PCMD MicroCT Imaging Core Learning Lunch Series

In Vivo μCT Imaging of Rodents

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October 18th, 2017

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Why *in vivo* µCT?

- 3D imaging with sufficient spatial resolution for the assessment of rodent bone microarchitecture
- Longitudinal studies of bone morphology Waarsing+2006 Brouwers+2007, Brouwers+2008, Brouwers+2009, Klinck+2008, Bouxsein+2010, Lan+2013, Boyd+2006, Campbell+2008, Buie+2008, Lambers+2011, Schulte+2011
  - Skeletal responses to various diseases and treatments
    - Bone loss associated with disuse or surgery
    - Increased bone mass owing to pharmacologic treatment or mechanical loading
- Input to micro finite element (µFE) models to estimate the mechanical properties of bone van Rietbergen+1998, Schulte+2011
- Increased statistical power
  - Reduction in number of animals Bouxsein+ 2010

Micro Computed Tomography

- *In vivo* µCT scanner
  - Scanco vivaCT 40
  - Best resolution:
    10.5 µm isotropic voxel size
  - X-Ray Source
    - 30 - 70 kVp
  - Max Scan Size
    - 38.9 x 145 mm (Ø x L)

In Vivo µCT Imaging

Animal Model (Rats, mice)

↓

Scanning Resolution

↓

Sample Holder

↓

Before Scanning:
Anesthesia

↓

During Scanning:
Animal breathing

↓

After Scanning:
Animal waking up

How to Choose Image Resolution

- Image resolution is determined by FOV and number of projections

Field of View (mm)	Proj./180°	Resolution (μm)
21.5	1000	10.5
21.5	500	21
25.6	1000	12.5
25.6	500	25
30.7	1000	15
30.7	500	30
35.8	1000	17.5
35.8	500	35
38.9	1000	19
38.9	500	38

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Radiation Dose – VivaCT 40

- Computed Tomography Dose Index (CTDI)
  - proportional to the integration time (s), with the same current (µA) and number of projections

Energy (KV)	Integration time (ms)	Current (µA)	Field of View (mm)	Proj./180°	CTDI (mGy)	Resolution (µm)
55	300	109	21.5	1000	720	10.5
55	300	109	30.7	1000	350	15
55	300	109	38.9	1000	220	19

- Radiation dose on current scanning protocol
  - 10.5µm for rat tibia, mouse distal femur, proximal tibia and tibial midshaft
  - → CTDI = **639 mGy**
  - 15µm for mouse vertebrae
  - → CTDI = **310 mGy**
  - 19µm for rat femur midshaft
  - → CTDI = **195 mGy**

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Concerns – Radiation Exposure

- *In vivo* scan on Wistar rats Klinck+ 2008
  - 8 month old, female rats
  - 12.5 µm isotropic voxel size, 55 kV voltage, 109 µA current, 200 ms integration time, 2000 projections
  - Scanned right tibia at wk0, 2, 4, 6, 8, 12
  - Radiation dose: 502.5 mGy

  → *No radiation effect*

- *In vivo* scan on Wistar rats Brouwers+ 2007
  - 30 week old, female rats
  - 15 µm isotropic voxel size, 70 kV voltage, 85 µA current, 350 ms integration time, 2000 projections
  - Scanned right tibia at wk0, 1, 2, 3, 4, 5, 6, 8; left tibia at wk0 and 8
  - Radiation dose: 939 mGy
  - Determined cell radiation damage using a cell viability test

  → *No radiation effects on bone microarchitecture and marrow cells*

Concerns – Radiation Exposure

- *In vivo* scan on BL6 mice Laperre+2011
  - 10 weeks old, male mice
  - 9 µm isotropic voxel size
  - *In vivo* scanned left tibia at wk0, 2, 4; ex vivo scanned on both tibia after sacrifice (wk4)
  - Radiation dose: 776 mGy
  - → *Negative effects on BV/TV and Tb.N and increased Oc.S/BS*

- *In vivo* scan on BL6 mice Laperre+2011
  - 4 and 16 weeks old, male mice
  - 9 µm and 18 µm isotropic voxel size
  - *In vivo* scanned left tibia at wk0, 2, 4; ex vivo scanned on both tibia after sacrifice (wk4)
  - Radiation dose: 434 mGy (9 µm) and 166 mGy (18 µm)
  - → *No radiation effect on both trabecular and cortical bone architecture in pre-pubertal or adult mice*

Concerns – Radiation Exposure

- *In vivo* scan on C3H, BL6, and BAL mice Klinck+ 2008
  - 8-10 weeks old, female mice
  - 10.5 µm isotropic voxel size, 55 kV voltage, 109 µA current, 200 ms integration time, 2000 projections
  - Scanned right tibia at wk0, 1, 2, 3
  - Radiation dose: 712.4 mGy
  - → *Negative effects on trabecular microarchitecture*

- *In vivo* scan on BL6 mice Zhao+ 2016
  - 12 weeks old, female mice
  - 10.5 µm isotropic voxel size, 55 kV voltage, 109 µA current, 200 ms integration time, 2000 projections
  - *In vivo* scanned right femur and L4 at wk0, 3, 6; *ex vivo* scan on both femurs, L3 and L4 after sacrifice (wk9)
  - Radiation dose: 639 mGy (femur) and 310 mGy (vertebra)
  - → *No effect on BV/TV and cellular activities; Negative effects on trabecular microarchitecture (~10-20%)*

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Conclusion: Radiation Exposure

- Minimal impact on ***rat*** bone mass and bone microarchitecture
- Compared to rats, ***mice*** are more sensitive to radiation exposure
  - High resolution scans (10-15 µm) leading to 10-20% deterioration of trabecular bone microarchitecture compared to non-radiated sites
  - *Suggestion:* Reduce radiation exposure by
    - Reduction in scan frequency and Increase in interval time between repeat scans
    - Reduction in scan resolution

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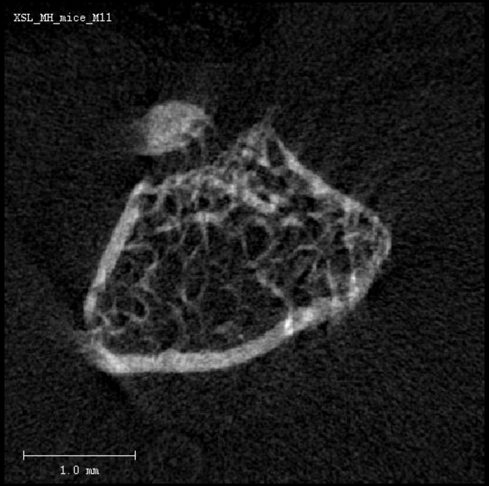
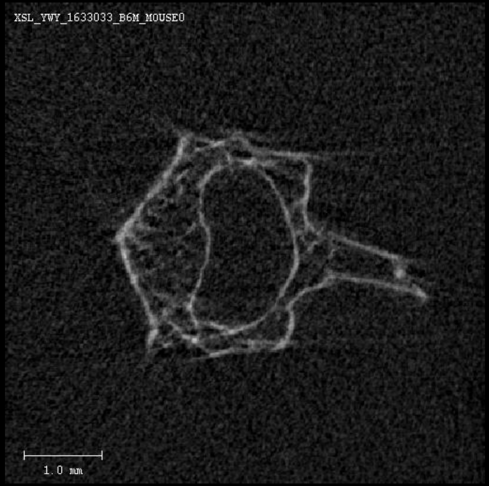
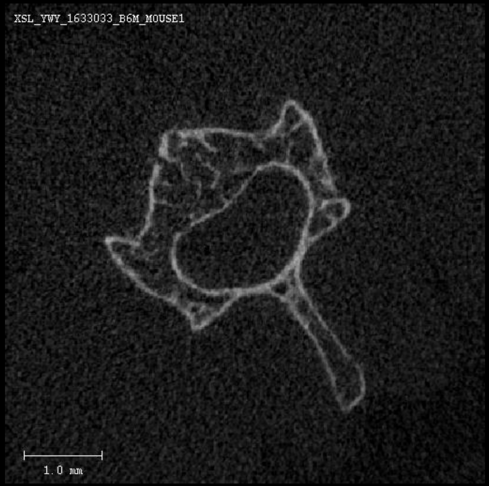
Concerns – Movement Artifacts

- Movement Artifacts due to animal breathing

Distal Femur | **L2 Vertebrae**







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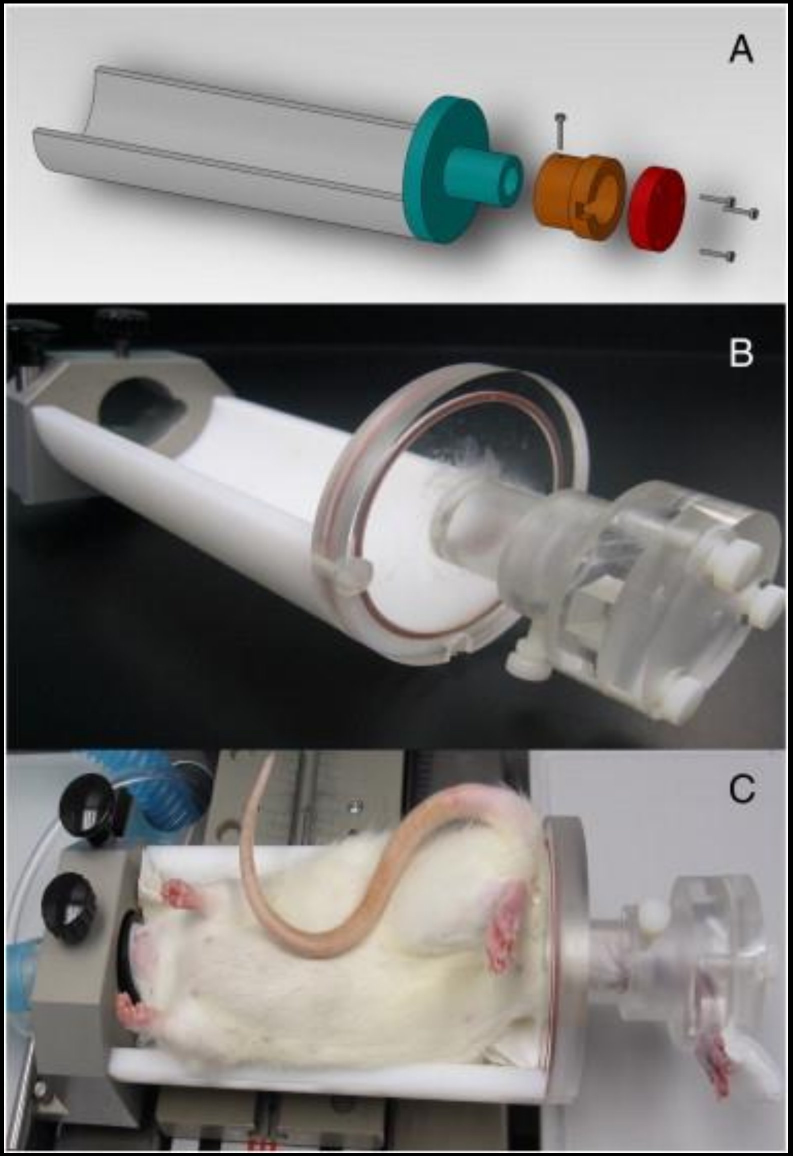
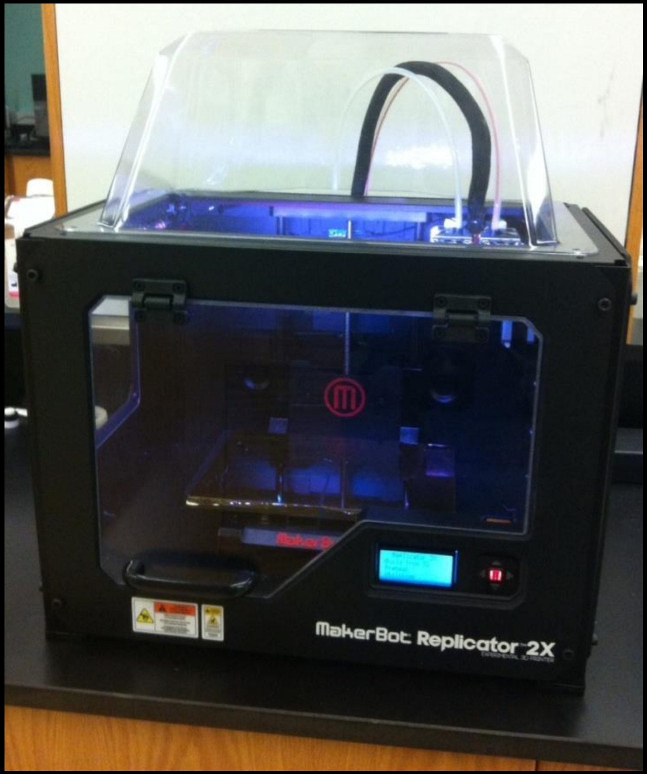
Customized Holders - Machining

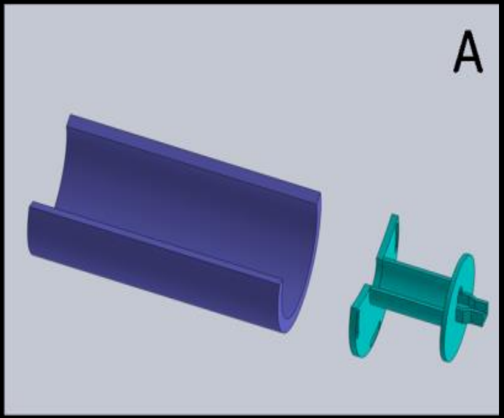
- Minimize the movement of the skeletal site of interest
- Minimize the reposition error induced by repeat scans



Lan+2013

Customized Holders – 3D printing





Chang+2016 SB3C

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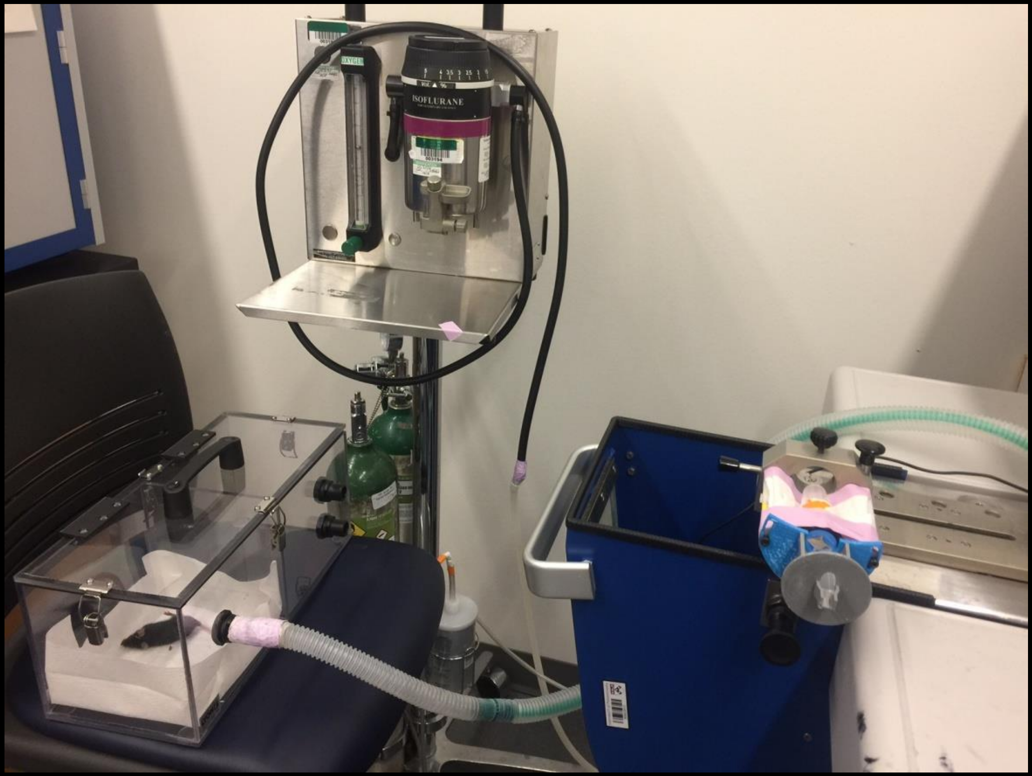
Before Scanning - Anesthesia

- Non-painful procedures (Penn IACUC Guideline)
  - Isoflurane
    - Mice: 3-4% for induction and 1-3% for maintenance
    - Rats: 3-5% for induction and 1-3% for maintenance



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http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/IACUCGuideline-MouseAndRatAnesthesiaAndAnalgesia.pdf

Before Scanning - Anesthesia

- Non-painful procedures (Penn IACUC Guideline)
  - Isoflurane
    - Mice: 3-4% for induction and 1-3% for maintenance
    - Rats: 3-5% for induction and 1-3% for maintenance
  - Ketamine/xylazine
    - Mice: 70-100 mg/kg ketamine (IP) + 5-12 mg/kg xylazine. If animals appear to be responding to touch or awakening, redose with up to 50% of the initial dose of ketamine only.
    - Rat: 40-100mg/kg ketamine (IP) + 5-10mg/kg xylazine. If the animal appears to be responding to touch or awakening, re-dose with up to 50% of the initial dose of ketamine

http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/IACUCGuideline-MouseAndRatAnesthesiaAndAnalgesia.pdf

Before Scanning - Anesthesia

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During Scanning

- Checking animal's breathing

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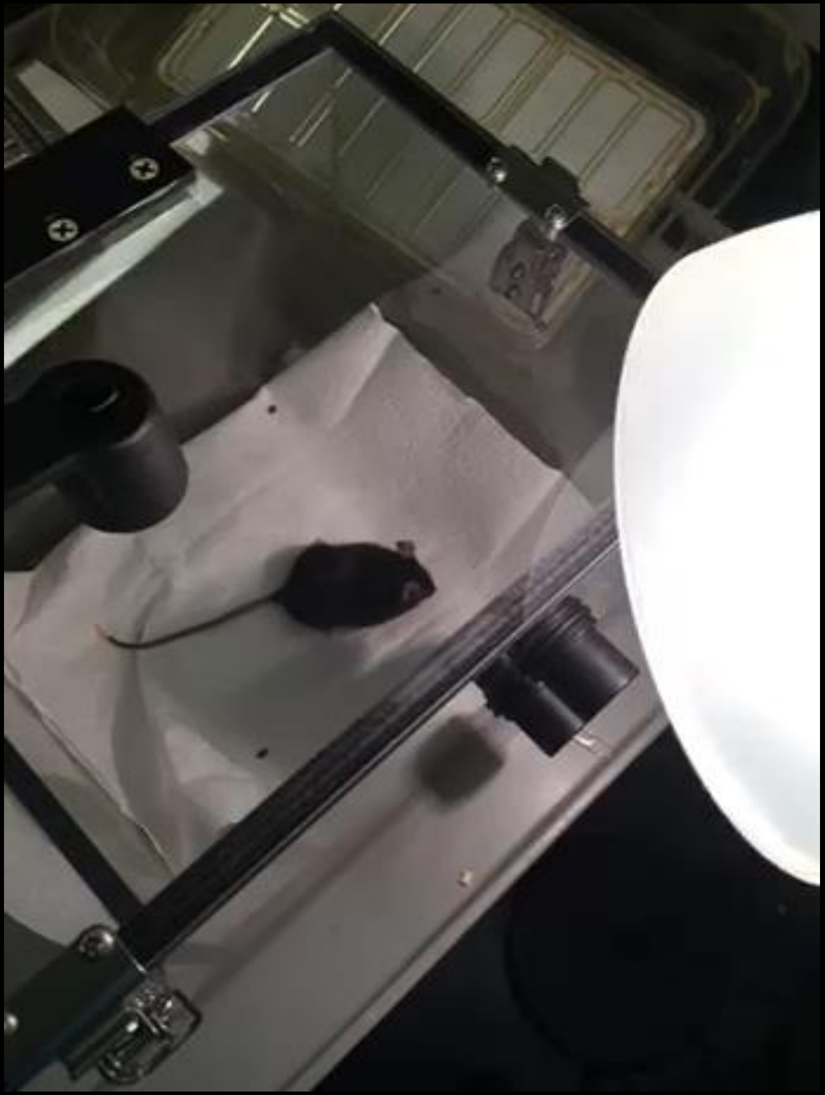
After Scanning

- Waking up the animal
  - Heat pad
  - Light



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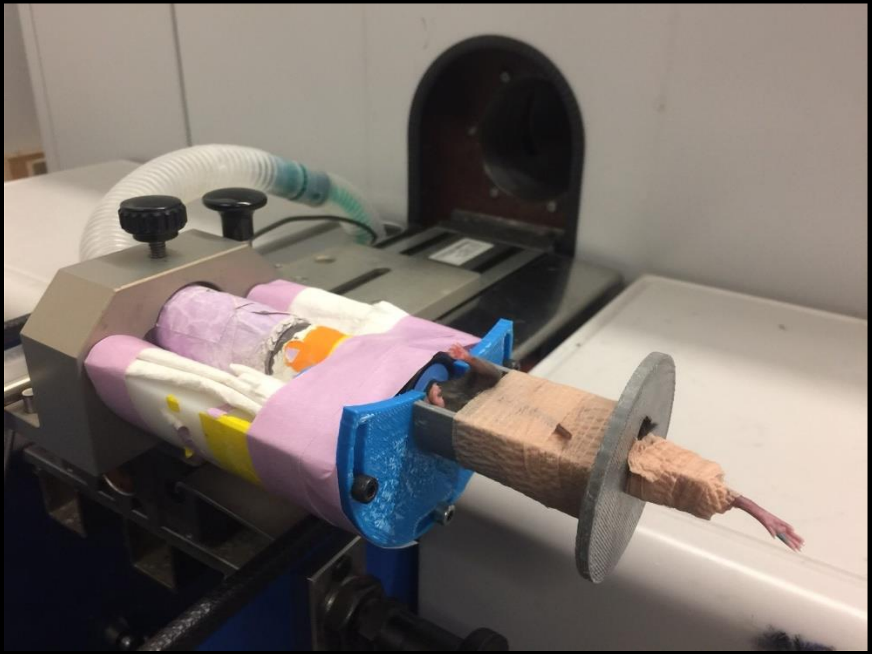
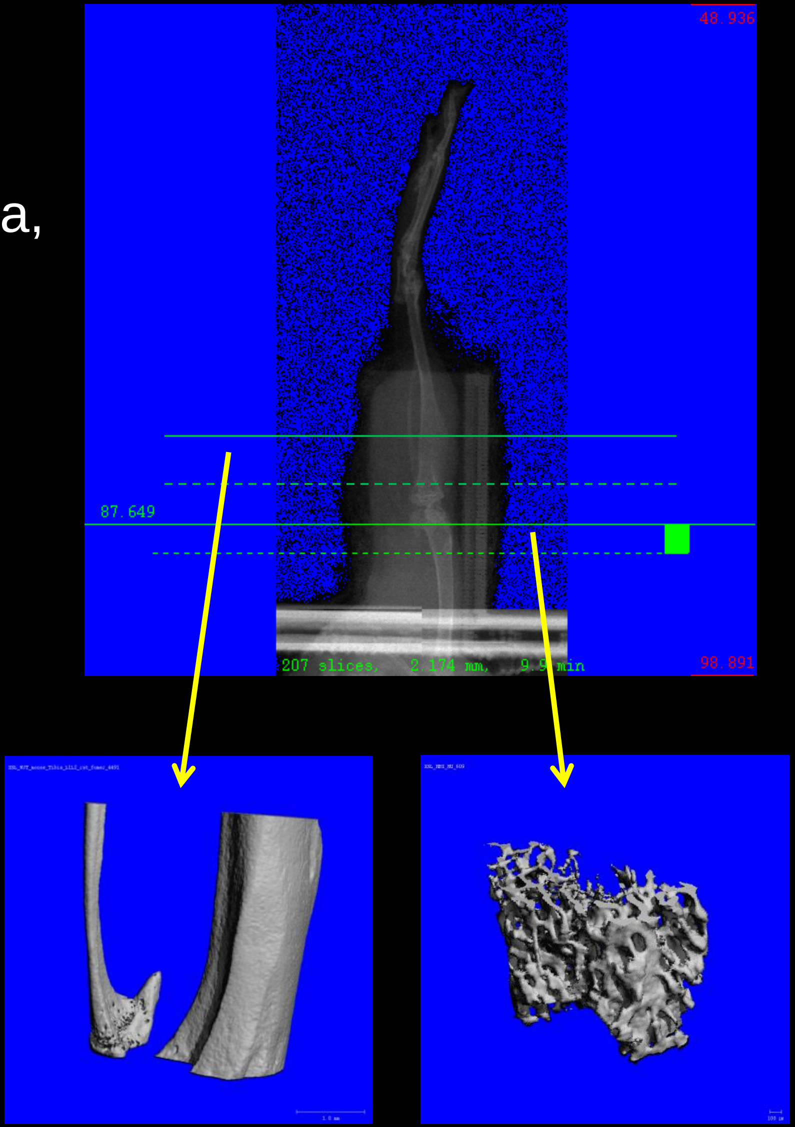
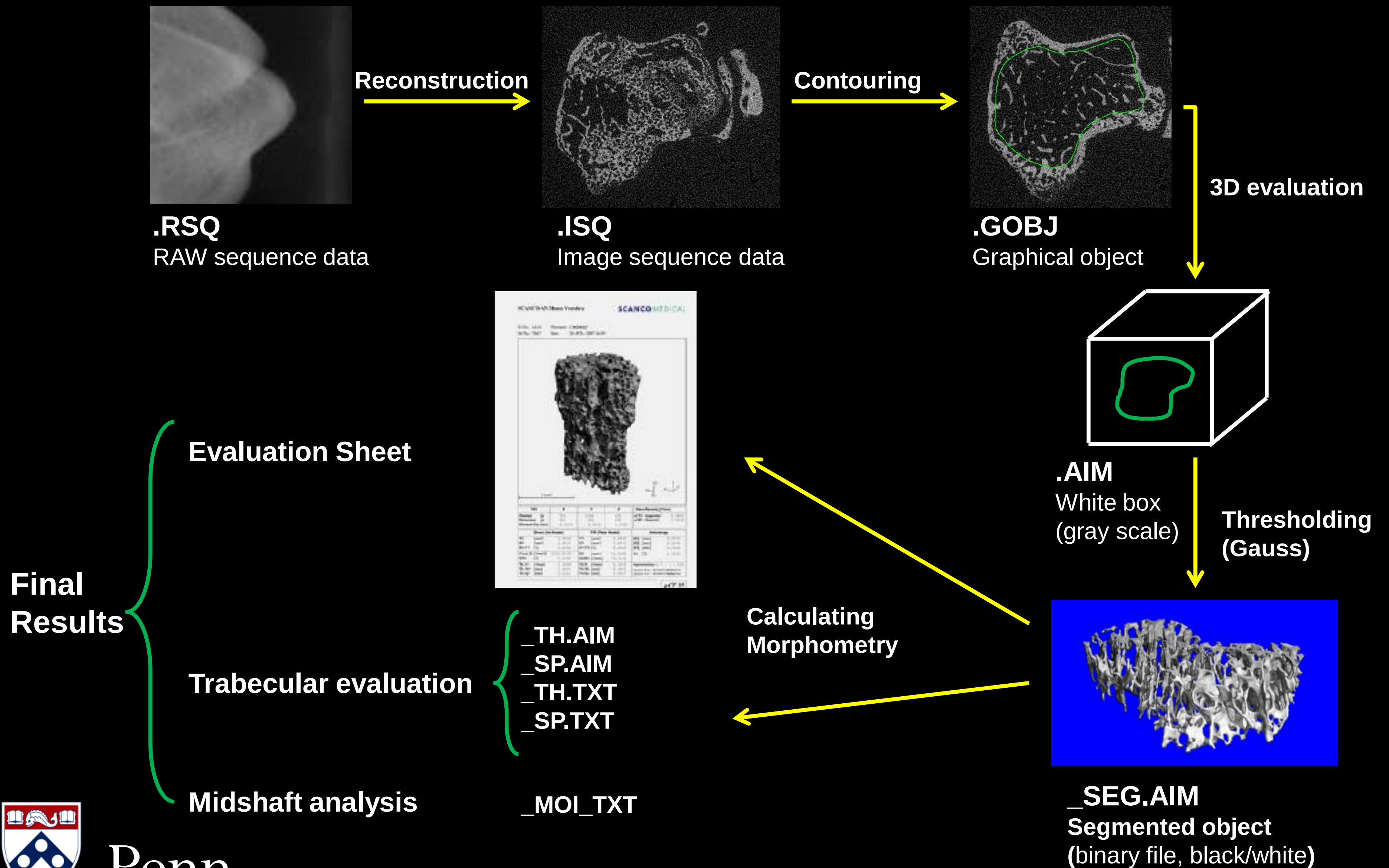
Bone Microarchitecture – Mouse Tibia & Femur

- *In vivo* µCT scan
  - 10.5 µm isotropic voxel size
  - 2 mm bone segment of proximal tibia, distal femur
  - Average scan time: 10 mins





From Scan to Results



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Concerns – Reposition error

- Precision affected by reposition of animals at each follow-up scan
  - Short term precision study (same day, multiple scans)
    - 12.5 µm, Precision: 1-6% in rats Nishiyama+2010
    - 10.5 µm, Precision: 1%-7% in rat tibia Lan+2013
    - 10.5 µm, Precision: 1-8% in BL6 or C3H mice tibia Nishiyama+2010
    - 10.5 µm, Precision: 4-12% in femur and 6.5-17.6% in L4 of BL6 mice Chang+2016 SB3C

  → *In vivo* precision in rodent bone measurements satisfy studies that expect to observe >5% change in bone mass and >10% change in bone microarchitecture

- Reduction in the reposition error
  - Customized animal holders during the scan
  - 3D image registration

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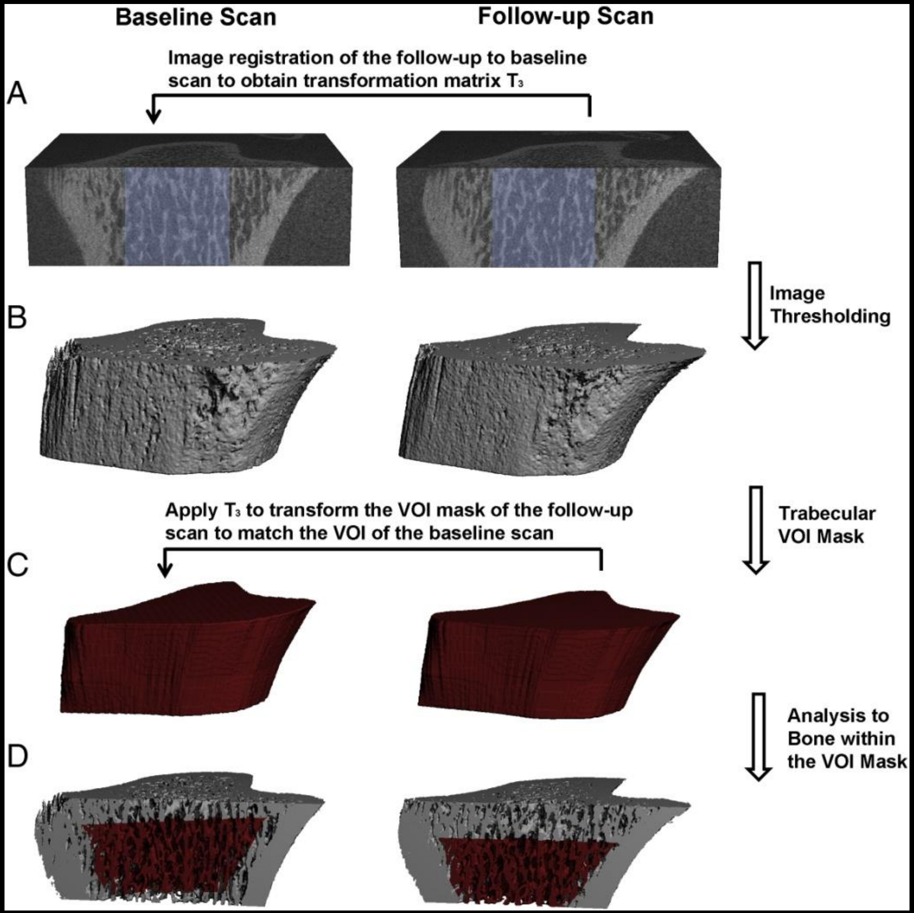
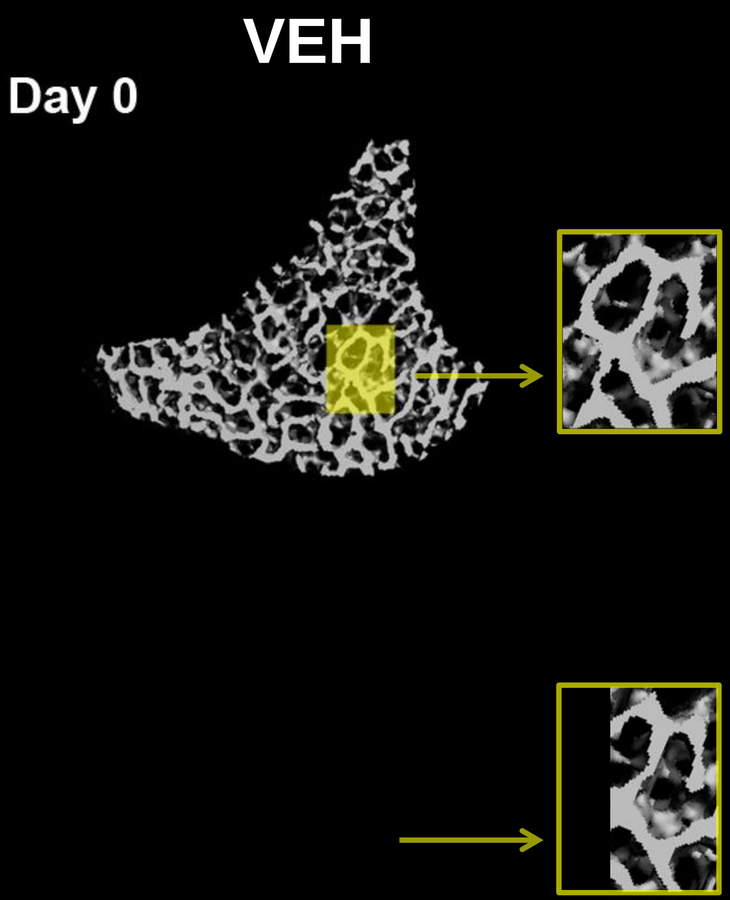
Image Registration and Analysis

- Same trabecular volume of interest (VOI) identified and subjected to analysis in the baseline and subsequent scans





Lan *et al.* 2013

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Concerns – Reposition error

- Significant but moderate improvement in precision error in all morphology and density measurements

  - Short term precision study (same day, multiple scans)
    - 12.5 µm, Precision: 1-6% in rats Nishiyama+2010

      → 1-4%
    - 10.5 µm, Precision: 1-8% in BL6 or C3H mice tibia Nishiyama+2010

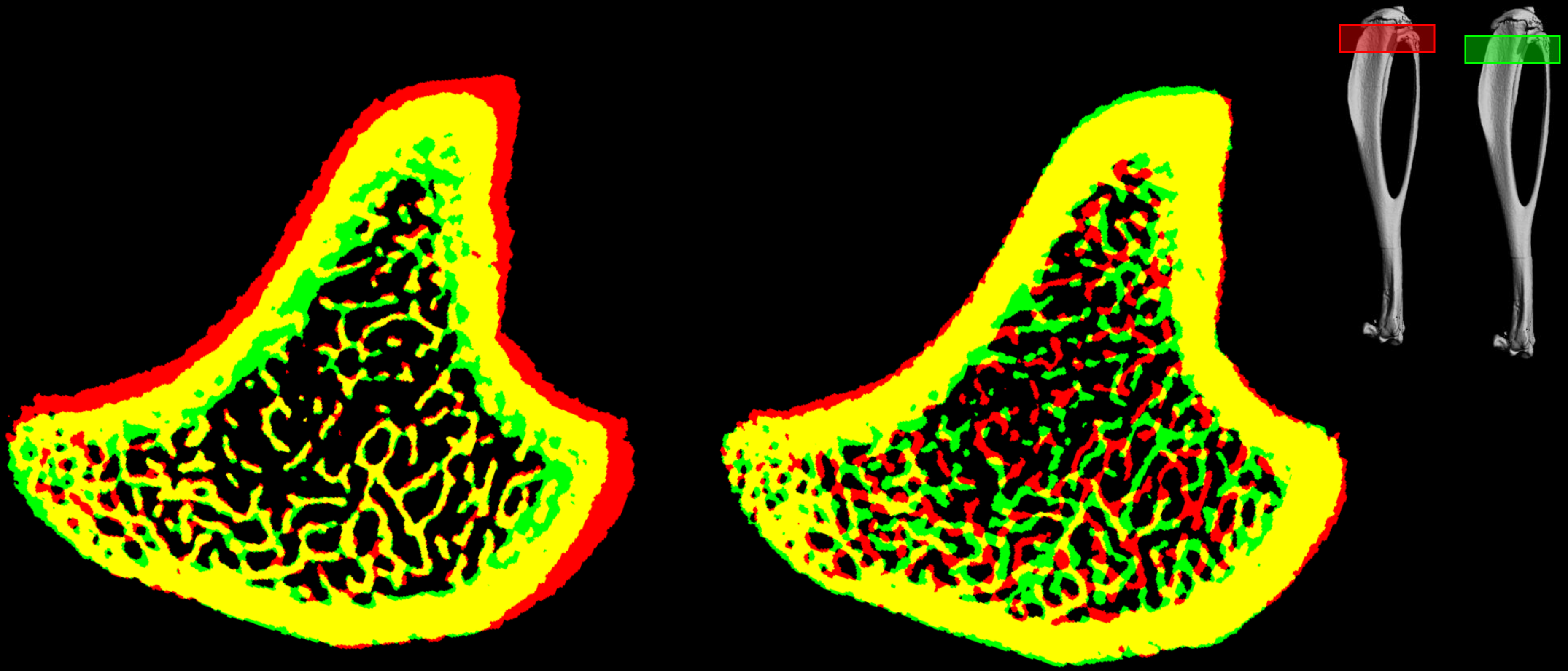
      → 1-5%
    - 10.5 µm, Precision: 0.85%-7.49% in rat tibia Lan+2013

      → 0.75%-7.01%
    - 10.5 µm, Precision: 4-12.4% in femur and 6.5-17.6% in L4 of BL6 mice

      Chang+2016 SB3C → 2.9-5.01% in femur and 3.11-8.55% in L4

Concerns – Long-Term Reposition error

- Continuous endochondral ossification in adult rats and mice



Repeated baseline scans and 14 day follow-up scans

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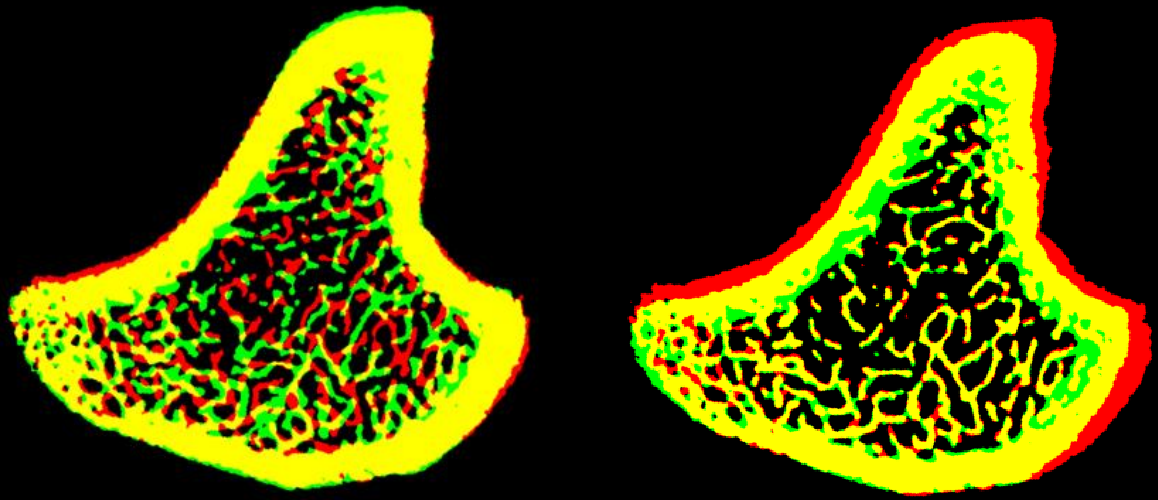
Lan *et al.* 2013

Concerns – Long-Term Reposition error



- *In vivo* µCT long-term precision based on 14-day follow-up scans
  - Significant difference between registered and unregistered comparisons
  - Results of unregistered comparisons are biased by global growth effect

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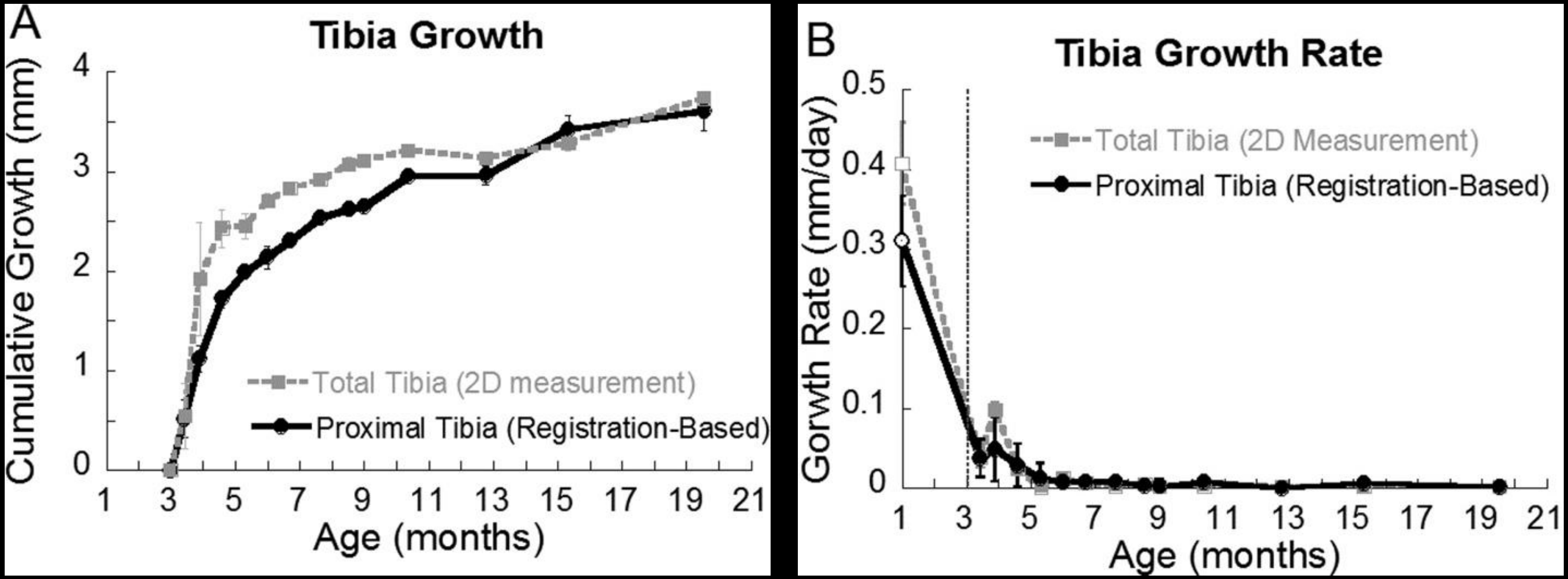
Age-Dependent Long Bone Growth



- Suggestion: rat age > 4 months for longitudinal study of changes in rat long bone

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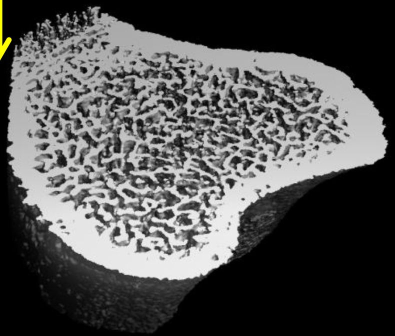
Lan *et al.* 2013

Bone Microarchitecture – Rat Proximal Tibia

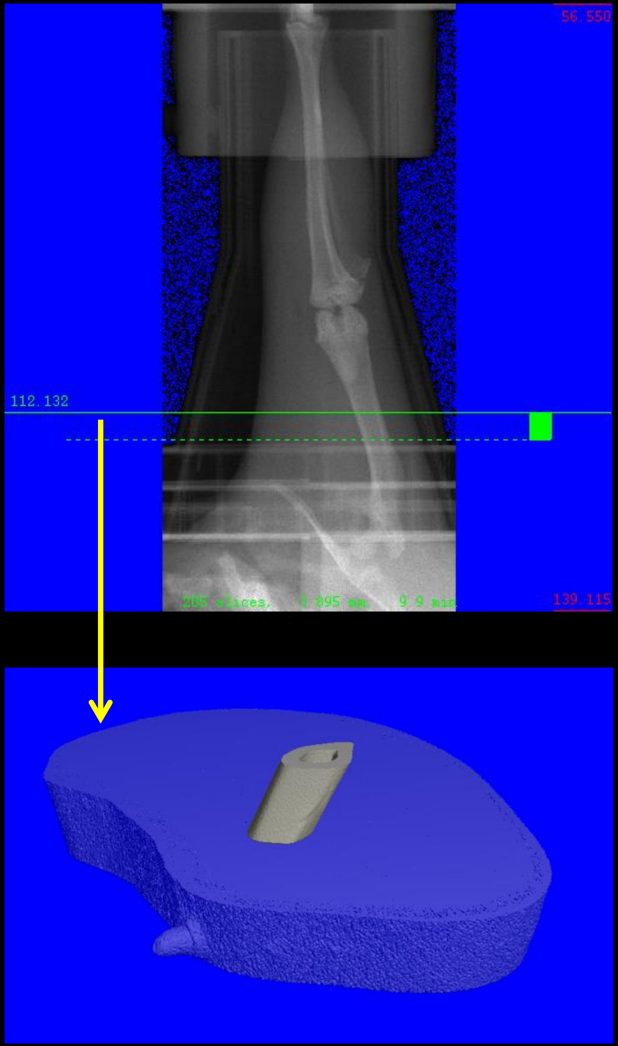
- *In vivo* µCT scan
  - 10.5 µm isotropic voxel size
  - 4 mm bone segment of proximal tibia below growth plate
  - Average scan time: 20 mins

Lan et al., Bone. 2013;56(1):83-90



Bone Microarchitecture – Rat Femur

- *In vivo* µCT scan
  - 19 µm isotropic voxel size
  - 2 mm bone segment of femur midshaft and muscle
  - Average scan time: 10 mins



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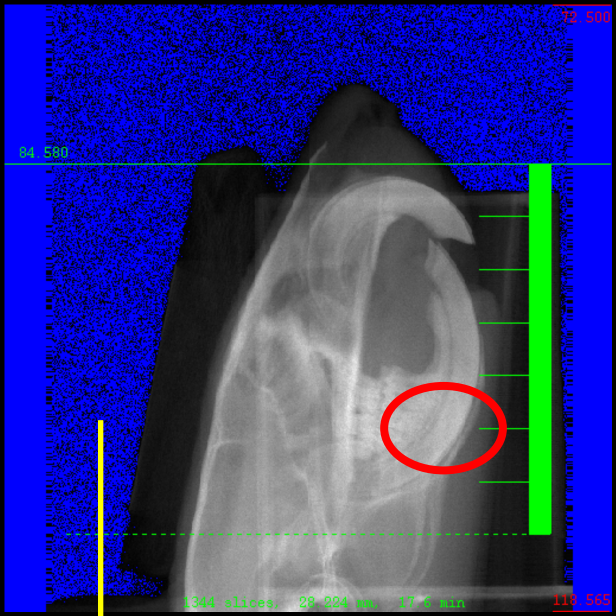
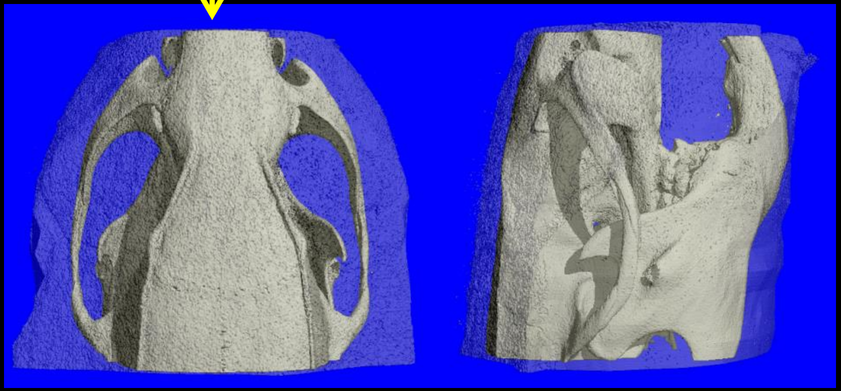
Bone Microarchitecture – Rat Mandible

- *In vivo* µCT scan
  - 19 µm isotropic voxel size
  - 28 mm bone segment of Mandible
  - Average scan time: 18 mins





Dr. Sunday Akintoye
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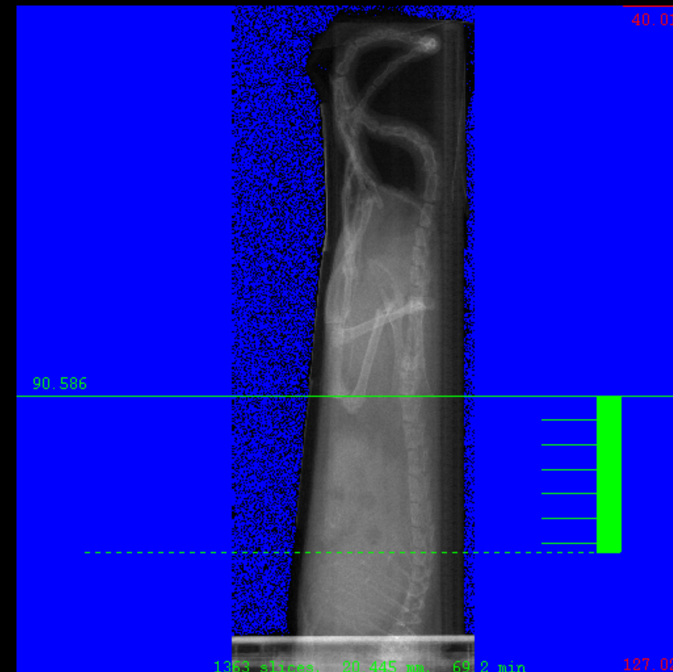
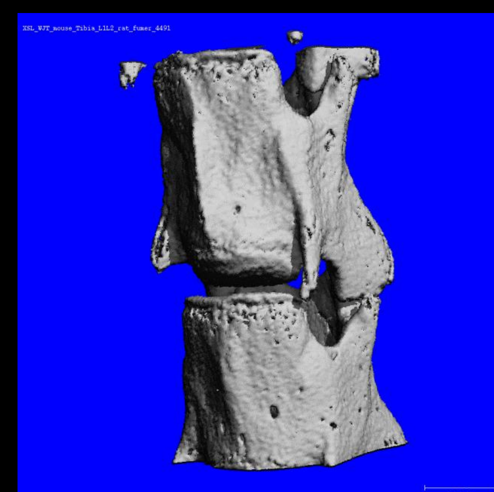
Bone Microarchitecture – Mouse Vertebrae

- *In vivo* µCT scan
  - 15 µm isotropic voxel size
  - 4 mm bone segment of L1, L2
  - Average scan time: 15 mins





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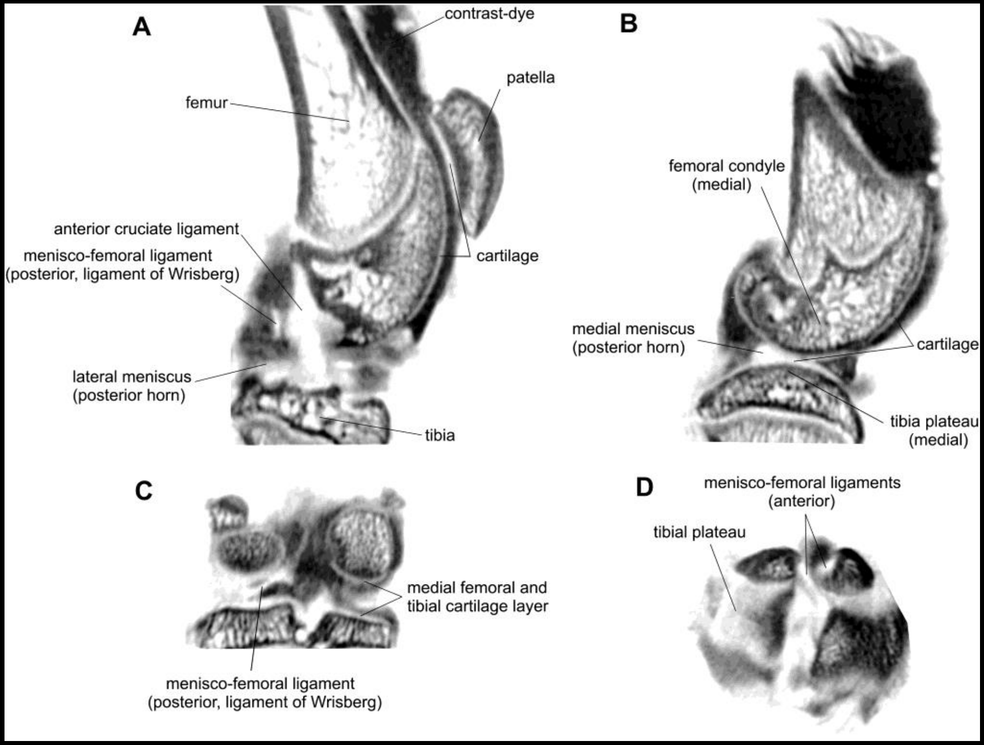
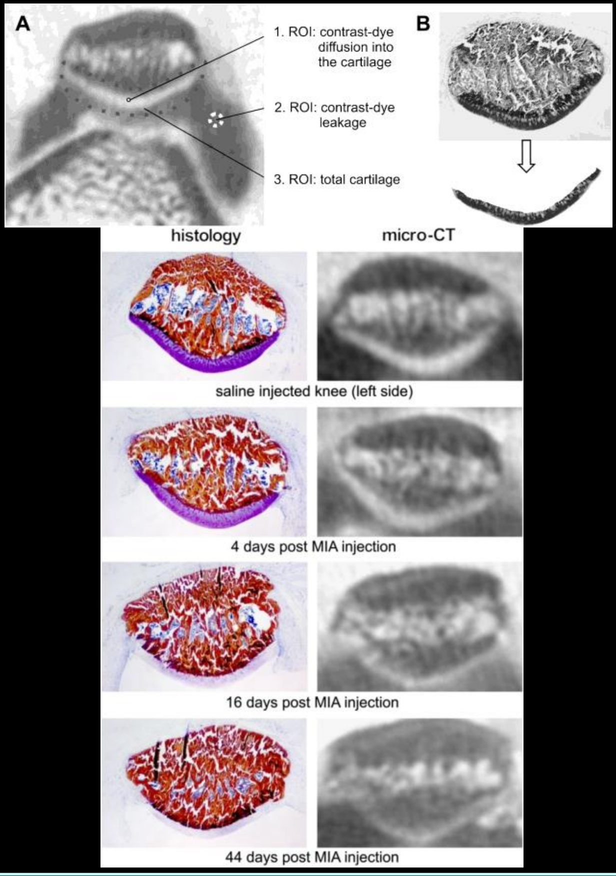
In vivo imaging of cartilage

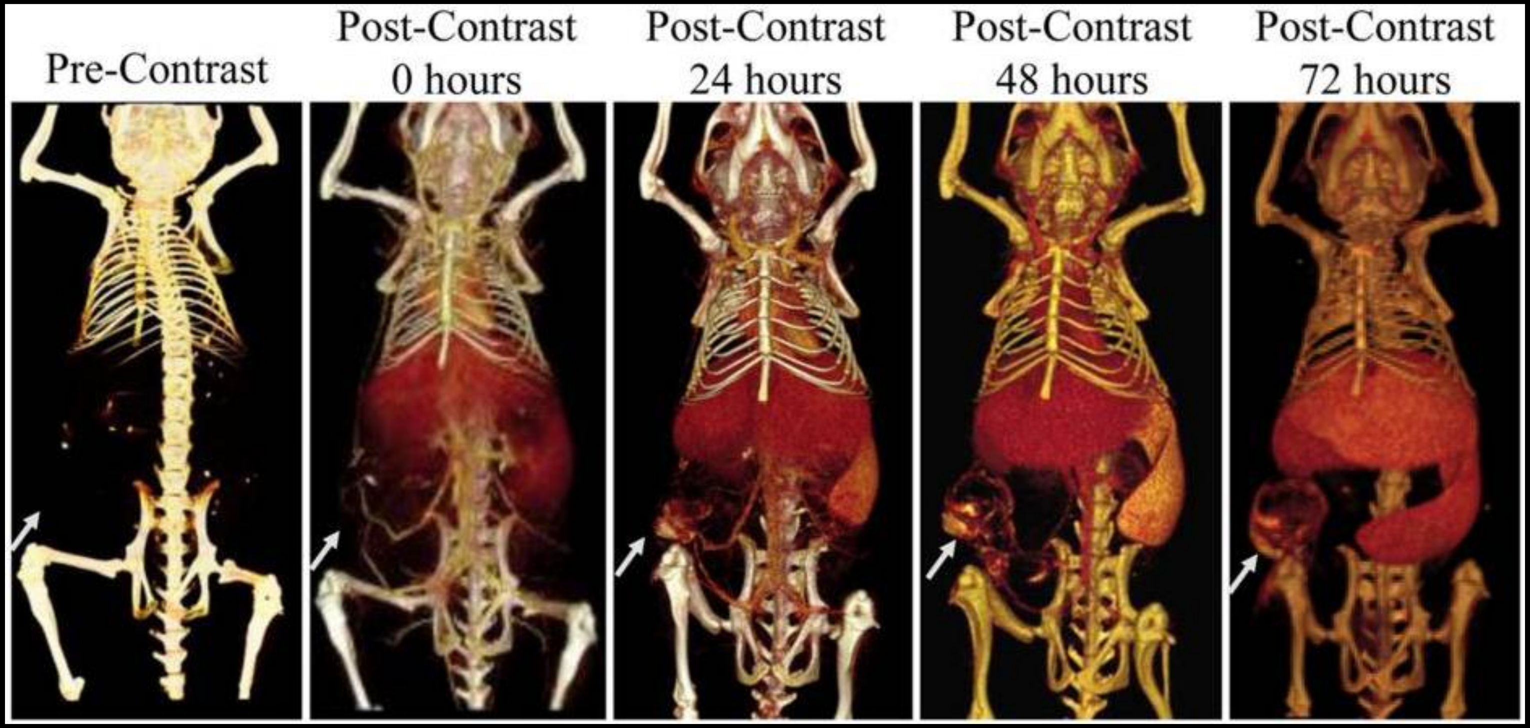
- μCT-arthrography Piscaer+ 2008
  - 35 μm isotropic voxel size (55 kV, 177 μA, FOV: 35 mm)
  - Injected Non-diluted Hexabrix320 (100 μl) into the knee cavity
- May monitor cartilage changes *in vivo* Piscaer+ 2008, Siebelt+ 2011





In vivo imaging of cancer

- Nanoparticles Ghaghada+ 2011, Ashton+ 2015
  - 80 µm isotropic voxel size (60 kV, 500 µA, 250ms/exposure)
  - Injected Liposomal iodine contrast agent
- To enhance the signal to locate the tumor *in vivo*



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Next generation *in vivo* µCT scanner

- *In vivo* µCT scanner
  - Scanco vivaCT 80
  - Best resolution:
    - 10.5 µm isotropic voxel size
  - X-Ray Source
    - 30 - 70 kVp
  - Max Scan Size
    - 80 x 145 m (Ø x L)
- Capacity to scan rat vertebrae

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Questions?

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