Biomarker Research

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Biomarkers

(National Institutes of Health)

"a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

- Adaptive vs pathologic process
- Diagnosis
- Monitoring of therapy
- Catastrophic injury prevention-an indication unique to horses

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2005

2009

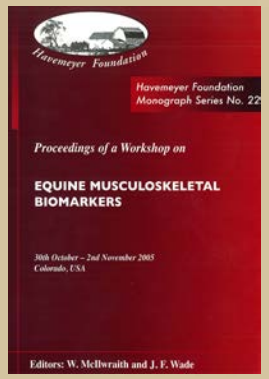
Havemeyer Foundation

2014

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12339

Science in brief: report on the Havemeyer Foundation workshop on equine musculoskeletal biomarkers - current knowledge and future needs

C. W. McIlwraith and P. D. Clegg†
Gail Holmes Equine Orthopaedic Research Center, Colorado State University, Fort Collins, USA
†Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Chester, UK.



The authors would like to thank Christopher Little, Sheila Laverty, David Frisbie, Mark Vaudin, Stina Ekman, Eva Skiöldebrand, Joanna Price, Christopher Riley, Christopher Kawcak, Roger Smith for assistance in writing this paper. The meeting was also attended by Troy Trumble, Jack Quinn, Elwyn Firth, Chris Riley and John Kisiday as well as prominent experts from the human biomarker research field Robin Poole, Dick Heinegård, Bruce Caterson and Virginia Byers Kraus. A full report for the meeting can be found at http://csu-cvmbs.colostate.edu/documents/research-equine-musculoskeletal-biomarkers-white-paper.pdf

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Clinical Injuries

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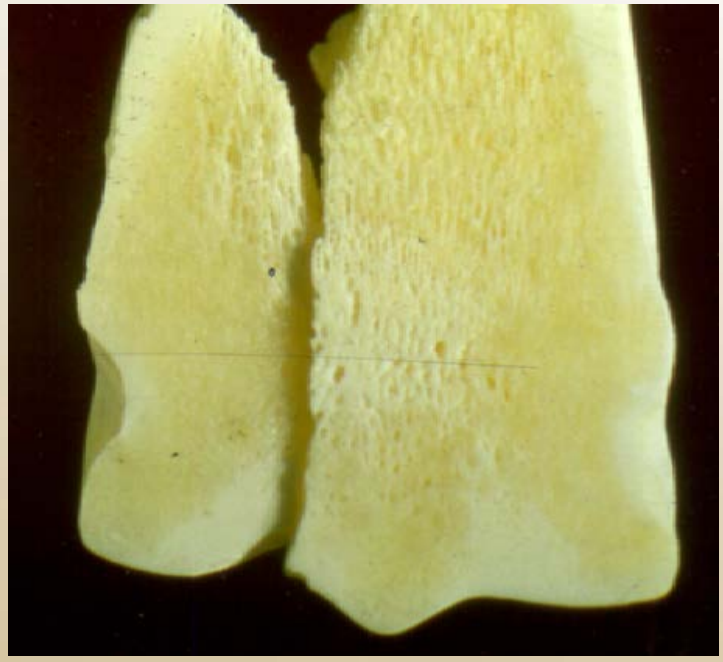

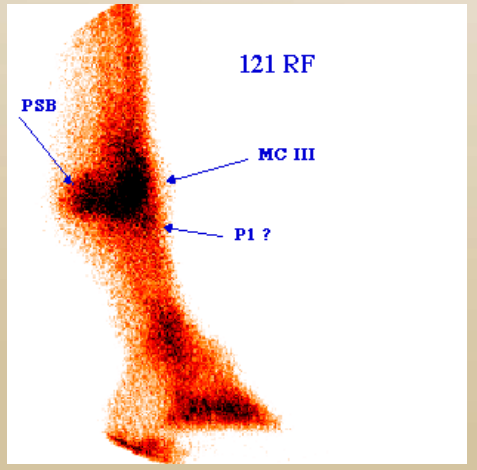

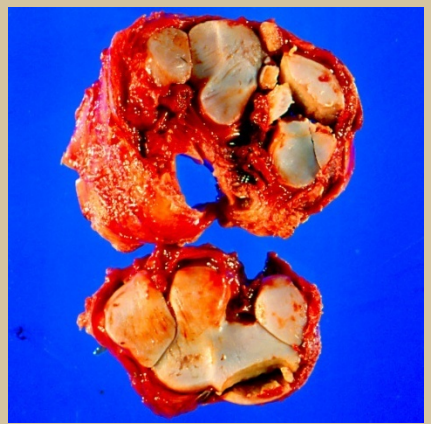

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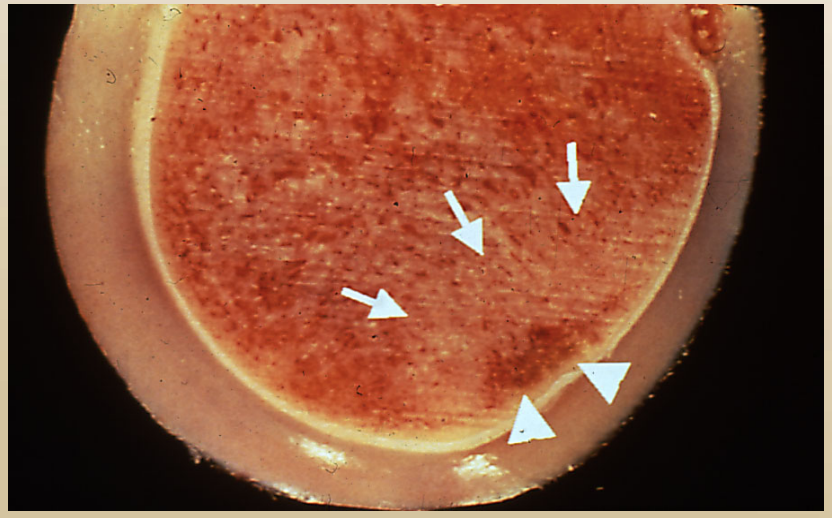
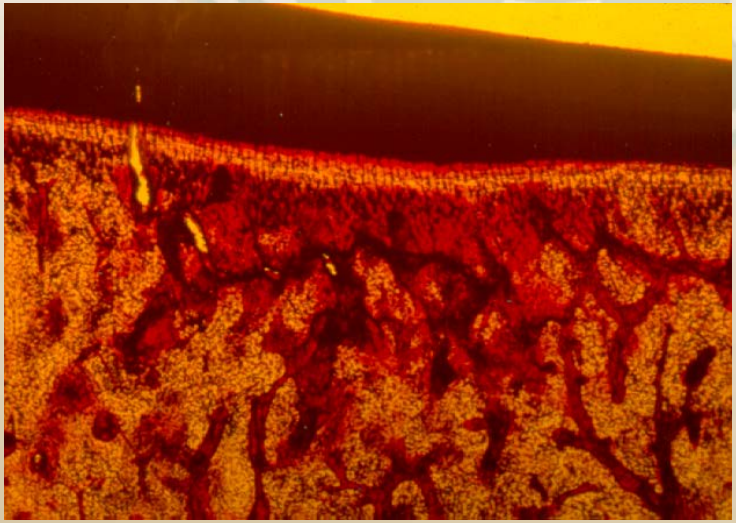
Articular Fractures of Metacarpophalangeal (fetlock) Joint




Courtesy LR Bramlage DVM

Subchondral bone disease precedes articular fractures





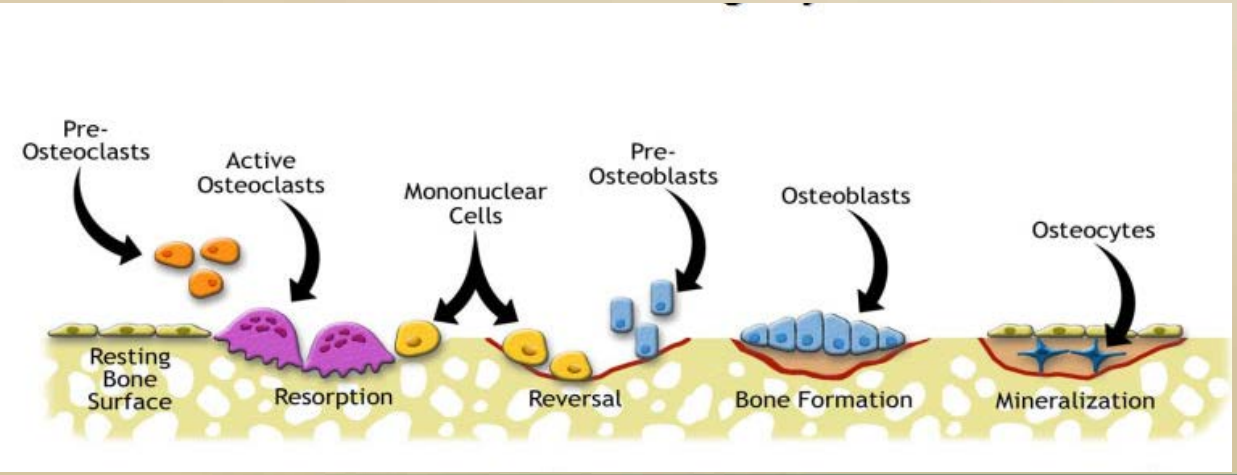
Norrdin et al Bone 1999

Biomarkers

- Fluids
  - Blood
  - Synovial Fluid
  - Urine
- Imaging
  - Structure
  - Physiologic response to adaptation or disease
- Movement
  - Inertial Measurement Units

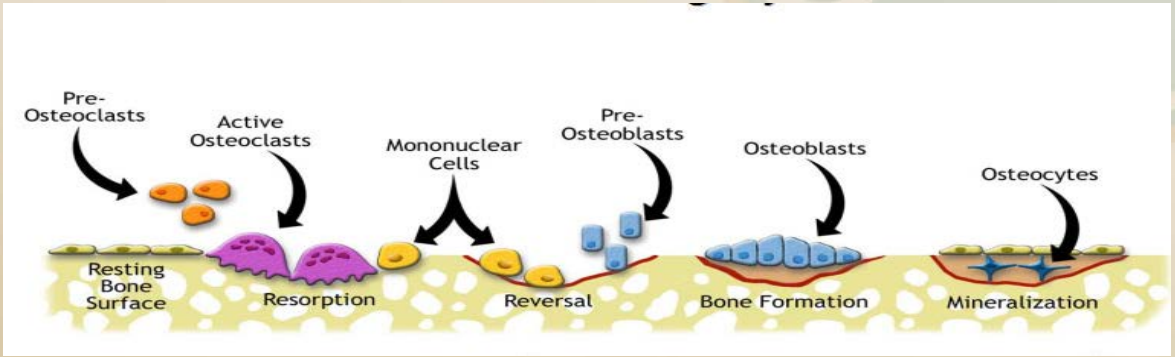
Normal Response to Repetitive Loading

- Normal remodeling cascade
  - Relatively rapid resorption followed by relatively delayed bone formation.
  - Creates reversal lines that form strong integration with parent bone.



Factors That Can Influence Response to Loading

Microdamage



Inappropriate Training
Too High
Too Low

Inappropriate Bone Formation

Inappropriate Mineralization

Need to understand normal adaptive changes

Fluid Biomarkers

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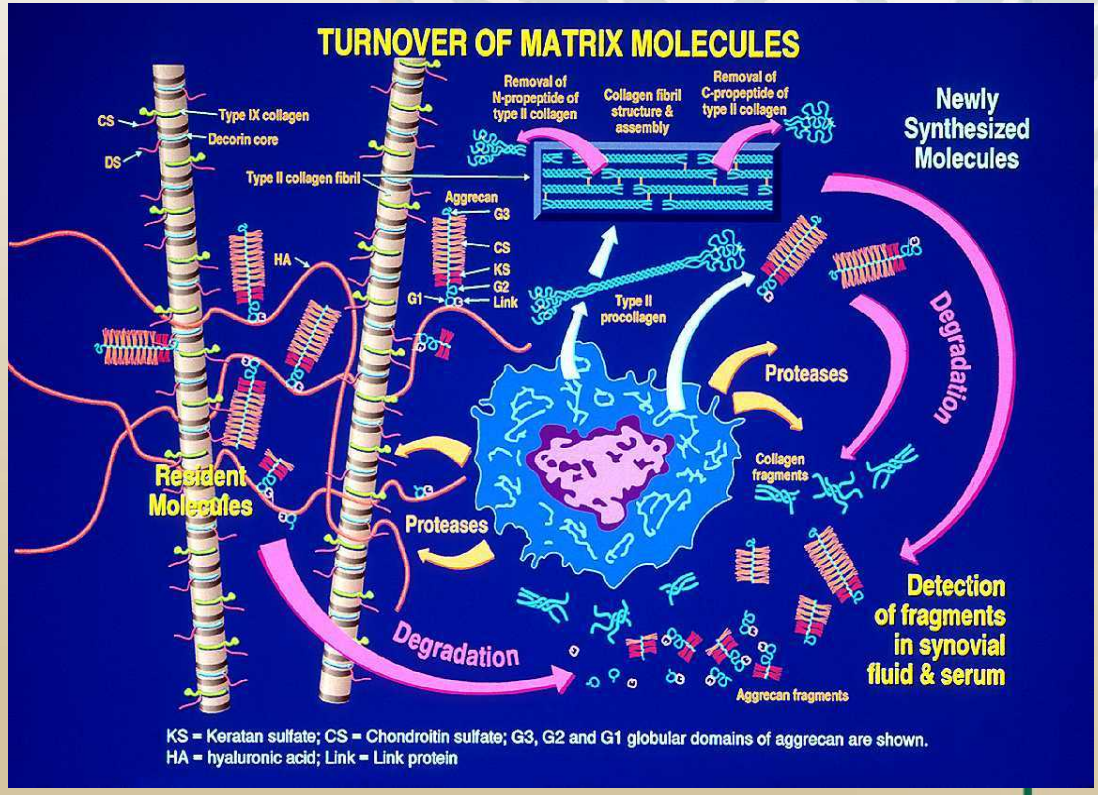
- Direct or indirect indicators of abnormal skeletal turnover
- Often molecules that are the normal products and byproducts of metabolic processes occurring within the skeletal tissues
- Concentrations may increase or decrease



Courtesy Poole 2000

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First Study of Prediction of Disease in Horses - SF Biomarker Changes with Osteochondral Fragmentation

- TP significantly higher (p=0.0001)
- CS846 significantly higher (p=0.0290)
- CPII not significantly higher (p=0.0653)
- KS not significantly higher (p=0.2841)
- WBC not significantly higher (p=0.3425)

Am J Vet Res 1999

Measurement of synovial fluid and serum concentrations of the 846 epitope of chondroitin sulfate and of carboxy propeptides of type II procollagen for diagnosis of osteochondral fragmentation in horses

David D. Frisbie, DVM, MS; Christopher S. Ray, DVM, MS; Mirela Ionescu, MSc; A. Robin Poole, PhD, DSc; Phillip L. Chapman, PhD; C. Wayne McIlwraith, BVSc, PhD

Serum Concentrations of CS Epitope 846 & CPII in Horses with Osteochondral Fragmentation

- CS 846 + CPII concentrations were significantly higher in horses with OC than in control (KS not)
- CS 846 + CPII concentrations not linearly related to grade of OC but significantly higher with grade 1 & grade 2 OC
- 79% correct in estimating presence of osteochondral fragmentation based on serum CS 846 & CPII

*Frisbie et al. Am J Vet Res 1999;**60**:306-309*

Biomarkers Change with Exercise

- Need to differentiate from disease in equine athlete

OsteoArthritis and Cartilage (2003) **11**, 760–769

doi:10.1016/S1063-4584(03)00152-3

Osteoarthritis and Cartilage

ICRS International Cartilage Repair Society

OARSI Osteoarthritis Research Society International

Significant exercise-related changes in the serum levels of two biomarkers of collagen metabolism in young horses

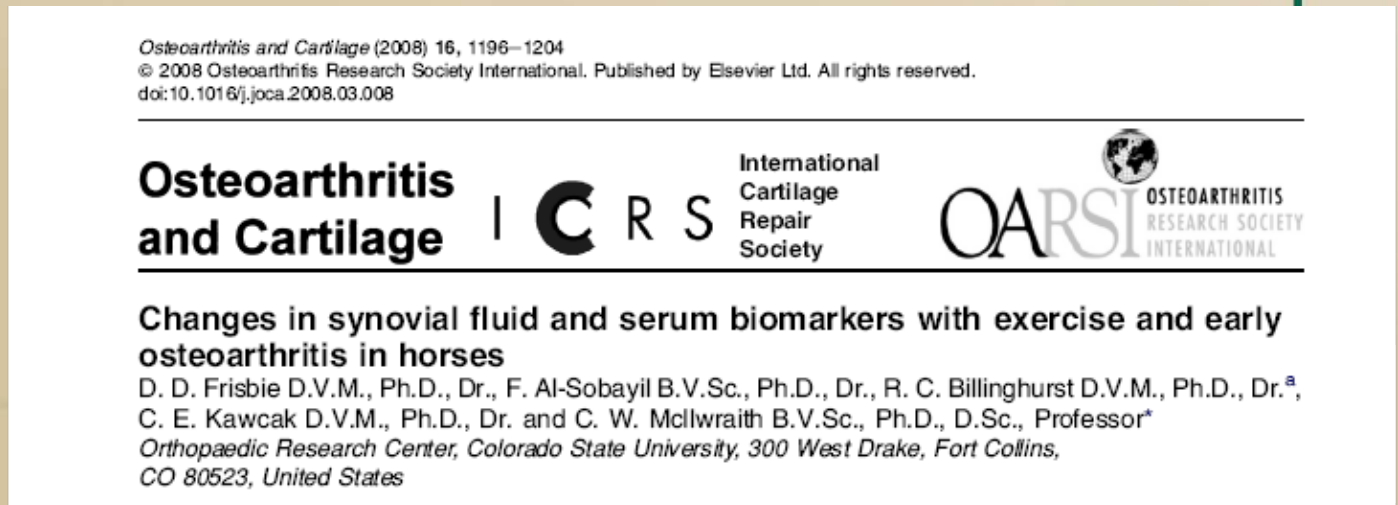
R. C. Billinghurst D.V.M., Ph.D. Associate Professor†*, P. A. J. Brama D.V.M., Ph.D. Assistant Professor‡, P. R. van Weeren D.V.M., Ph.D. Professor‡, M. S. Knowlton B.S. Research Associate† and C. W. McIlwraith B.V.Sc., Ph.D. Professor†

† *Department of Clinical Sciences, Colorado State University, Fort Collins, CO 80523, USA*

‡ *Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*

Differentiation of Biomarker Changes with Exercise versus Disease

- Treadmill study
- Horses with exercise alone and horses with exercise plus osteochondral fragmentation model of OA



Osteoarthritis and Cartilage (2008) **16**, 1196–1204

doi:10.1016/j.joca.2008.03.008

Osteoarthritis and Cartilage

ICRS International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Changes in synovial fluid and serum biomarkers with exercise and early osteoarthritis in horses

D. D. Frisbie D.V.M., Ph.D., Dr., F. Al-Sobayil B.V.Sc., Ph.D., Dr., R. C. Billinghurst D.V.M., Ph.D., Dr.[a], C. E. Kawcak D.V.M., Ph.D., Dr. and C. W. McIlwraith B.V.Sc., Ph.D., D.Sc., Professor*

Orthopaedic Research Center, Colorado State University, 300 West Drake, Fort Collins, CO 80523, United States

Serum Biomarkers - Exercise and OA

- Concentration of serum CS846, CPII, GAG, osteocalcin, C1,2C and Col I increased with exercise
- For each of these biomarkers, there was also a statistically significant increase in levels in OA-affected horses compared to exercise-alone horses
- Six SF & serum biomarkers were useful in separating early experimental OA from exercise alone but SF CTX1 and serum Col CEQ & CTX1 were not

Prospective collection of serum samples to examine prediction of injury with biomarkers

- 2-3 year old racing Thoroughbred in training/racing in Southern California

Serum biomarker levels for musculoskeletal disease in two- and three-year-old racing Thoroughbred horses: A prospective study of 130 horses

D. D. FRISBIE*, C. W. McILWRAITH, R. M. ARTHUR†, J. BLEA‡, V. A. BAKER§ and R. C. BILLINGHURST¶

Equine vet J. (2010) 42 (7) 643-651
doi: 10.1111/j.2042-3306.2010.00123.x

Prospective collection of seven biomarkers

- 238 racing TBs
- Exit criteria were lack of training for > 30 days, or completion of 10 study months
- Horses with solitary MS injury & completion of >2 months training were analyzed
- Musculoskeletal injury
  - Intraarticular fragmentation (IAF)
  - Tendon or ligamentous injury (TL)
  - Stress fracture (SF)
  - Dorsal metacarpal disease (DMD)

Results

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- 59 horses sustained single injury
- 71 acted as untreated controls
  - 16 (27%) IAF
  - 17 (29%) TL
  - 7 (12%) SF
  - 19 (32%) DMD
- Comparisons entry or injury time NSD
- There were significant changes seen in biomarkers based on injury incurred when longitudinal samples were assessed

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Results

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- Controls showed longitudinal increase in GAG & decrease in OC
- IAF showed decrease in CS 846 & an increase in OC & CTX-1 compared to controls
- TL horses demonstrated decrease in GAG & increase in CTX-1
- SF horses showed increase in CTX-1
- DMD showed decrease in CS 846 & GAG as well as increase in OC & CTX-1
- Based on serum biomarkers collected prior to injury, horses could be correctly identified as injured or noninjured 73.8% of the time

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Other Molecular Markers of Musculoskeletal Disease

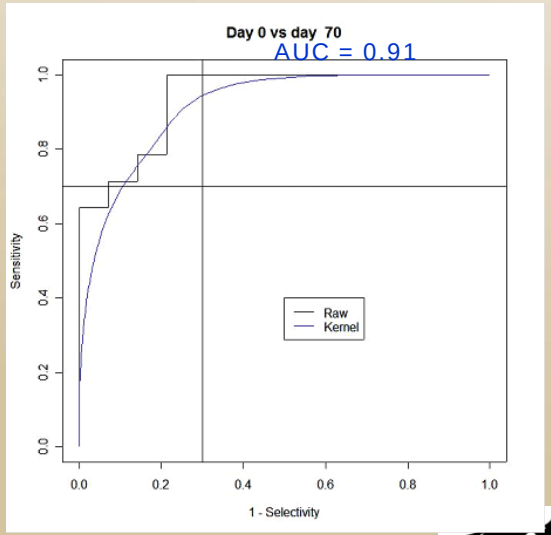
- 2005 Colorado State University Study
- Examined the differential expression of ~3100 equine gene sequences using Affymetrix GeneChip (1st Gen)
  - RNA from the peripheral blood of horses using the OA fragment model at
    - Day 0
    - Day 7
    - Day14 (2 weeks post OA induction)
    - Day 42
    - Day 70
  - Found 22 genes with a upregulated expression profile that matched those of serum biomarkers

Molecular Markers of Musculoskeletal Disease

- Six Genes Used for Day 0 to Day 70 ROC Analysis

Sensitivity	Specificity	Success	Genes					
0.972	0.882	0.943	BM781378_unkn	WBC032E04	WBC026F09	WBC003G03	WBC009B11	WBC419
0.972	0.882	0.943	WBC419	BM781378_unkn	WBC003G03	WBC026F09	WBC012E07	WBC032E04
0.972	0.882	0.943	BM781165	WBC003G03	WBC026F09	WBC419	BM735265	WBC018F02

Currently completing study in reining horses



D Frisbie et al. 2005 A Diagnostic Test for Equine Osteoarthritis Using Peripheral Blood Molecular Biomarkers Evaluated In an Experimental Model AAEP 2005

Imaging Biomarkers

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- Imaging Limitations
  - Trained human eye can reliably detect structural and textural changes in tissues for individual diagnosis and treatment plan development
    - Good once disease process has physically changed the tissues
    - Sometimes unlikely that tissues will revert back to normal
  - Are there changes below the resolution of the human eye that can be used?

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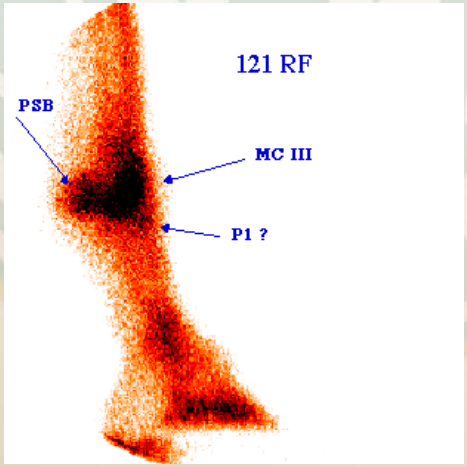
NUCLEAR SCINTIGRAPHY

- Need to interpret in face of normal remodeling / modeling response
- Often most sensitive method of characterizing problem since physical bone changes may not be apparent



Bone Sclerosis

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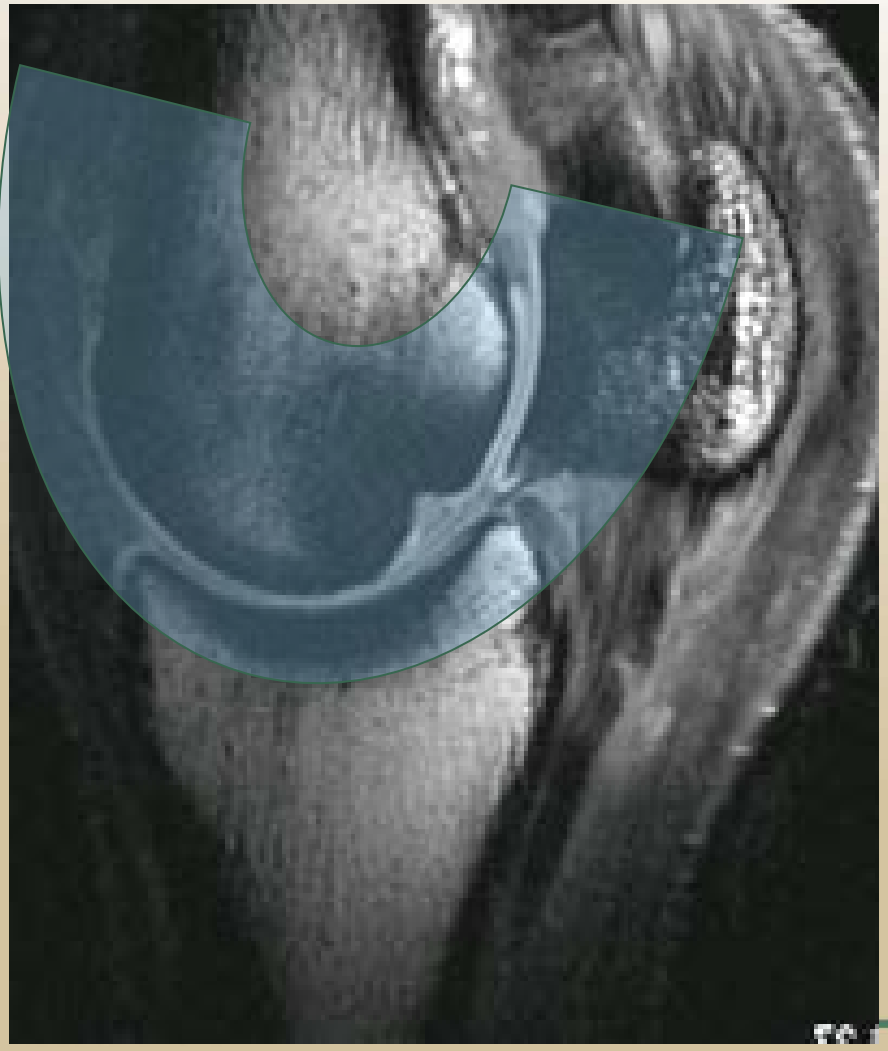

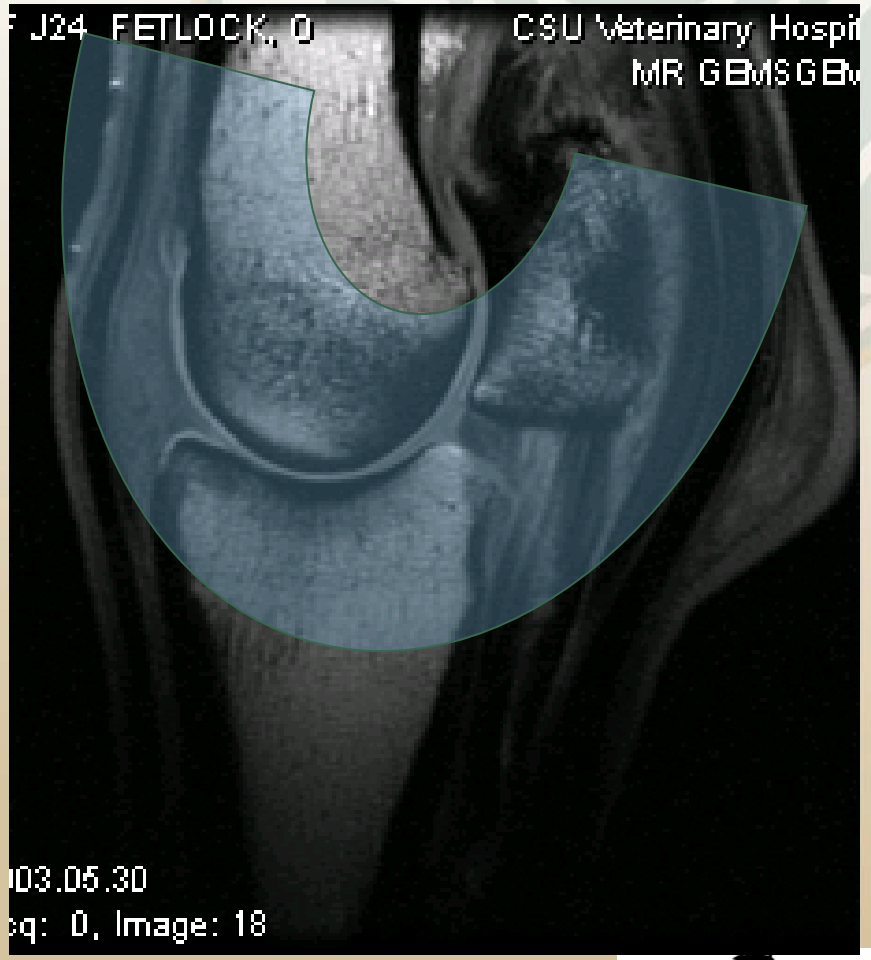

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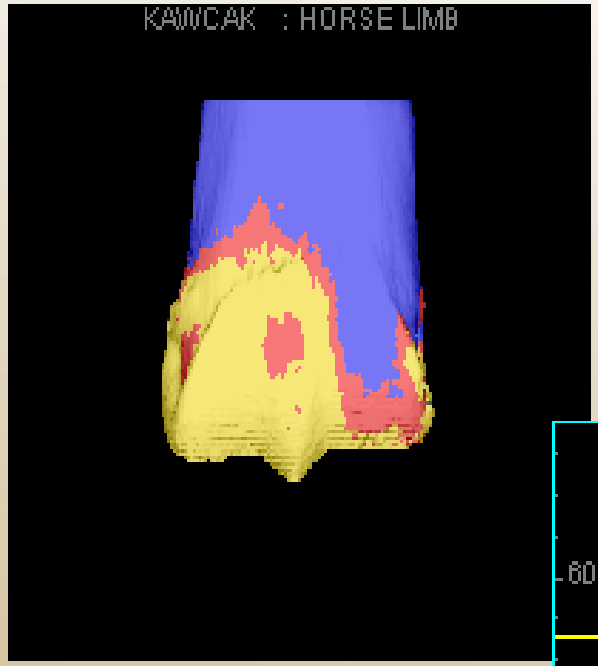
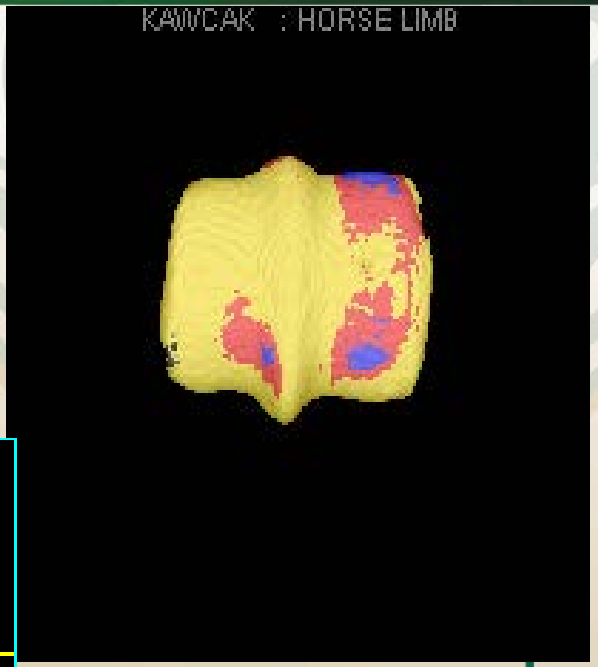
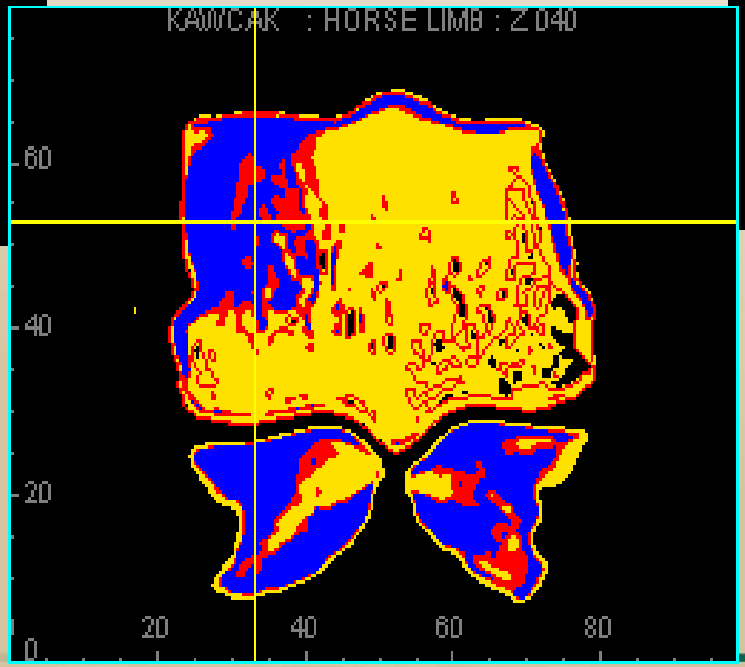
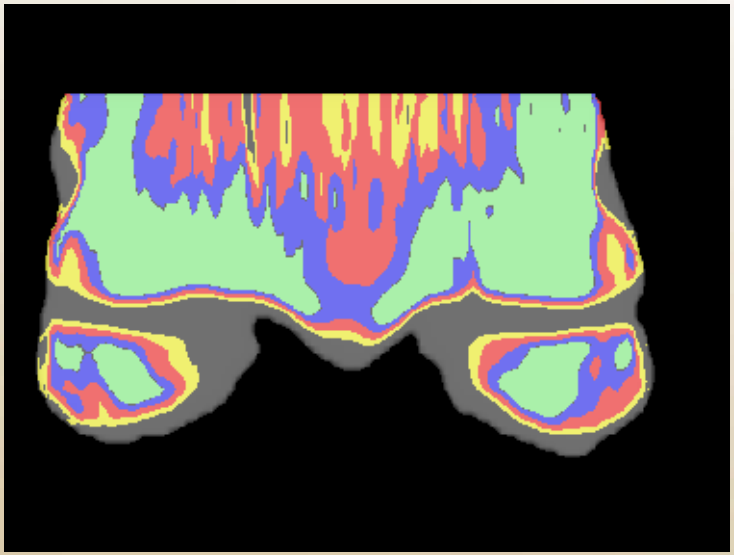
COMPUTED TOMOGRAPHY

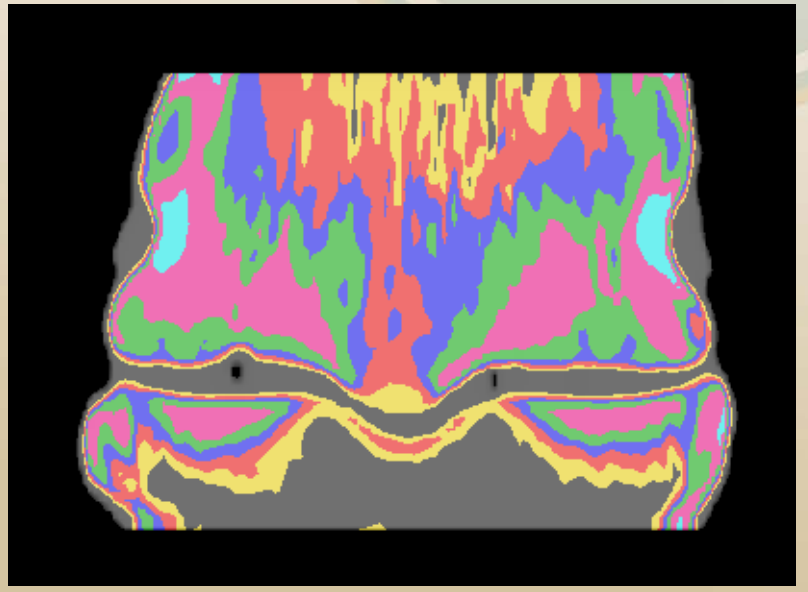
- High resolution
- Reformat into any plane
- Contrast CT
  - Venous
  - Arterial
  - Intraarticular
    - Plane contrast
    - CA4+


KAWCAK : HORSE LIMB

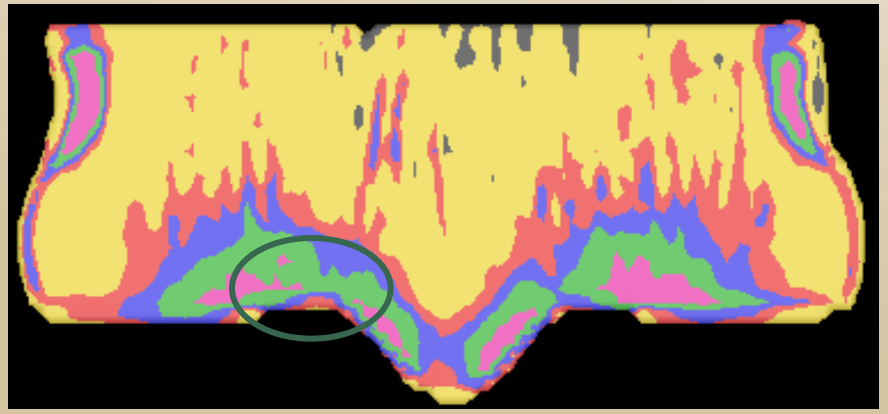

KAWCAK : HORSE LIMB

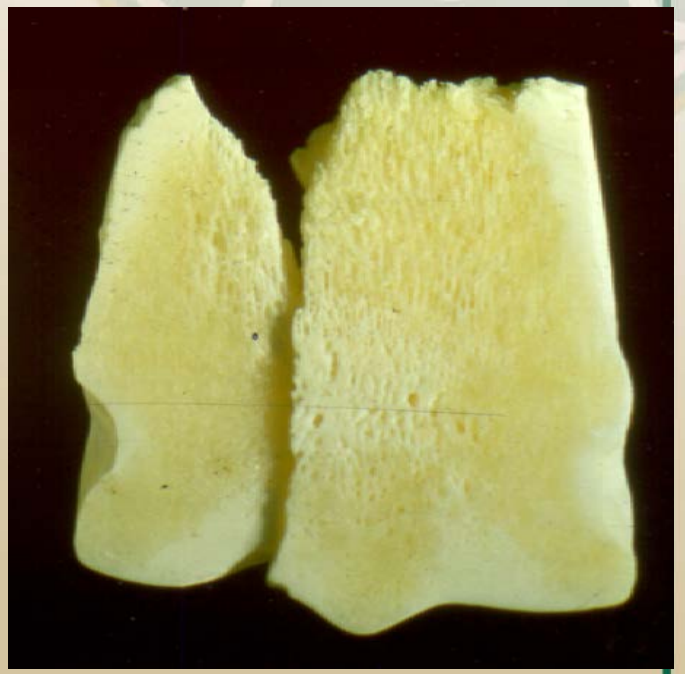

KAWCAK : HORSE LIMB : Z 040
60
40
20
0
20
40
60
80





- Density gradient between axial condyle and abaxial aspect of the sagittal ridge
  - Area involved in condylar fractures





General density patterns

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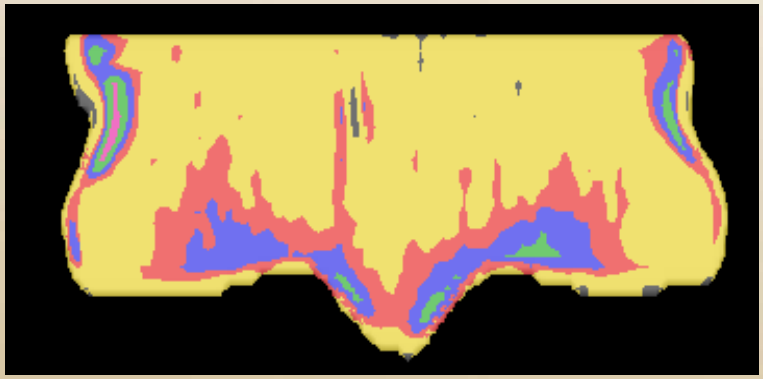
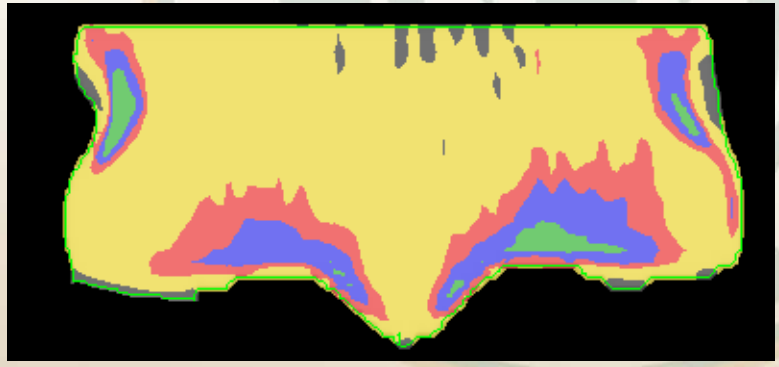
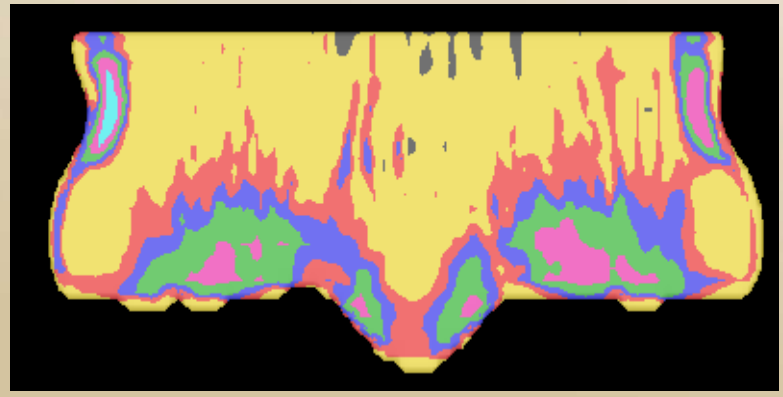


Exercised horse – palmar 30º



Exercised horse – palmar 30º



Control horse – palmar 30º

bone density

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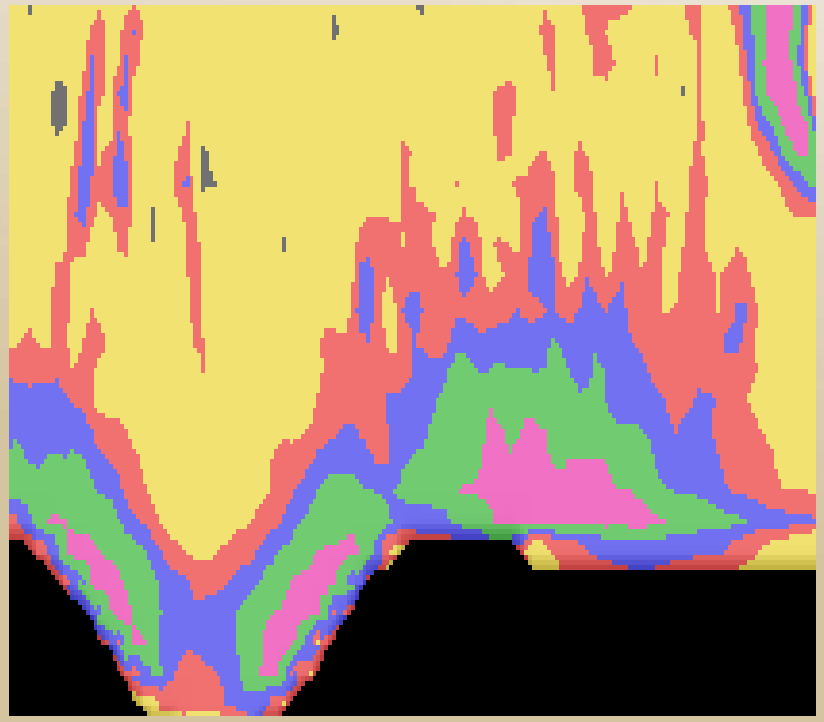
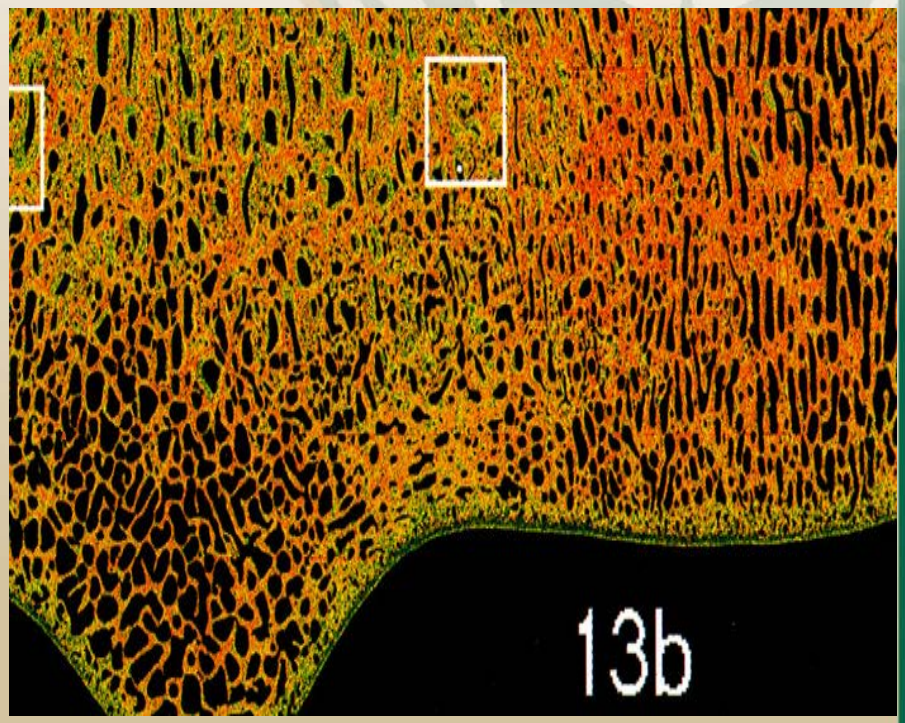
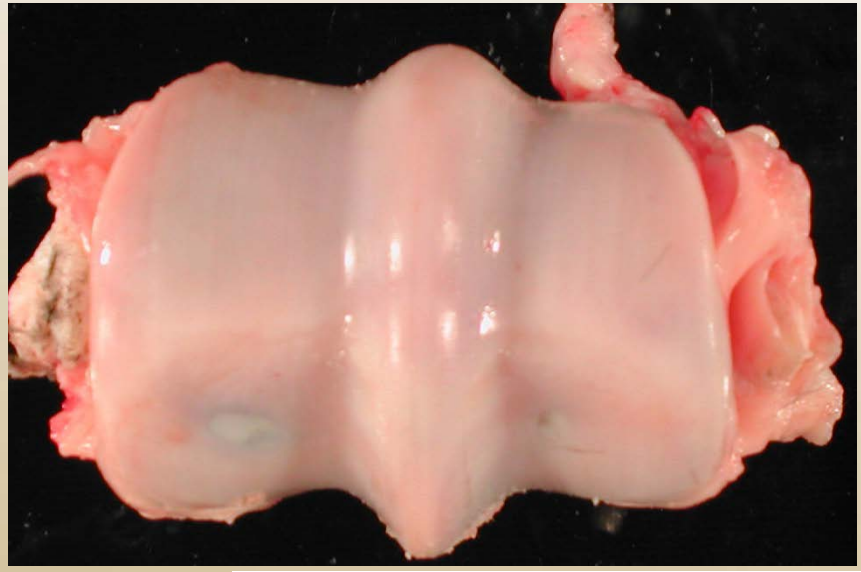
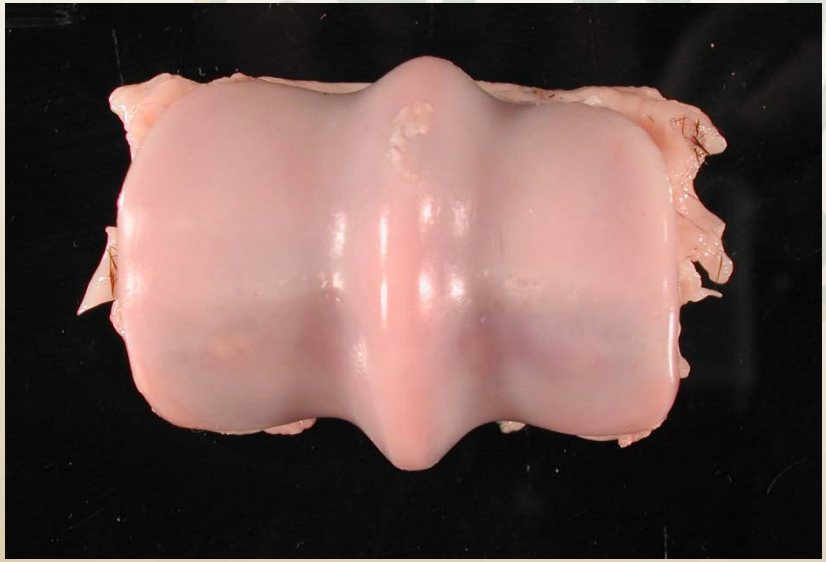
Can we detect this change in bone character?





Shape Variability





Effects of Third Metacarpal Geometry on the Incidence of Condylar Fractures in Thoroughbred Racehorses

Chris E. Kawcak, DVM, PhD, Diplomate ACVS; Chelsea A. Zimmerman, BS;
Katrina L. Easton, BS;
C. Wayne McIlwraith, BVSc, PhD, DSc, FRCVS, Diplomate ACVS; and
Tim D. Parkin, BSc, BVSc, Diplomate ECVPH, MRCVS

Results

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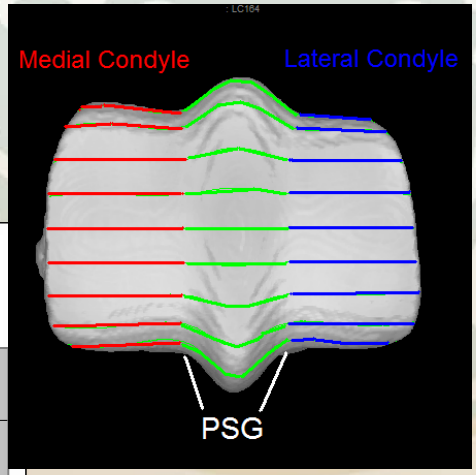

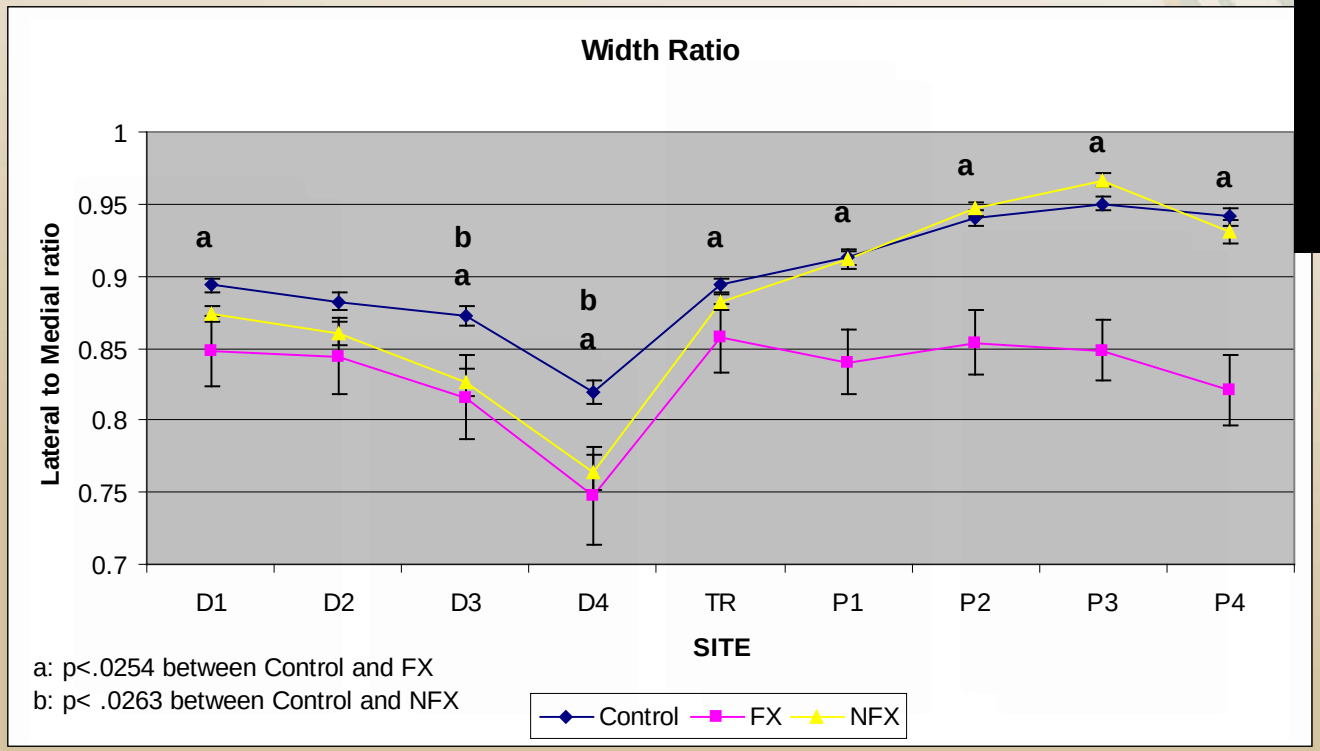

a: p<.0254 between Control and FX
b: p< .0263 between Control and NFX

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Statistical Shape Modeling

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Journal of Biomechanics 43 (2010) 1780–1786

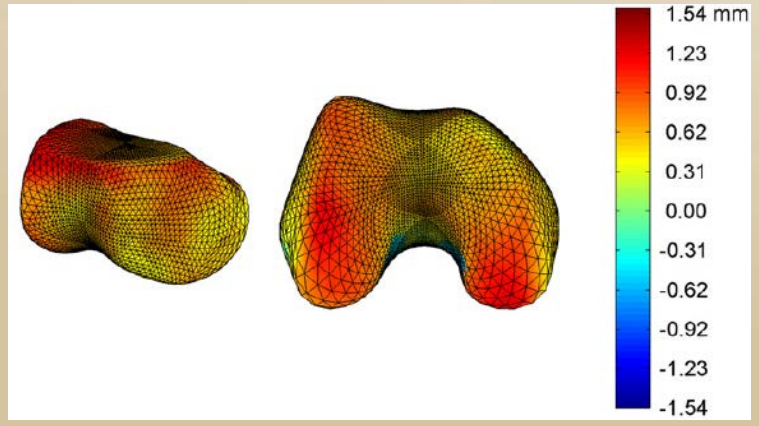
Contents lists available at ScienceDirect

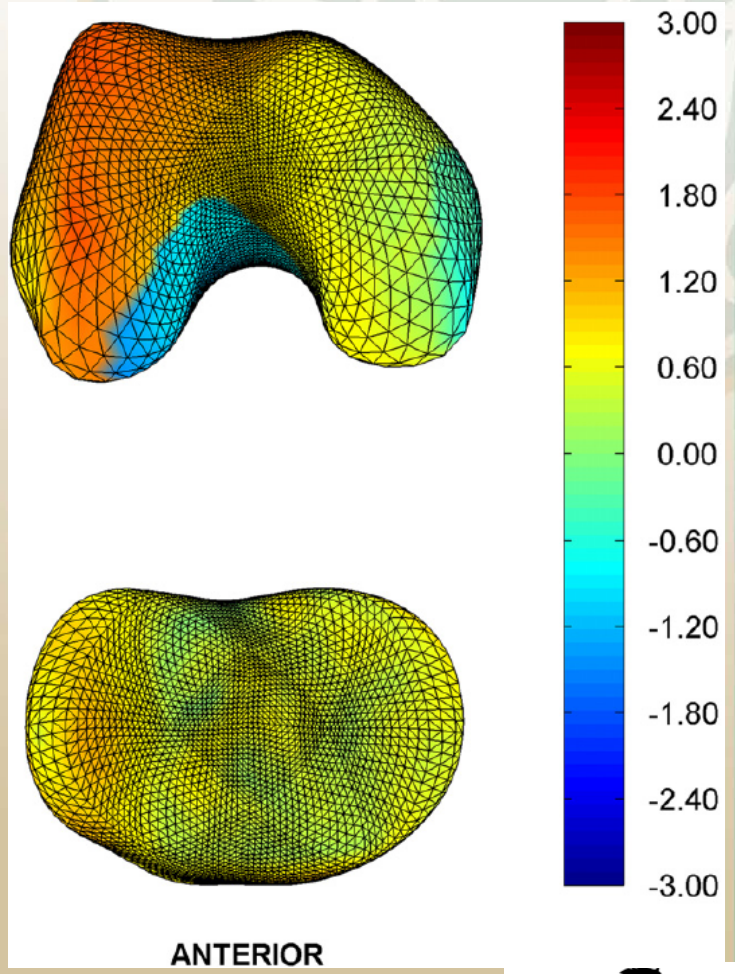
Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Statistical shape modeling describes variation in tibia and femur surface geometry between Control and Incidence groups from the Osteoarthritis Initiative database

Todd L. Bredbenner [a,*], Travis D. Eliason [a], Ryan S. Potter [a], Robert L. Mason [a], Lorena M. Havill [b], Daniel P. Nicolella [a]





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Fracture Risk Discriminators based on Statistical Shape and Density Modeling of the Proximal Femur

[1]Todd L. Bredbenner, [1]Ryan S. Potter, [1]Robert L. Mason, [2]Lorena M. Havill, [3]Eric S. Orwoll, and [1]Daniel P. Nicolella for the Osteoporotic Fractures in Men (MrOS) Study

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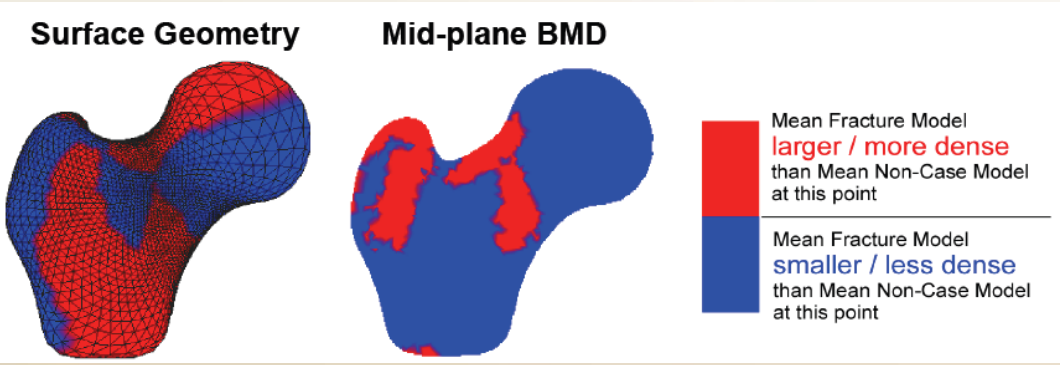
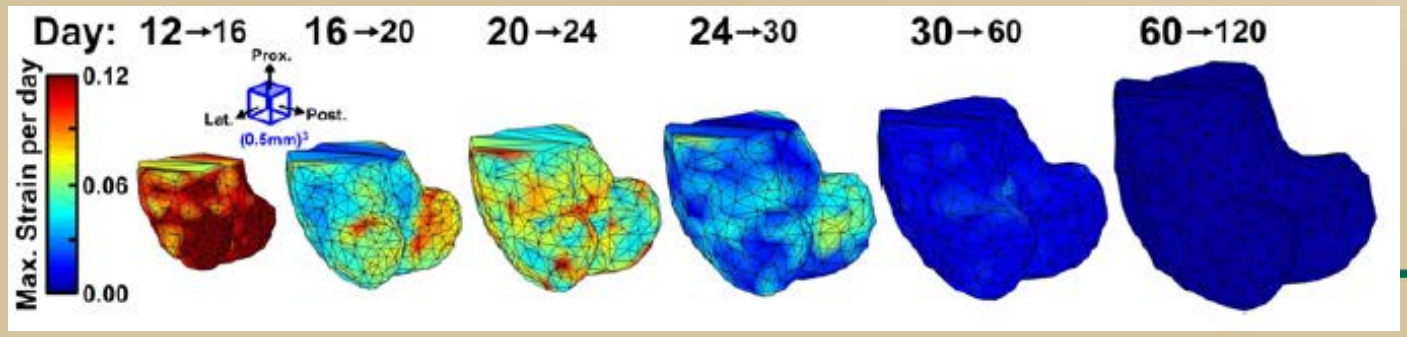


Structural and Functional Maturation of Distal Femoral Cartilage and Bone During Postnatal Development and Growth in Humans and Mice

Elaine F. Chan, MS[a], Ricky Harjanto, BS[a], Hiroshi Asahara, MD, PhD[d], Nozomu Inoue, MD, PhD[e,f], Koichi Masuda, MD[c], William D. Bugbee, MD[g], Gary S. Firestein, MD[b], Harish S. Hosalkar, MD[h], Martin K. Lotz, MD[d], Robert L. Sah, MD, ScD[a,i,*]



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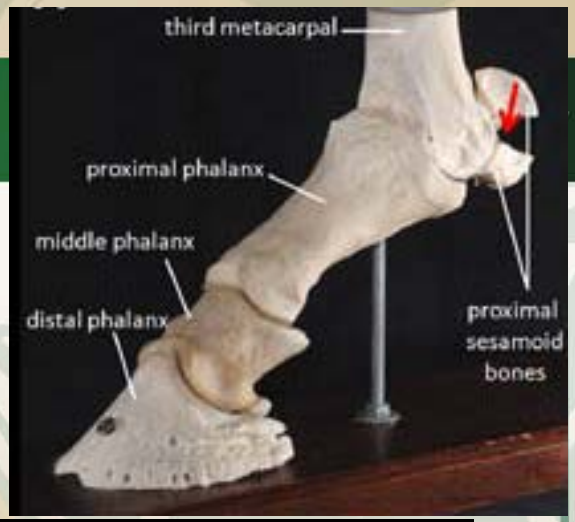
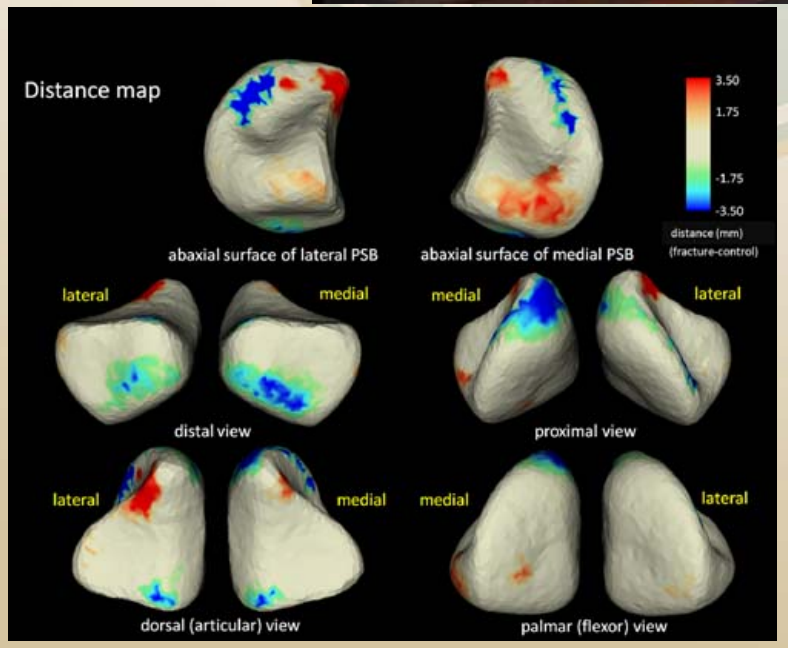
Recent statistical shape model (SSM) study of horse proximal sesamoid bones



- Related fracture risk to bone geometry in forelimbs of Thoroughbred racehorses
- Created average surfaces (gray)
- Colors show locations of differences between fracture group and control group
  - Red up to 3.5mm prominence of fracture group
  - Blue up to 3.5mm prominence of the control group



Journal of Orthopaedic Research
Volume 30, Issue 8, pages 1277-1284, 17 JAN 2012

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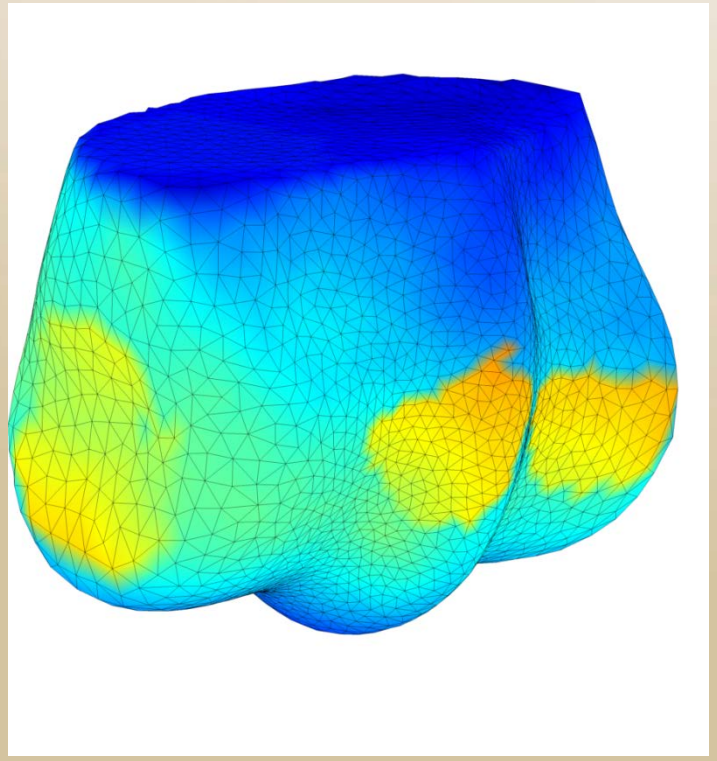
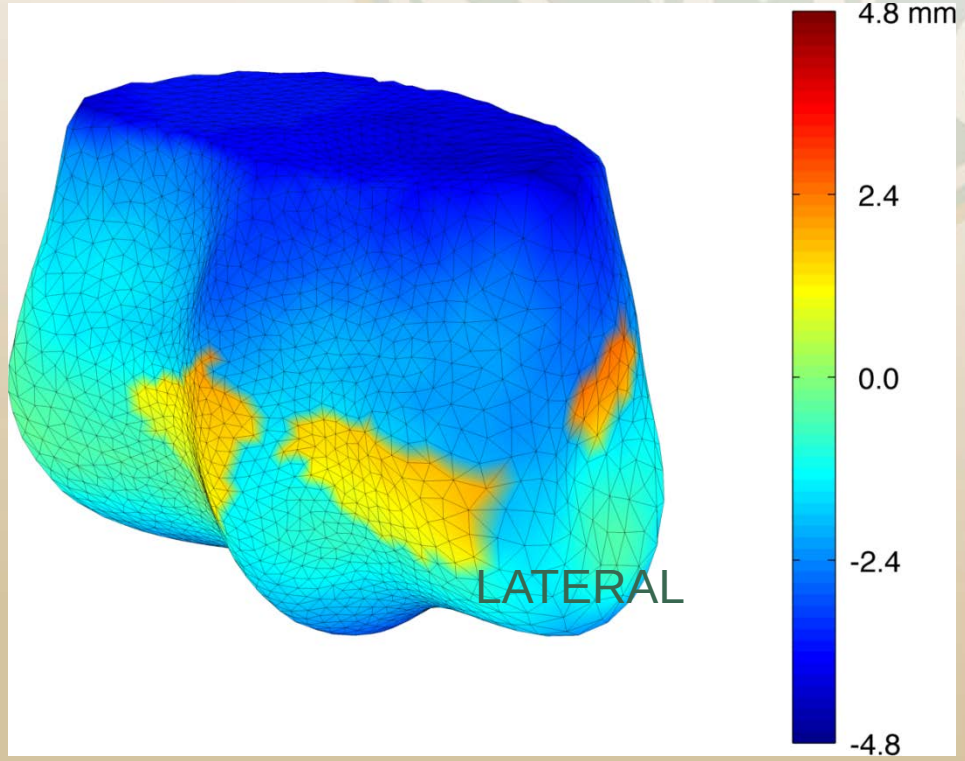
Statistical Shape Model Example

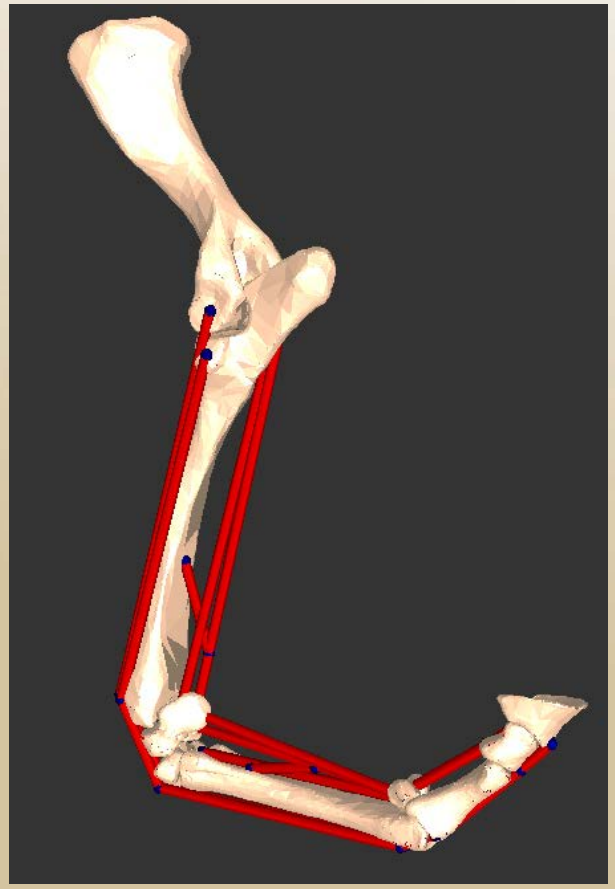


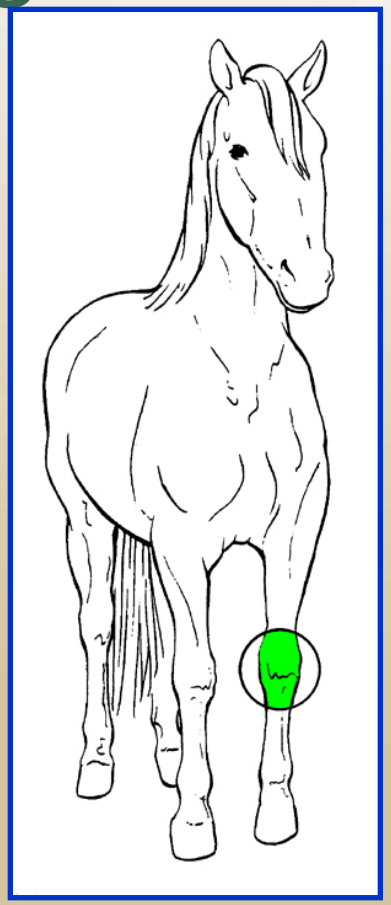


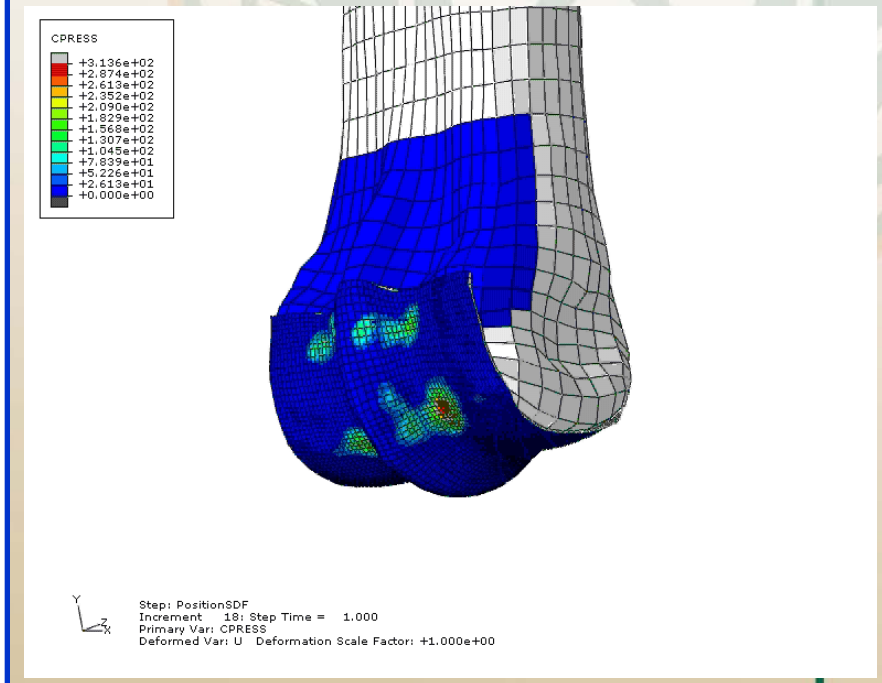
Statistical Shape and Trait Modeling

- Allows investigation of the importance of variation in a broad range of measurable or estimable musculoskeletal traits, as well as the interaction between traits
  - Example applications (cross-sectional or longitudinal studies)
    - Prediction of likelihood of onset of MCP osteoarthritis
    - Prediction of metacarpal fracture
    - Determination of developmental effects
  - Example traits
    - Bone geometry and overall BMD distribution (i.e. SSDM)
    - Limb / Joint alignment
    - Cartilage thickness
    - Compositional, microstructural, and material properties of bone, cartilage, ligaments, tendons, etc…

Pathogenesis - Modeling







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Market Factors

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- Cost
  - Low cost for repeated use
- Ease of use
- Development
  - Relatively small market limits development of novel biomarkers
    - Imaging and fluid markers
- Objective data to verify use
  - Large clinical studies
  - Limited funding opportunities

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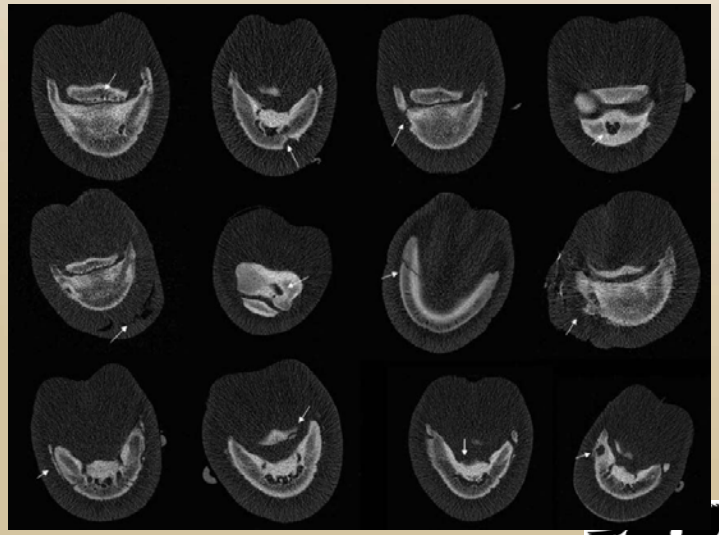
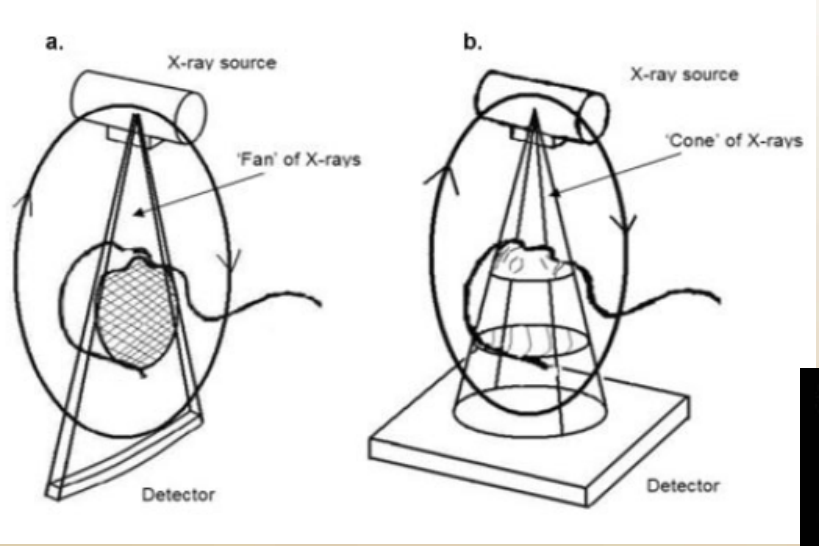
EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2008) **20** (2) 93-98
doi: 10.2746/095777308X272085

Original Article

A technique for computed tomography (CT) of the foot in the standing horse

F. G. Desbrosse, J.-M. E. F. Vandeweerd*, R. A. R. Perrin, P. D. Clegg†, M. T. Launois, L. Brogniez and S. P. Gehin




a.
X-ray source
'Fan' of X-rays
Detector
b.
X-ray source
'Cone' of X-rays
Detector


a.
b.

Standing CBCT

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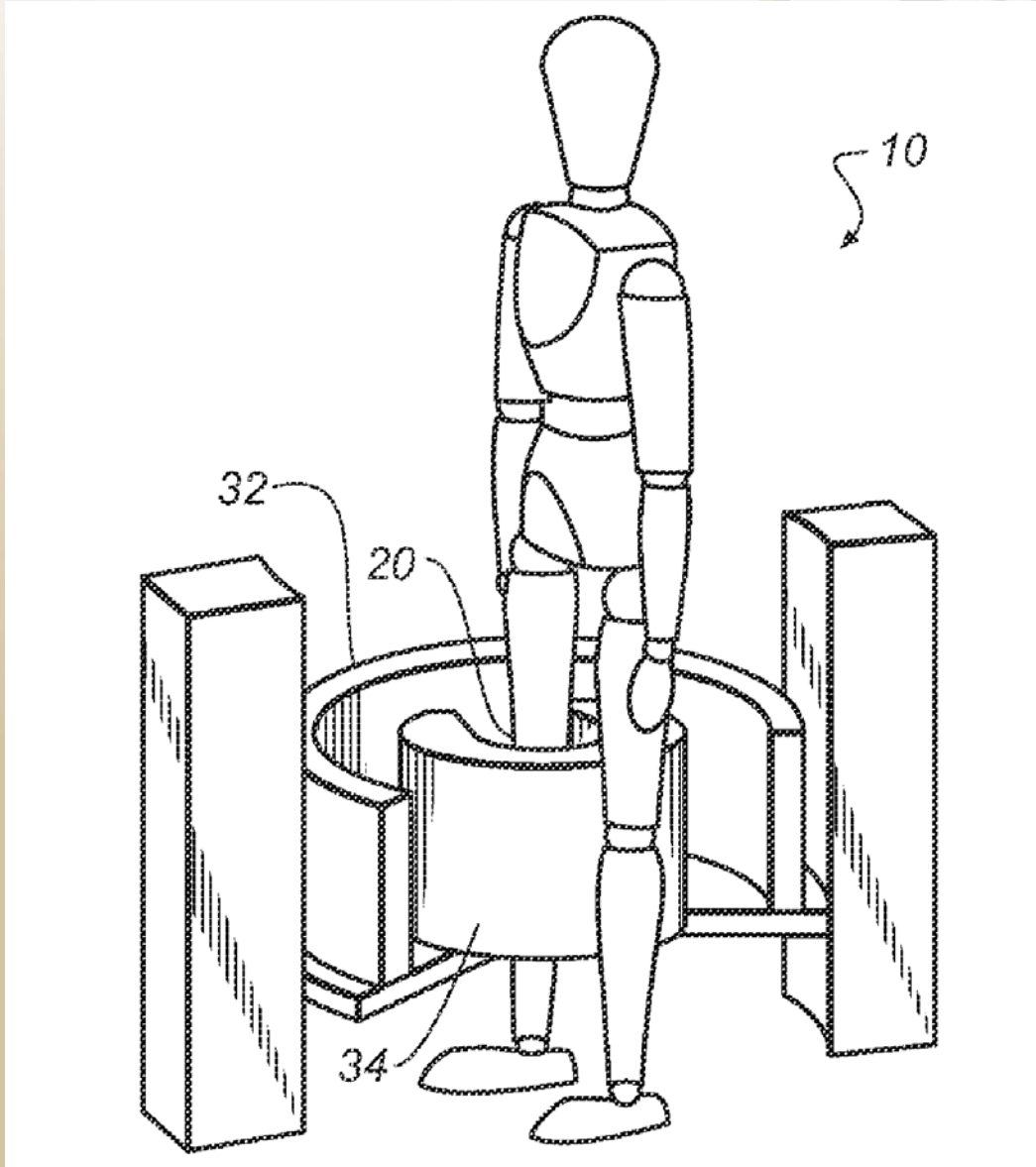

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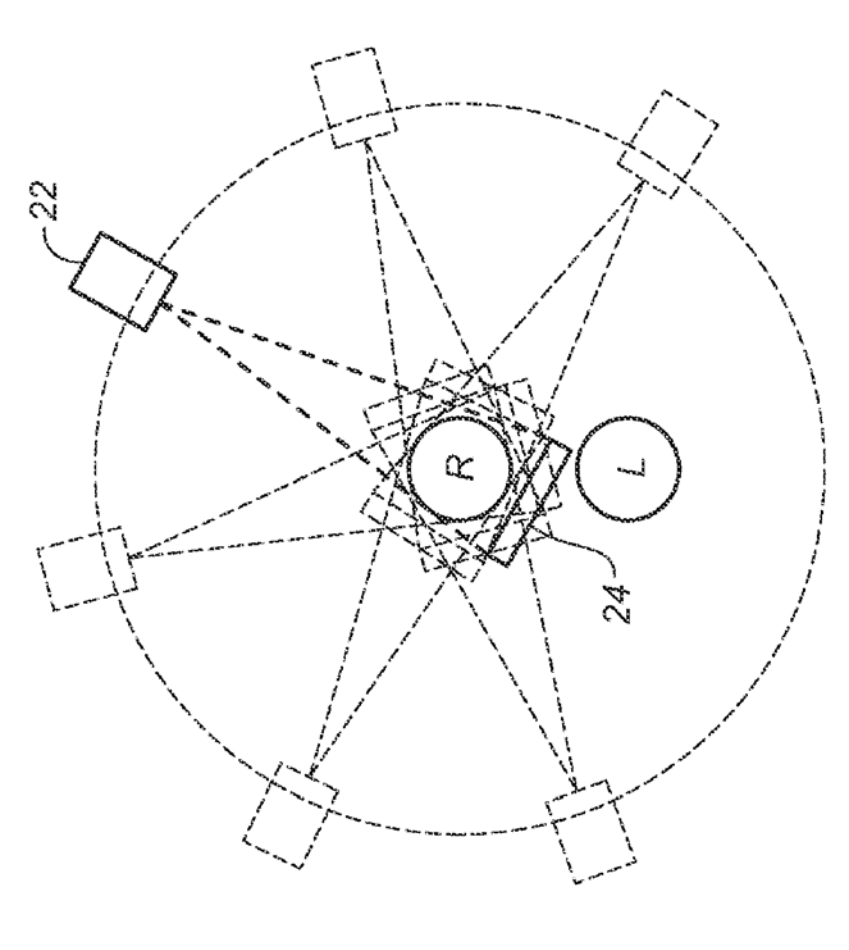
22
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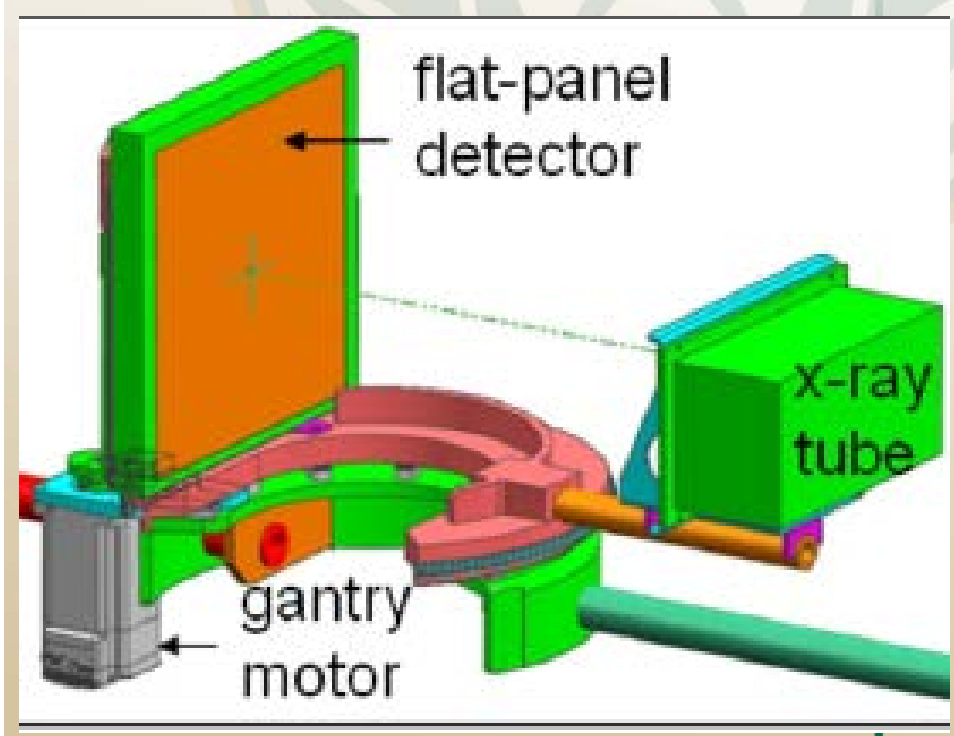
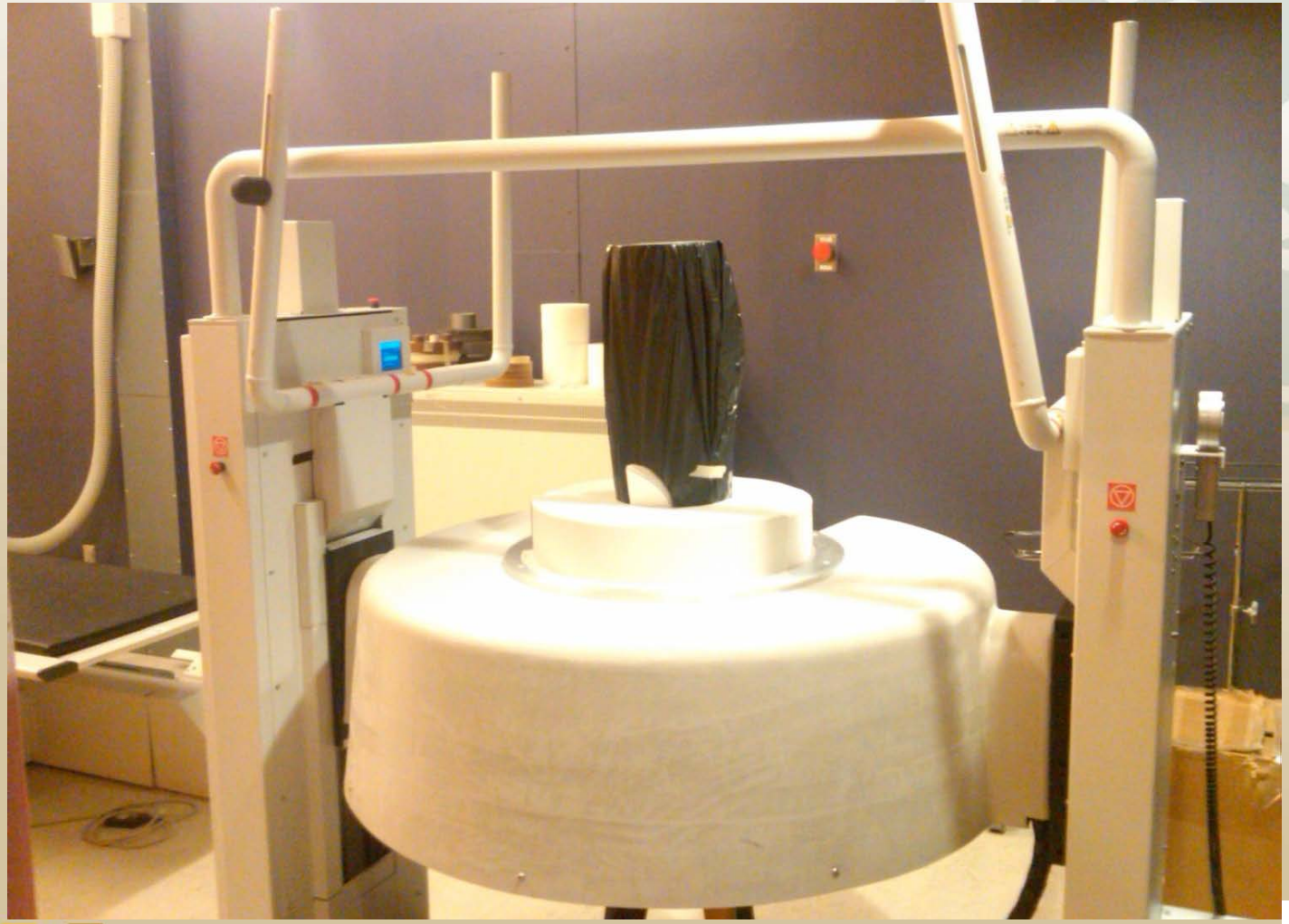

flat-panel
detector
x-ray
tube
gantry
motor



Philips vs CBCT

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Philips .08 bone window

Cbct hann .03 bone window

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SVR: Visual
OW 409
OL 295


OW 409
OL 295


SVR: Visual
OW 409
OL 295

Dual Energy CBCT

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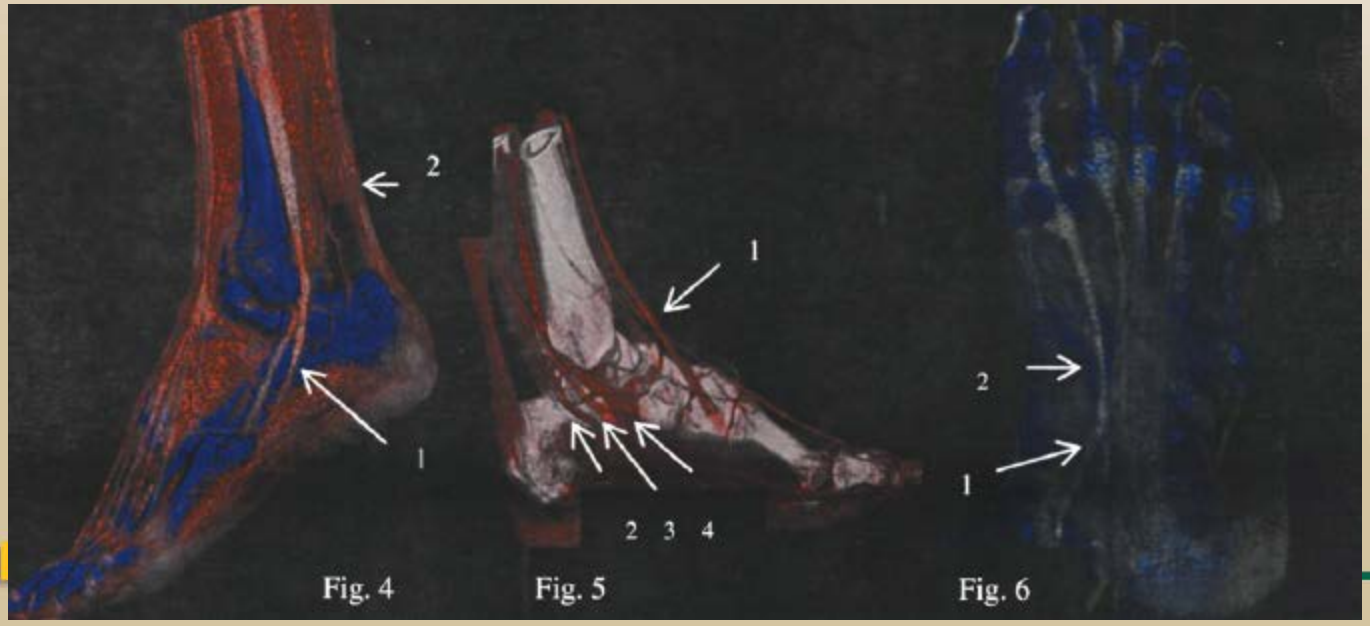
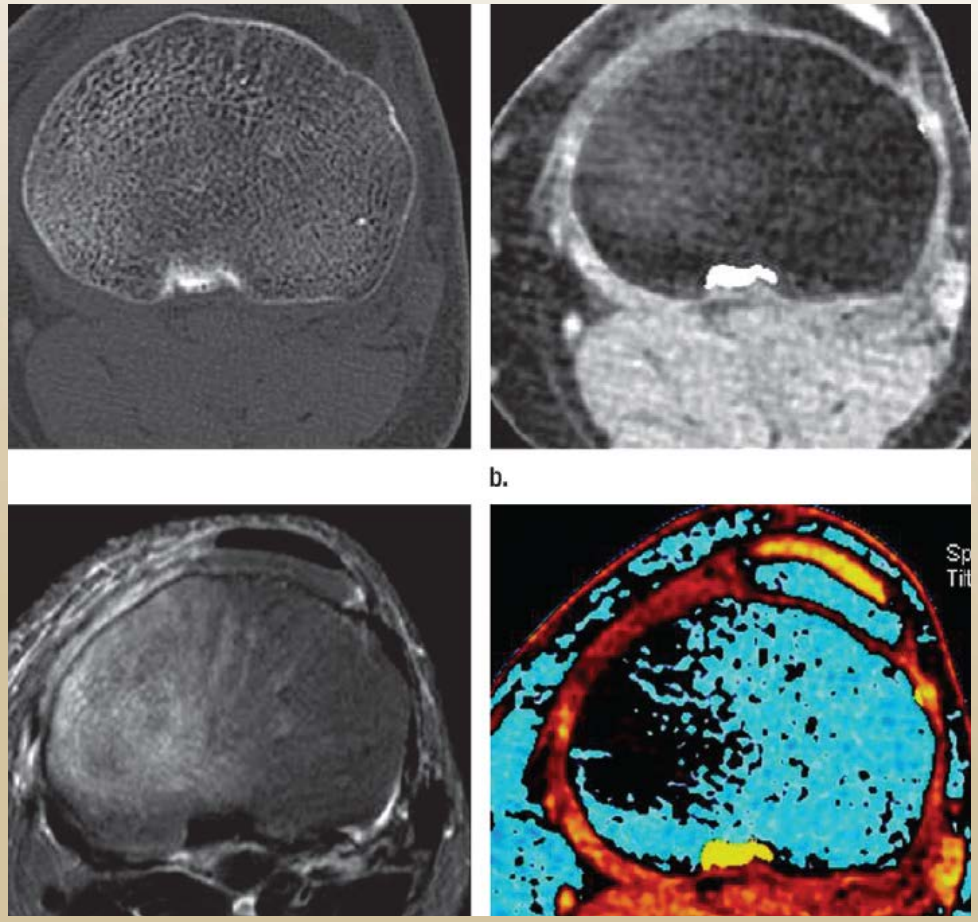
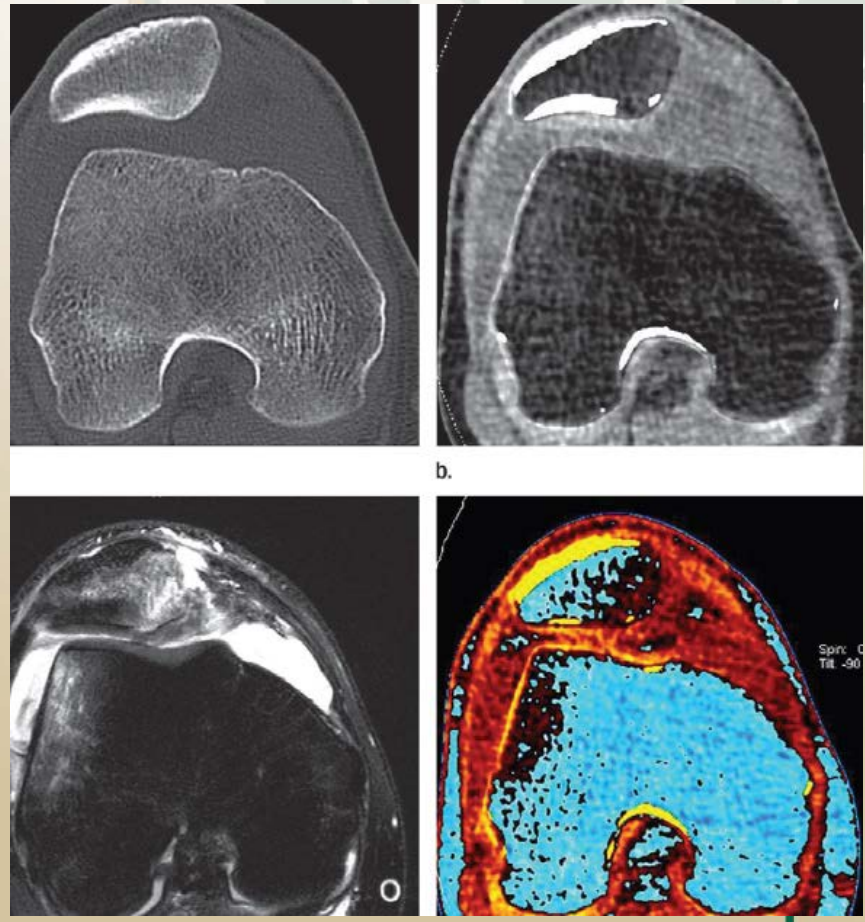


Fig. 4 Fig. 5 Fig. 6


b.


b.

Current Cone Beam Devices

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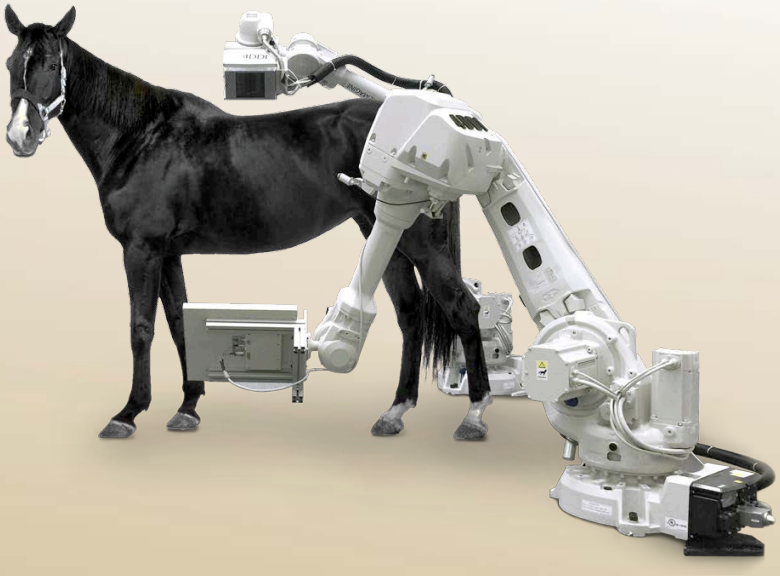

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PET Imaging

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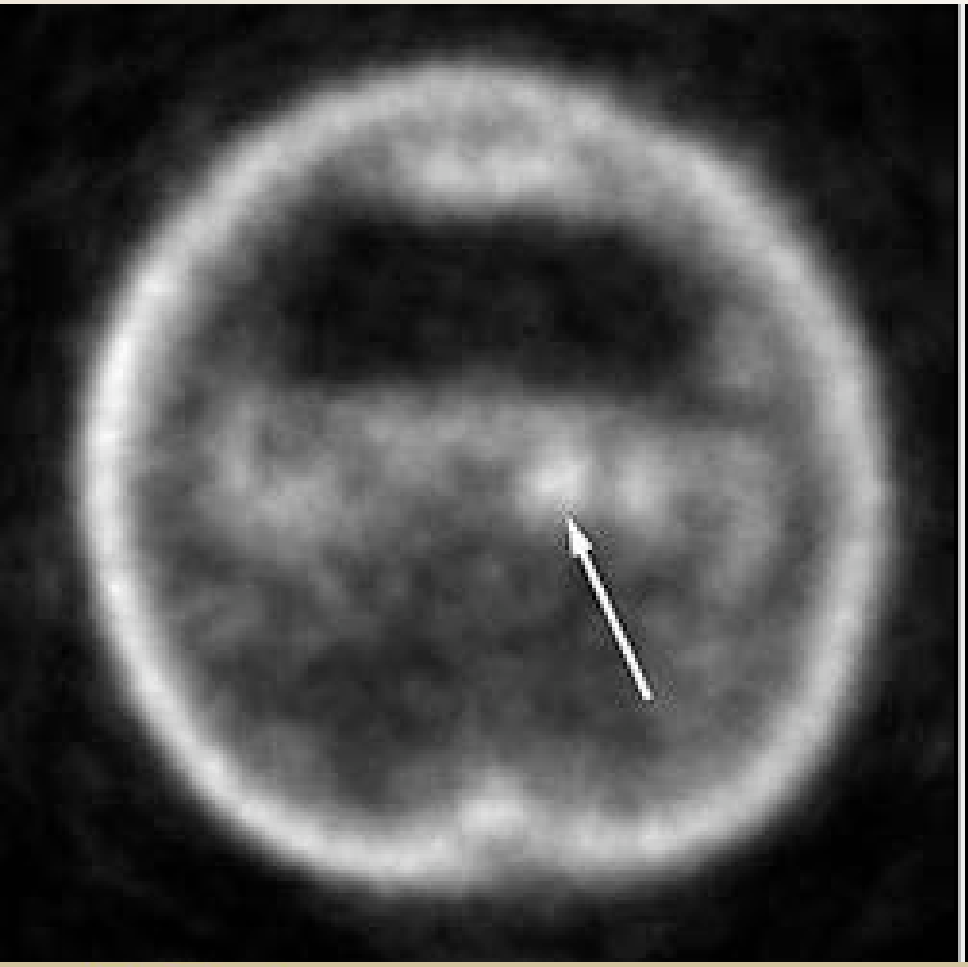

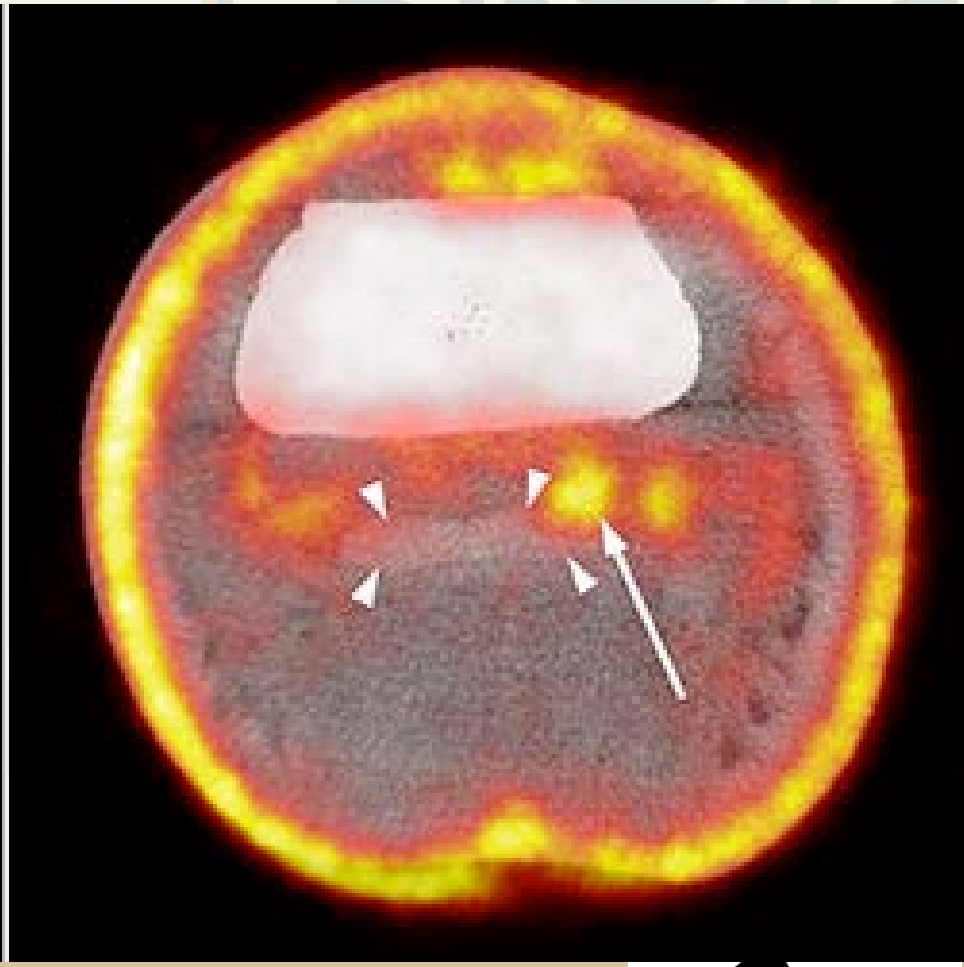

M. Spriet. UC Davis

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Spectral CT

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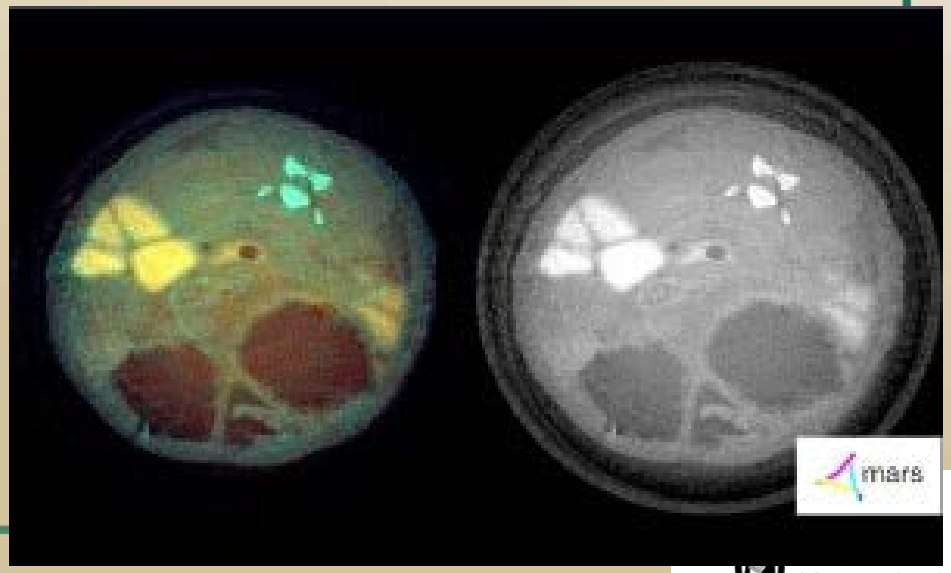
- Discriminates both Density and Atomic Structure



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Economics of Biomarkers

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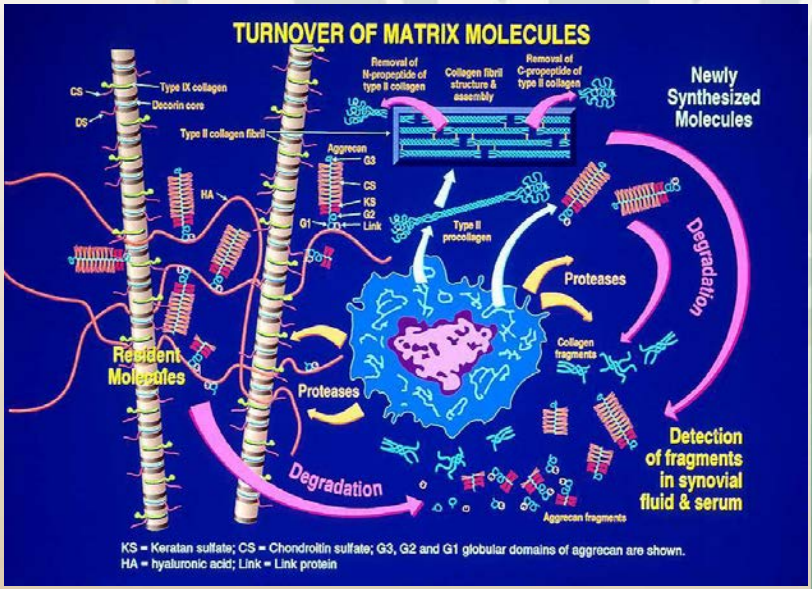
- Require sequential imaging
- Industry must identify value in sequential imaging
- Technologies exist, but equine industry is considered a low priority for companies that operate in the human space

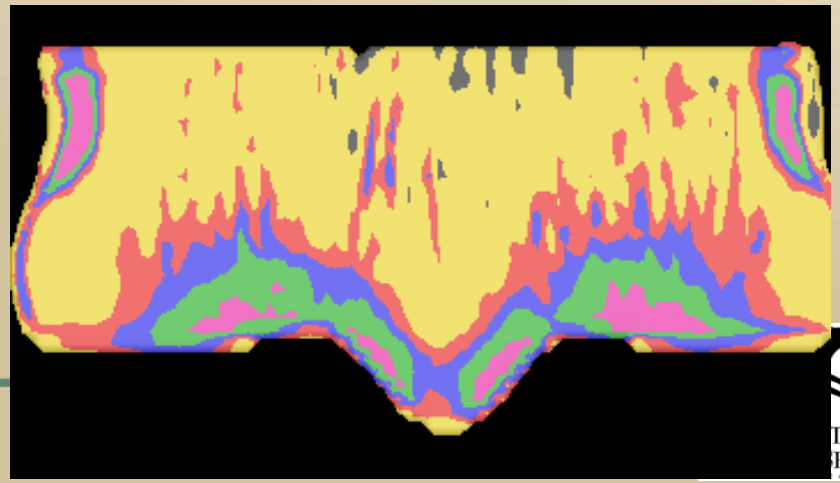
Development of a biomarker sample bank

It was agreed by the Havemeyer group that this should be a major priority. While Biobanking is emerging as an important research tool in the human field, it is now also gaining momentum in veterinary medicine [12,13]. A Biobank is a repository of biological material that has been collected and stored in a standardised fashion and whose phenotype, origin, date of collection and location can be easily determined [13]. These specimens can be stored at one or more site and distributed to the Biobank users based on preset guidelines. Not only sample collection and storage methods but also data recording (quality, completeness, consistency) relating to samples at different storage sites must be harmonised [14,15] and a powerful informatics programme that permits efficient and reliable management of all the Biobank's specimens is essential for its success [15]. A key element of a Biobank is that all necessary legal and ethical permissions are in place to allow appropriate use of materials for research purposes. This is obviously complicated in the case of an international Biobank where different legislative frameworks and cultural issues may have an impact [16].

A Vision We Have at CSU ORC

- Screening of horses with serum biomarkers
- Imaging of horses at risk
  - Nuclear scintigraphy
  - CT
  - MRI





Acknowledgements

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- College Research Council, CVMBS, Colorado State University.
- Lufkin Foundation
- Grayson Jockey Club Research Foundation.
- Horseracing Betting Levy Board, UK.
- Global Equine Research Alliance
- Marilyn Simpson Trust
- Southern California Equine Foundation
- Chalk Racing – Rick Westerman
- Jeff Seiwerdsen and I-STAR Lab
- Carestream Health
- Todd Bredbenner

Carestream