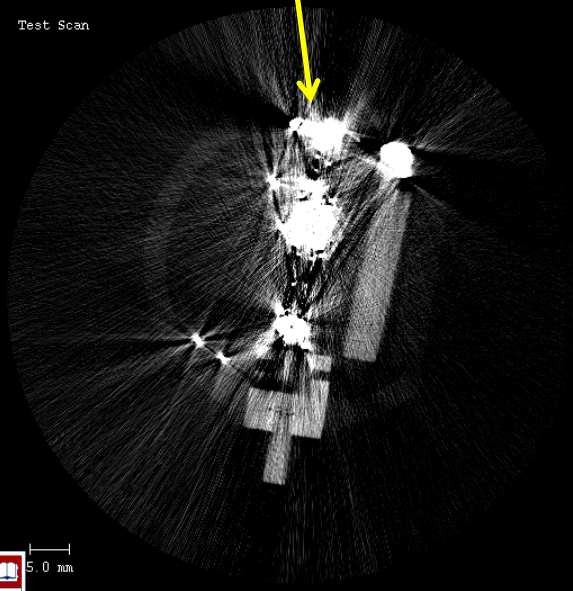
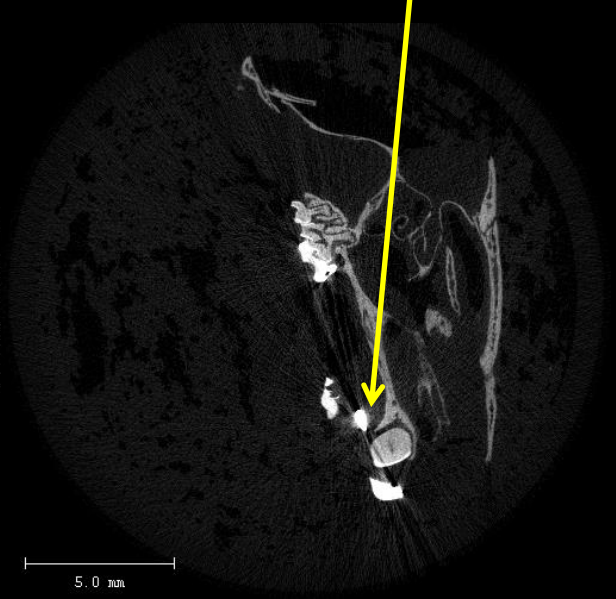
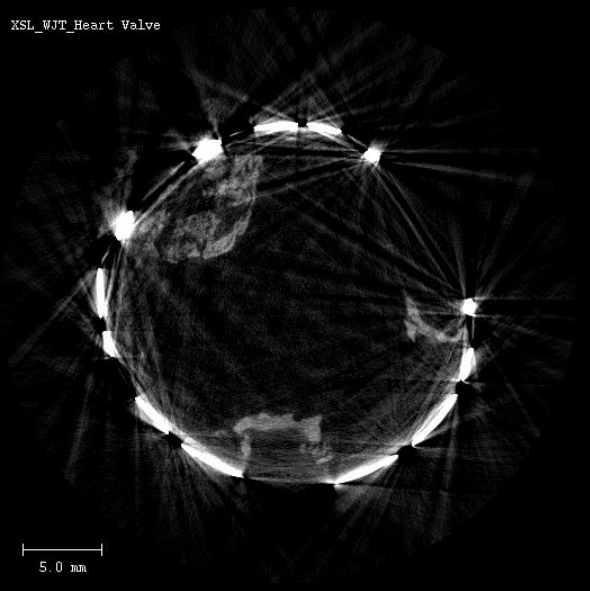
# Metal Artifact



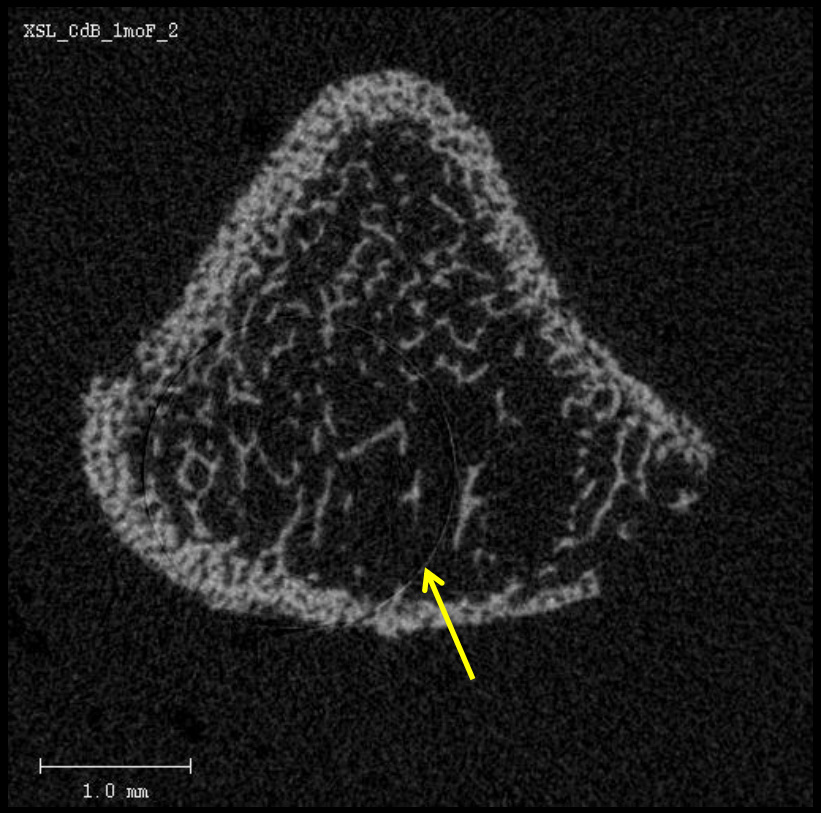


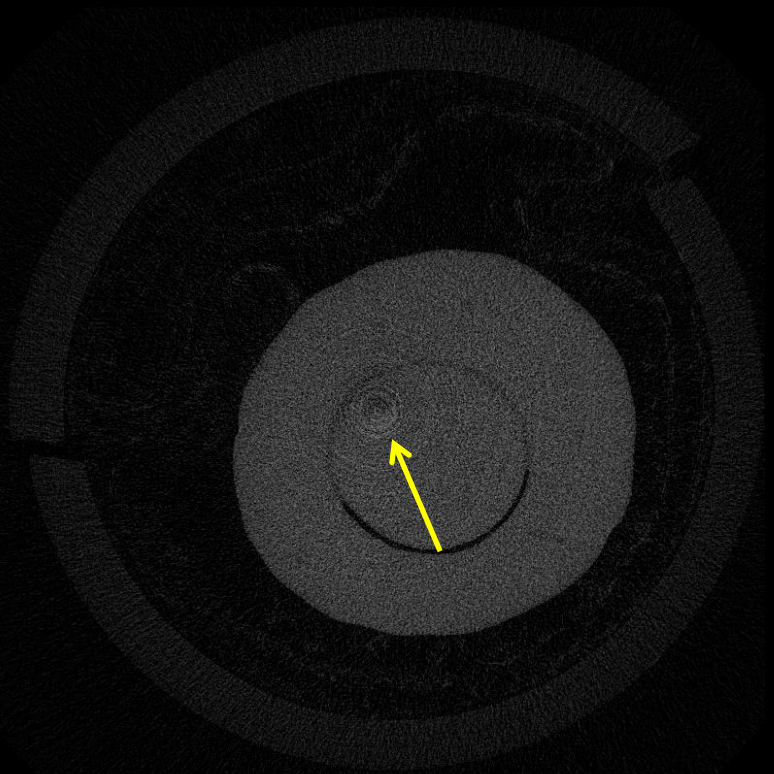


# Ring Artifact



Report to the core manager with sample and measurement #

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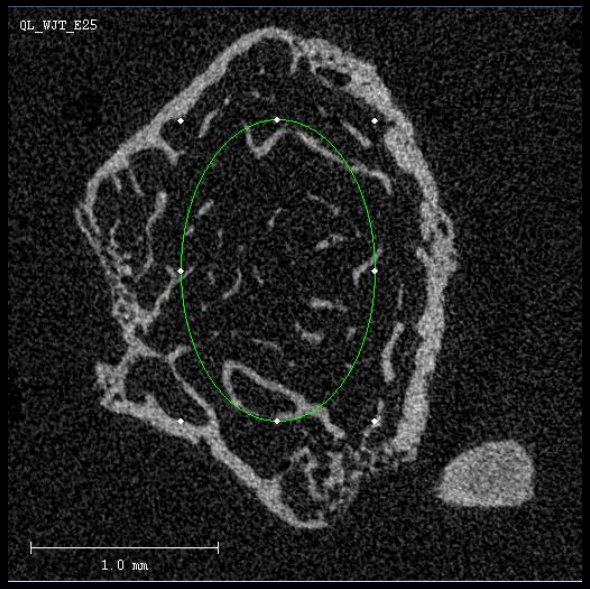
# After Scanning

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

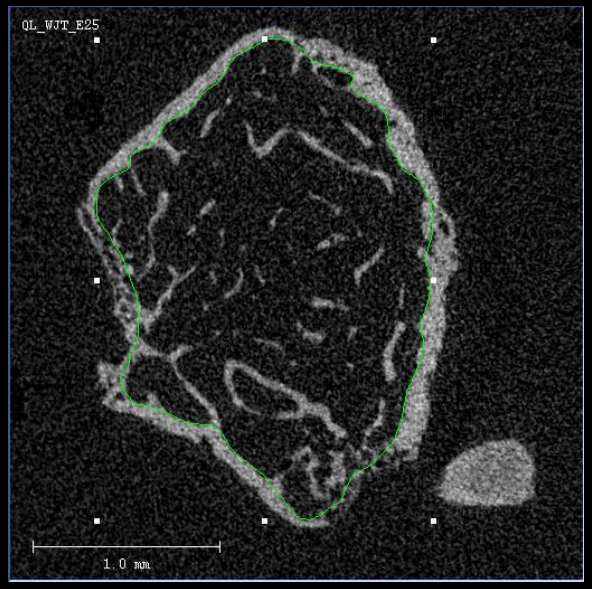
# Contouring

- Choose Volume of Interest (VOI)

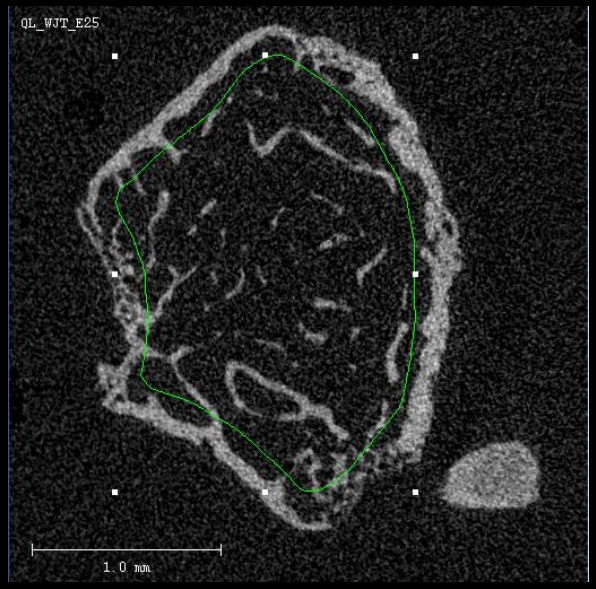
X




X

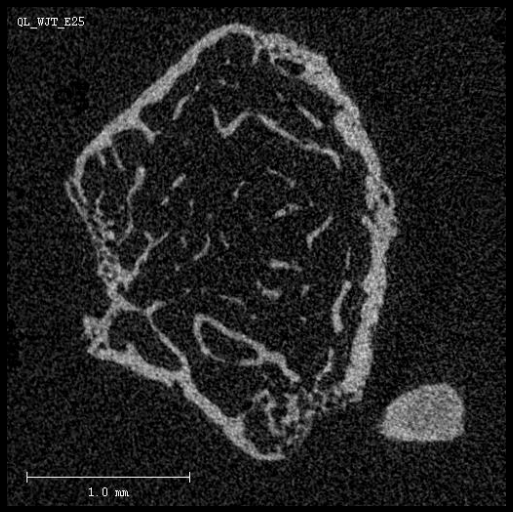



√

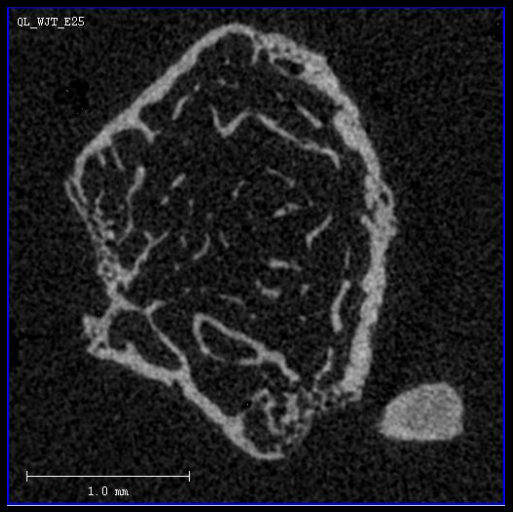


# Filtration

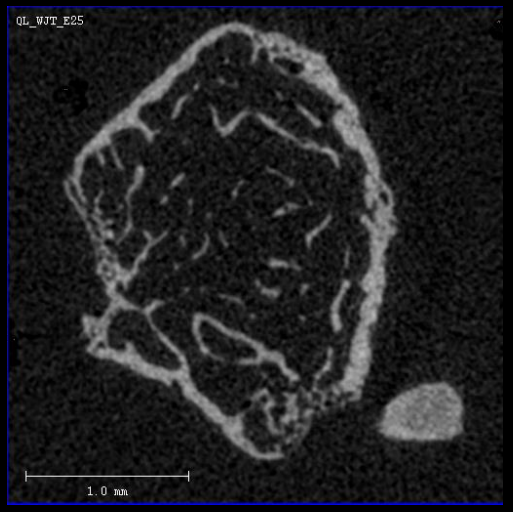
- Gaussian filter to suppress image noise
  - σ : Extent of the kernel or degree of smoothing
  - Support: the size of the discrete kernel (# of neighboring voxels that contributes to the weighted mean)




unfiltered




σ=1.2, support 2




σ=2, support 2

Recommended

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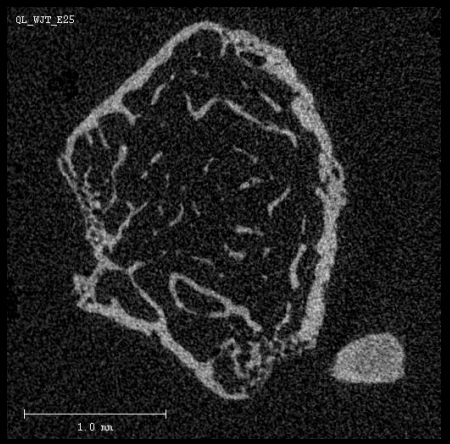
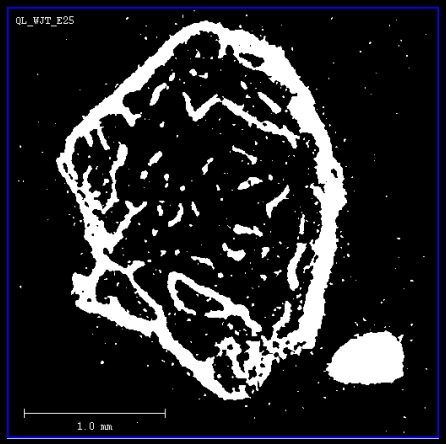
# Segmentation

- 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
  - Recommendation: low threshold: trabecular bone ~330; cortical bone ~360

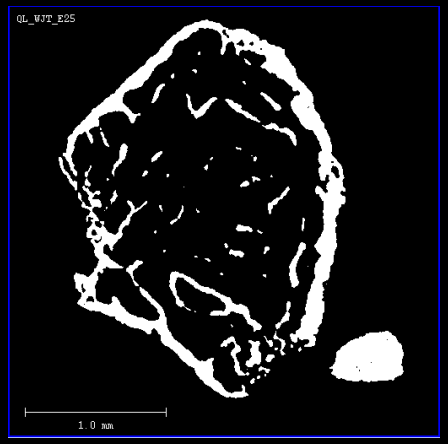
Grayscale



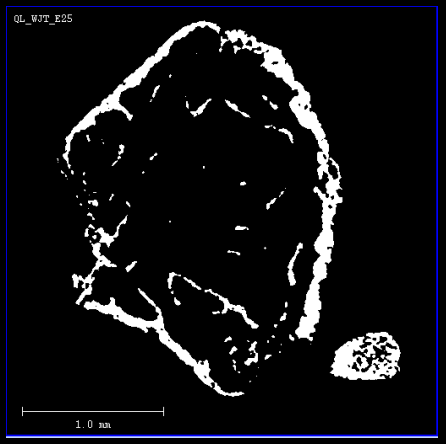

200-1000




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/$cm^3$ |

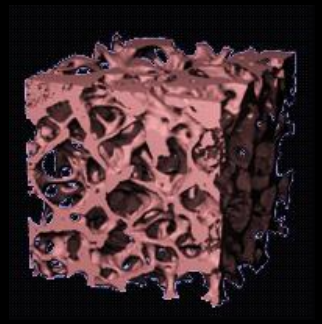
# Trabecular Bone Density and Microstructure

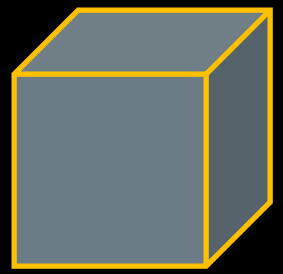
- Scale
  - Bone Volume Fraction (BV/TV)
  - Trabecular Number (Tb.N)
  - Trabecular Spacing (Tb.Sp)
  - Trabecular Thickness (Tb.Th) (Hildebrand & Ruegsegger, 1995)
- Topology
  - Connectivity (Conn.D) (Kinney and Labb, 1998; Kabel, *et al*. 1999)
  - Structural Model Index (SMI) (Wehrli *et al*., 2001;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

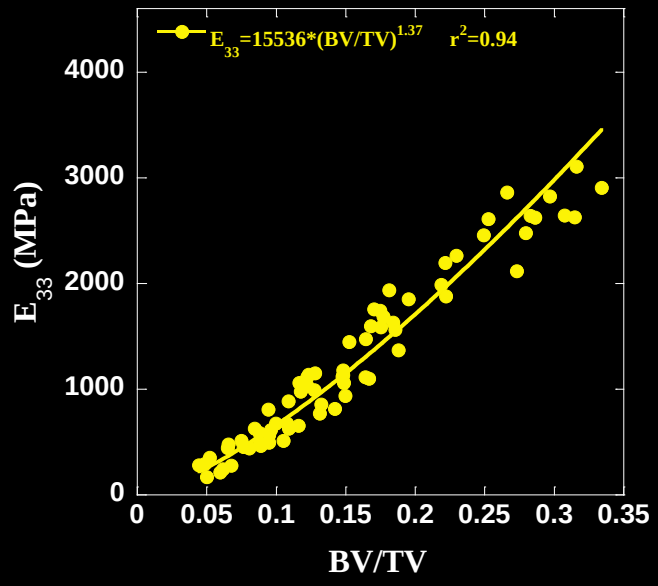
Bone Volume Fraction (BV/TV)

=

Bone Volume (BV) ÷ Total Volume (TV)





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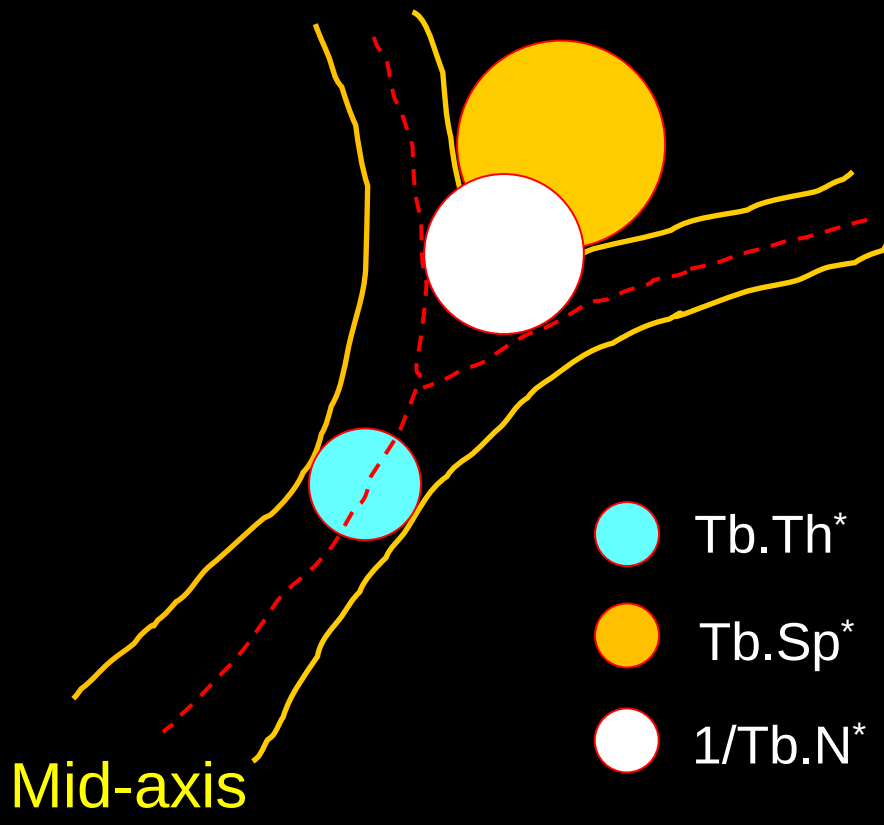
# Trabecular Bone Density and Microstructure

- Scale
  - 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)

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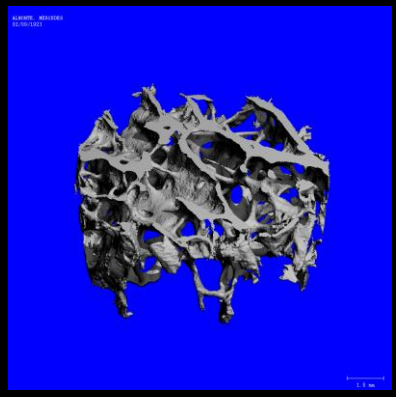
# Trabecular Bone Density and Microstructure

- Topology
  - Connectivity density (Conn.D) (Kinney and Labb, 1998; Kabel, *et al*. 1999)
    - Indication of the integrity of trabecular bone network
    - A 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 the 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

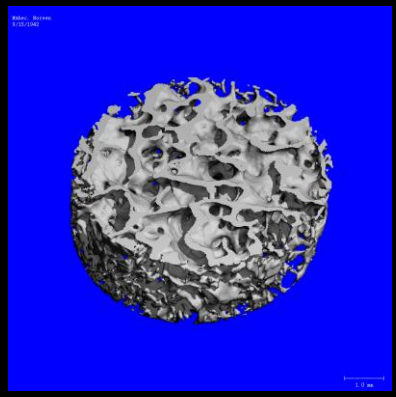


Plate-like structure

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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 the total 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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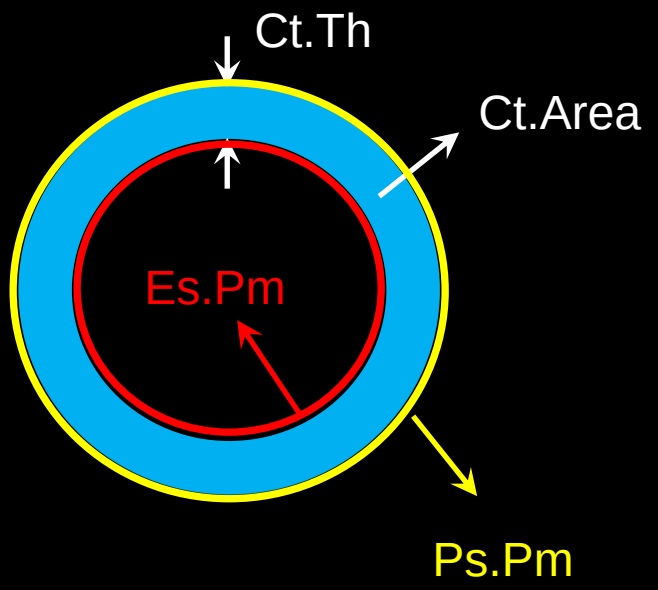
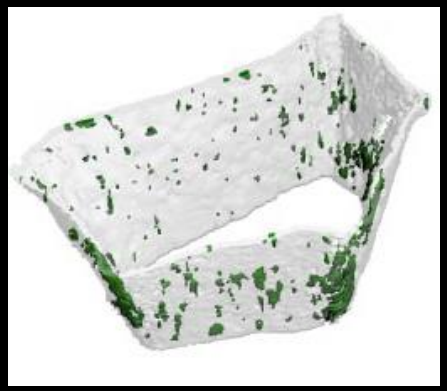
# Cortical Bone Morphometry

- 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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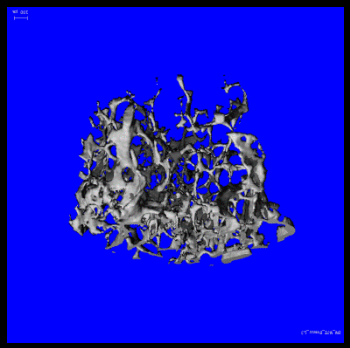
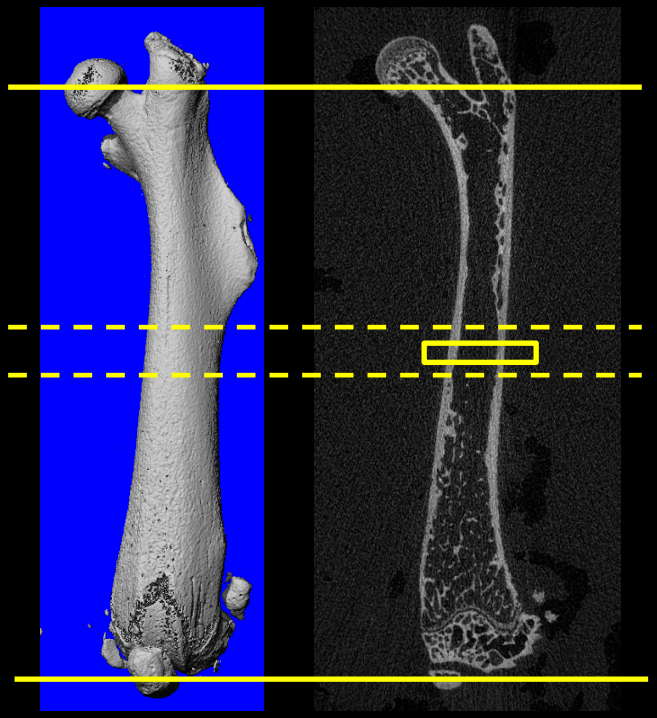
# Phenotyping

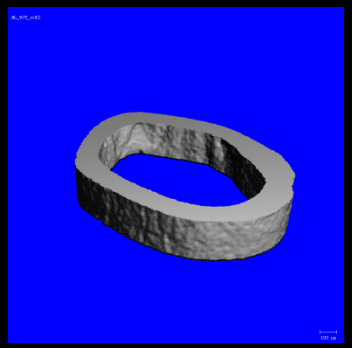
- Mouse trabecular bone (long bone)
  - Distal femur, proximal tibia
  - 6 µm isotropic voxel size
  - Scan Region
    - 1-2 stacks, ~210-420 slices
  - Average scan time: 24-48 mins
  - Analysis region
    - 100-200 slices,  0.5-1mm from the growth plate
  - Outcome measures: BV/TV, Tb.Th, Tb.N, Tb.Sp,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



# Phenotyping

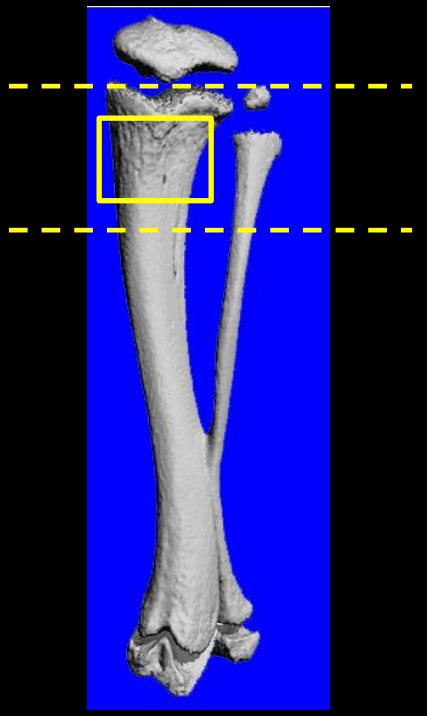
- Mouse cortical bone (long bone)
  - Midshaft of tibia or femur
  - 6-10 µm isotropic voxel size
  - Scan Region
    - 1 stacks, ~210 slices
  - Average scan time: 24 mins
  - Analysis region
    - Middle 50 slices
  - Outcome measures: Ct.Area, Ct.Th, pMOI, Ct.Po, TMD

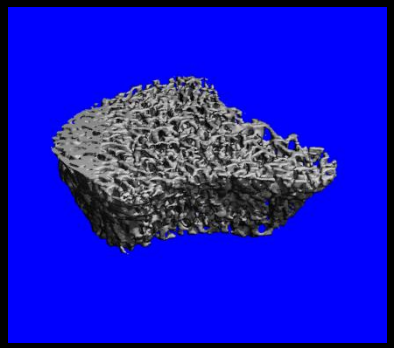




# Phenotyping

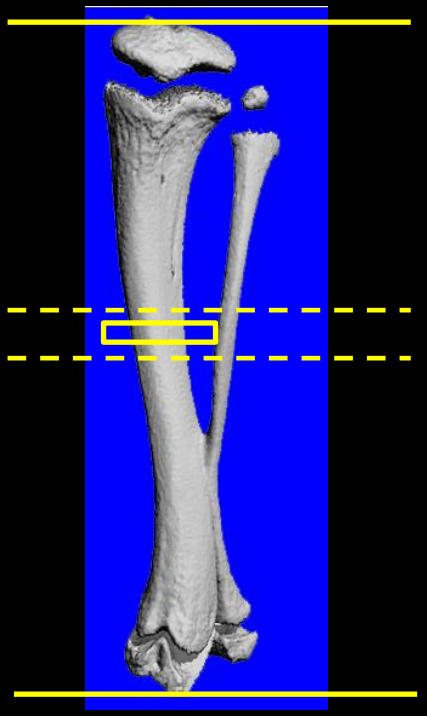
- Rat trabecular bone (long bone)
  - Proximal tibia
  - 6-10 µm isotropic voxel size
  - Scan Region
    - 2 stacks, ~420 slices
  - Average scan time: 24-48 mins
  - Analysis region
    - ~200 slices, 1-2mm distal to the growth plate
  - Outcome measures: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn.D, TMD

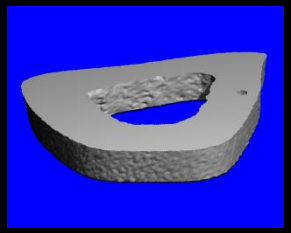




# Phenotyping

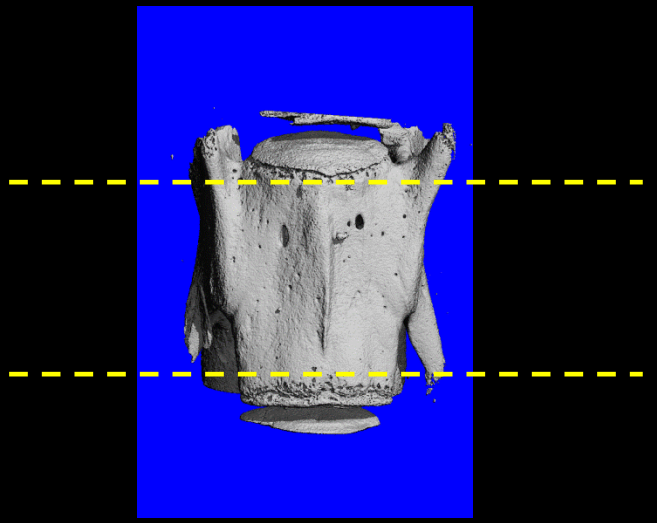
- Rat cortical bone (long bone)
  - Midshaft of tibia or femur
  - 6-10 μm isotropic voxel size
  - Scan Region
    - 1 stacks, ~210 slices
  - Average scan time: 24 mins
  - Analysis region
    - Middle 50 slices
  - Outcome measures: Ct.Area, Ct.Th, pMOI, Ct.Po, TMD

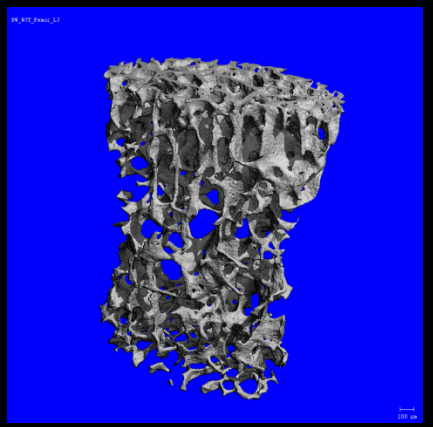




# Phenotyping

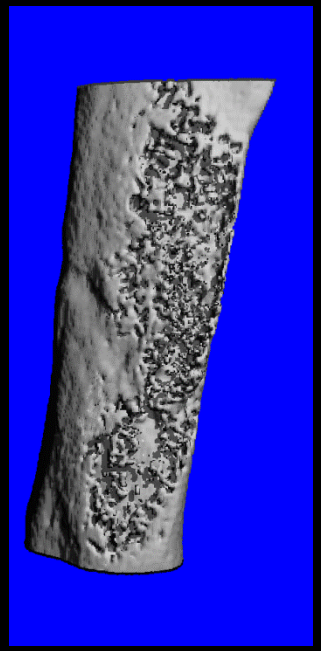
- Mouse/Rat vertebral trabecular bone
  - Lumbar vertebra L1-L4
  - 6 µm (mouse) / 6-10 µm (rat) isotropic voxel size
  - Scan Region
    - Between end plates
  - Average scan time: 48-72 mins
  - Analysis region
    - Between two end plates
    - Middle 150-200 slices
  - Outcome measures: BV/TV, Tb.Th,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

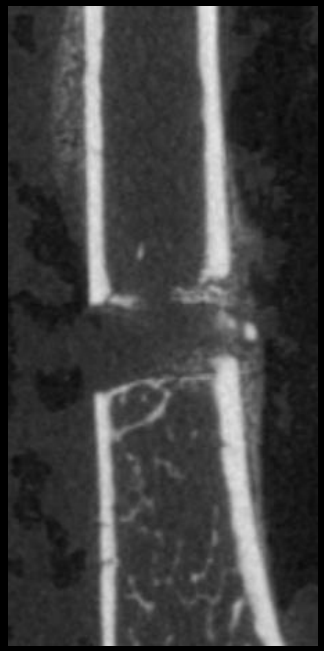


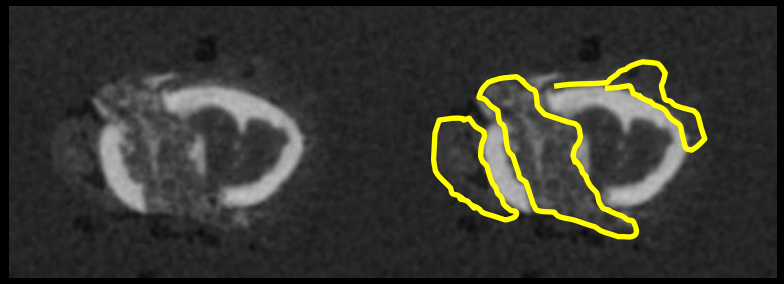


# Fracture Healing

- 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

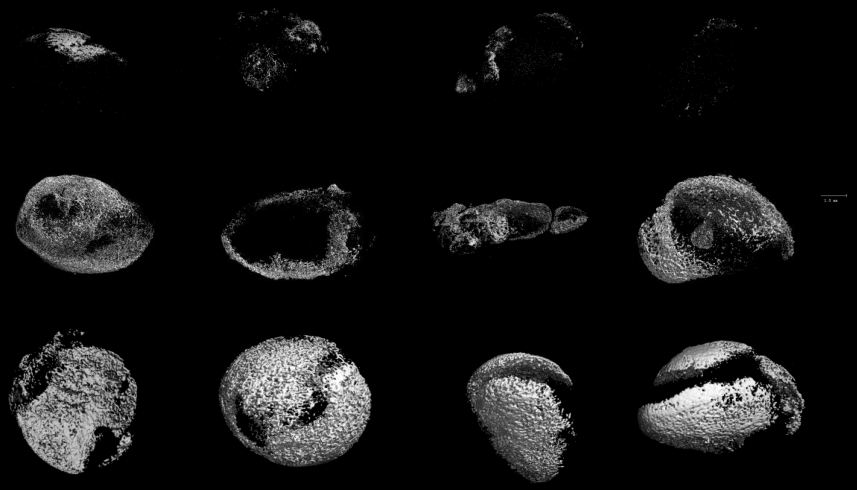






# Scaffolds

- 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)

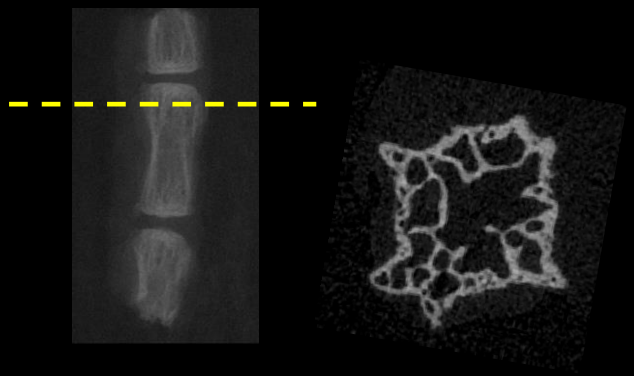


Courtesy of Dr. Masahiro and Dr. Kenta Uchibe

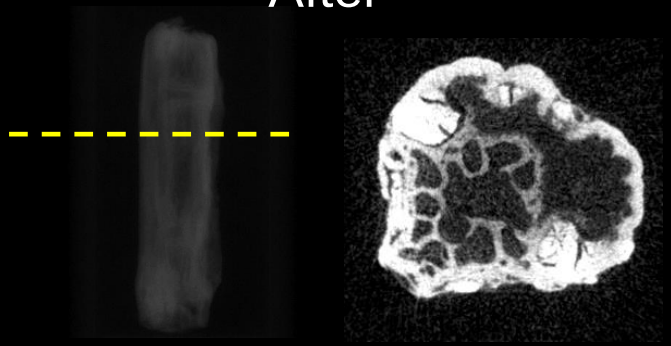
# Soft Tissue µCT Imaging

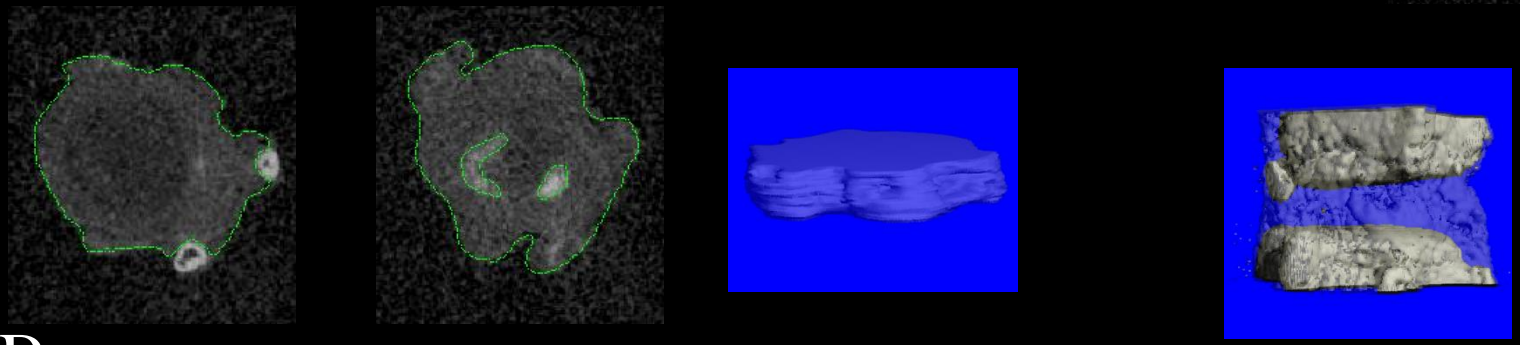
- Contrast-Enhanced µCT Imaging
  - Mouse Intervertebral disc
  - 19 µm or lower voxel size
  - Spine segment scanned before and after Lugols staining
  - Scan Region:
    - Whole segment
  - Average scan time:
    - 0.5-2 hours
  - Outcome measures
    - Disc thickness (Tb.Th)

Before



After





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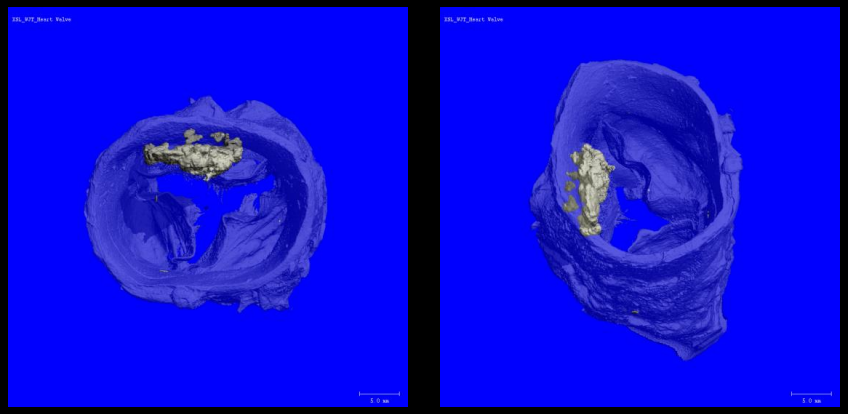
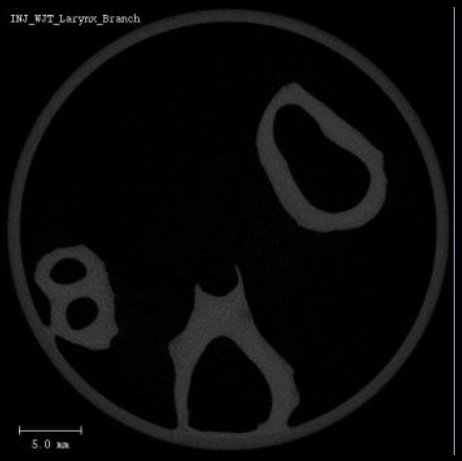
# Soft Tissue µCT Imaging

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



Heart Valve




Larynx Branch

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# Questions?

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