PCMD MicroCT Imaging Core Learning Lunch Series

In Vivo µCT Imaging of Live Rodents

Feb 23rd, 2023
PCMD MicroCT Imaging Core

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Penn Center for Musculoskeletal Disorders (P30-AR069619)

Outlines

- Brief introduction of our core facility
- Recent progress of our video tutorials, automated services
- In Vivo µCT Imaging of Live Rodents
- Q & A

µCT Imaging Core Resources

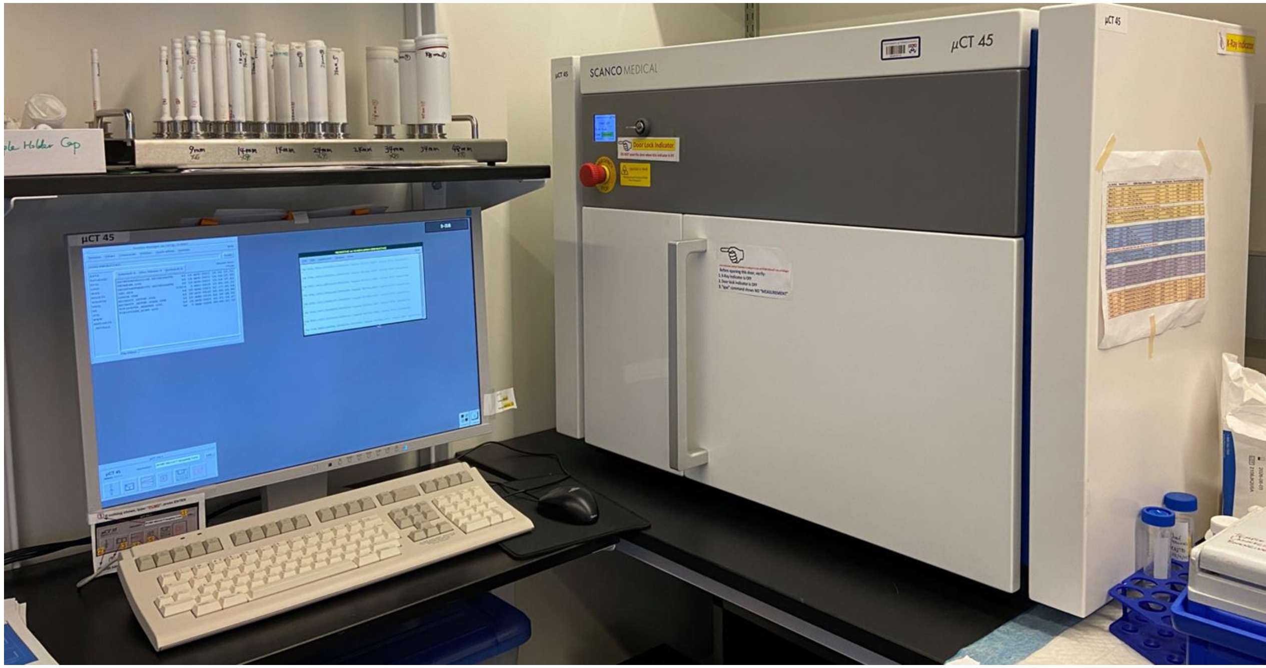
	Model	Location	Scan Size (ØxL;mm)	Voxel Size (µm)	Applications
1	µCT 35	Stemmler Hall	37.9 x 120	3.5-72	High resolution *ex vivo* scans
2	µCT 45	Stemmler Hall	50 x 120	3.0-100	High resolution *ex vivo* scans
3	vivaCT 40	Stemmler Hall	38.9 x 145	10.5-76	High resolution *in vivo* scans for small animals
4	vivaCT 80	Stemmler Hall	80 x 145	10.4-76	High resolution *in vivo* scans for small animals
5	µCT 50	PVAMC/TMRC	50 x 120	0.5-100	Ultra high resolution (sub-micron) *ex vivo* scans
6	vivaCT 75	PVAMC/TMRC	79.9 x 145	21-150	*In vivo* scans for small animals; *ex vivo* scans for large specimens
7	XtremeCT II	CTRC	140 x 200	60-82	Clinical scans for peripheral skeleton

Ex vivo (Specimen) Scanners

- Scanco µCT 35 (Purchased in 2012)
  - Native voxel sizes: 3.5 µm, 6 µm, 10 µm, 15 µm, 18.5 µm

Ex vivo (Specimen) Scanners

- Scanco µCT 45 (Purchased in 2019 ***new!***)
  - Native voxel sizes: 3 µm, 4.5 µm, 7.4 µm, 10.4 µm, 14.6 µm
  - Carousel system supporting 20 sample holders
  - "Air" filter for scanning low density materials
  - "Copper" filter for scanning specimen with metal implant



In vivo Scanners

- Scanco vivaCT 40 (Purchased in 2010)
  - Voxel sizes: 10.5 μm, 12.5 μm, 15 μm, 17.5 μm, 19 μm

In vivo Scanners

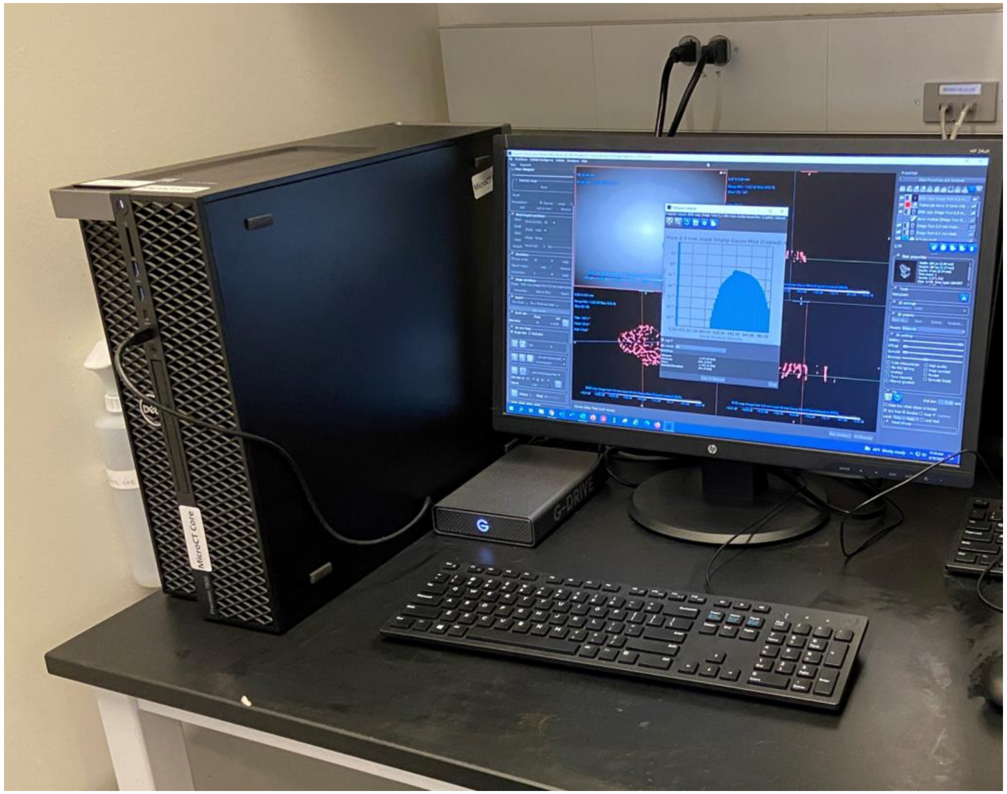
- Scanco vivaCT 80 (Purchased in 2018 ***new!***)
  - Voxel sizes: 10.4 μm, 11.6 μm, 13 μm, 16.1 μm, 20.8 μm, 26 μm
  - Internal heating device to keep animal warm
  - Internal camera to monitor animal's breathing
  - Ex vivo scan for specimen from large animals or human cadaver

MicroCT Analysis PC

- 2 PCs for MicroCT Analysis (315 Stemmler)
  - Windows 10 platform
  - Either remote or onsite access
  - Scanco software

Dragonfly Workstation

- Workstation for Dragonfly software (324 Stemmler)
  - Windows 10 platform
  - PMACS account required (either remote or onsite access)
  - Deep learning assisted analysis
  - Training videos https://www.theobjects.com/dragonfly/tutorials.html



Video Tutorials & Instruction Documents

https://www.med.upenn.edu/pcmd/mctimagingcore/user-tutorials.html

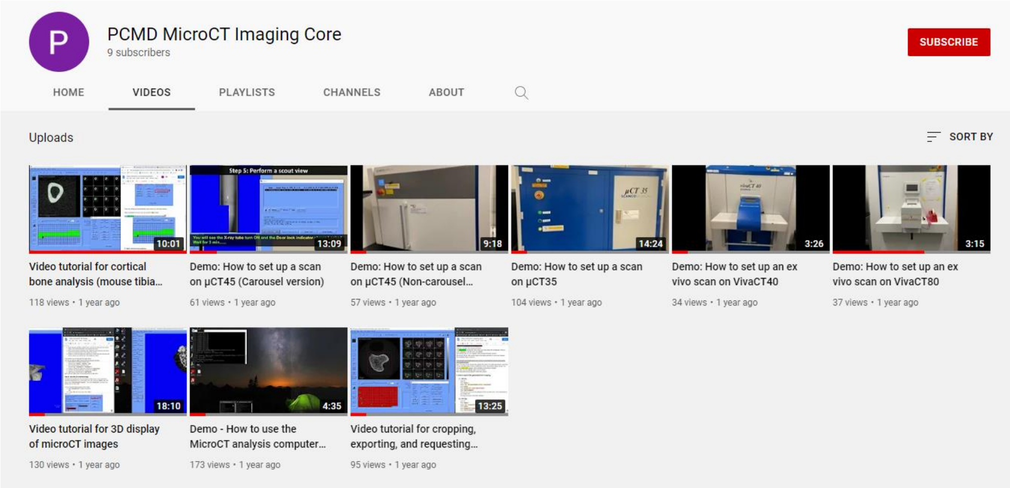
https://www.youtube.com/channel/UCzznR9Fdv-3kjEX7miwsi0A

Video Tutorials:

Our YouTube channel: https://www.youtube.com/channel/UCzznR9Fdv-3kjEX7miwsioA/

µCT scan setup:

1. How to set up a scan on µCT35 (PDF download) (Video download)
   https://www.youtube.com/watch?v=QUtoQqlYJ8o
2. Demo: How to set up a scan on µCT45 (Recommended: Carousel version)
   (PDF download) (Video download)
   Note: To use this Carousel version, please remove the sample holder on the rotation stage.
   https://www.youtube.com/watch?v=fzlfffR5XyE
3. Demo: How to set up a scan on µCT45 (Non-carousel version) (PDF download) (Video download)
   Note: To use this Non-carousel version, please remove all sample holders on the carousel.
   https://www.youtube.com/watch?v=JEoLn1igEjE
4. How to set up an ex vivo scan on VivaCT40 (PDF download) (Video download)
   https://www.youtube.com/watch?v=sxvTV4bvosw
5. How to set up an ex vivo scan on VivaCT80 (PDF download) (Video download)
   https://www.youtube.com/watch?v=HdQYWwjulXM



µCT viewing & analysis:

1. How to use "microCT Analysis" computers (PDF download) (Video download)
   https://www.youtube.com/watch?v=qHHcB6KJJe4
2. Tutorial for cropping, exporting, and requesting microCT images (PDF download) (Video download)
   https://www.youtube.com/watch?v=umRF6ODcQqQ
3. Tutorial for 3D display of microCT images (PDF download) (Video download)
   https://www.youtube.com/watch?v=YdQSo41rgR8
4. Tutorial for cortical bone analysis (mouse tibia midshaft) (PDF download) (Video download)
   https://www.youtube.com/watch?v=B4OE9X8Bkwg

Publications from our users

- Our users have published over 250 journal articles on their µCT projects.
- Selected publications with detailed µCT protocols for other users to cross reference:

https://www.med.upenn.edu/pcmd/mctimagingcore/publications.html

1. Calified Tissue Imaging

1.1. Skeletal Phenotyping

1.1.1.Rodents

OA study (gene therapy): Proximal tibia of Sprague-D

Mason, J.B., et al., Wnt10b and Dkk-1 gene therapy diffe and osteophytosis in a skeletally mature rat model of o

OA study (DMM Model): Medial epiphysis of the mice

Sambamurthy, N., et al., Chemokine receptor-7 (CCR7) deficits in a murine model of osteoarthritis. J Orthop R

Sambamurthy, N., et al., Deficiency of the pattern-reco decline in a murine model of osteoarthritis. PLoS One,

2. Non-calcified Tissue Imaging

2.1. Cartilage Imaging

Cartilage repair: Osteochondral specimens from t

Friedman, J.M., et al., Comparison of Fixation Techni Weightbearing Porcine Large Animal Model. Cartila

Pfeifer, C.G., et al., Age-Dependent Subchondral Bo Part C Methods, 2017. 23(11): p. 745-753.

Patel, JM., et al., Resorbable pins to enhance scaffol 1947603520962568.

3. In Vivo Small Animal Imaging

Reproducibility and Radiation study: Mice distal fe

Zhao, H., et al., Reproducibility and Radiation Effect Mouse Lumbar Vertebra and Long Bone. Ann Biome

Bone remodeling study: Longitudinal *in vivo* scan

de Bakker, C.M.J., et al., Minimizing Interpolation Bia Structure and Dynamics. Ann Biomed Eng, 2016. 44

Reproduction cycles study: Longitudinal *in vivo* sc

de Bakker, C.M., et al., Adaptations in the Microarchi Response to Multiple Reproductive Cycles in Rats. J

4. Clinical Imaging

HR-pQCT scanner (XtremeCT II), human tibia

Zhao, X., et al., Feasibility of assessing bone mat One, 2017. 12(3): p. e0173995.

5. Other Imaging

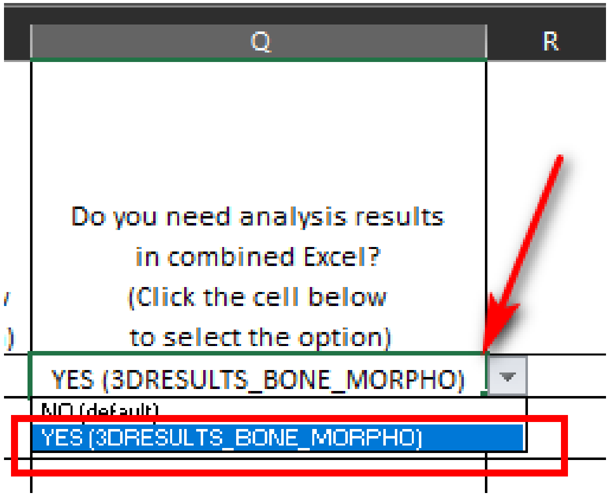
Metal implants in rat brain (90 kVp with a copper filter

Burton A, et al., Wireless, battery-free, and fully implantat Nanoeng. 2021;7:62.

Fully Automated Services

- File request: fully automated service sharing MicroCT files to users (running 7/24)
- Auto compiling microCT results into Excel sheet

https://www.med.upenn.edu/orl/uct/assets/user-content/secure/User_file_request%20(v2020.01).xlsx



- Sample realignment/reorientation request: fully automated service help users to do sample realignment (running 7/24)

https://www.med.upenn.edu/orl/uct/assets/user-content/secure/Sample_Realignment_request(v2020.01).xlsx

µCT Troubleshooting Guide

https://www.med.upenn.edu/orl/uct/assets/user-content/documents/microct-troubleshooting-guide.pdf

Why *in vivo* µCT?

- µCT provides 3D imaging with sufficient spatial resolution for the assessment of rodent bone microarchitecture
- *In vivo* µCT: 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 due to pharmacologic treatment or mechanical loading
- Input to micro finite element (µFE) models to track the mechanical properties of bone van Rietbergen+1998, Schulte+2011
- Increased statistical power
- Reduction in number of animals Bouxsein+ 2010

vivaCT 40

- vivaCT 40 (Purchased in 2010)
  - 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)
  - Capacity to scan:
    All tissues on mice
    Rat tibia

vivaCT 80

- vivaCT 80 (Purchased in 2018)
  - Best resolution:
    10.4 µm isotropic voxel size
  - X-Ray Source:
    30 - 70 kVp
  - Max Scan Size:
    80 x 145 mm (Ø x L)
  - Capacity to scan:
    All tissues on mice
    All tissues on rat
    (body weight < 700g)

In Vivo µCT Imaging

1. Animal Model (Rats, mice)
2. Scanning Resolution
3. Sample Holder
4. Before Scanning: Anesthesia
5. During Scanning: Monitor animal breathing
6. After Scanning: Animal waking up

In Vivo μCT Imaging

Animal Model (Rats, mice)

↓

Scanning Resolution

↓

Sample Holder

↓

Before Scanning:
Anesthesia

↓

During Scanning:
Monitor animal breathing

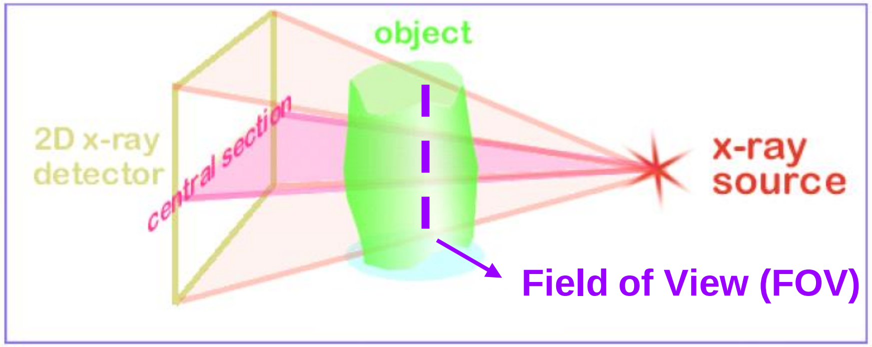
↓

After Scanning:
Animal waking up

How to Choose Image Resolution (vivaCT 40)

- Image resolution is determined by FOV and number of projections

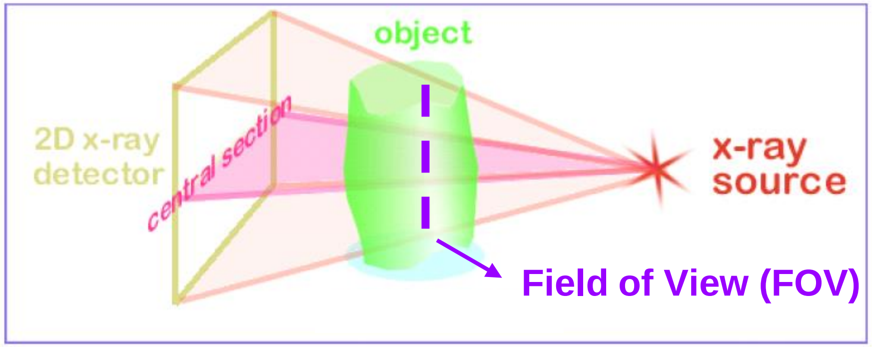
vivaCT40 Field of View (mm)	Proj./180°	Best Resolution (µm)
21.5	1000	10.5
25.6	1000	12.5
30.7	1000	15
35.8	1000	17.5
38.9	1000	19



How to Choose Image Resolution (vivaCT 80)

- Image resolution is determined by FOV and number of projections

vivaCT80 Field of View (mm)	Proj./180°	Best Resolution (µm)
31.9	1500	10.4
35.9	1500	11.6
39.9	1500	13.0
49.8	1500	16.1
63.9	1500	20.8
79.9	1500	26.0



Radiation Dose – VivaCT 40

- Computed Tomography Dose Index (CTDI): Proportional to the integration time (s), 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

Radiation Dose – VivaCT 80

- Computed Tomography Dose Index (CTDI): Proportional to the integration time (s), 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	145	32	1500	1537	10.4
55	300	145	40	1500	998	13.0
55	300	145	50	1500	615	16.1
55	300	145	64	1500	368	20.8
55	300	145	80	1500	230	26.0

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 all 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%)

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*** to reduce radiation exposure:
    - Reduction in scan frequency and Increase in interval time between repeated scans
    - Reduction in scan resolution

In Vivo µCT Imaging

1. Animal Model (Rats, mice)
2. Scanning Resolution
3. Sample Holder
4. Before Scanning: Anesthesia
5. During Scanning: Monitor animal breathing
6. After Scanning: Animal waking up

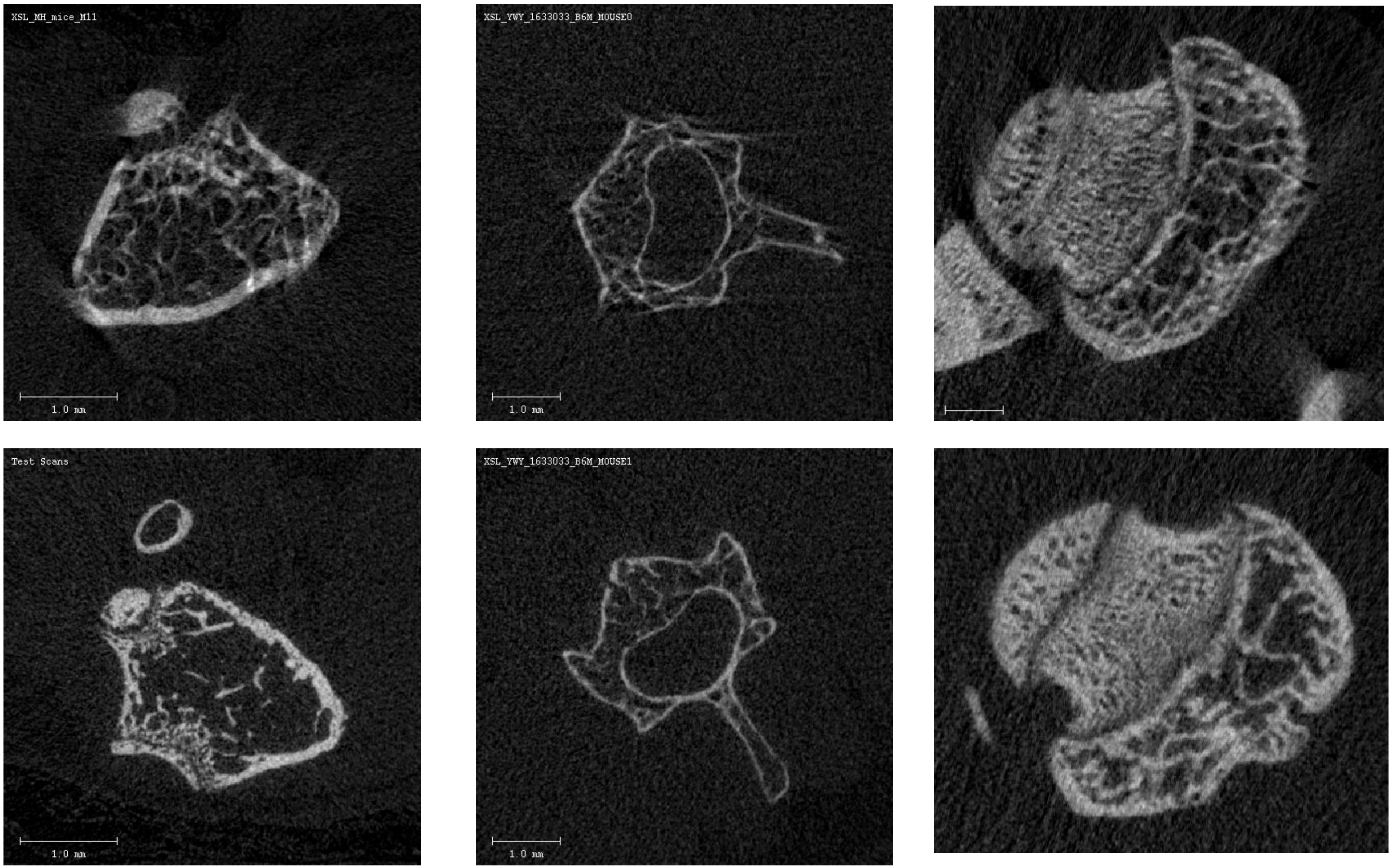
Why Need Holder? Movement Artifacts

- Movement Artifacts caused by **animal breathing**

Why Need Holder? Movement Artifacts

- Movement Artifacts due to **animal breathing**

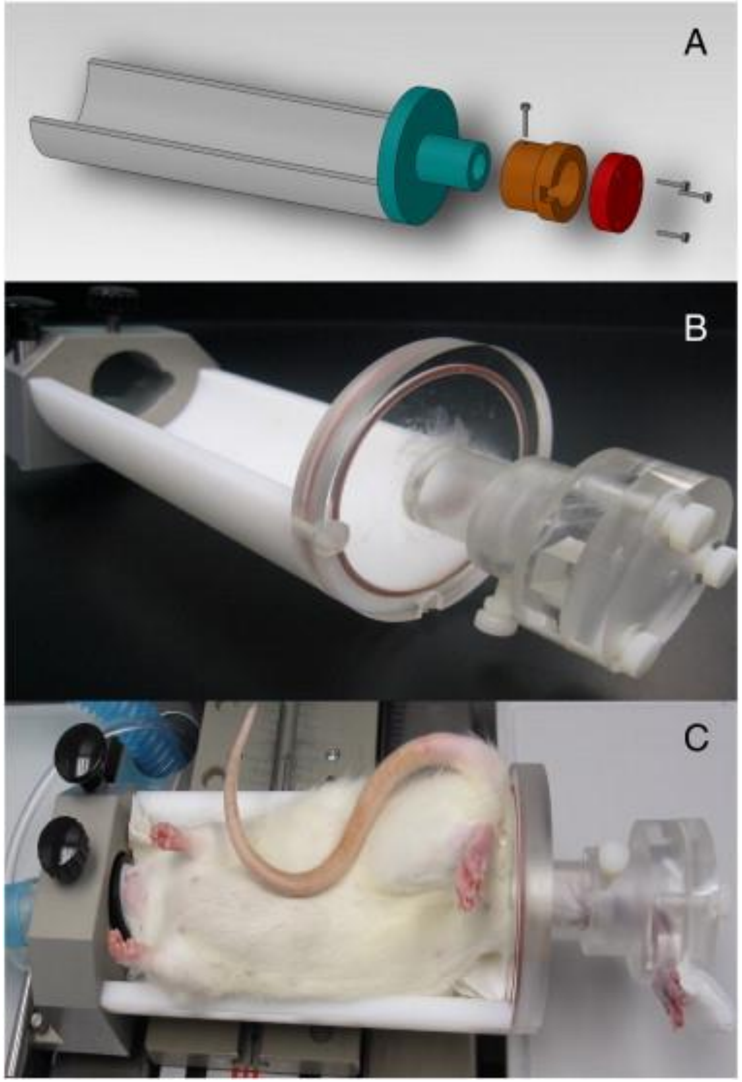
Distal Femur | **L2 Vertebrae** | **Humerus**



Customized Holders - 3D Printing

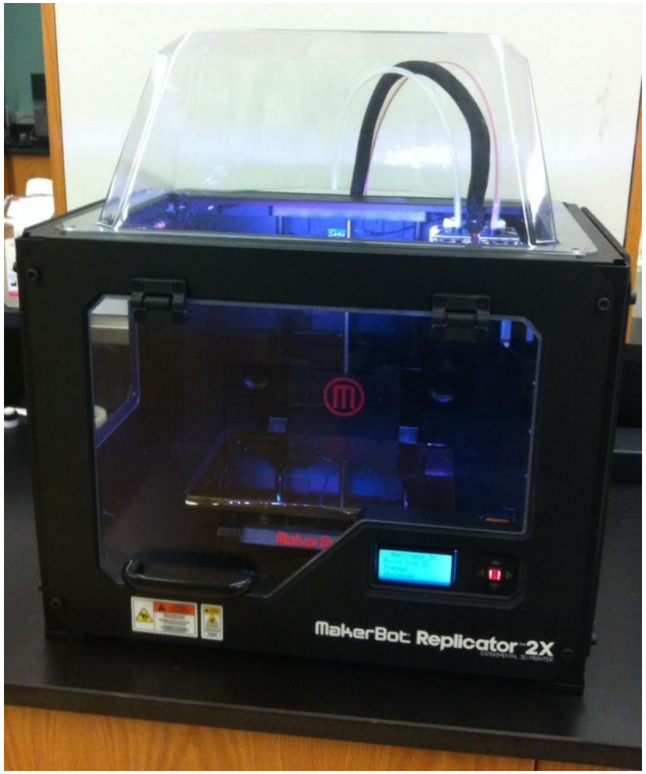
- Minimize the movement
  of the skeletal site of interest

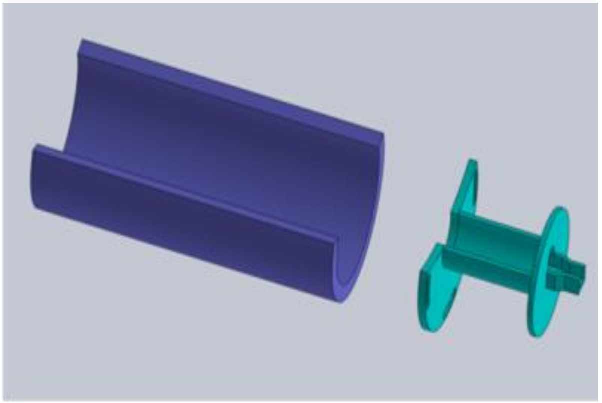
- Minimize the reposition error
  induced by repeat scans

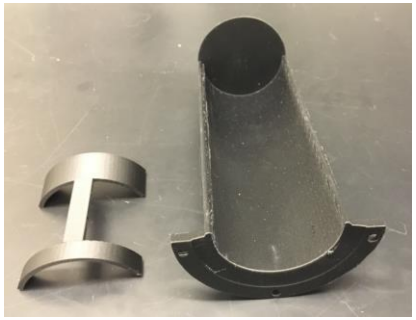


Rat tibia holder

Customized Holders - 3D Printing







In Vivo μCT Imaging

Animal Model (Rats, mice)

↓

Scanning Resolution

↓

Sample Holder

↓

Before Scanning:
Anesthesia

↓

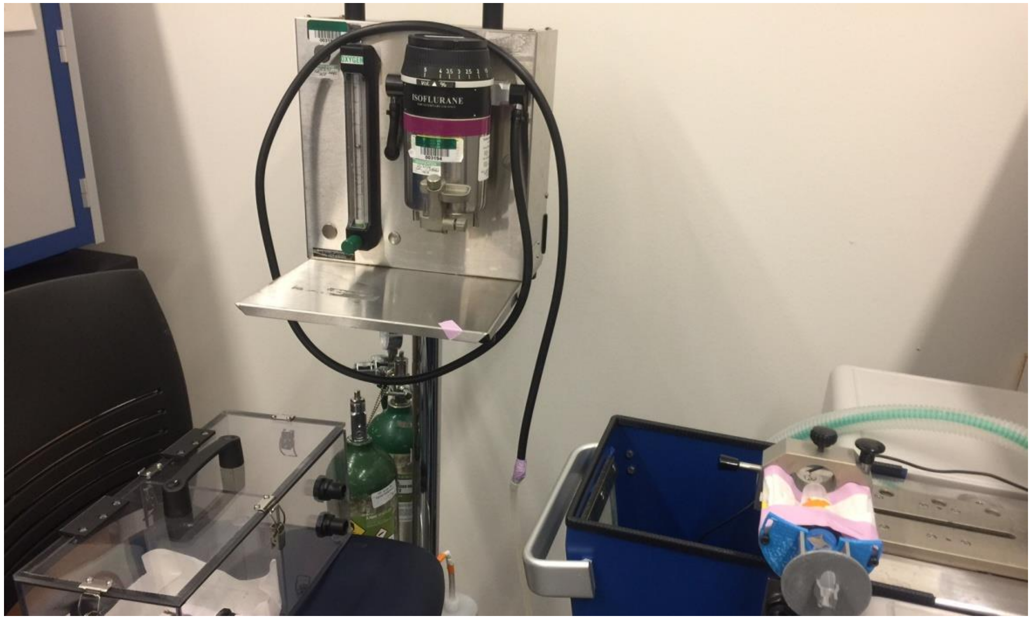
During Scanning:
Monitor animal breathing

↓

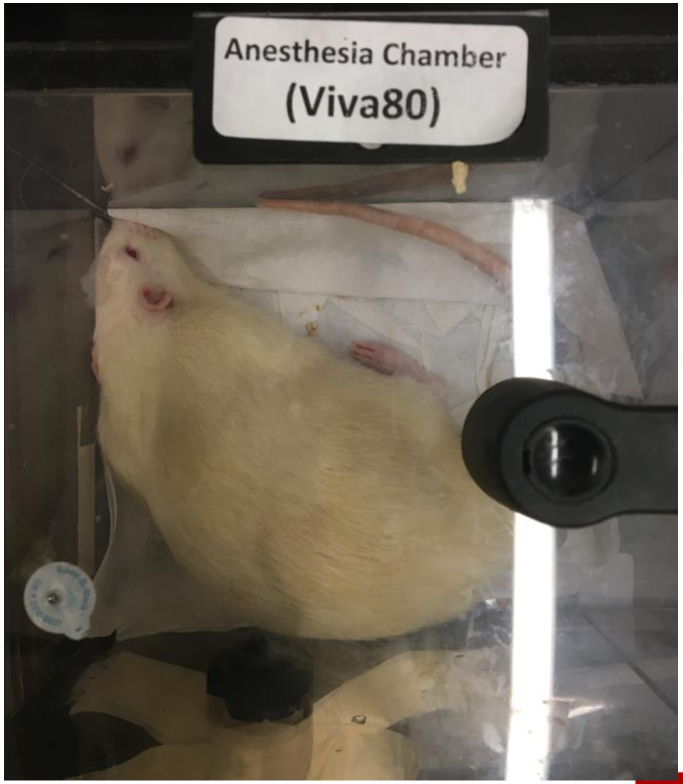
After Scanning:
Animal waking up

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



Anesthesia chamber



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

- Advantages of Isoflurane (vs. Ketamine/xylazine)
  - Safer
  - Faster (induction, adjusting depth and recovery)
  - No need for reversal agents

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

During Scanning

- Monitor animal's breathing

After Scanning

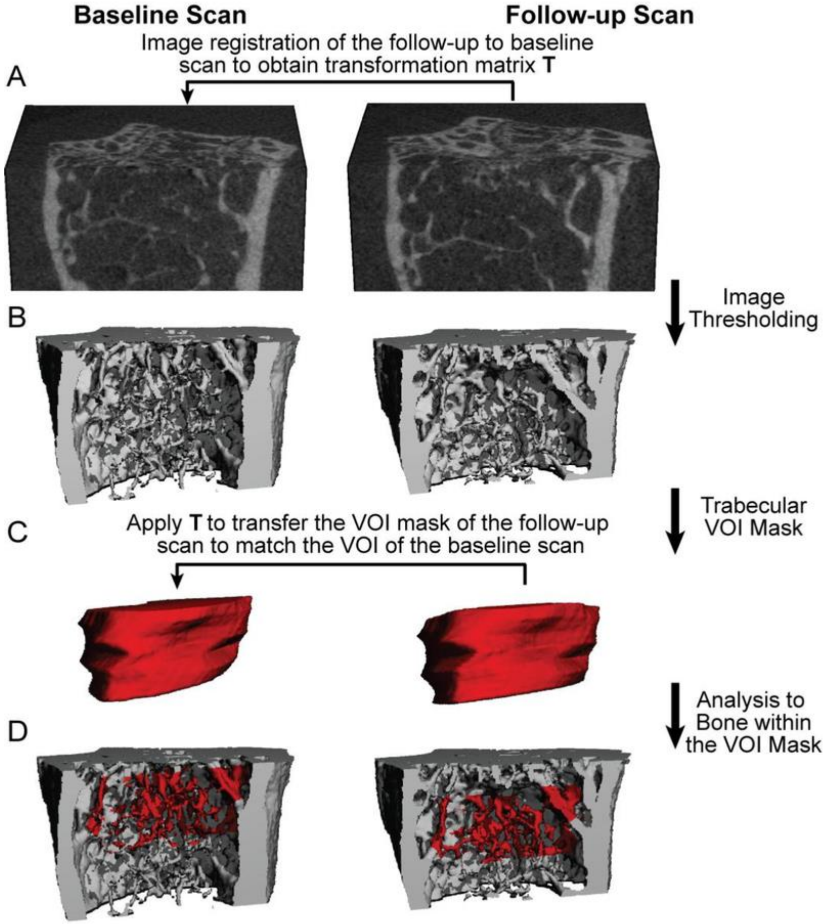
- Waking up the animal: Heating lamp

Precision error & Reposition error

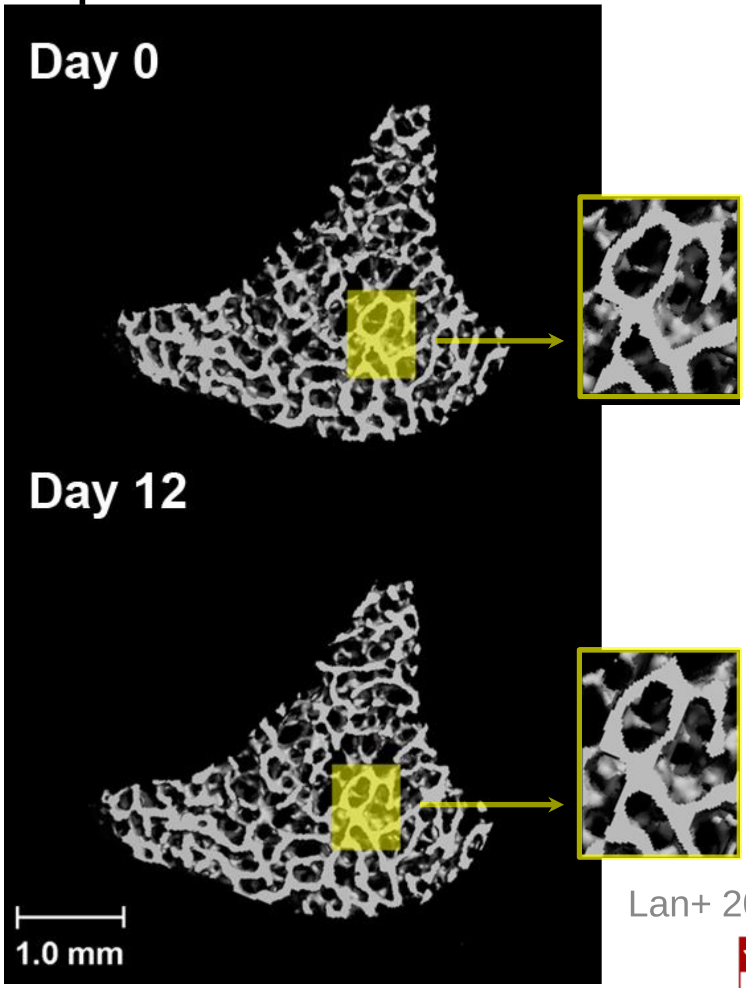
- Precision error: Measurement error between repeated scans of the same sample
- Precision affected by reposition of animals at each follow-up scan
  - Short term precision study (same day, multiple scans)
    - 12.5 µm, Precision: 1-6% in rats Nishiyama+2010
    - 10.5 µm, Precision: 1%-7% in rat tibia 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
- Reduction in the reposition error
  - Customized animal holders for the scan
  - Image registration

Image Registration for Analysis

- To identify the same trabecular volume of interest (VOI) for analysis in the baseline and follow-up scans



Lan+ 2013



Lan+ 2013

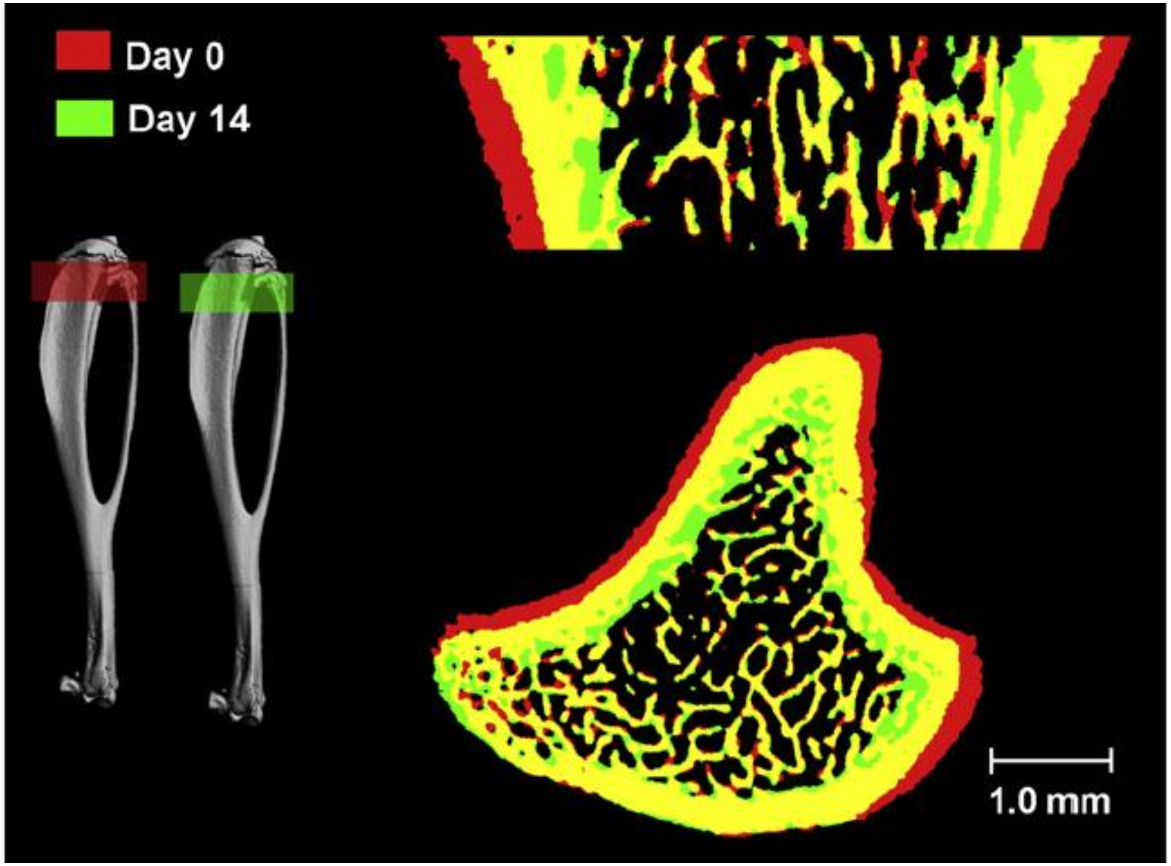
After Image Registration

- 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

Long-Term Precision After Image Registration

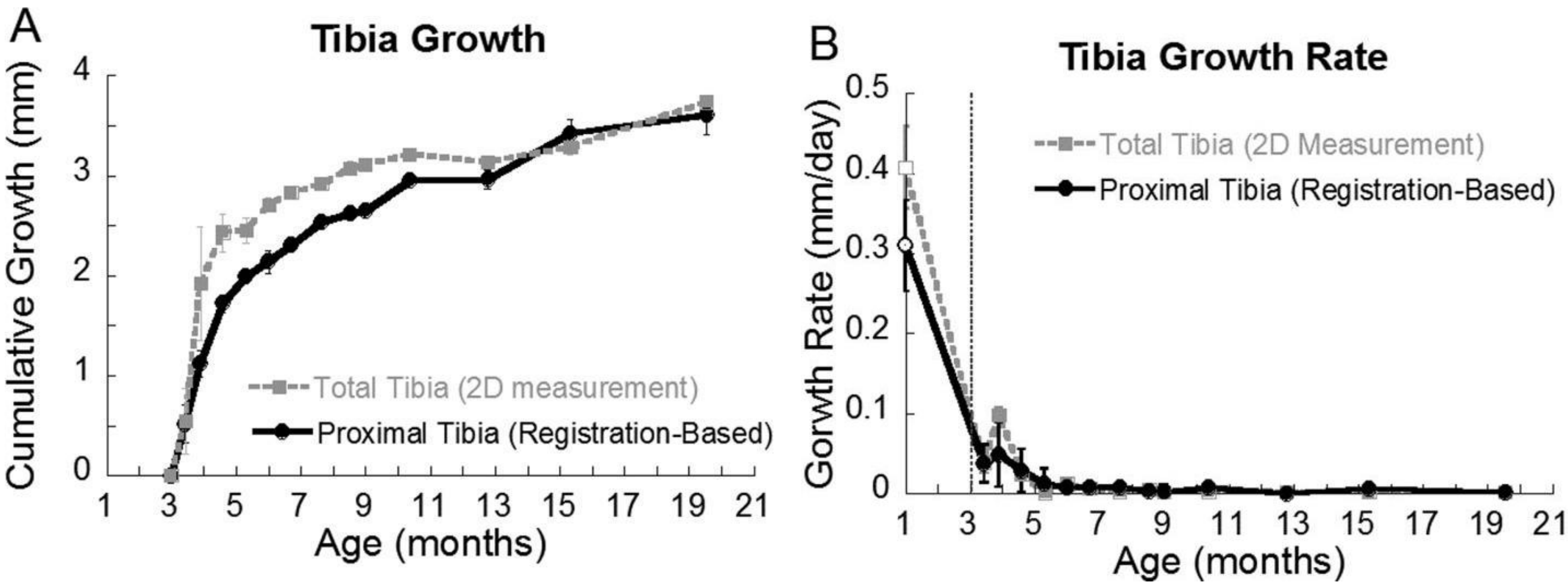
- Continuous bone resorption at the periosteum, bone formation at the endocortical surface.



Lan+ 2013

Baseline scan overlaid with 14 day follow-up scan

Age Selection to Study Long Bone Changes

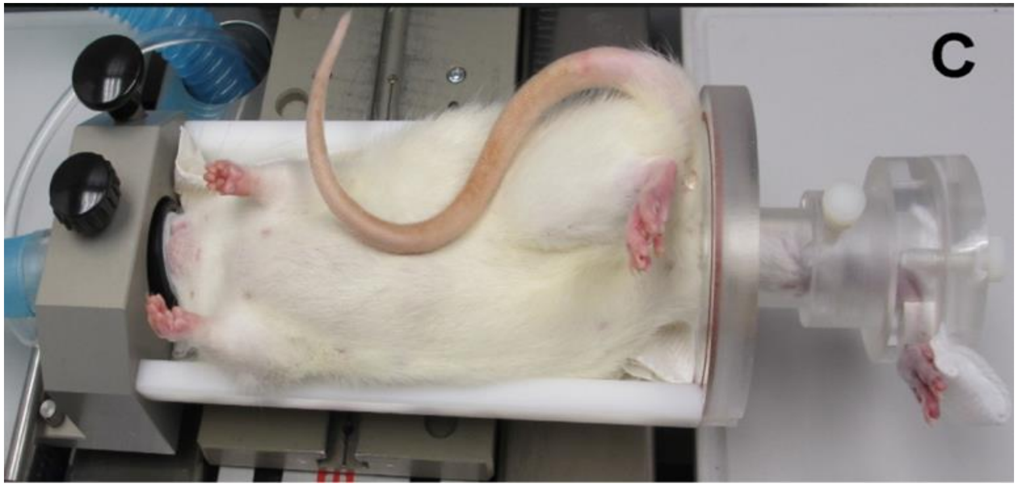


Altman+ 2015

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

User Application – Rat Proximal Tibia

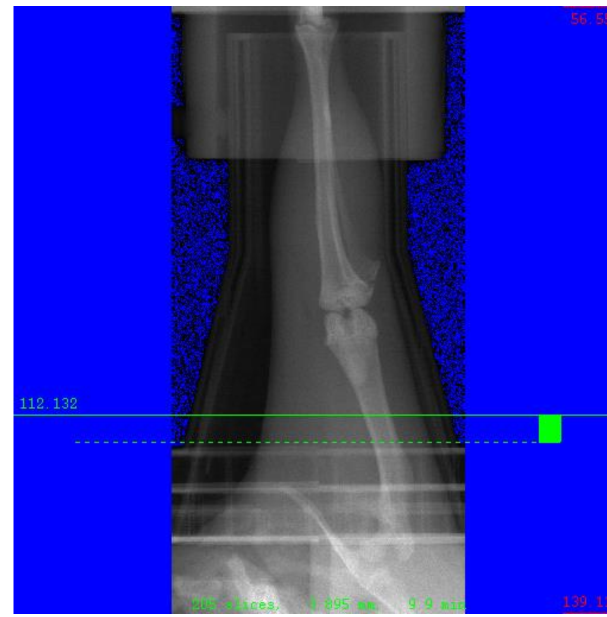
- In vivo µCT scan
  - 10.5 µm isotropic voxel size
  - 4 mm bone segment of proximal tibia below growth plate
  - Average scan time: 20 mins



Lan+ 2013

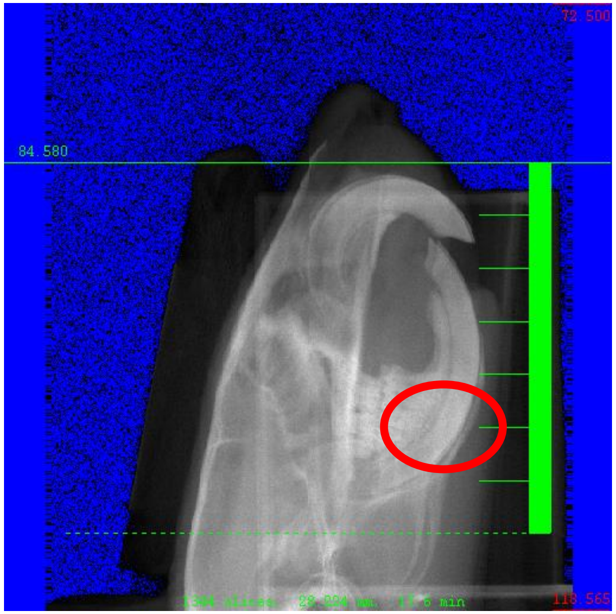
User Application – Rat Femur

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



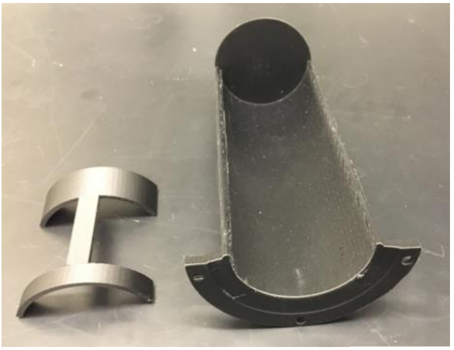
User Application – Rat Mandible

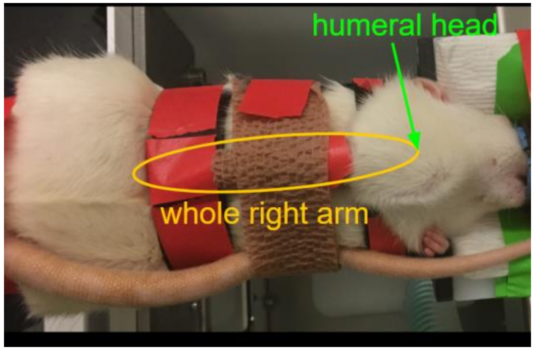
- In vivo µCT scan
  - 19 µm isotropic voxel size
  - 28 mm bone segment of Mandible
  - Average scan time: 18 mins

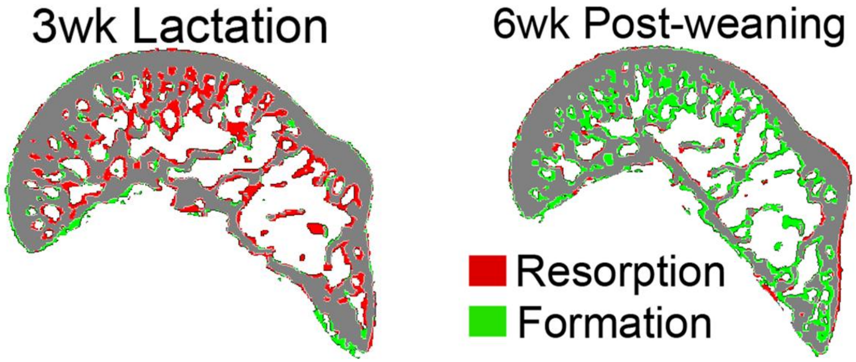


User Application – Rat Humerus

- In vivo µCT scan
  - 20.8 µm isotropic voxel size
  - 6.82 mm bone segment of humerus bone
  - Average scan time: 20 mins



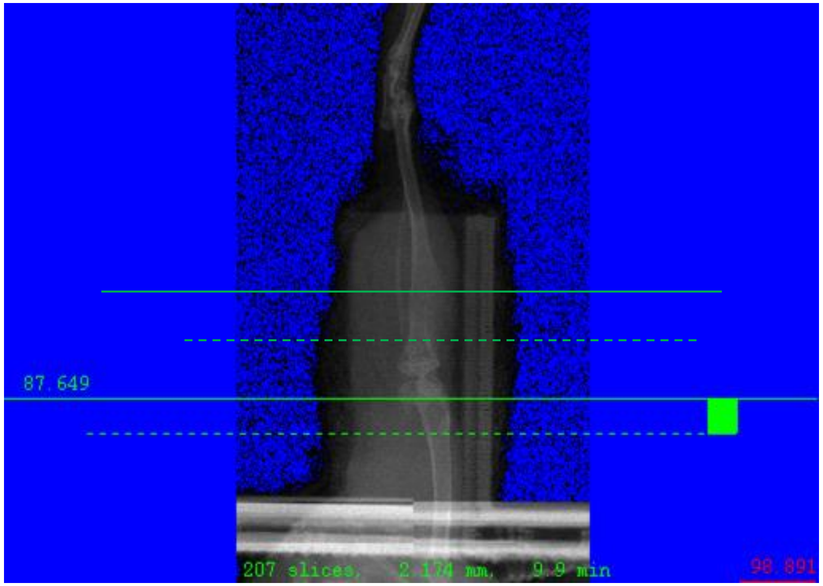




User Application – Mouse Tibia & Femur

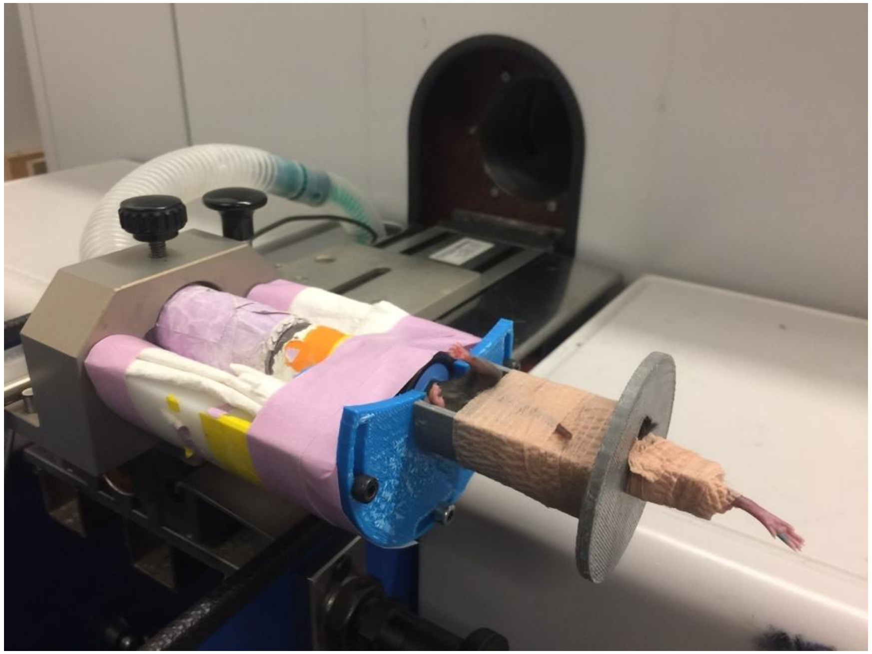
- In vivo µCT scan
  - 10.5 µm isotropic voxel size
  - 2 mm bone segment of proximal tibia, distal femur
  - Average scan time: 10 mins

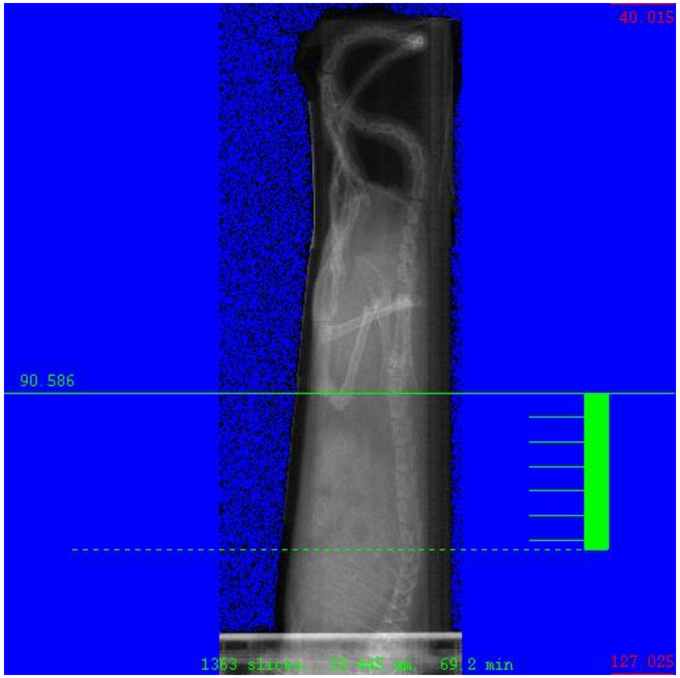
Zhao+ 2020



User Application – Mouse Vertebrae

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





Questions?