# Study of Effects of Microgravity on Disc Height and Compositional Analysis

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UCSD-Doshisha Medical Imaging Research Center 2<sup>nd</sup>
Symposium
08/24/2010



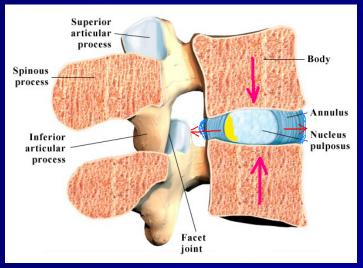




### Disc Herniation and Back Pain

- Commonly occurs in astronauts
- Increases the injury likelihood and inhibits daily life
- Underlying factor: intervertebral disc (IVD) degeneration (NASA)
- Endplates
  - Separates disc and vertebral body
  - Structural and compositional changes during space travel
    - Spinal elongation
    - Imbalance of collagenproteoglycan ratio (Pedrini-Mille)





## **Gravity Effects on Spine**

- Spine compressed during return to Earth
- Intervertebral discs compressed and may cause disc herniation (Maynard)
- Compositional and structural changes in trabecular bone (LeBlanc)



### **Objectives**

- To investigate structural and compositional changes of mice intervertebral discs under microgravity by examining
  - Disc heights
  - Trabecular analyses
- Methods
  - Micro computed tomography (µCT)
  - Tri 3D Bon software and CT Analyser software
  - Mimics 13.0 and 13.1
- Analyses parameters
  - Bone volume ratio (BV/TV)
  - Trabecular number (Tb.N)
  - Trabecular thickness (Tb.Th)
  - Bone mineral density (BMD)
  - Disc height

## Samples

NASA Discovery STS-131 mission mice

Strain: C57BI/6

CD45.1 congenic mice

Weigh 25g



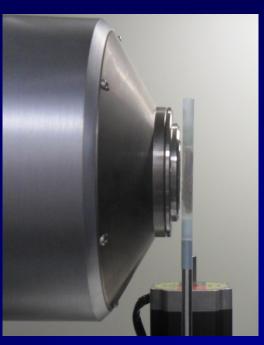


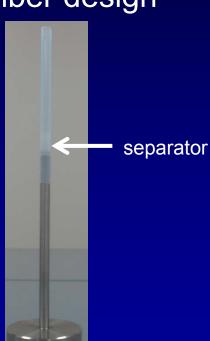
## **Image Acquisition**

Shimadzu SMX-160CTS

Chamber design







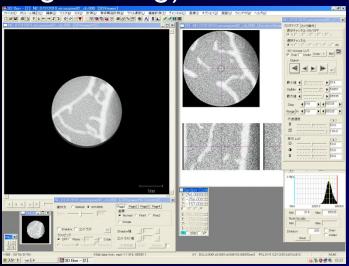
## Micro CT Scanning: High Resolution Imaging

#### Conditions

- 1.59 microns isotropic
- source-to-object distance (SOD) of 3.5mm
- Voltage: 65 kV 72 kV
- Reconstruction matrix size 512 x 512
- Field of view (FOV XY) approximately 0.8 mm
- Limitation: incomplete view of endplate

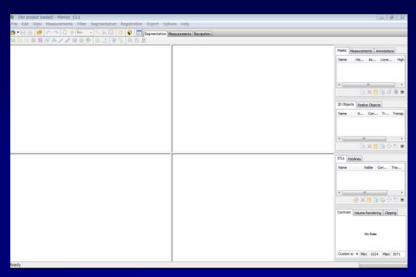
### **Image Reconstruction**

1. Tri/3D Bon (Ratoc System Engineering)



- Import raw data from µCT
- Noise reduction and filter
- Generate 3D model
- Export files as bitmap

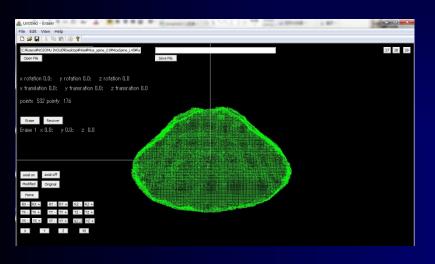
2. Mimics 13.1 ® (Materialise)



- Import bitmap files
- Threshold and segment
- Generate reconstruction 3D model
- Export point cloud (with Mimics 13.0)

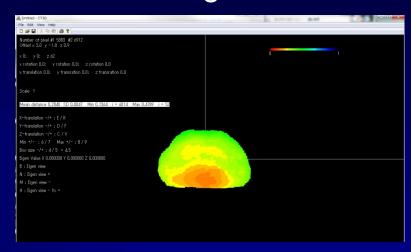
### Disc Height Calculation

### 1. Eraser Program



- Open point cloud data from Mimics
- Erase unnecessary regions
- Generate endplate
- Save files as .txt

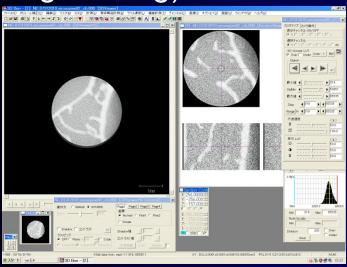
#### 2. DHD Program



- Open caudal and cranial erased text files
- Calculate disc height

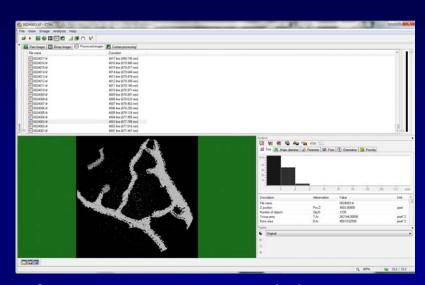
### Trabecular Analysis

1. Tri/3D Bon (Ratoc System Engineering)



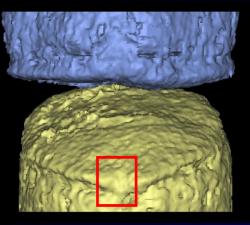
- Import raw data from µCT
- Noise reduction and filter
- Generate 3D model
- Export files as bmp

2. CT Analyser Software

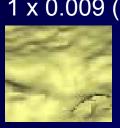


- Import reconstructed data
- Select region of interest (ROI)
- Calculate trabecular parameters
- Export analyses as text file

### Results: Disc Height Practice



Resolution level 2 X/Y: 2 x 0.009 (mm) Z: 1 x 0.009 (mm)

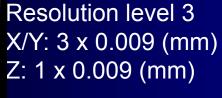


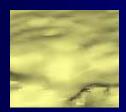
Resolution level 4 X/Y: 4 x 0.009 (mm) Z: 1 x 0.009 (mm)

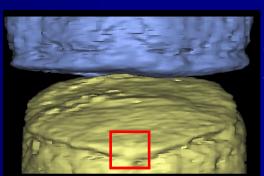


40.8 KB









Resolution level 5 X/Y: 5 x 0.009 (mm) Z: 1 x 0.009 (mm)



62.5 KB

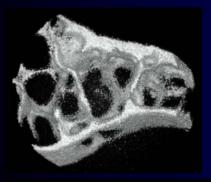
46.4 KB

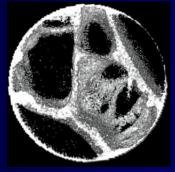
# X-Y resolution reduction by 2-5x appears appropriate for estimating IVD height in mice

resolution	mean	minimum	maximum	
1	n/a	n/a	n/a	
2	0.2887	0.1383	0.5271	
3	0.284	0.1364	0.4789	
4	0.2849	0.136	0.5103	
5	0.2898	0.136	0.5469	
STDEV	0.00283	0.00110	0.0288	

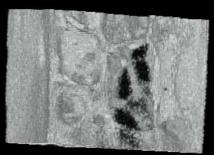
Table 03. Disc heights of L45 of mice spine sample 1 at various resolutions

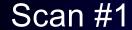
#	Date	type	voltage (kV)	brightness (kV)	FOV XY (mm)	FOV Z (mm)	SOD (mm)	Resolution (microns)
1	07/31/2010	cancellous bone	62	0	3.098	1.895	8.6	512; (6.050)
2	08/04/2010	cancellous bone	60	0	0.873	0.825	3.9	512; (1.705)





•Trabecular bone images of the fourth vertebrate at two different resolutions.







Scan #2

**Bone Volume Ratio over Vertebrae Levels** 

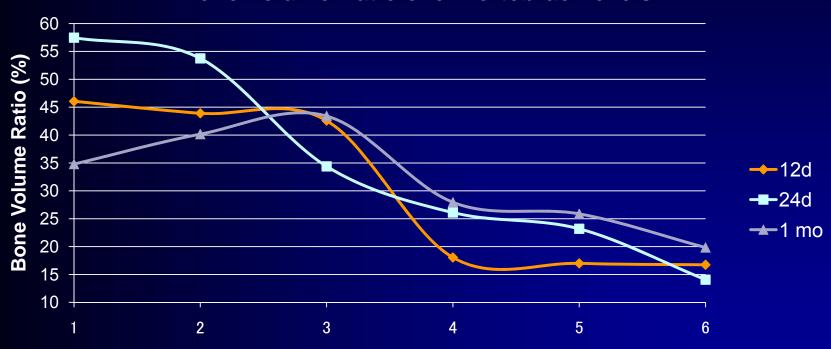


Figure 01. Bone volume ratio of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.

Vertebrae Levels

**Bone Surface Density over Vertebrae Levels** 

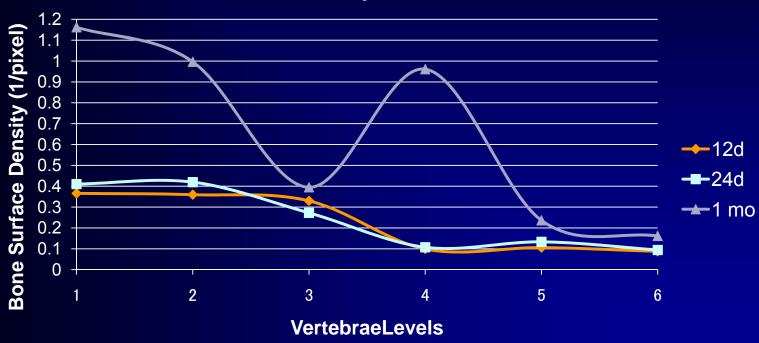


Figure 02. Bone surface density of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.



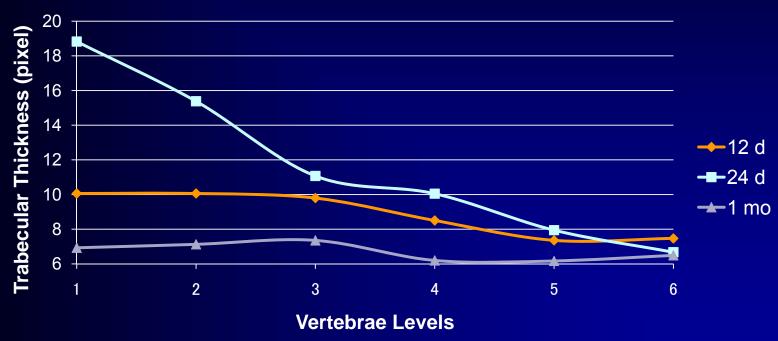


Figure 03. Trabecular thickness of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.



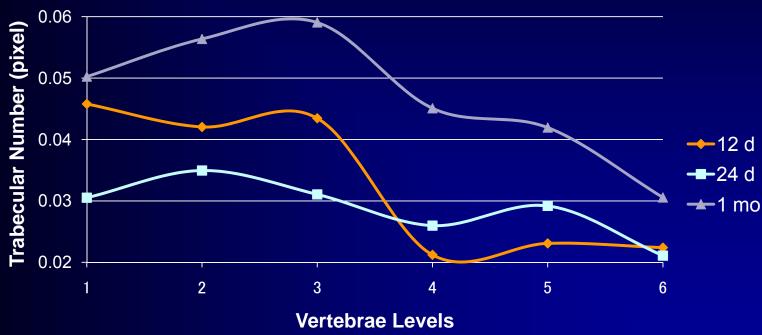


Figure 04. Trabecular number of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.

### Results: Endplate Imaging

#	date.,	voltage-	brightness	FOV:XY	FOV:Z	resolution.	
		(kV).1	(power).	(mm).1	(mm).,	(microns).1	А
1*.,	2010/6/8.,	80.1	20.1	0.834.,	0.730.,	512; (1.629)	
2.,	2010/5/8.,	72.1	0.,	0.817.,	0.743.,	512; (1.595)	
3.1	2010/5/8.,	72.,	0.,	0.750.,	0.750.,	512; (1.465)	

Table 04. micro CT scanning results of endplates at various voltage-power settings with a source-to-object distance (SOD) of 3.5 mm and a matrix of 512 x 512.

### Results: Endplate Imaging

4*	2010/5/8.,	65.1	55.,	0.813.,	0.743.,	512; (1.588)	
5.1	2010/6/8.,	65.1	0.1	0.834.,	0.730.,	512; (1.629)	
6.1	2010/6/8.,	60.1	0.1	0.834.,	0.730.,	512; (1.629)	

<sup>\*</sup> oscillograph at 50% - 60% ₽

Table 05. micro CT scanning results of endplates at various voltage-power settings with a source-to-object distance (SOD) of 3.5 mm and a matrix of 512 x 512.

### **Discussion**

#### Accomplished/Established:

- Trabecular and endplate high resolution scanning protocol
- Mimics software protocol
- Micro CT scanning and reconstruction practice

#### Future Goals

- Space mice disc heights calculation and analysis
- Further assessment of parameters of trabecular analyses
- Biochemical analyses of collagen-proteoglycan ratio

### Acknowledgements

#### Laboratories and People

- Prof. Nozomu Inoue, Tissue Engineering Lab, Doshisha University
- Prof. Noriko Koizumi, Research Center for Inflammation and Regenerative Medicine, Doshisha University
- Prof. Robert L Sah, Cartilage Tissue Engineering Lab, UCSD
- Prof. Koichi Masuda, Skeletal Translational Research Lab, UCSD
- Dr. Gabriele Wienhausen, Associate Dean of Education, Division of Biology, UCSD
- Dr. Peter Arzberger, Principal Investigator, Pacific Rim Application and Grid Middleware Assembly (PRAGMA)

#### Programs and Supporting Agencies

- UCSD PRIME
- National Science Foundation, IOSE-0710726