Penn Center for Musculoskeletal Disorders

PCMD Integrated Cores Learning Lunch Series
(MicroCT and Biomechanics)

Working with Rodent in vivo Dynamic Loading Systems

CTPros FEA Module Update

3D-Printed Animal Holders for in vivo MicroCT Scans

Xiaoyu Xu

Wen Sang

Fen 17 2025
PCMD MicroCT Imaging Core

Penn Center for Musculoskeletal Disorders (P30-AR069619)

Outlines

- Brief introduction of our core facility
- Rodent in vivo dynamic loading systems
- CTPros update and 3D-printed holders
- Q & A

μCT Imaging Core Resources

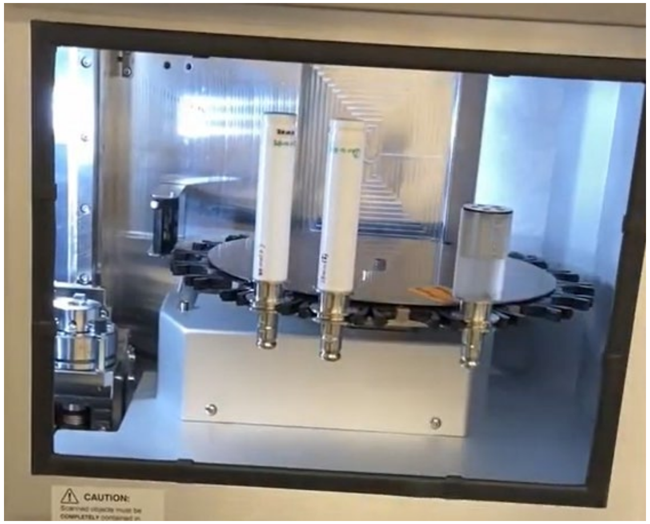
Model	Location	Scan Size (ØxL)	Voxel Size (μm)	Applications
μCT 35	Stemmler 335A	37.9 x 120	3.5-72	High resolution *ex vivo* scans
μCT 45	Stemmler 335A	50 x 120	3.0-100	High resolution *ex vivo* scans
vivaCT 80	Stemmler 368B	80 x 145	10.4-76	High resolution *in vivo* scans
DXA	Stemmler 368C	165 x 255	100	Full body 2D scan and analysis
μCT 50	PVAMC/TMRC	50 x 120	0.5-100	Ultra high resolution (sub-micron) *ex vivo* scans
vivaCT 75	PVAMC/TMRC	79.9 x 145	21-150	*In vivo* scans for small animals; *Ex vivo* scans for large specimens
XtremeCT II	CTRC	140 x 200	60-82	Clinical scans for peripheral skeleton

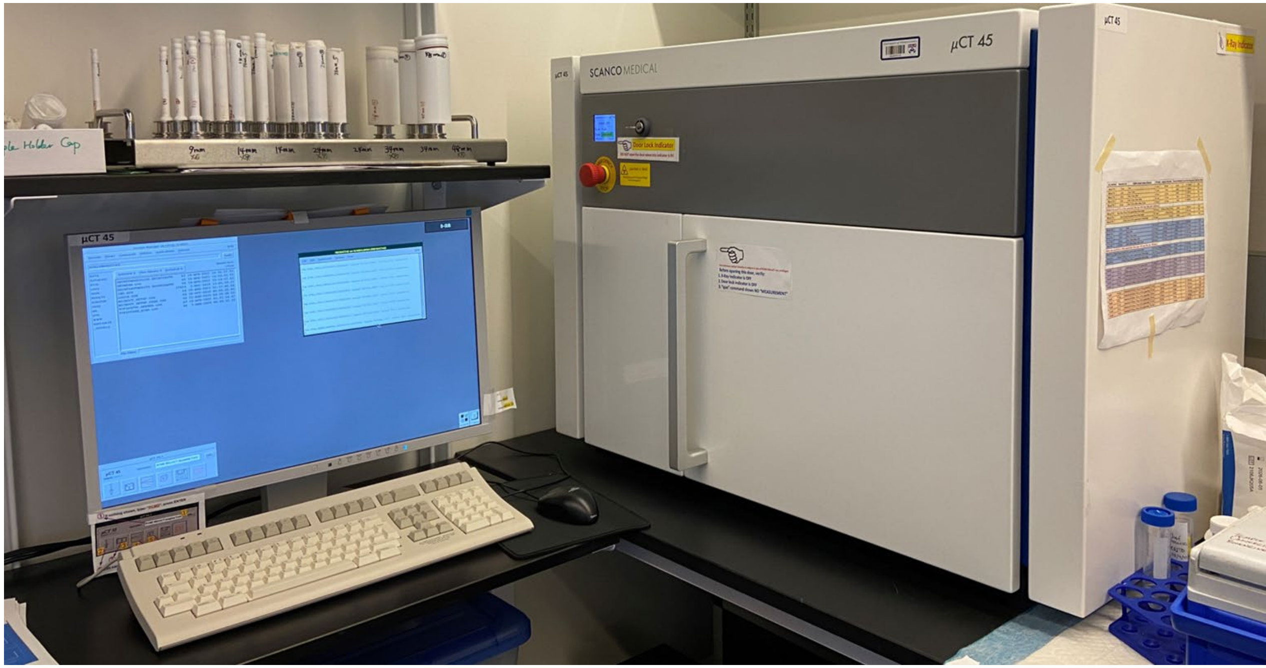
Ex vivo (Specimen) Scanners

- 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)
  - 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 implants





In vivo Scanner

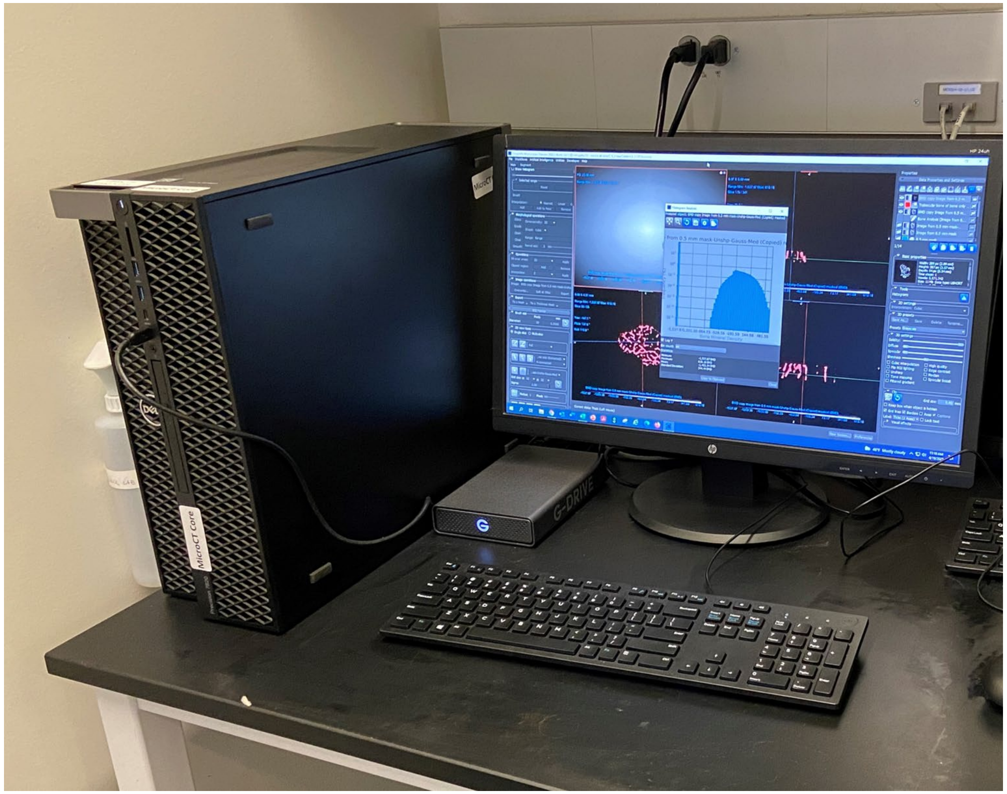
- Scanco vivaCT 80 (Purchased in 2018)
  - Voxel sizes: 10.4 µm, 11.6 µm, 13 µm, 16.1 µm, 20.8 µm, 26 µm
  - Internal heating device to keep animal warm
  - Internal camera to monitor animal's breathing
  - Ex vivo scan for specimen from large animals or human cadaver

MicroCT Analysis PC

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

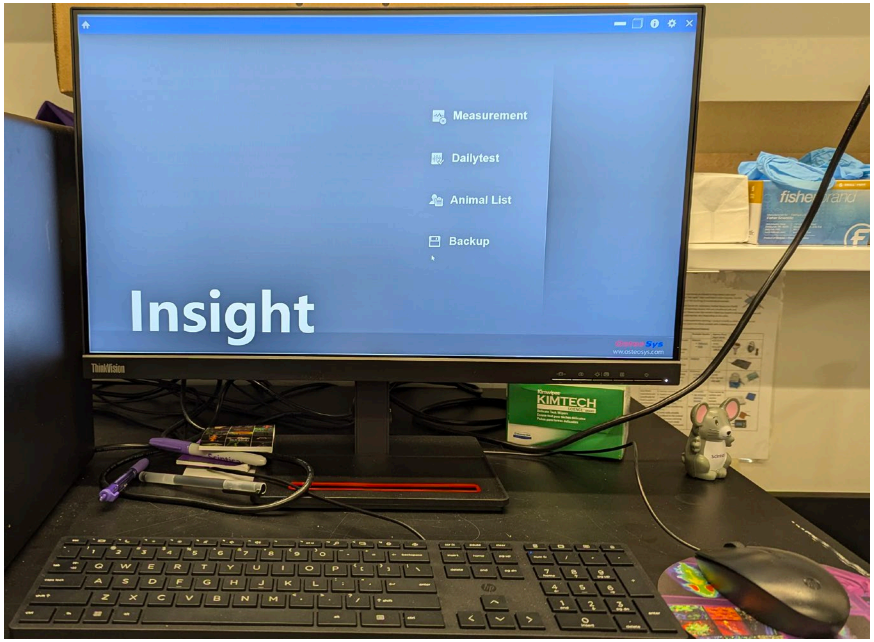
Dragonfly Workstation

- 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



Scintica Insight DXA

- DXA system (368B Stemmler)
  - Windows 10 platform
  - In vivo / Ex vivo 2D scan and analysis
  - 25 sec per scan
  - Video tutorial: https://www.youtube.com/watch?v=ZRWOm7NnK1g



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Rodent *In Vivo* Loading System

PCMD Micro-CT Learning Lunch

Xiaoyu Xu
02/18/2024

McKay Orthopaedic Research Laboratory
Department of Orthopaedic Surgery
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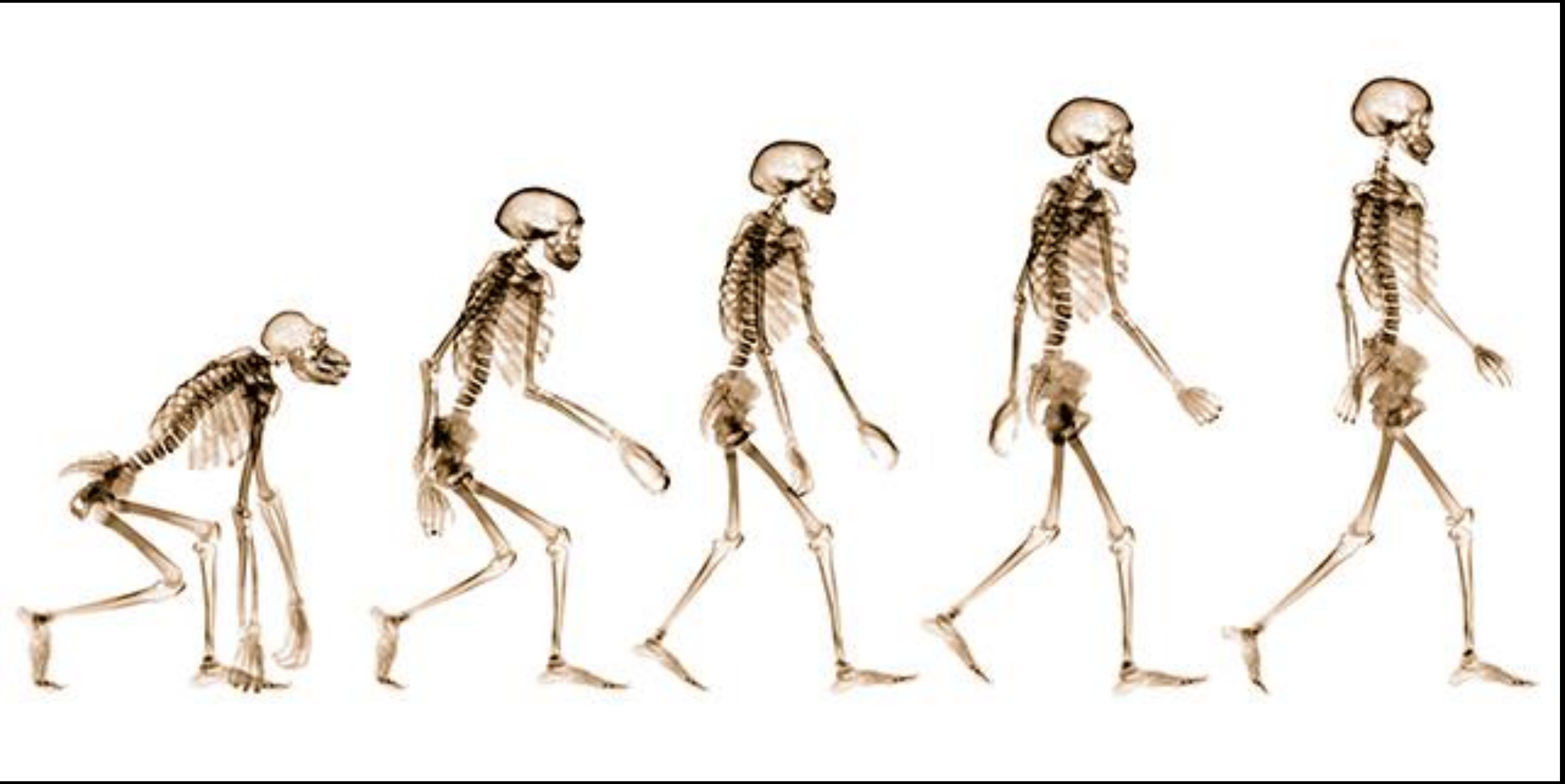
University of Pennsylvania
Philadelphia, PA

Skeletal Mechanoadaptation

- Skeleton is **a dynamic tissue** that can react to the forces applied to it --- '**Mechanoadaptation**'



Pew Research Center

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The History of Skeletal Mechanoadaptation

In early 17th Century: Surgeons and anatomists

- Bones adapt their shape and size to the loads they bear
- The first published comment on the mechanical function and shape of bone (Galileo Galilei,1638)

In the mid-late 19th Century:

- The relationship between mechanical stress and internal skeletal structures was firstly detailed described.

In late 19th Century: Wolff's Law (1892)

- As a dynamic tissue, bone adapts its structure and volume mass in response to the mechanical loads placed upon it
- ***'Use it or lose it'***

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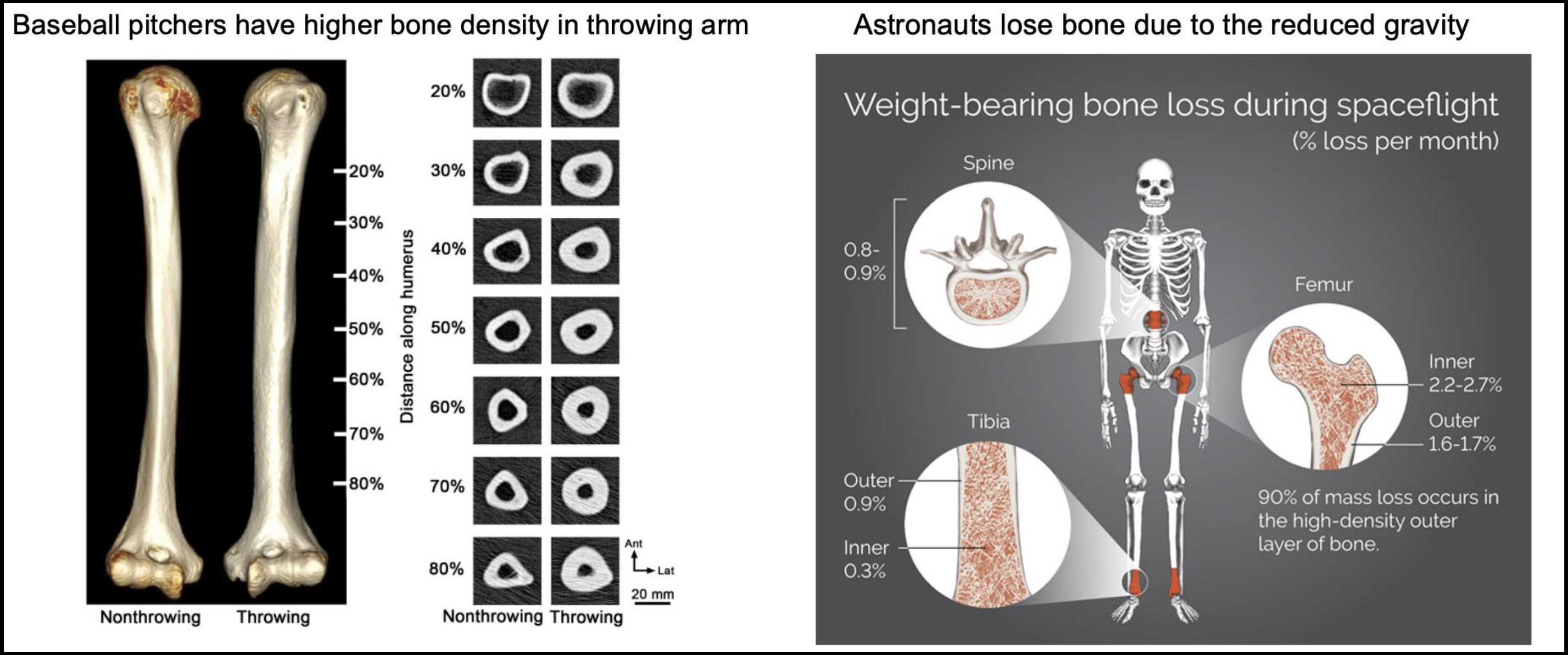
The History of Skeletal Mechanoadaptation



In late 19th Century: Wolff's Law (1892)

- As a dynamic tissue, bone adapts its structure and volume mass in response to the mechanical loads placed upon it
- ***'Use it or lose it'***

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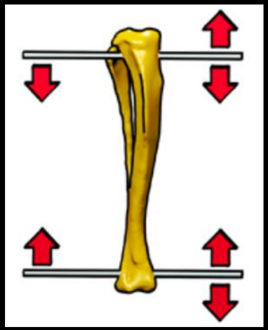
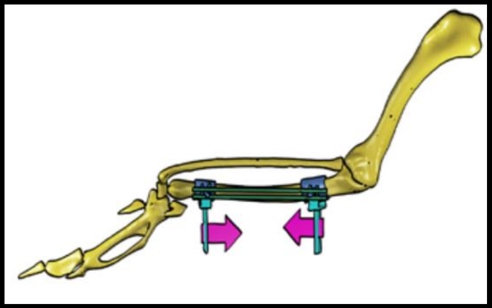
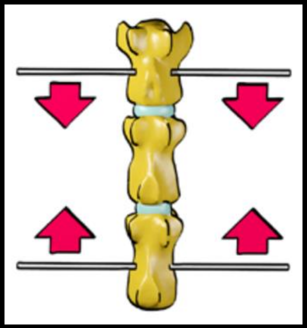
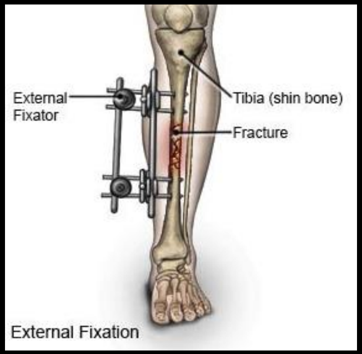
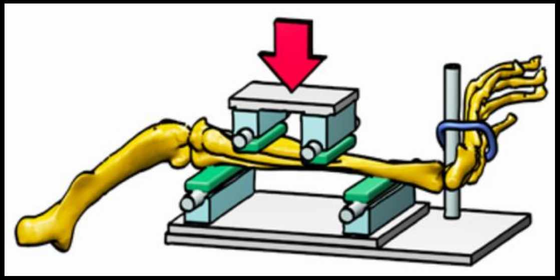
Development of *In Vivo* Loading Systems

In 20th Century: to validate and expand the Wolff's Law

- Early Approaches: for fracture healing and fixation
  - Surgical methods: *highly invasive, caused significant trauma, and did not allow for precise control of loading*

- Breakthrough : Non-invasive models for **dynamic** loading
  - Ulna Axial Loading Model (1970s)
  - Four-Point Bending Model (1980s)
  - Tibial Compressive Model (1990s)

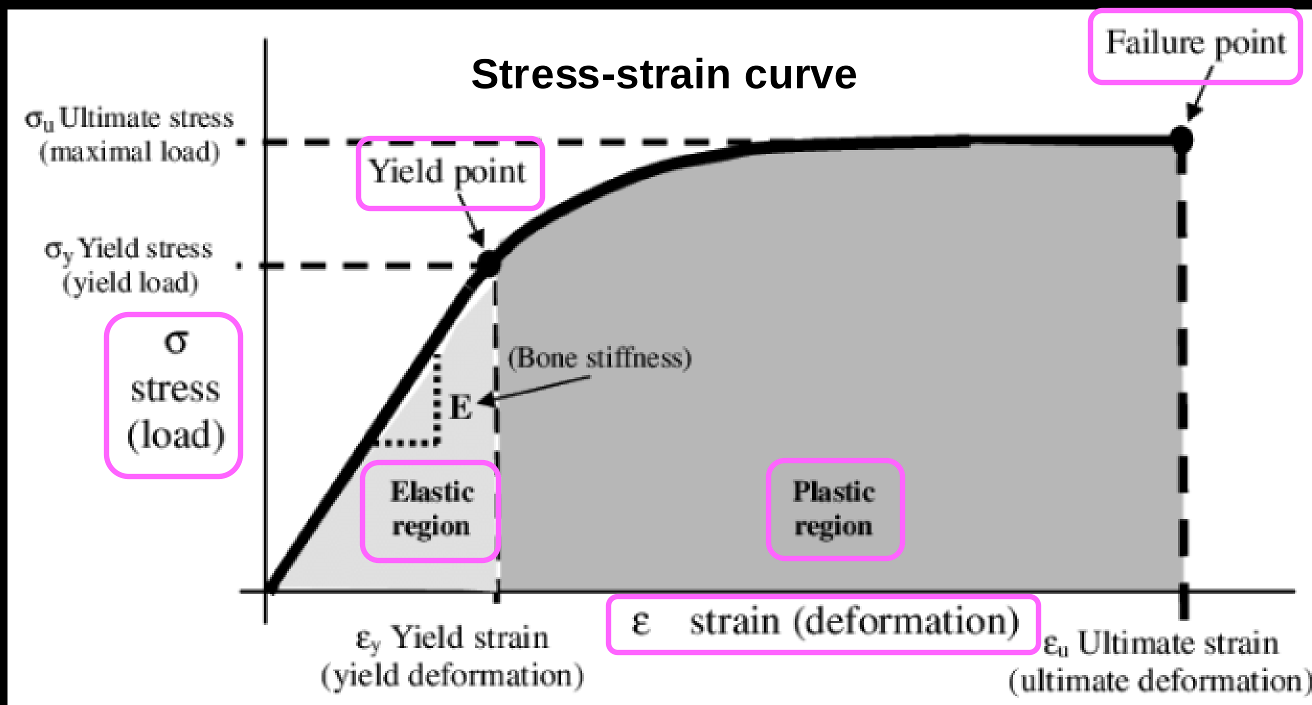




Main et al., 2020

Mechanical Property of Bone

In Late 20th Century: with the non-invasive loading models

- The mechanical property of bone was systematically investigated with the ***stress-strain curve***



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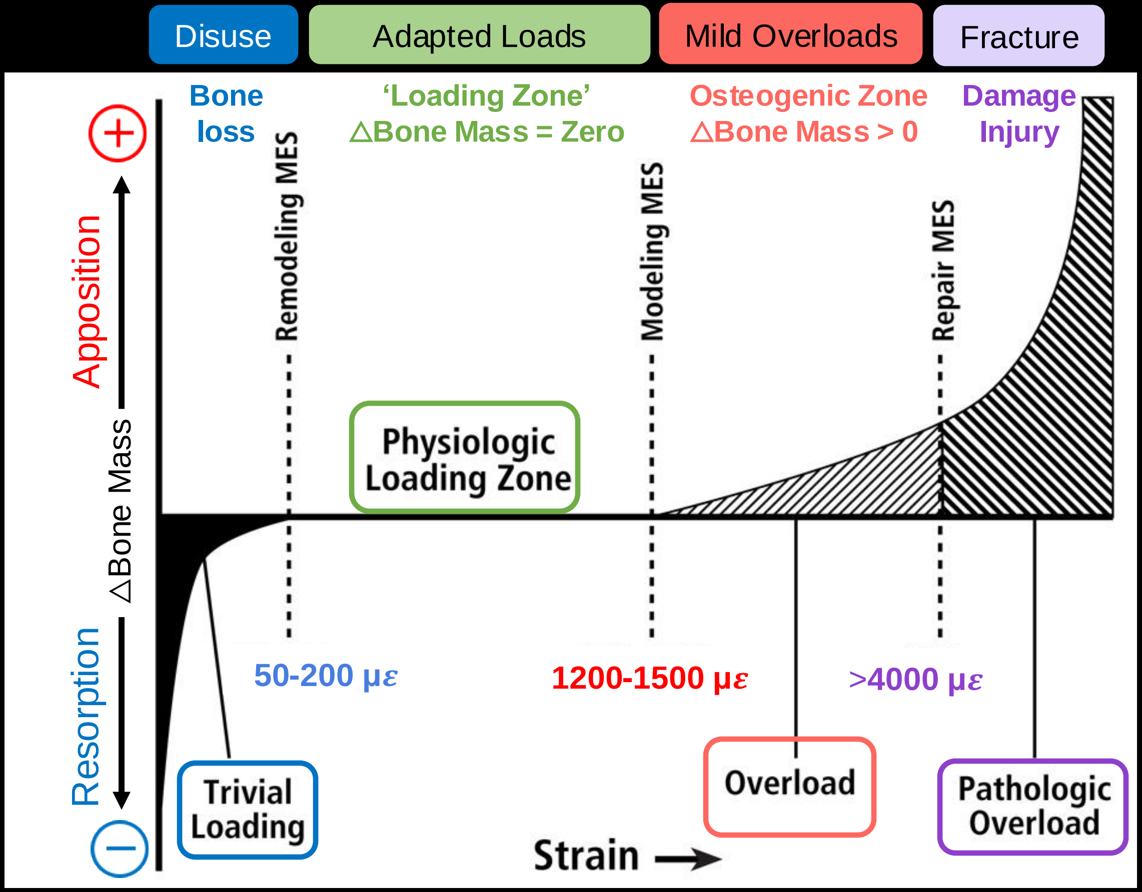
Mechanostat – Strain Threshold

In Late 20th Century: Mechanostat hypothesis by Harold Frost

- The concept of ***strain thresholds***, the minimum effective strain (**MES**), that determines how bone responds to mechanical force
- Mechanical environment (*strain*) ←→ Biological response (*modeling & remodeling; anabolic & catabolic net bone mass*)



Burr et al., 1992

Role of Osteocytes as Mechanosensors

In early 21st Century: Mechanosensation by Dr. Lynda Bonewald

- The interstitial fluid flow (IFF) within the lacunocanalicular system (LCS)
- Sense the *extracellular biophysical stimuli* (shear stress of fluid flow) → convert to *internal biochemical signals* → transduce *biological functions*
- Expressions of osteocyte markers, DMP1 (↑), E11(↑), and SOST(↓), in response to mechanical stimuli *using the non-invasive loading model*

ELSEVIER

BONE

Bone 42 (2008) 606–615

www.elsevier.com/locate/bone

Review

Osteocytes, mechanosensing and Wnt signaling

Lynda F. Bonewald *, Mark L. Johnson

University of Missouri — Kansas City School of Dentistry, Department of Oral Biology, 650 East 25th Street, Kansas City, MO 64108, USA

Received 12 November 2007; revised 20 December 2007; accepted 27 December 2007
Available online 12 January 2008

Qin et al., 2020

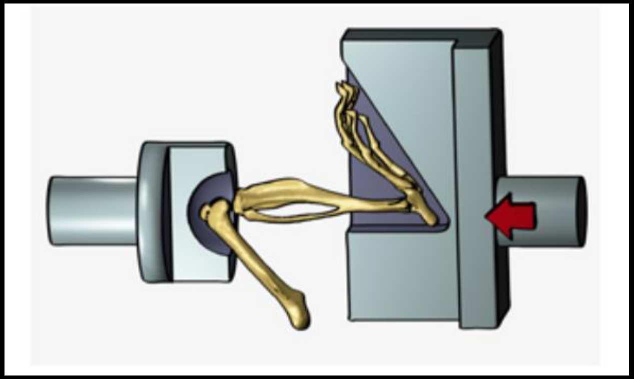
Development of *In Vivo* Loading Systems

In 21st Century: 2000s-present:

- Applications: shifted from learning mechanical property to:
  - induce <u>the osteogenic response</u> for bone mass preservation
  - investigate <u>osteocyte mechanotransduction</u>
- Loading devices need to be miniaturized and automated

The Axial Compressive Loading System: Most widely used today

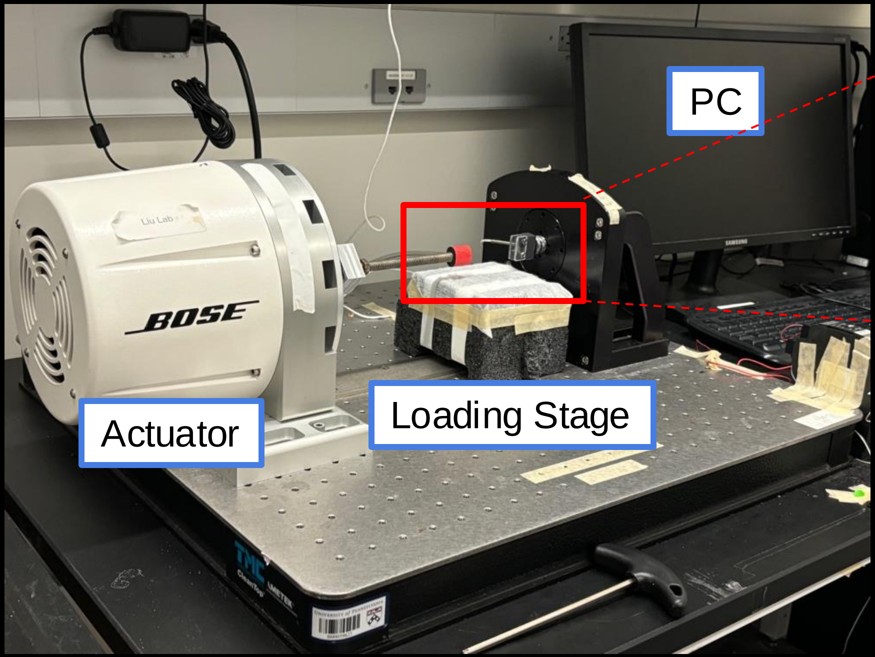
- *In vivo* and non-invasive; highly reproducible; longitudinal study
- Mimics physiological dynamic loading patterns (e.g., walking or running)
- Controllable load magnitude; high-frequency loading; adjustable protocol
- Applicable on ulna, tibia, and femur in rodents, rabbit, and chicken

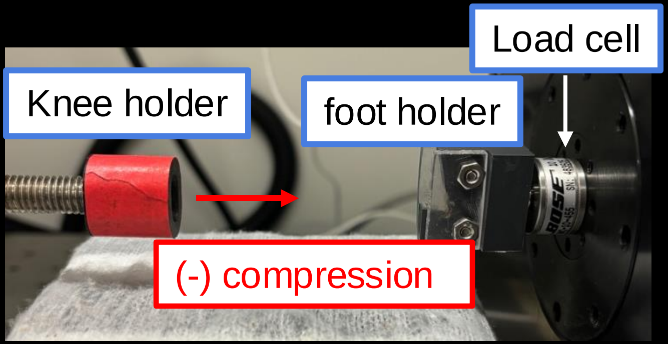


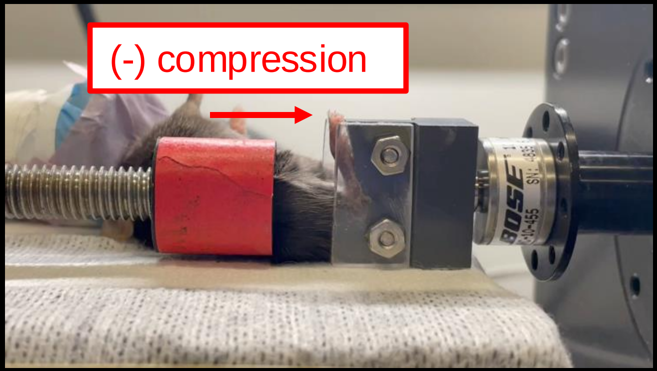
Main et al., 2020

In vivo Axial Compression Loading System

- Loading Device: Commercial linear actuators, Load cells, software-based force feedback control (TA Instruments/Bose)
- Location: MicroCT Viva80 room (368B)
- Types of Loading: Primarily axial dynamic compression

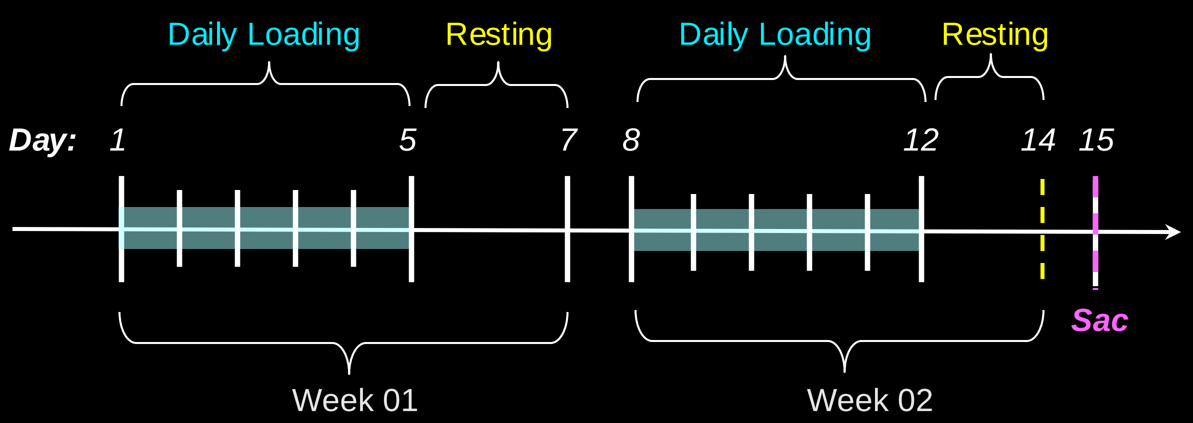


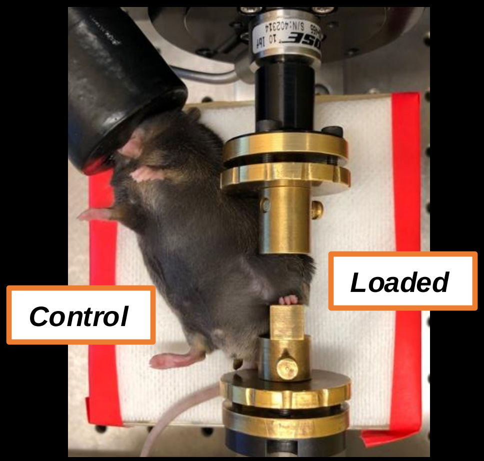




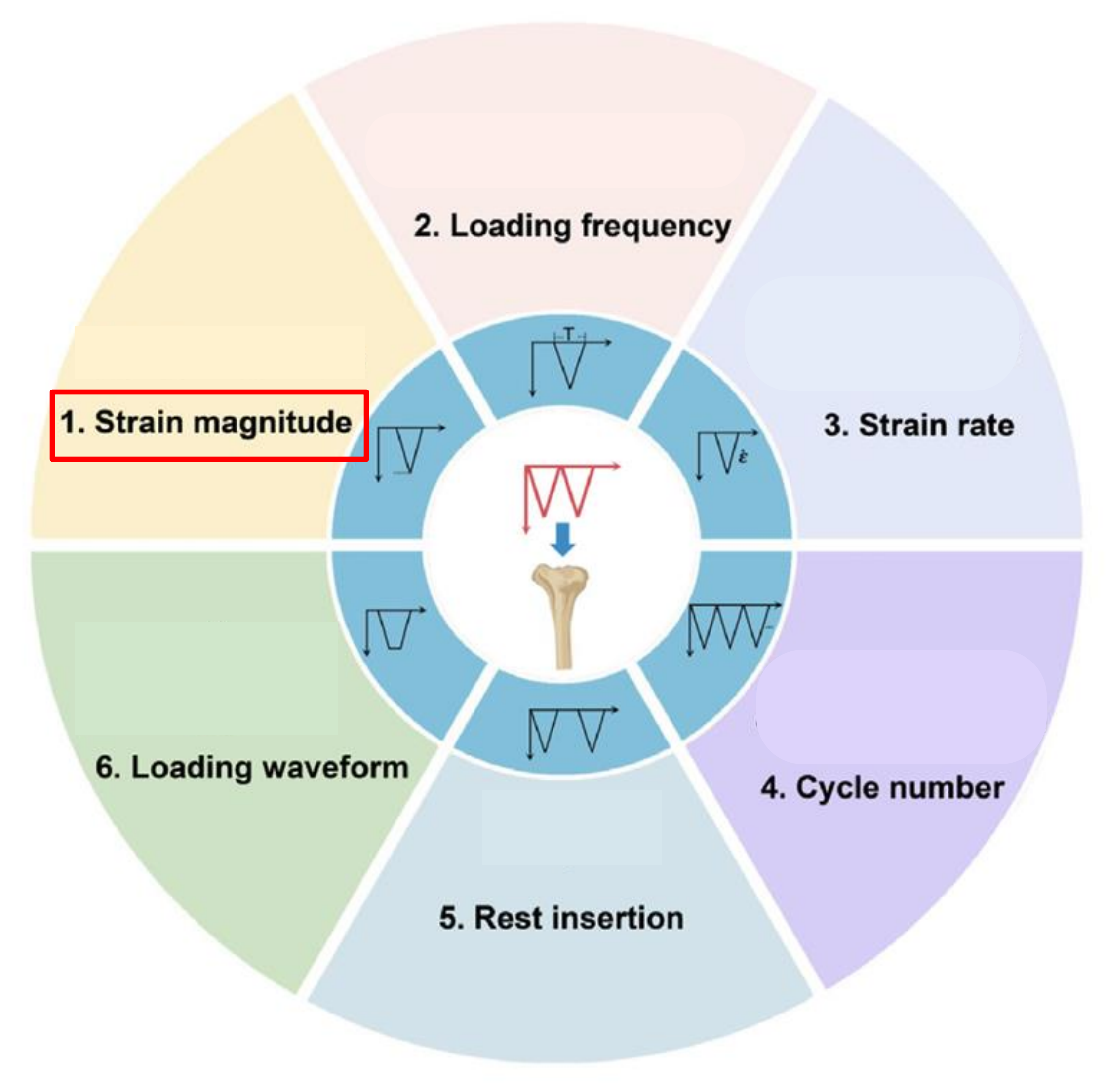
In vivo Axial Compression Loading System

- Loading Device: Commercial linear actuators, Load cells, software-based force feedback control (TA Instruments/Bose)
- Location: MicroCT Viva80 room (368B)
- Types of Loading: Primarily axial dynamic compression
- Species and bone type: Rat and mouse; Tibia (*contralateral control*)
- Common experimental timeline:
  - Loading plan: 5 times a week (Mon to Fri) over 2 weeks
  - Adjustable for short-term loading study (3 or 5 days)





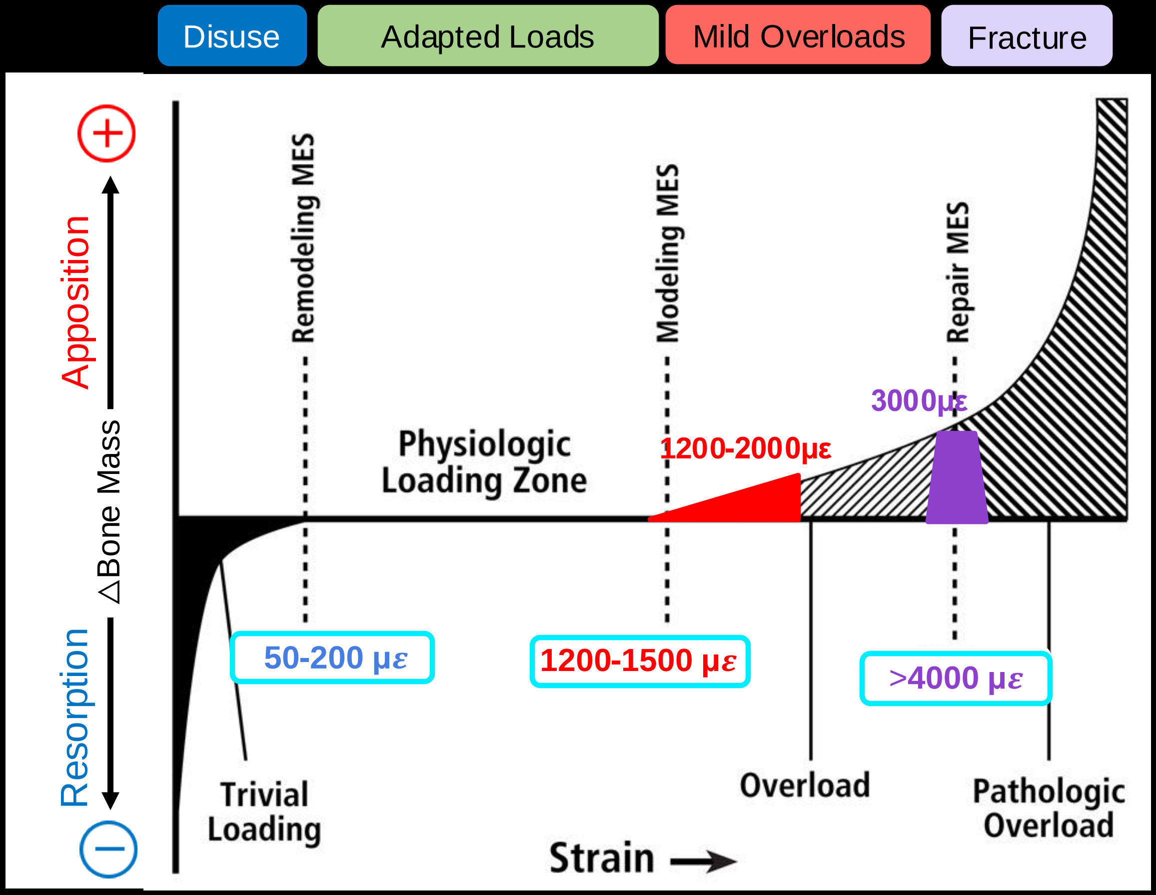
Mechanical Loading Parameters



Wang et al., 2025

Load-Strain Calibration

- **Under a given load, the stresses and strains may vary** across bones with *different size, geometry, or material properties due to animal's sex, age, or genotype*
- **Main concern**: How to match the aim strains to the given load?



Burr et al., 1992

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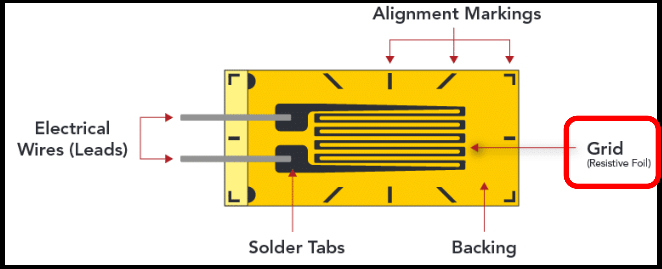
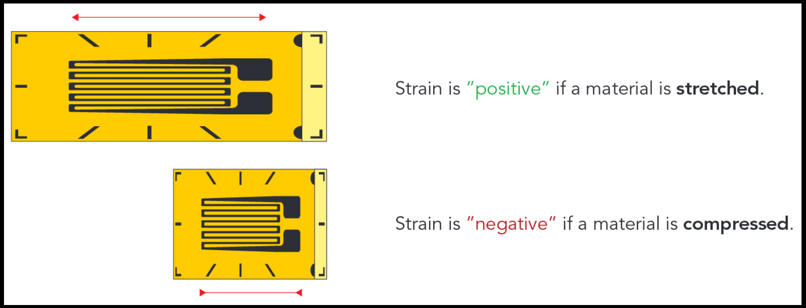
- **Strain matching**: Load-strain calibration by the strain gauge

Single strain gauge with conductive grid



Grid length:1-3mm

Gauge thickness: 0.01-0.035 mm



https://www.anyload.com/strain-gauge/

Strain/deformation (ε) (stretched + or compressed -) → Change in Resistance (ΔR) (increase or decrease) → $GF = \left(\frac{\Delta R}{R}\right)/\varepsilon$

GF : gauge factor (given)

ε: strain (%) can be output

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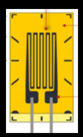
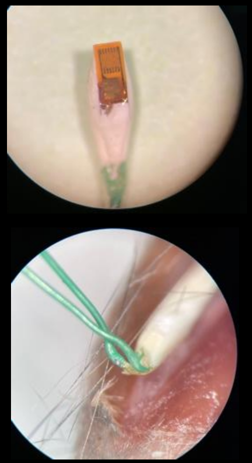
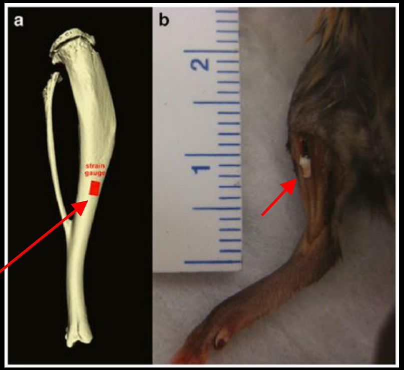
Single gauge



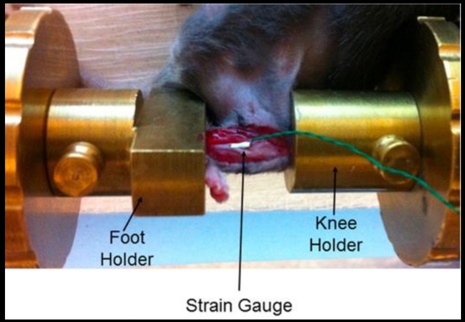


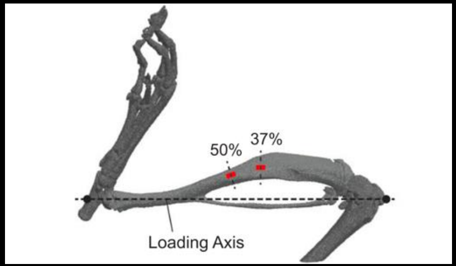
Attach to the medial surface of tibial midshaft



Melville et al. 2015

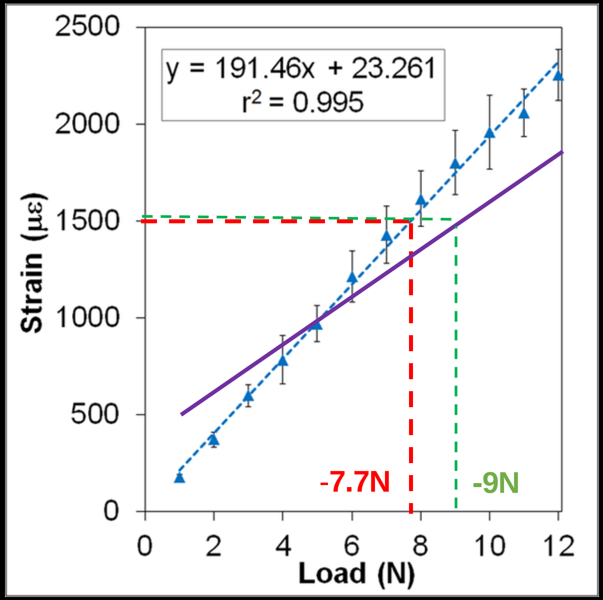
Apply serial gradient compressive loads
-2, -4, -6, -8, -10, -12 N





Main et al. 2020

Load-strain relationship



Berman et al. 2015

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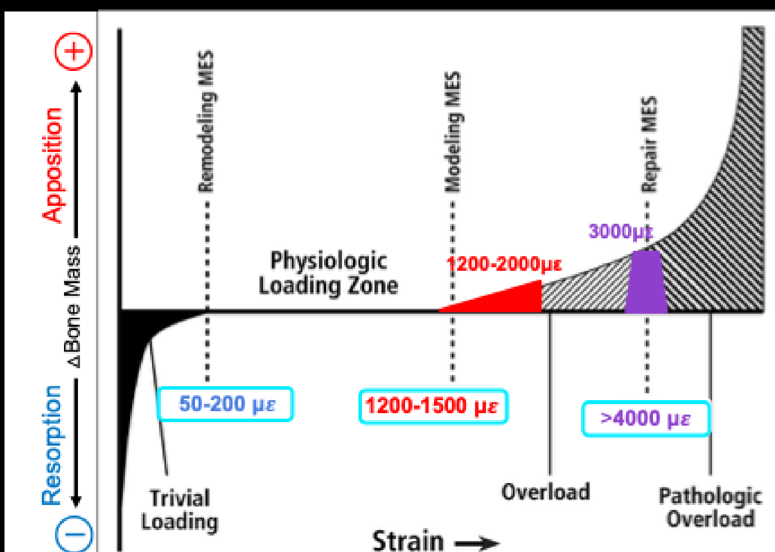
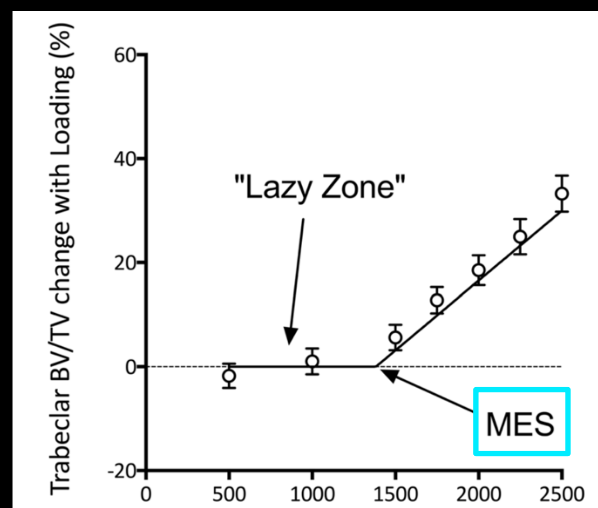
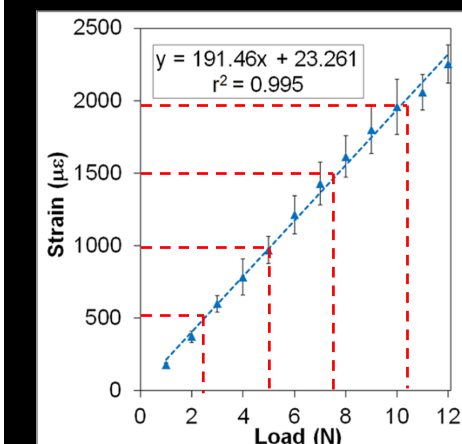


Burr et al., 1992



Meakin et al., 2014

Load-strain relationship



Berman et al., 2015

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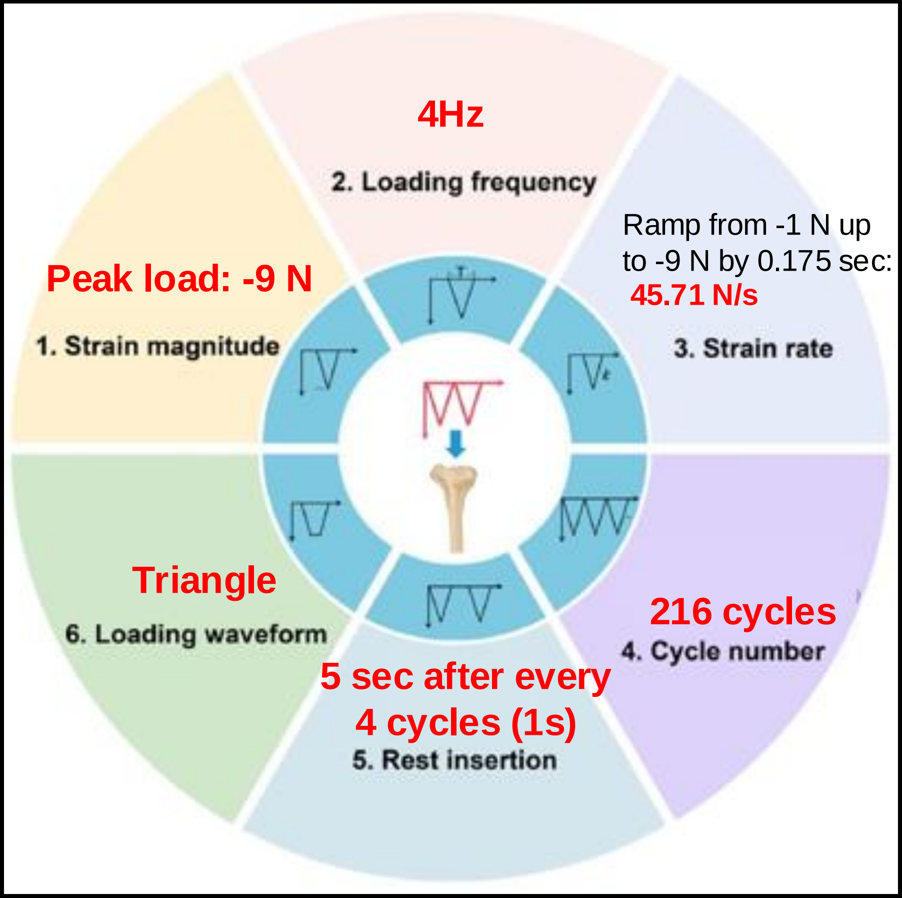
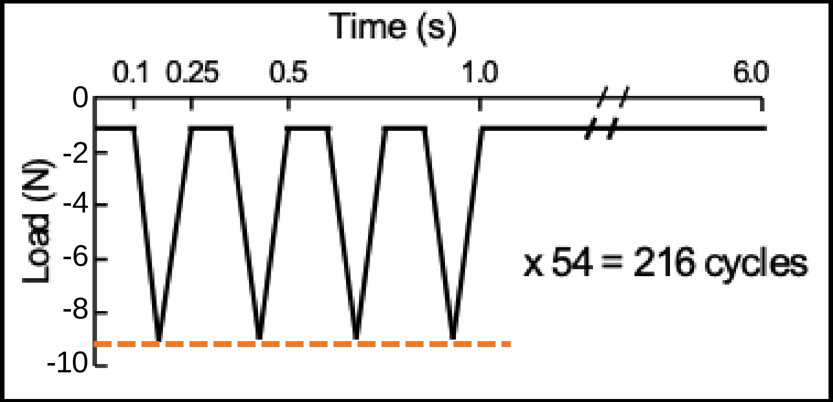
Mechanical Loading Parameters

- **Triangle waveform**: the most used waveform
- Pre-load: -1N



Wang et al., 2025



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Experiment Procedure of Daily Loading

Instrument setup:
loading device, force control software, and ***loading protocol***

↓

Animal Knockdown:
Anesthesia via isoflurane machine; Eye lubricant

↓

Loaded limb fixation:
Carefully place the knee and ankle to the fixtures and align the tibia along its anatomical axis

↓

Start the loading by click-running the protocol:
Record the real-time wavelength, load, voltage, and displacement

↓

Release the loaded limb and animal wake-up:
Carefully check-up the stability of knee and ankle;
Wake up the animal under the heating lamp

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Significance of the Loading Study

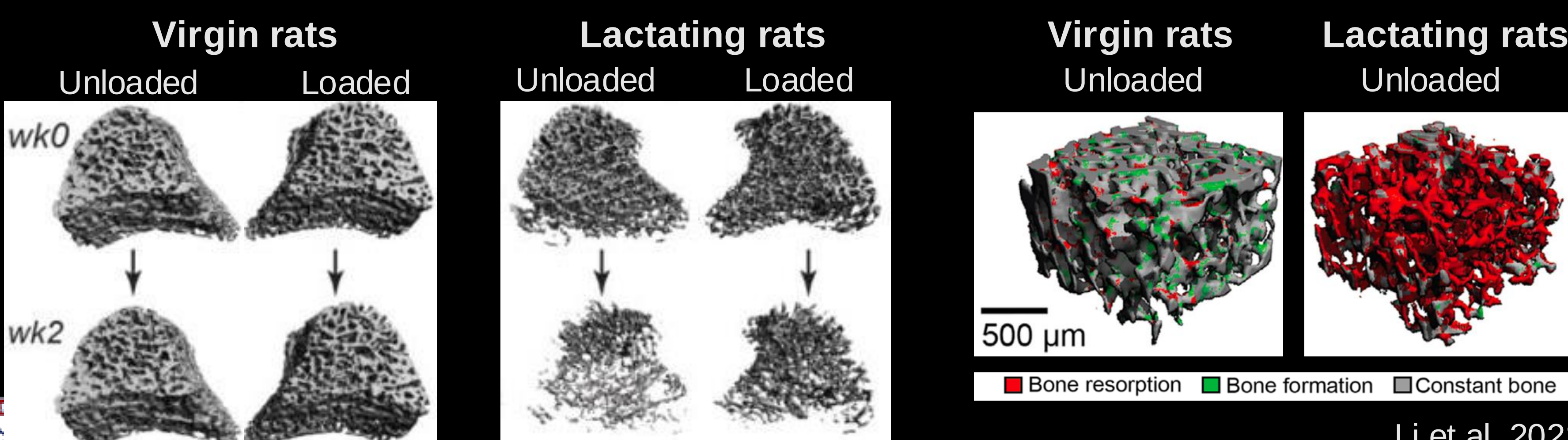
- Previous purposes: understanding bone adaptation (1800s-1900s)
- Current goals (2000s-present): indispensable tools
  - Tissue level
    - Modeling/remodeling under different strain environment
    - Material properties across different treatments or conditions
  - Bone cells' mechanotransduction (mainly osteocytes)
    - Osteocyte mechanobiology: LCS structure, differentiation, signaling pathways
    - Advancing basic science: cellular and molecular mechanisms in response to mechanical force
  - Bone diseases
    - Osteoporosis and disuse osteopenia (disuse vs. loading)
    - Potential treatment or therapeutic intervention for bone diseases
    - Fracture healing and bone repair; ACL rupture for OA model
  - From bench to bed
    - Mechanical devices to enhance strength (e.g., vibration platform)

Integration with *In Vivo* Imaging

In vivo Micro-CT Imaging (Viva80)

- Longitudinal tracking on the bone structural adaptations
  - Minimize the animal sample numbers
  - Adjustable imaging VOIs with high resolution
- Image Registration Analysis
  - Continuously traces the consistent region of bone structure
  - Investigate the <u>local dynamic changes</u> during skeletal adaptation in response to the mechanical loading

DEXA Scanning for Bone Mineral Density



Li et al, 2021

Applications in Bone Research

Combining with other technologies

- Tissue- and whole bone-levels
  - **Micro-CT**: bone mass and microstructural changes
  - **Finite element analysis**: strain distribution and mechanical property
- Cellular level
  - **Live confocal imaging**: mechanosensitivity
  - **Histomorphometry**: bone modeling/remodeling; bone cells' activities
  - **TEM/SEM**: osteocytes' LCS structure
- Molecular level
  - **Gene/protein analysis**: mechanotransduction (*Wnt/β-catenin signaling and SOST*)
- Animals: surgery/sex/age/genotype
  - OVX/ORX, iPTH, etc.
  - Physiological conditions, e.g., aging, pregnancy, or lactation
  - Transgenic animals with different ages

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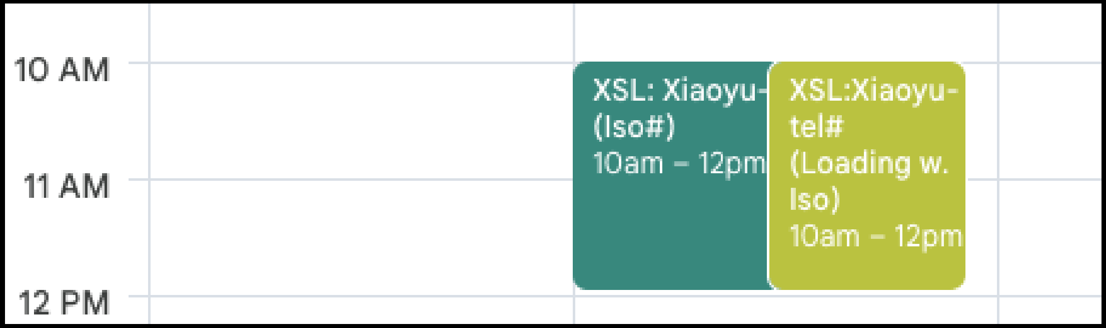
Training & Scheduling

- **Two sessions of training are required (in-person):**
  - 1) Basic introductions of the function of the instrument and safety operation; 2) Hands-on training
  - Consultation and customization of the loading protocol
  - Please contact via email: *xiaoyu.xu@pennmedicine.upenn.edu*

- **Scheduling your experiment window via Google Calendar**
  - Need to book your time on TWO calendars: Viva80 + Iso machine
    On **<u>Viva80 room</u>** calendar: Book the loading instrument
    XSL-Xiaoyu-Tel#-(Loading use)
    On **<u>Iso machine</u>** calendar: Book the Iso machine40
    XSL-Xiaoyu-Tel#-(Iso80)



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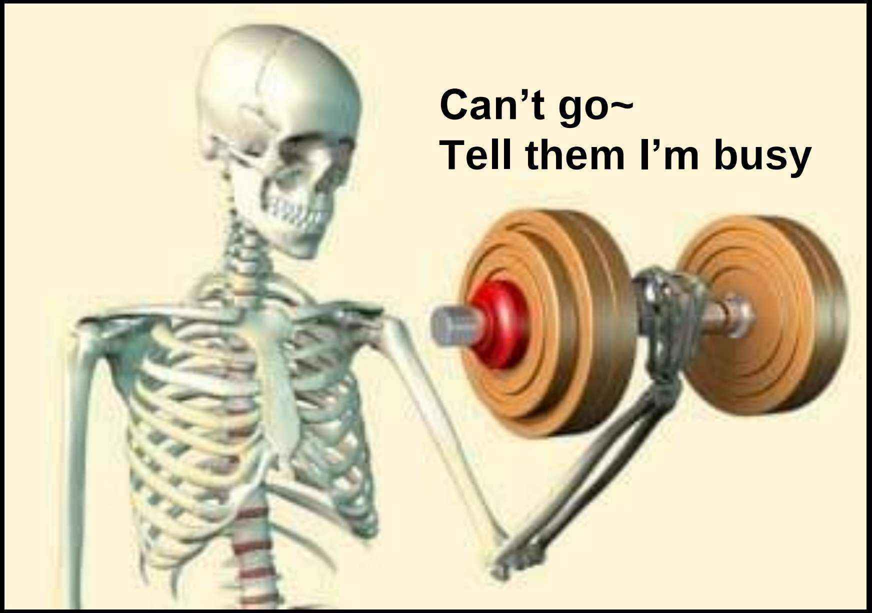
Thank you for listening!

Any questions?



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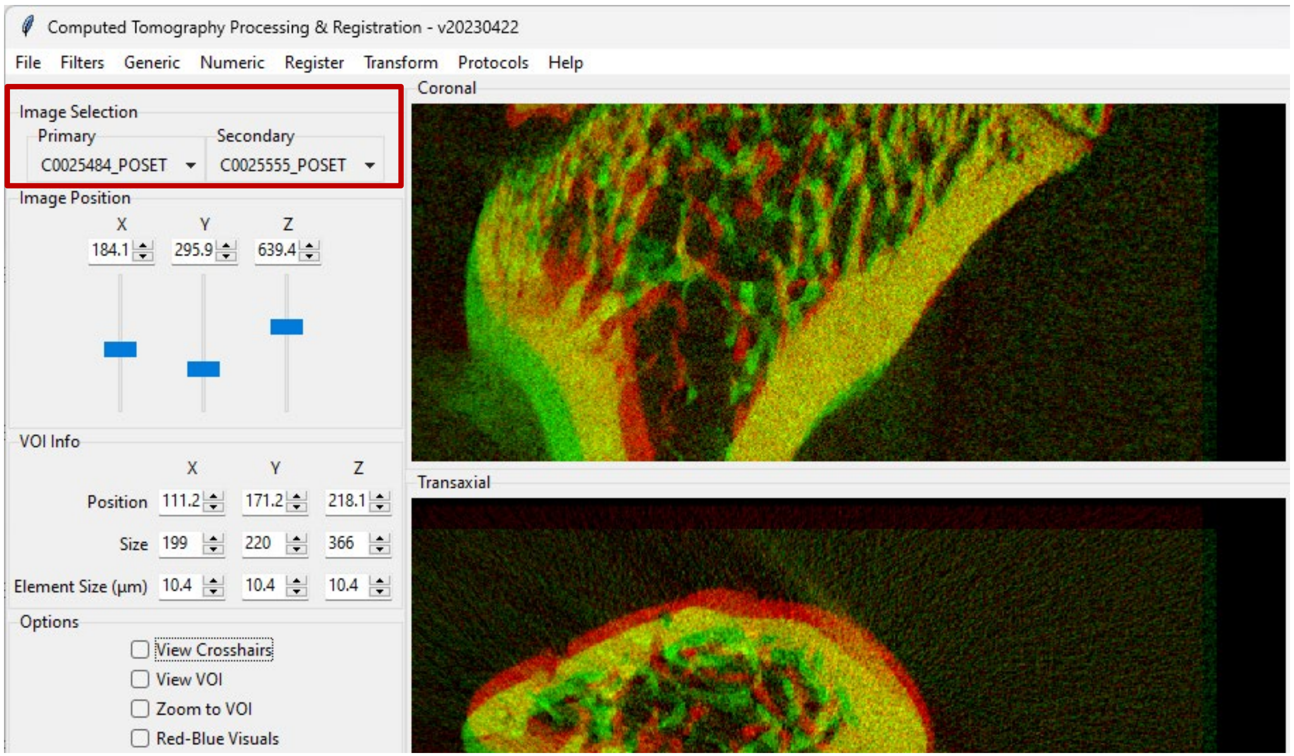
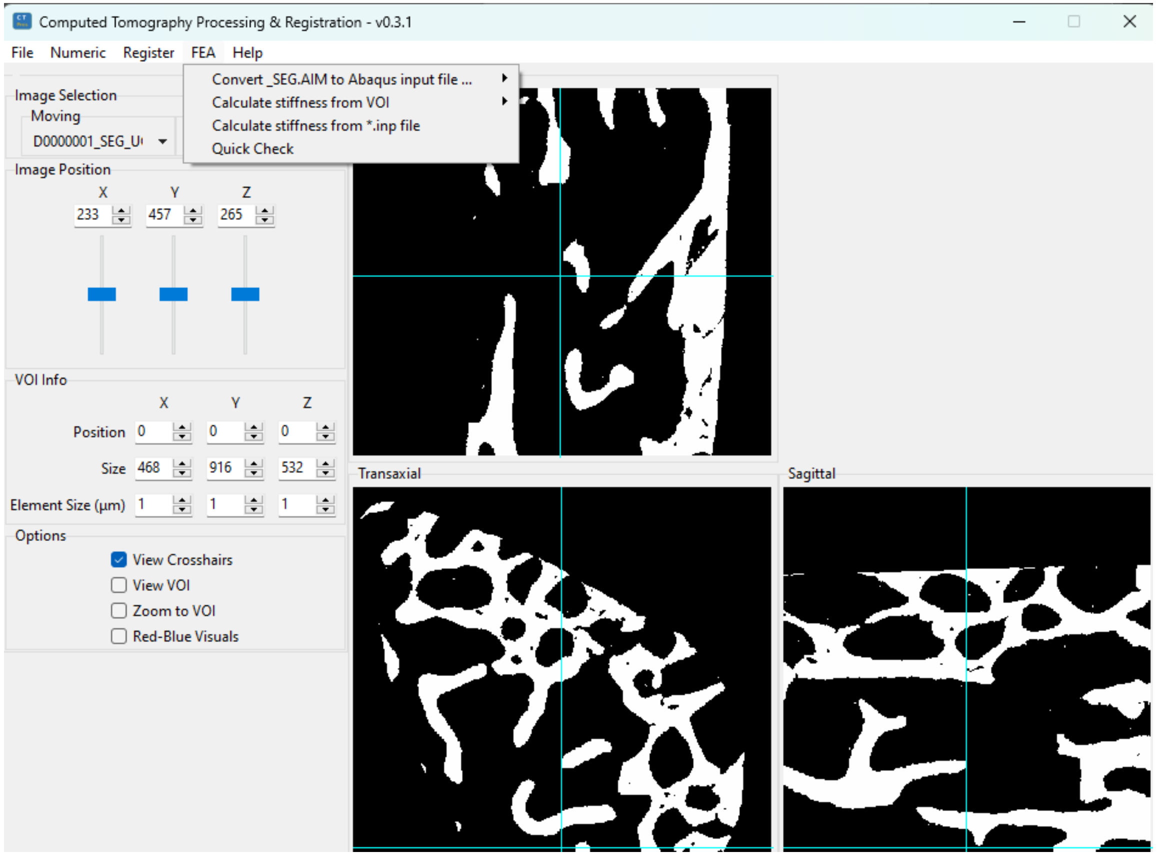
CTPros Update – FEA Module

Computed Tomography Processing & Registration



CTPros Update – FEA Module

- FEA Module
  - Convert VOI to Abaqus input file ✓
  - Calculate VOI stiffness using Homogenization Theory • • •



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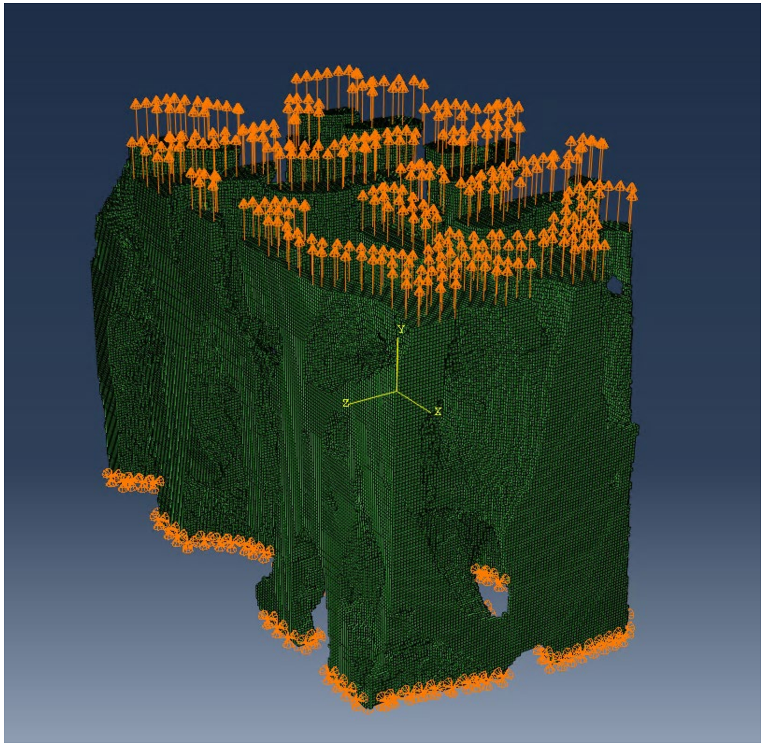

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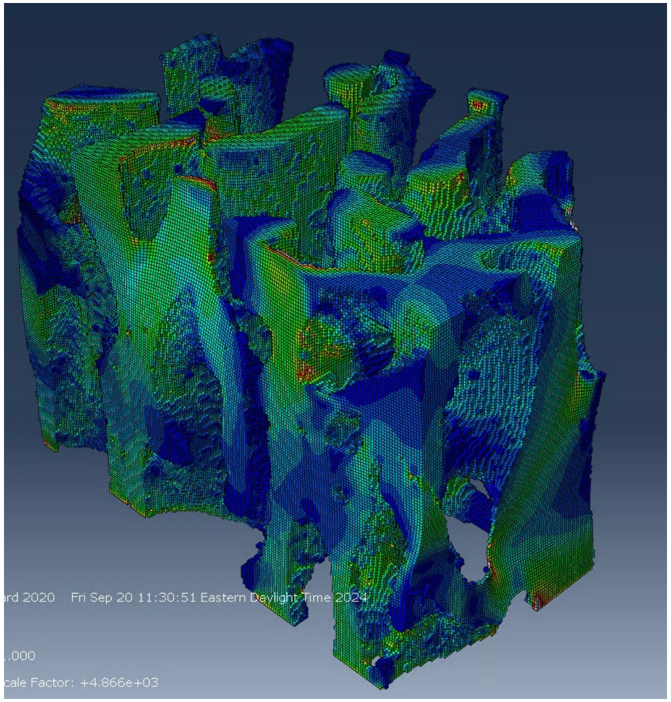

3D-printed Holders

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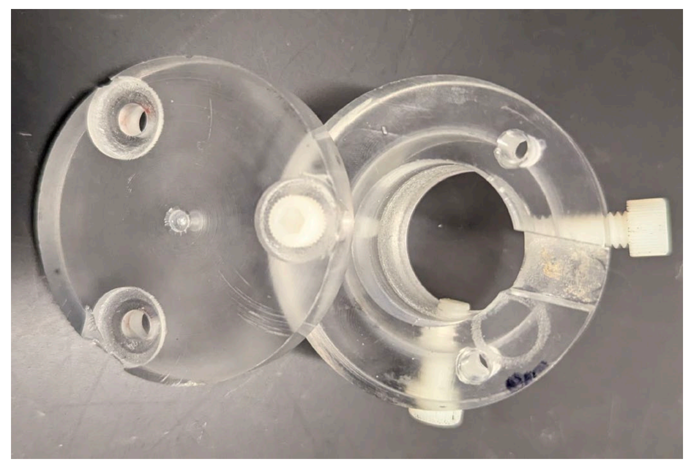
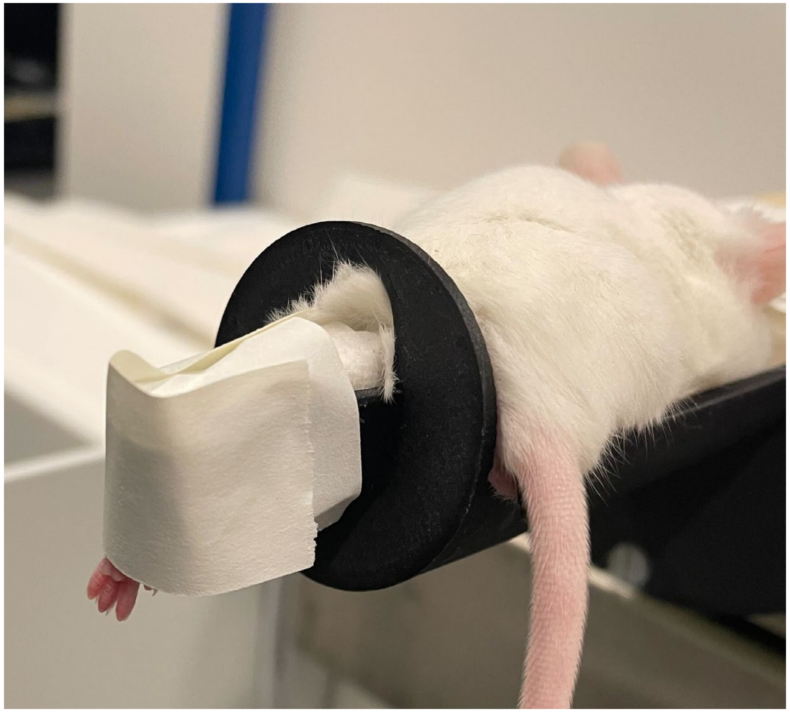
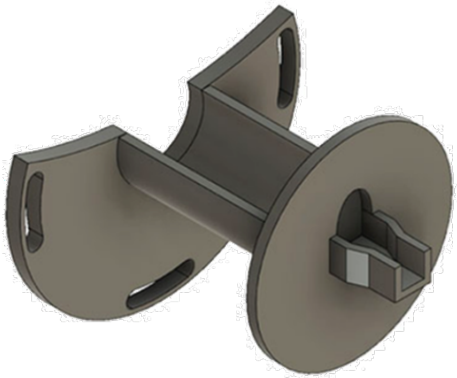
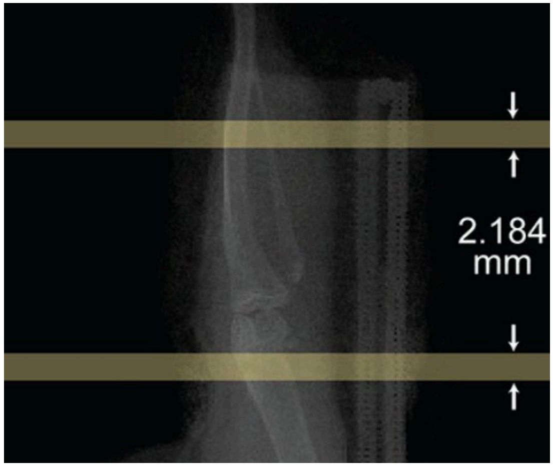


3D-printed Holders

- In vivo mouse scan
  - 10.4 µm isotropic voxel size (32 mm FOV)
  - 2.18 mm bone segment of femur midshaft

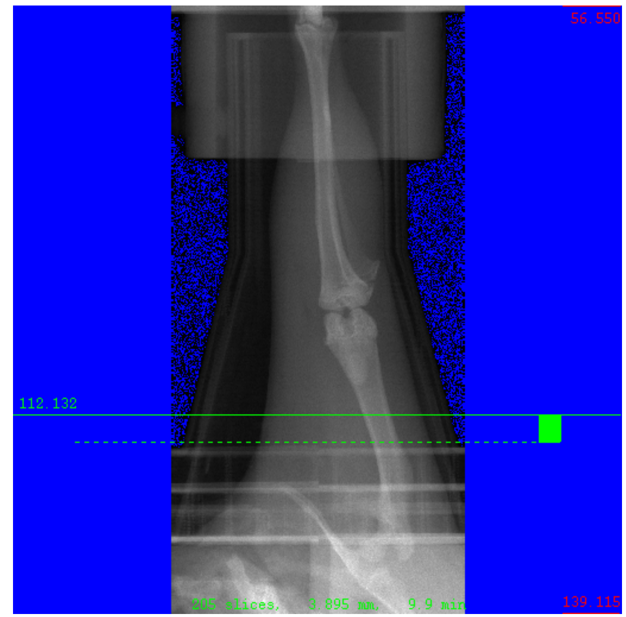






3D-printed Holders

- In vivo rat scan
  - 11.6 µm isotropic voxel size (36 mm FOV)
  - 2 mm bone segment of femur midshaft

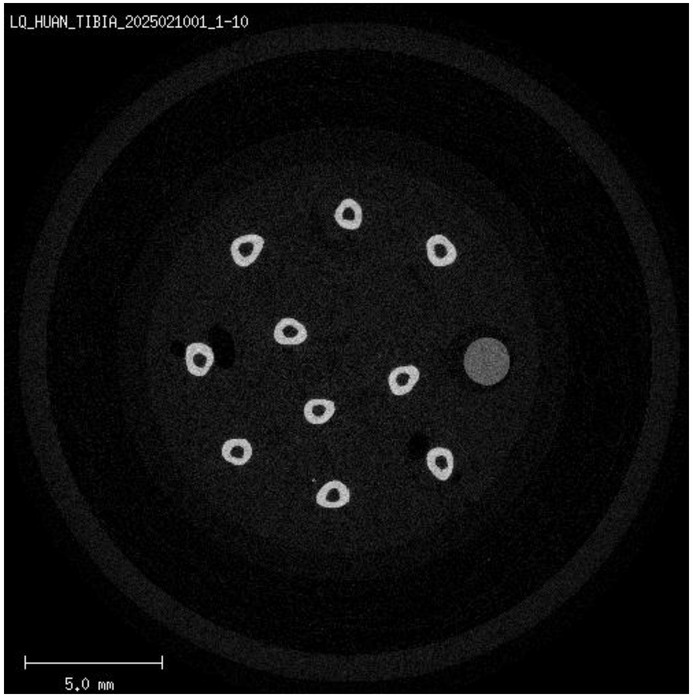


3D-printed Holders

- Ex vivo small samples
  - For 2.5 mm, 2.8 mm tibiae/femurs
  - Compatible with 15 mL lab tubes (7.4 µm resolution)

3D-printed Holders

- Ex vivo small samples
  - For 2.5 mm, 2.8 mm tibiae/femurs
  - Compatible with 15 mL lab tubes (7.4 µm resolution)



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