ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

IV. Applications: from statistics to quantitative bioimaging

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Bone structure and function

Motivation

i. Bone
   i. Hierarchical imaging
   ii. Anatomical imaging
   iii. Biomechanical
ii. Brain Imaging
iii. Visualizing Data

1. Motivation

Why do we do experiments?

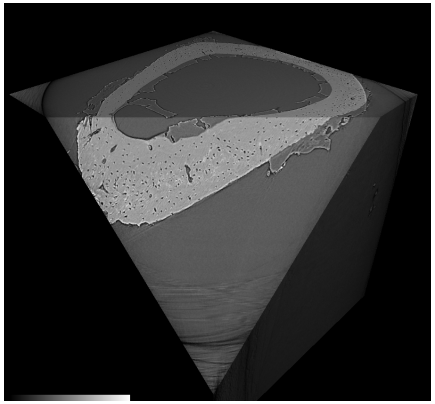
1. To get an idea of what is going on
2. To test a hypothesis
    1. Does temperature affect bubble size?
    2. Is this gene important for cell shape and thus mechanosensation in bone?
    3. Does higher canal volume make bones weaker?
    4. Does the granule shape affect battery life expectancy?

1. Motivation

Why do we do experiments?

1. To test a hypothesis

2560 (pixels in x)
x 2560 (pixels in y)
x 2160 (pixels in z)
x 32 bit
= 56GB / sample

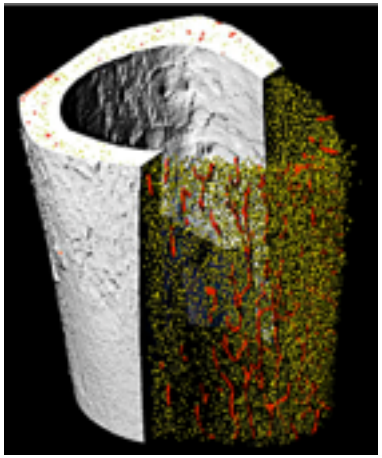


Highly Absorbing Regions

Weakly Absorbing Regions

Segmentation

…
x 1-2 bit
~ 2GB / sample



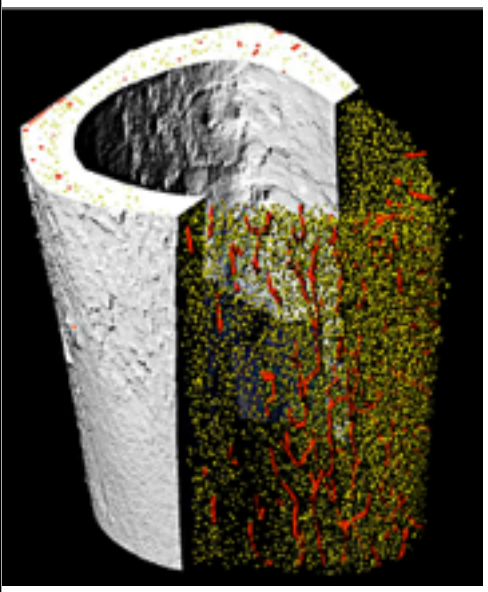
still a long way to go ….

1. Motivation

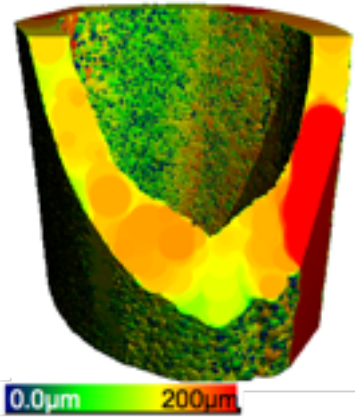
Why do we do experiments?

1. To test a hypothesis

Segmented Image



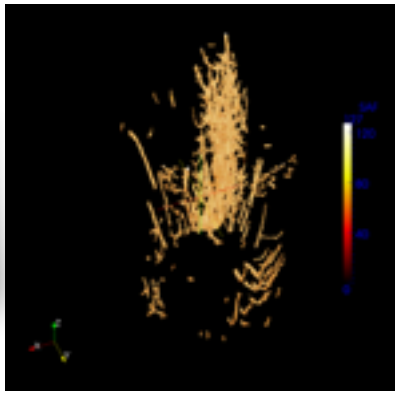
Cortical Bone



Mean Thickness
= 122.5μm
Std Thickness
= 20μm

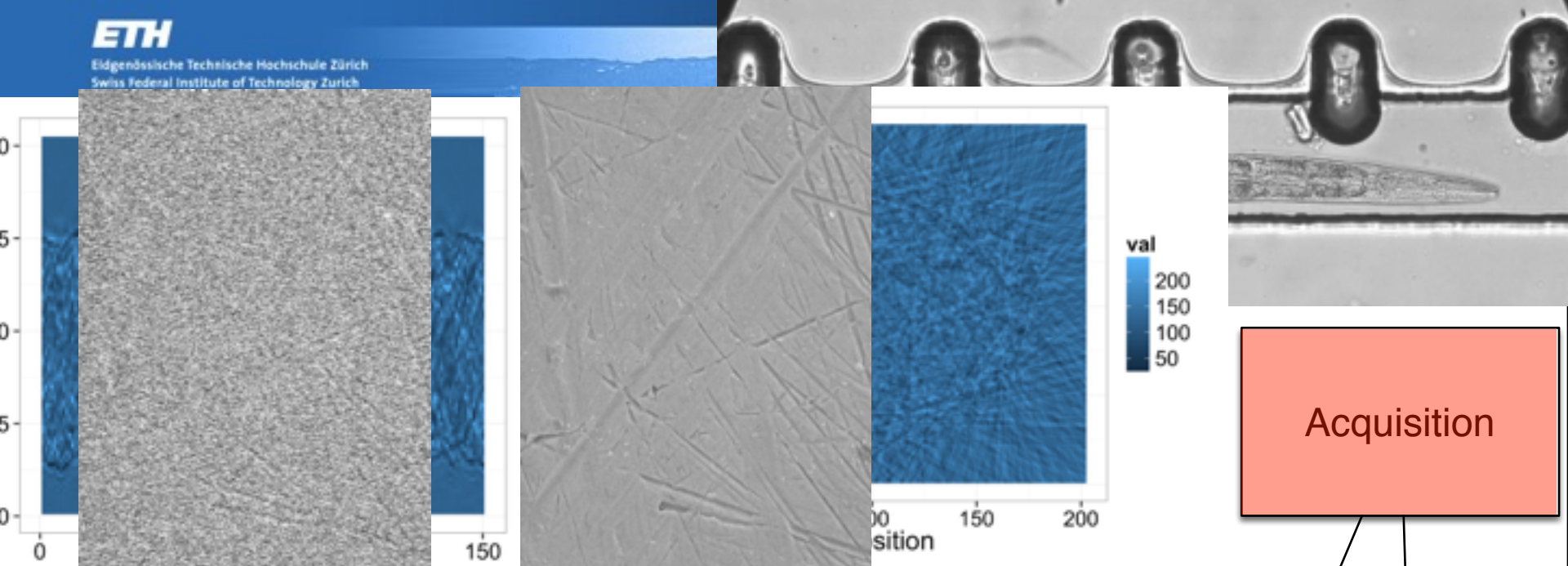
Morphology

Canal Network

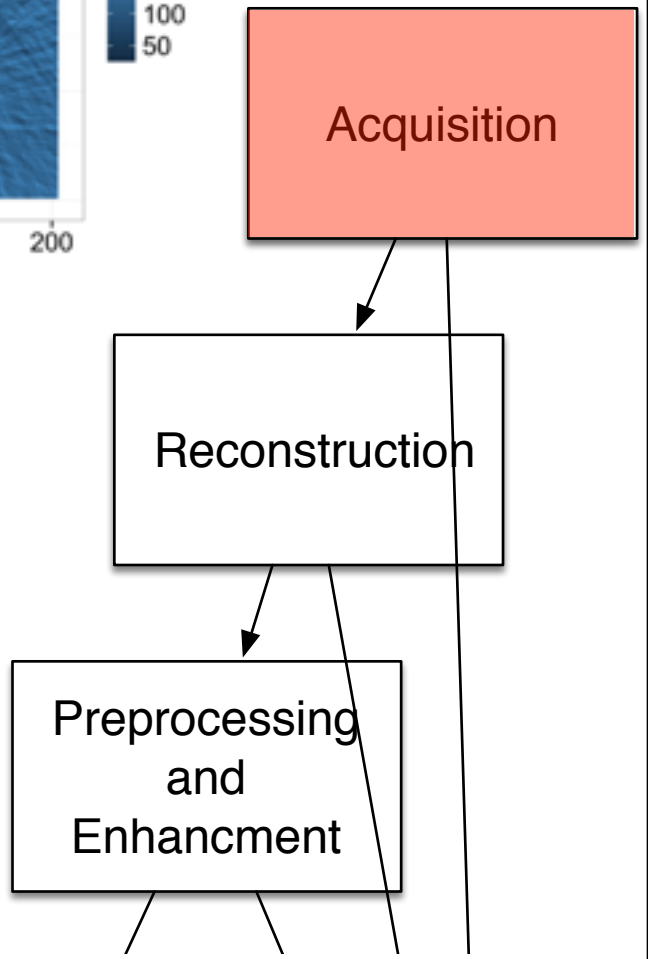


Component
Labeling

Canal Count = 30
Mean Canal Volume = 40,000μm^3
Canal Volume Fraction = 3%



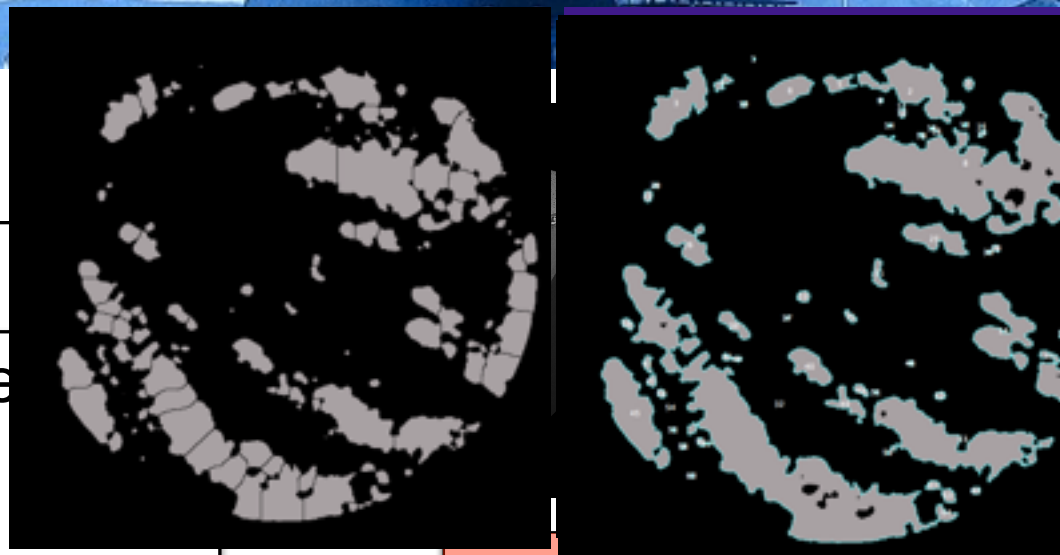
2. is t on from
   an
   ene
   ckpro
3. Preprocessing and Enhancement
   1. Gaussian filtering
   2. Edge enhancement

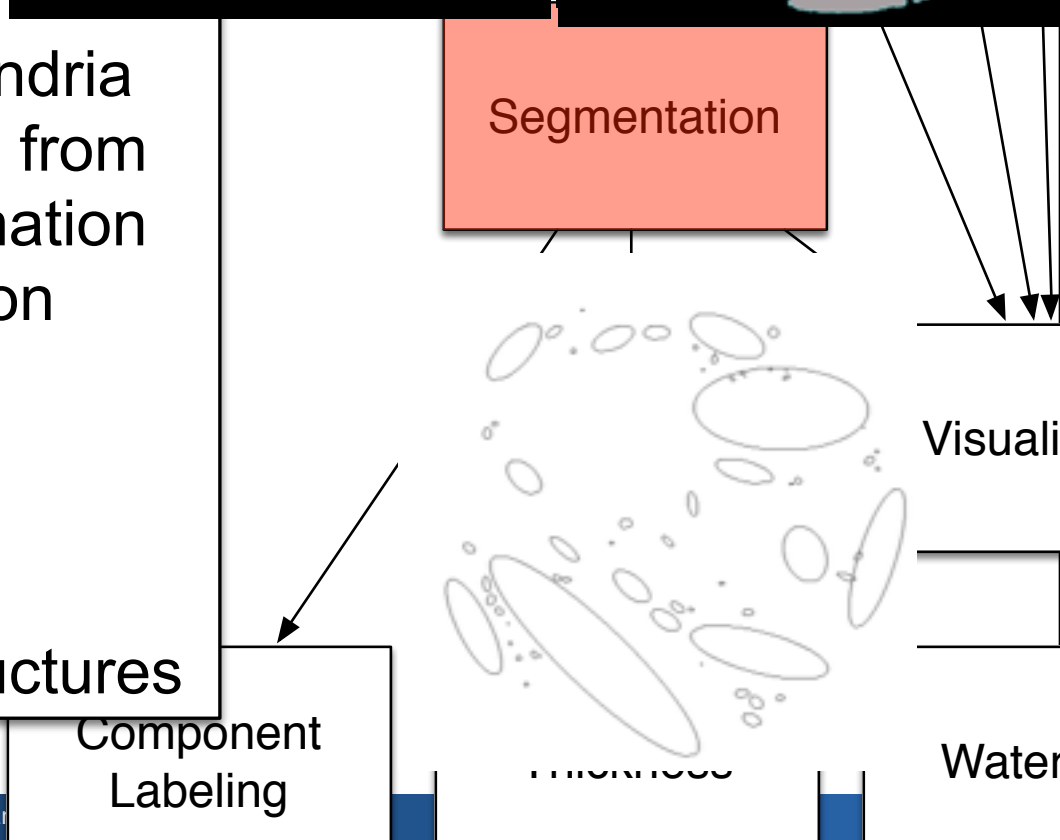


1. Motivation

Overview of the workflow

1. Segmentation extracts the regions from the image
   1. Bone tissue from air
   2. Nucleus from Mitochondria
2. Distance Map Generation from regions to distance information
   1. More spatial information
3. Component Labeling / Watershed
   1. Identify individual components
   2. Break apart larger structures





1. Motivation

How do we test hypothesis?

1. Declare ‘default’ assumption / null hypothesis
2. Establish the reliability of what we are measuring
3. Compare results to default using the reliability

Compare

Are the differences between the groups greater or less than my ‘default’ assumption?

Are the differences greater than the **reliability** in my experiments?

Are there **other possible** explanations for the results?

1. Motivation

Simple Example: Growth Hormone -> Osteocyte Count

1. Declare ‘default’ assumption / null hypothesis
2. Establish the reliability of what we are measuring
3. Compare results to default using the reliability

Compare

We then measure the growth-hormone group, again 3x3

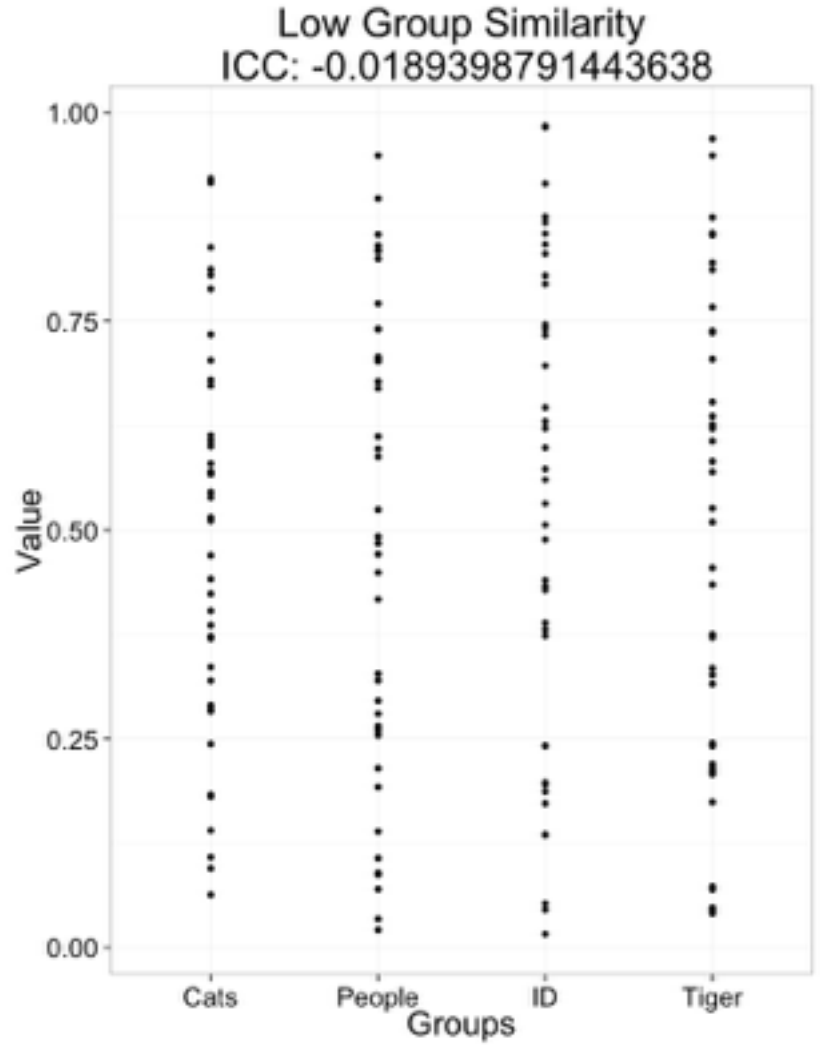
Sample 4=> 18000, 19000, 22000

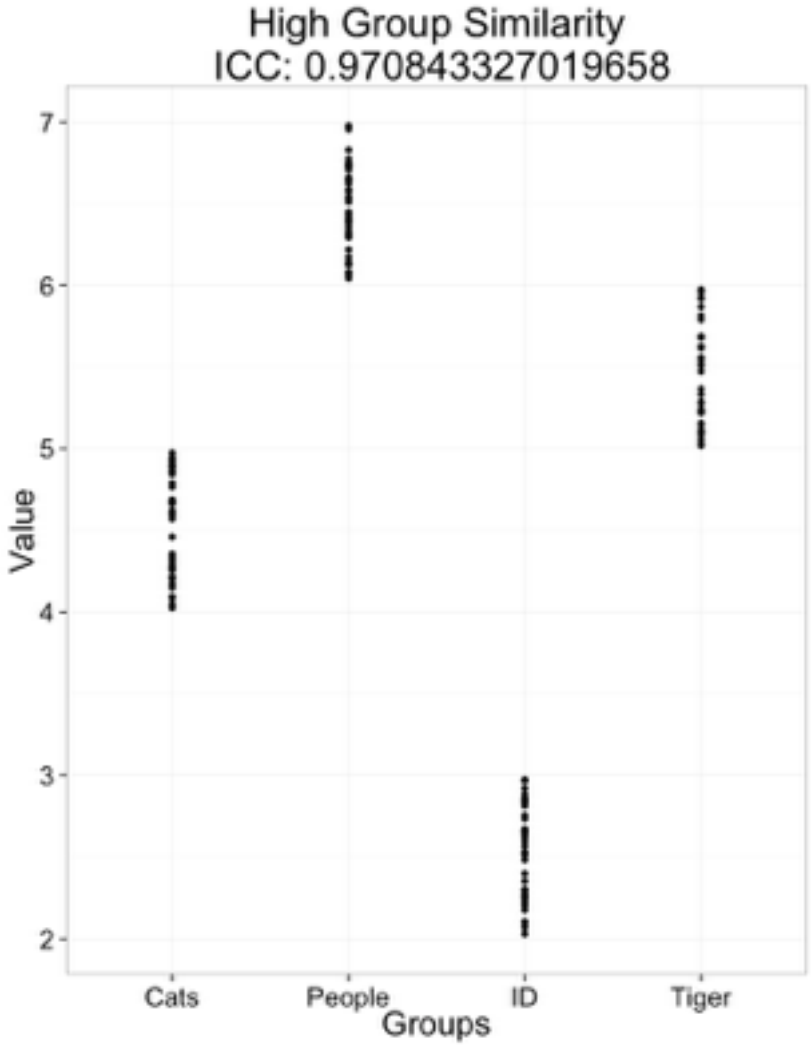
Sample 5=> 25000, 26000, 25000

Sample 6 => 28000, 22000, 27000

T-Test

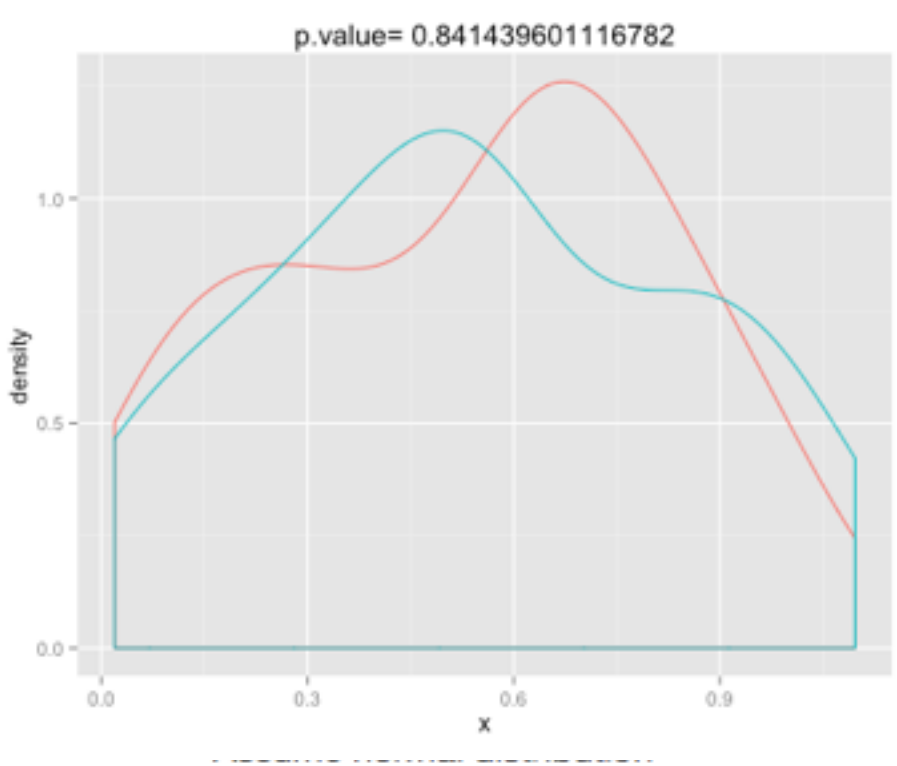
Statistics: Basics

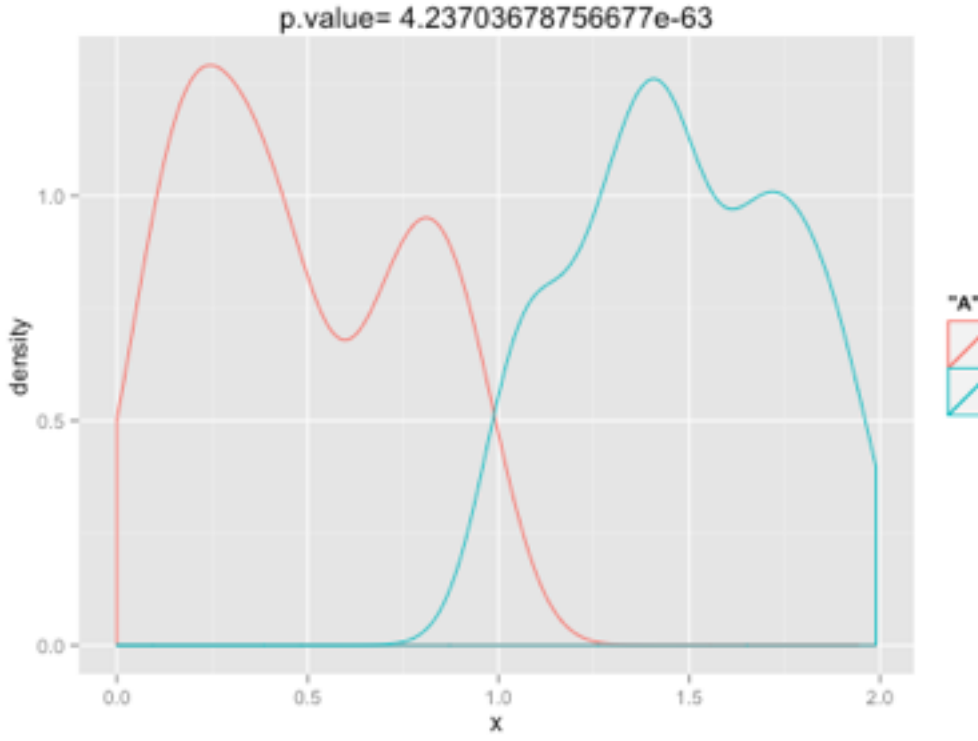




Statistics: Basics

Motivation





Bone structure and function

Motivation

Bone fractures

Bone disease

↓

Decreased bone strength

↓

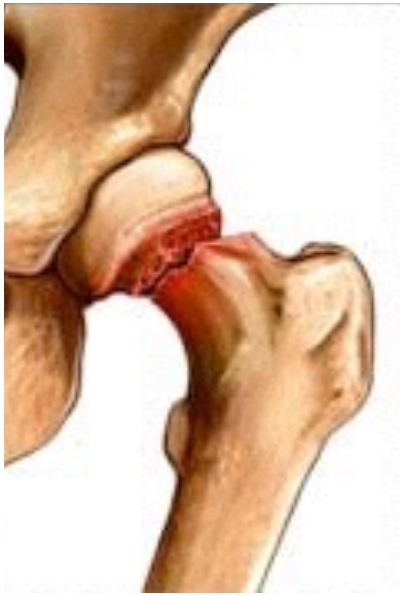
Fractures

Bone structure and function

Motivation

Osteoporosis

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to increased fracture risk.



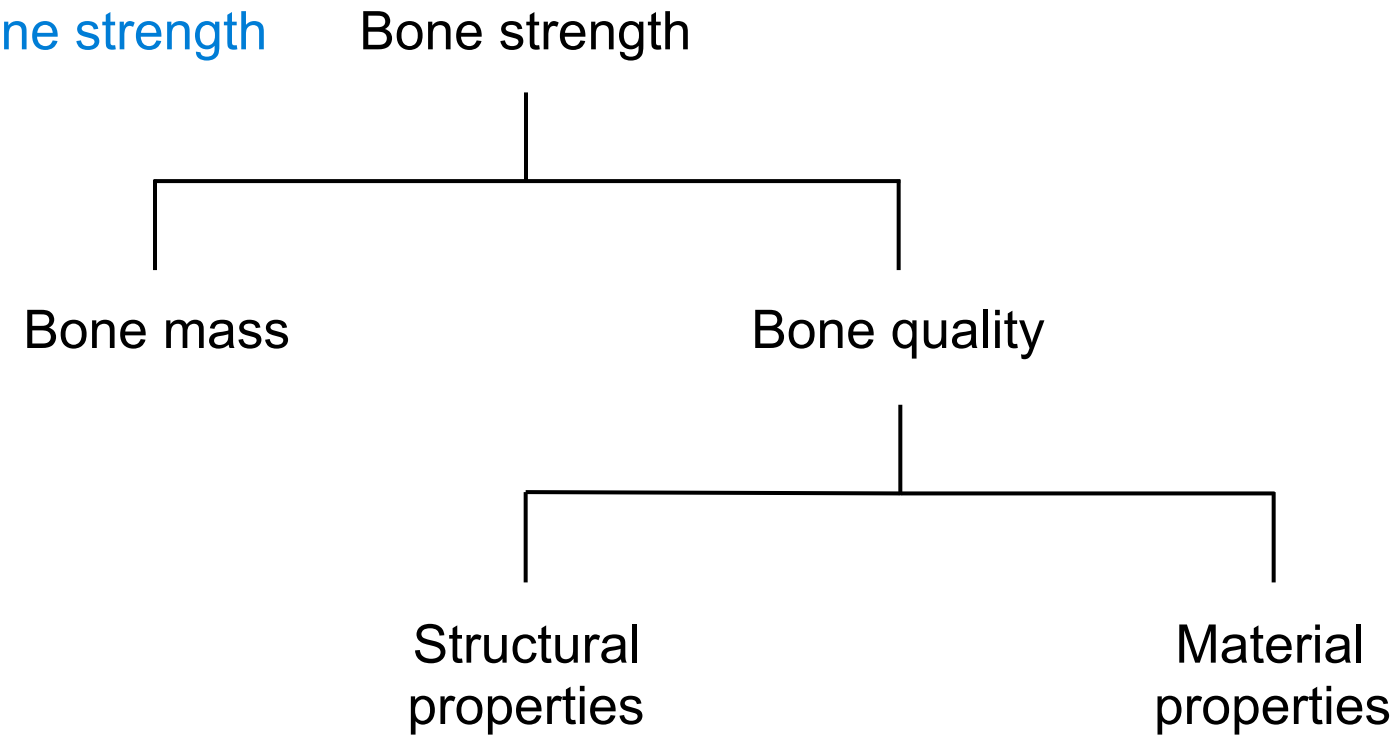
Lifetime fracture risk	Woman [%]	Men [%]
Proximal femur	17.5	6.0
Spine	15.6	5.0
Distal forearm	16.0	2.5
All three skeletal sites	39.7	13.1

Melton III LJ *et al*, J Bone Miner Res 20:886-92 (2005)

Bone structure and function

Motivation

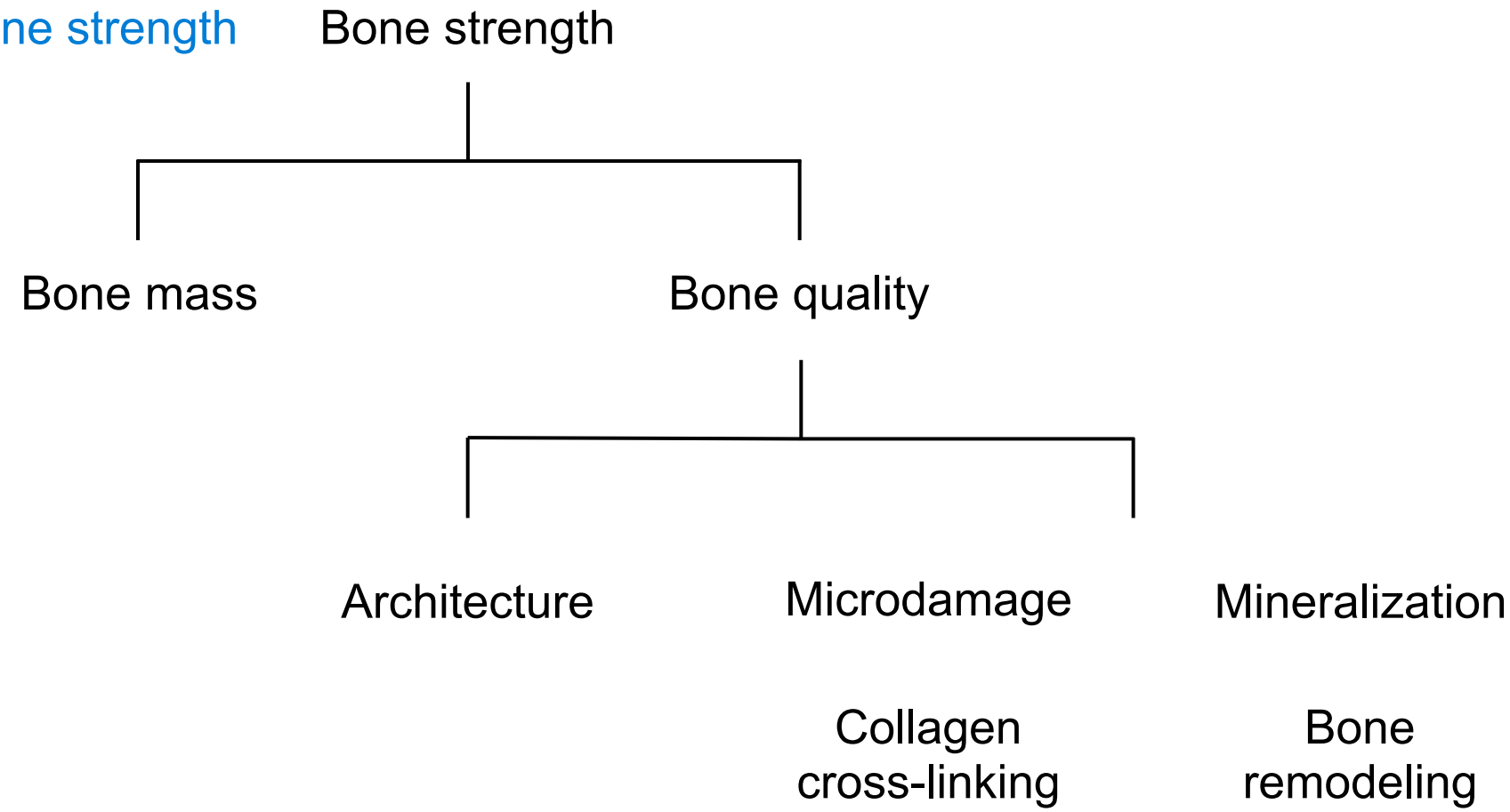
Bone strength



Bone structure and function

Motivation

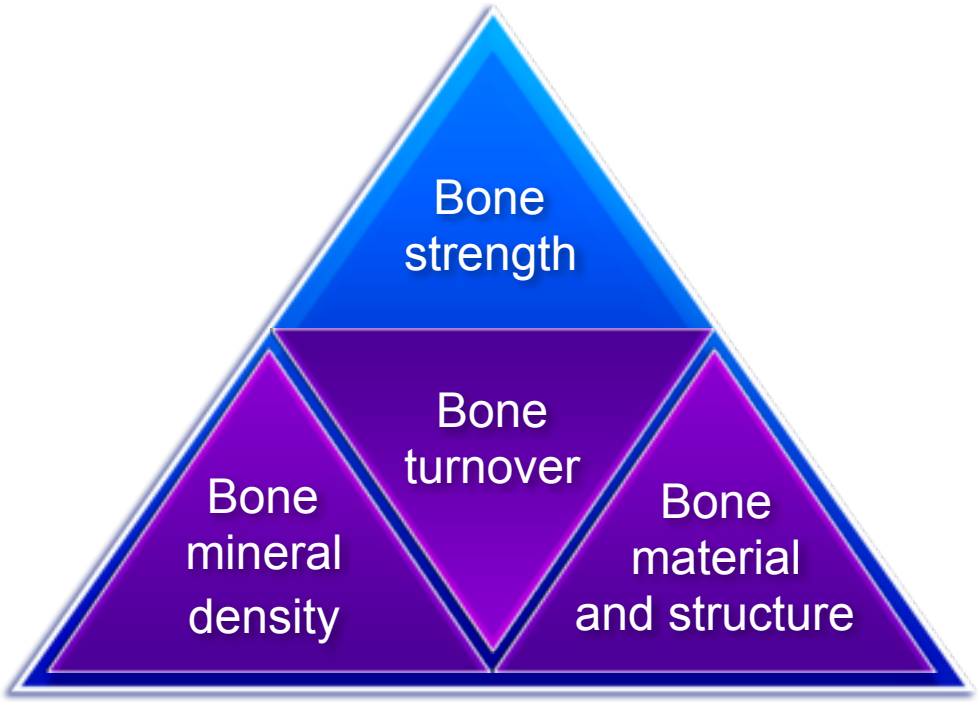
Bone strength



Bone structure and function

Motivation

Bone strength

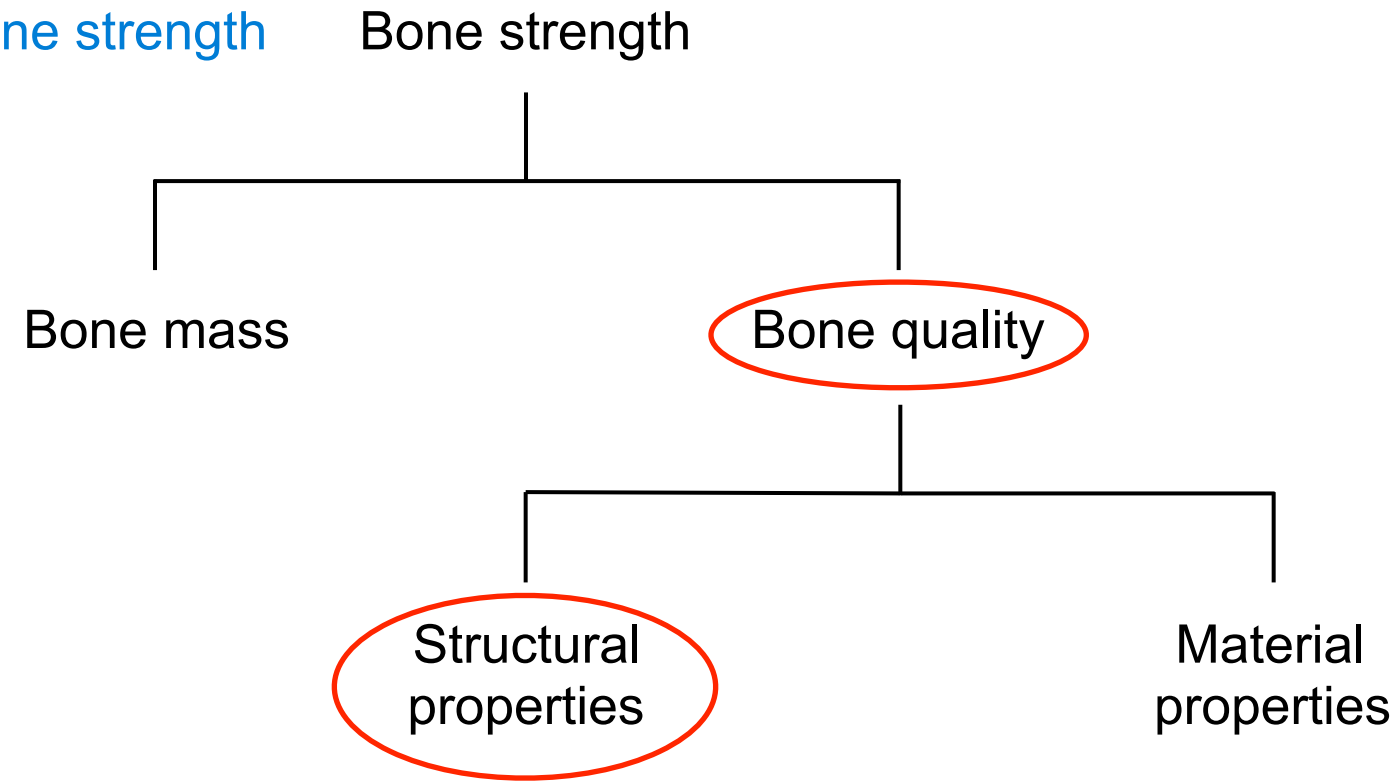


Genant HK *et al*, Horm Res 54(Suppl 1):24-30 (2000)

Bone structure and function

Motivation

Bone strength



Bone structure and function

Motivation

Hypothesis

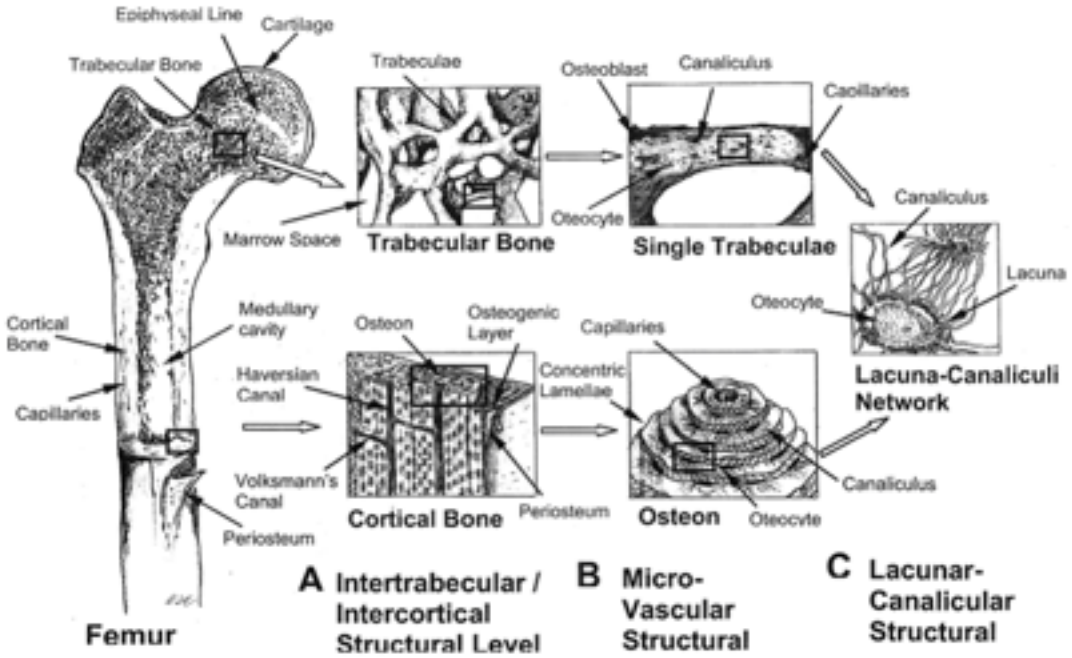
Quantitative assessment of bone structure using hierarchical imaging methods may improve our ability to estimate bone strength and failure behavior for

- **Prevention:** improve fracture risk prediction
- **Diagnosis:** clarify pathophysiology of skeletal disease
- **Therapy:** define skeletal response to treatment/therapy

Bone structure and function

Hierarchical Imaging

Hierarchical bone organization

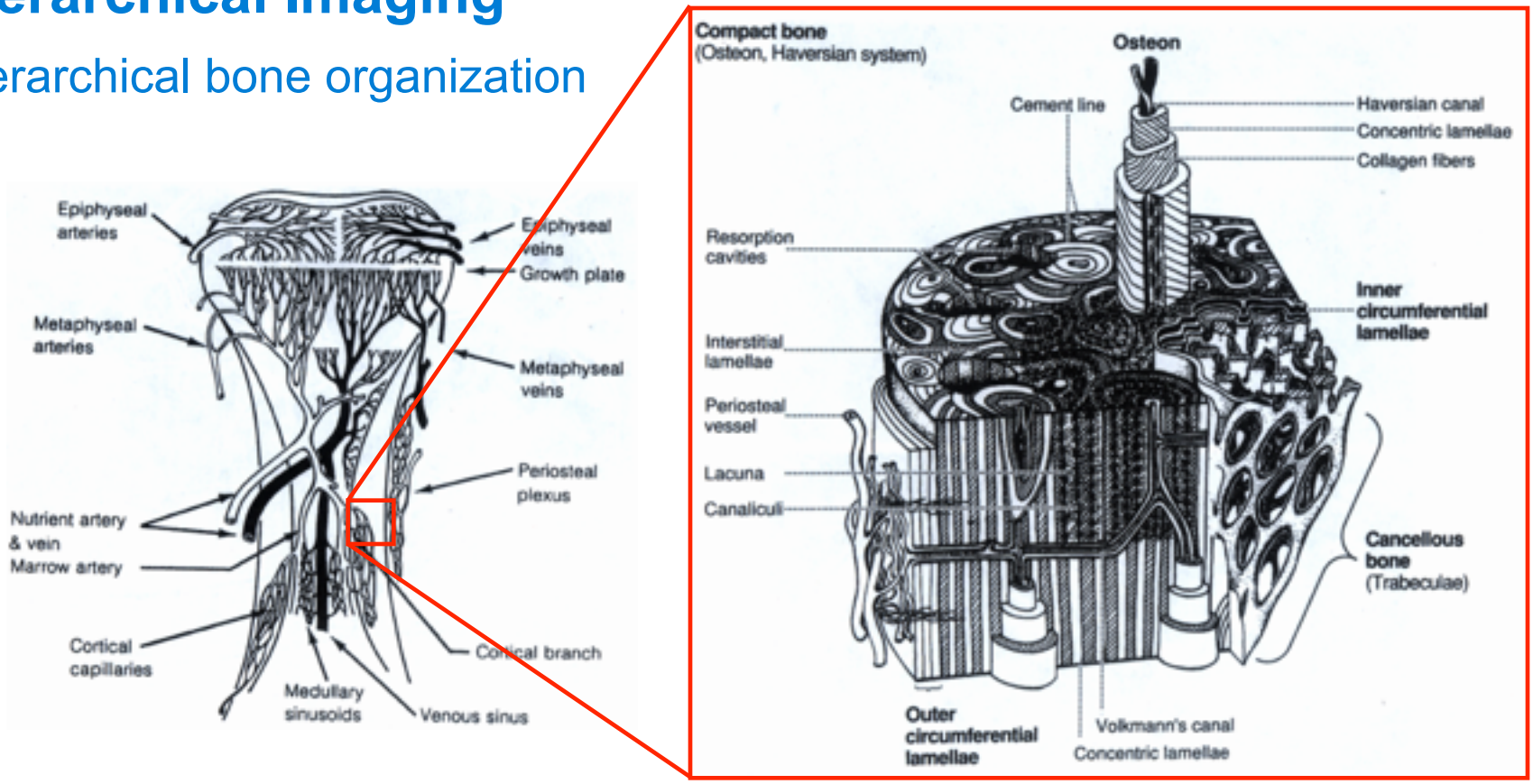


Liebschner MAK and Wettergreen MA, „Optimization of Bone Scaffold Engineering for Load Bearing Appications", in Topics In Tissue Engineering, Ashammakhi N and Ferretti P, Eds (2003)

Bone structure and function

Hierarchical Imaging

Hierarchical bone organization

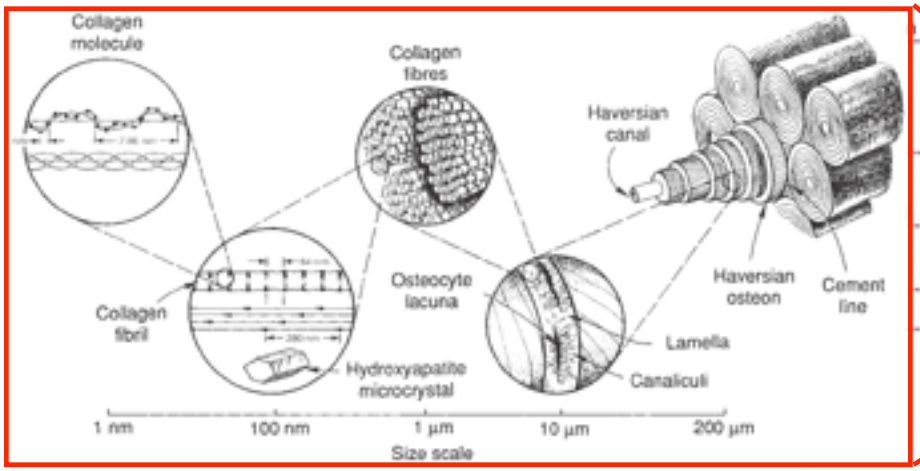


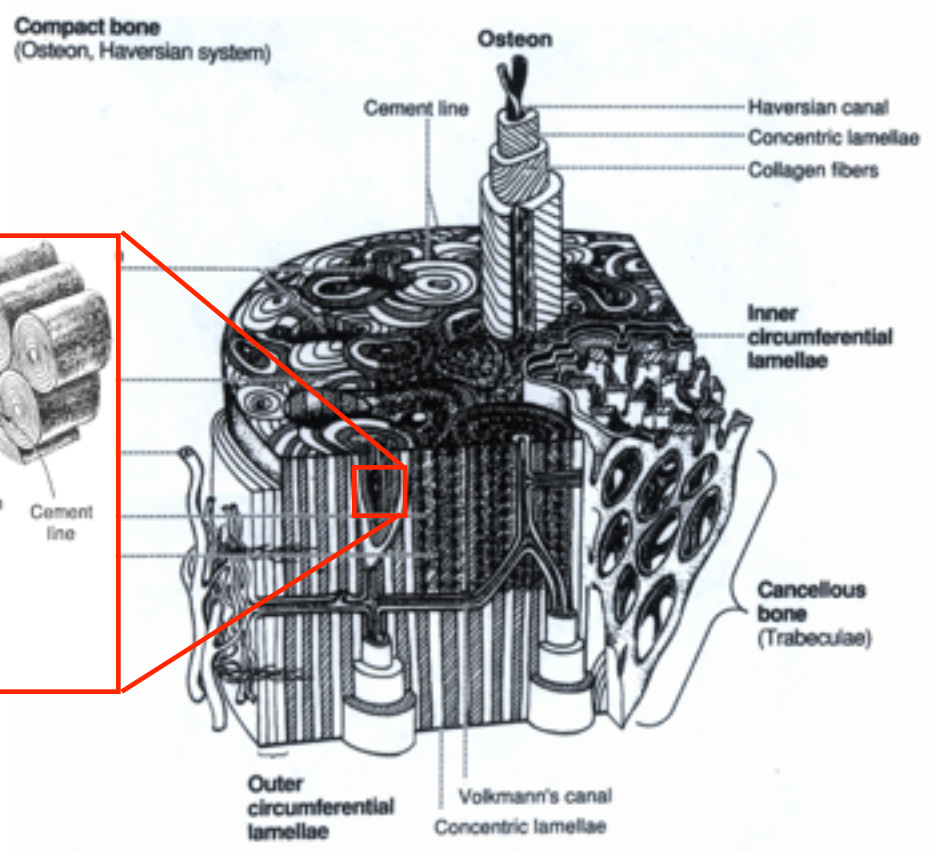
Weiss L, ed, “Cell and Tissue Biology, A Textbook of Histology”, Baltimore, MD: Urban and Schwarzenberg (1988)

Bone structure and function

Hierarchical Imaging

Hierarchical bone organization



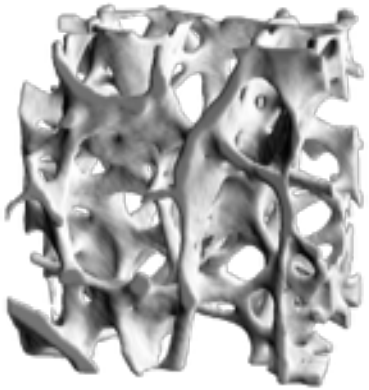


Lakes R, Nature 361:511-15 (1993)

Bone structure and function

Hierarchical Imaging

Bone macro-
& microstructure



Macrostructure
(> 100 µm)

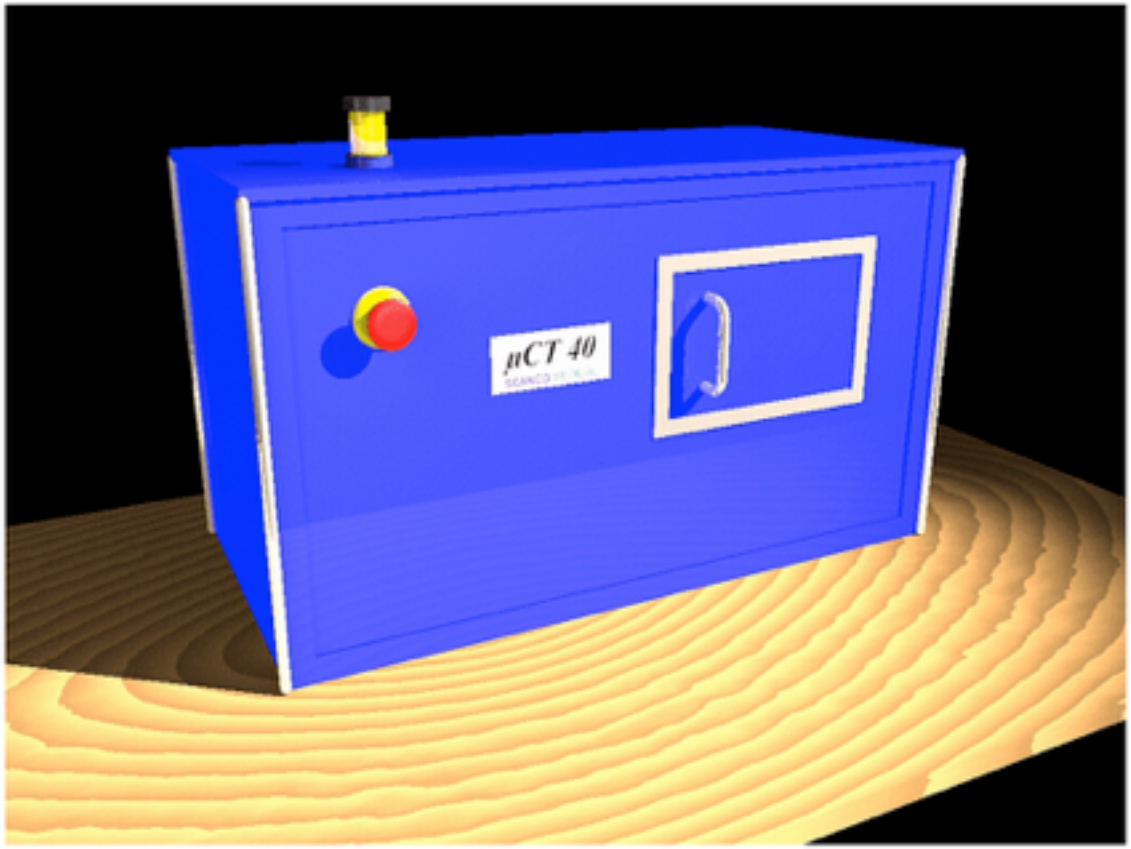
Microstructure
(10 – 100 µm)

(Desktop) µCT

Bone structure and function

Hierarchical Imaging

Desktop micro-computed tomography (µCT)

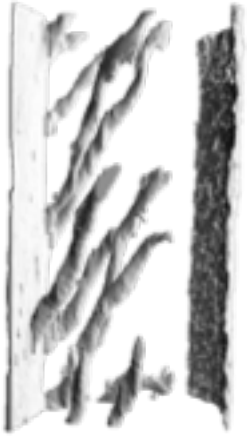


Stauber M, ETH Zurich (2002)

Bone structure and function

Hierarchical Imaging

Bone microstructure



Microstructure
(1 – 10 µm)
Tissue level

Microstructure
(≤ 1 µm)
Cellular level

Microstructure
(≤ 100 nm)
Subcellular level

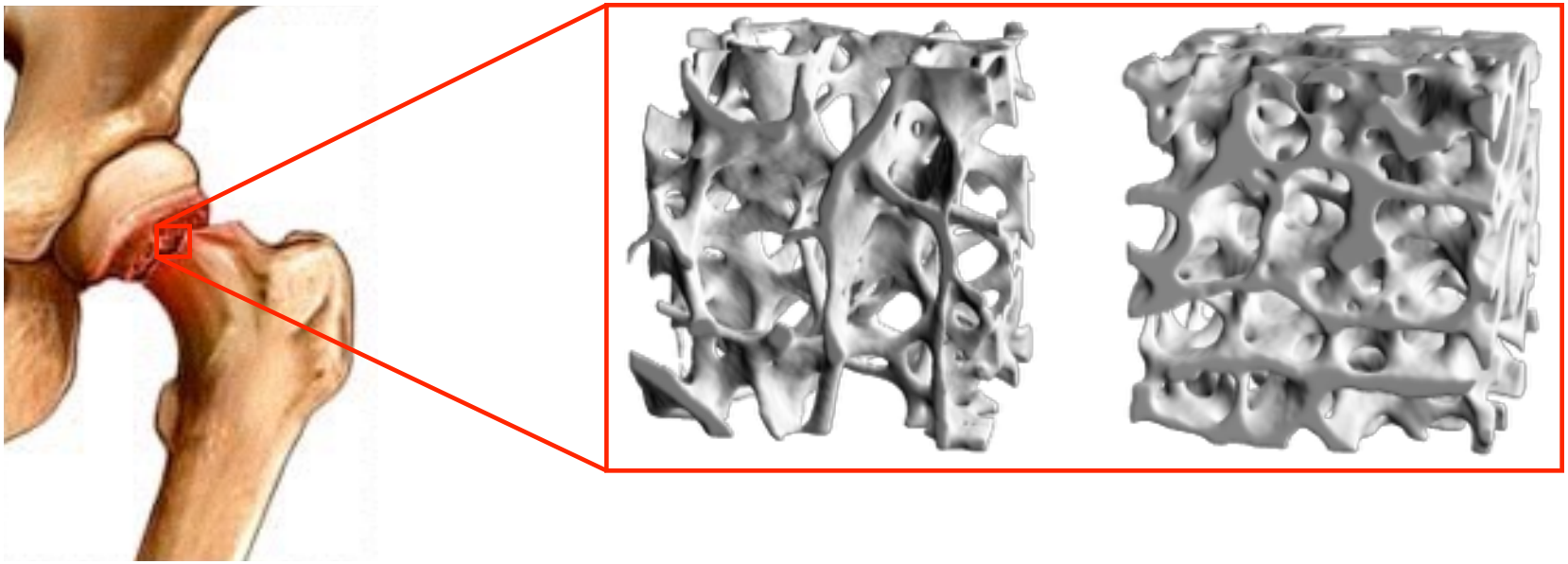
Synchrotron (SR) CT

Focused ion beam (FIB) &
scanning electron microscopy (SEM)

Bone structure and function

Anatomical imaging

Site dependency



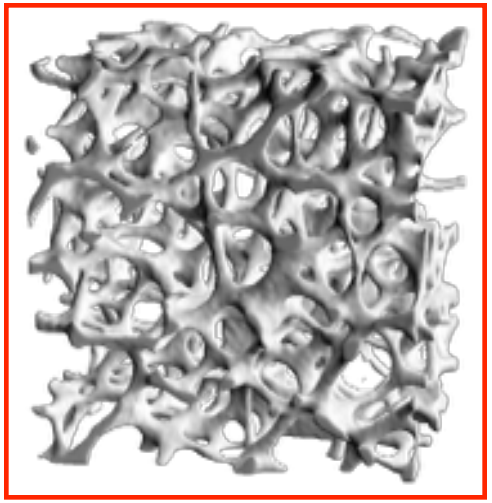
Osteoporotic

Young normal

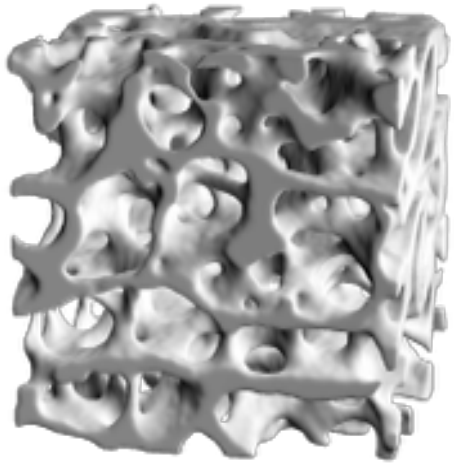
Bone structure and function

Anatomical imaging

Site dependency



Osteoporotic?

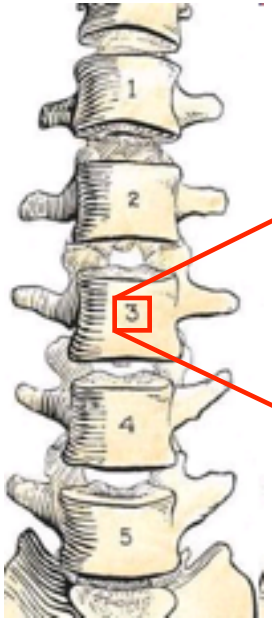


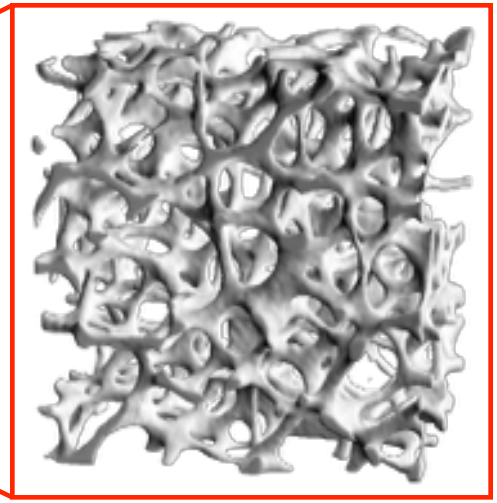
Young normal

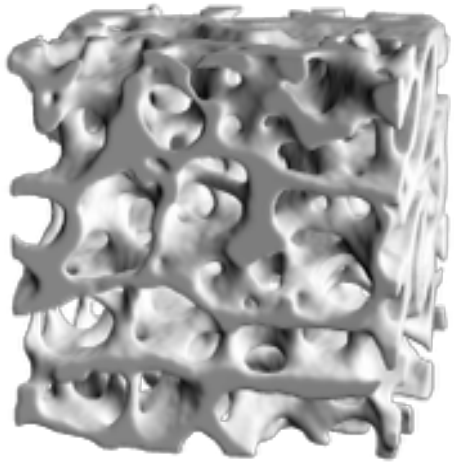
Bone structure and function

Anatomical imaging

Site dependency







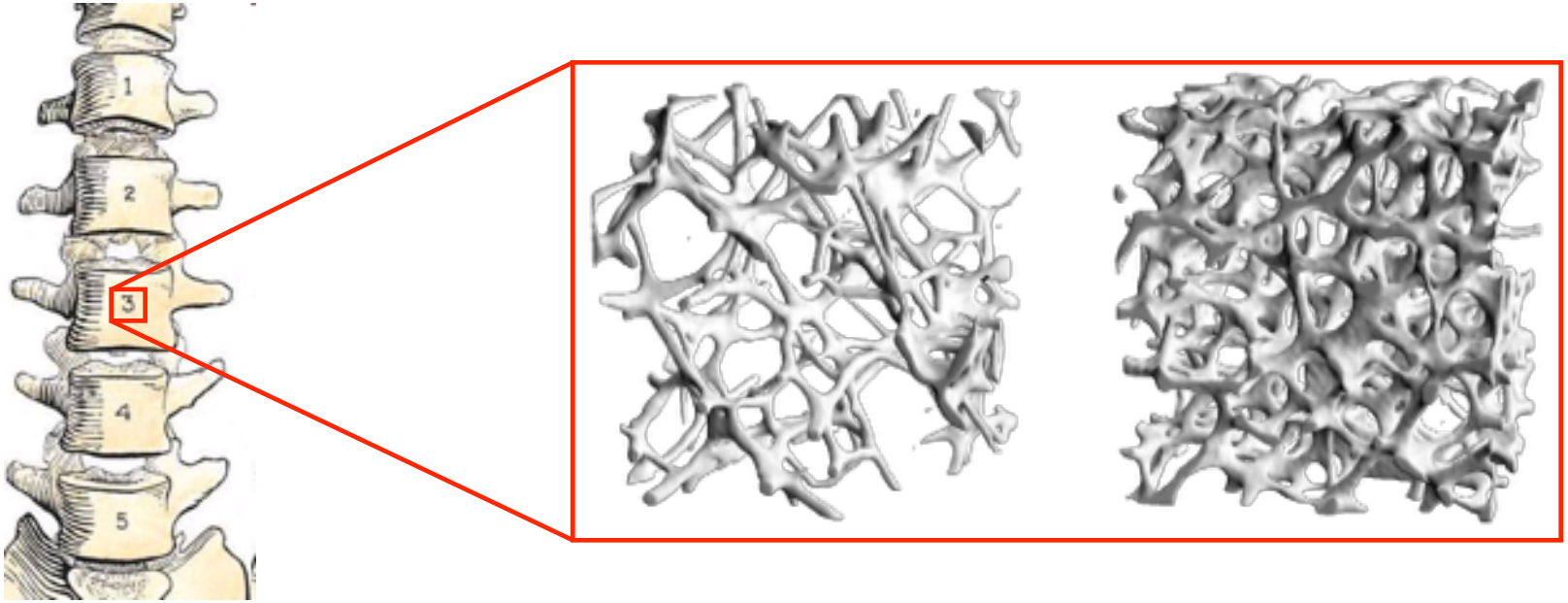
Lumbar spine

Femoral head

Bone structure and function

Anatomical imaging

Site dependency



Osteoporotic

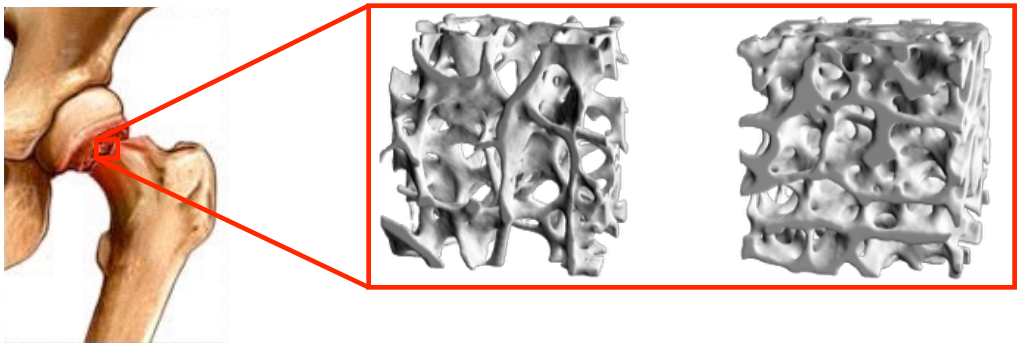
Young normal

Bone structure and function

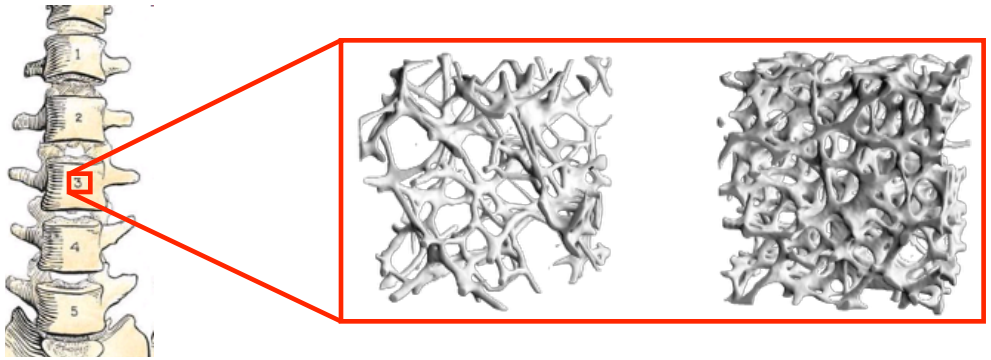
Anatomical imaging

Site dependency

Femoral head



Lumbar Spine



Osteoporotic Young normal

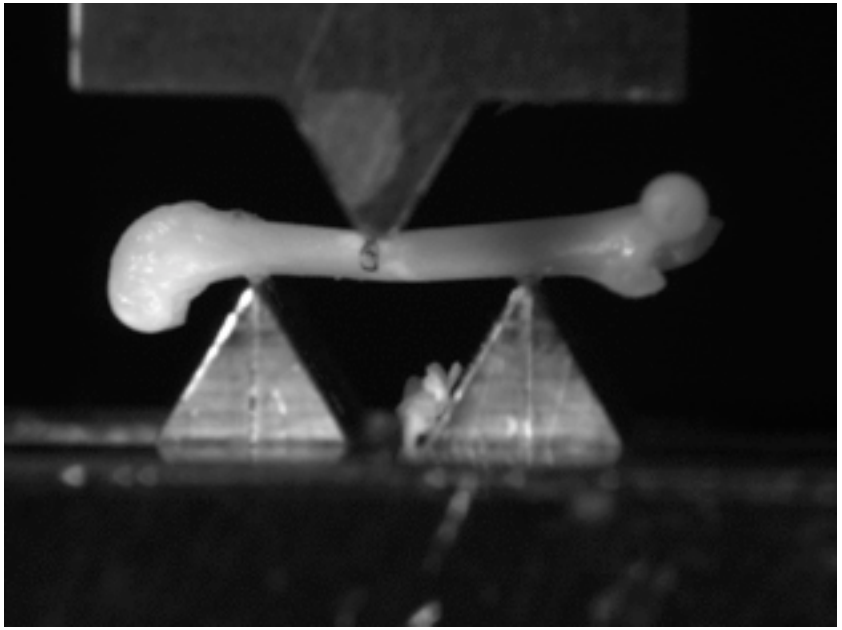
Bone structure and function

Biomechanical imaging

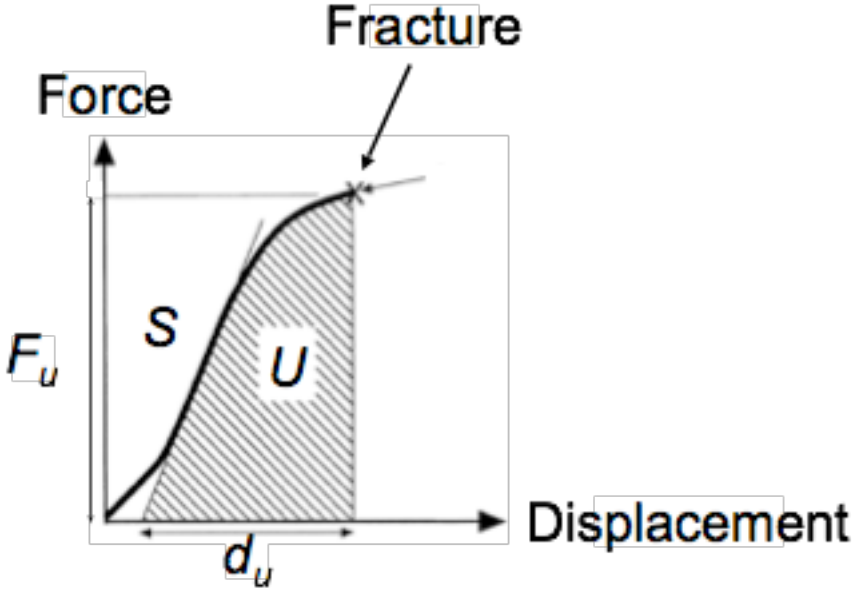
Biomechanical testing

Biomechanical testing is the gold standard for bone strength assessment:

- Provides detailed information on overall bone properties



Biomechanical testing



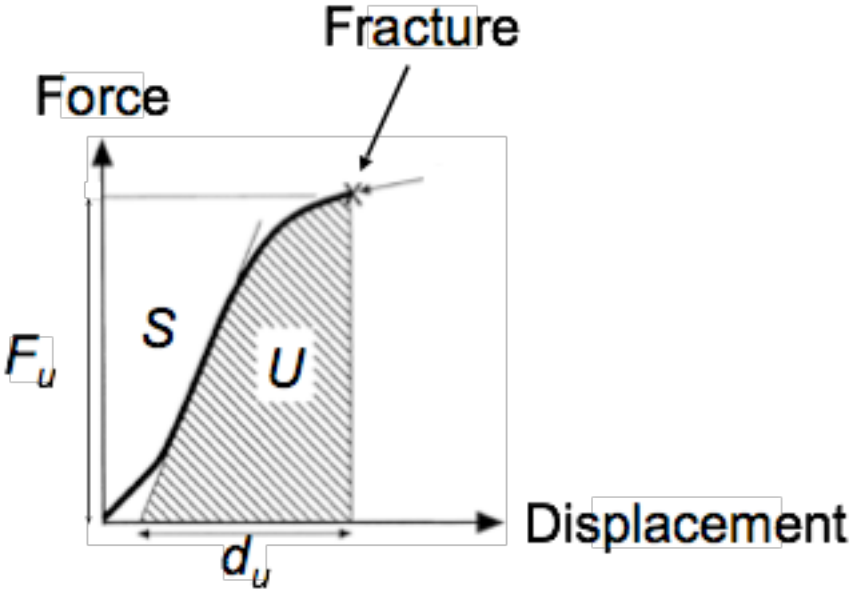
Bone structure and function

Biomechanical imaging

Biomechanical testing

Problems of biomechanical testing:

- Biomechanical testing is **invasive**
- **Only global mechanical signatures** are retrieved (no local information)

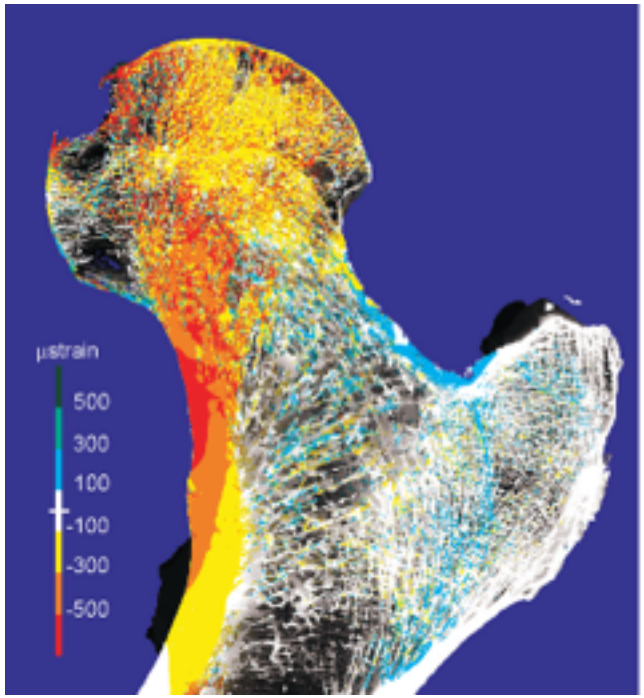


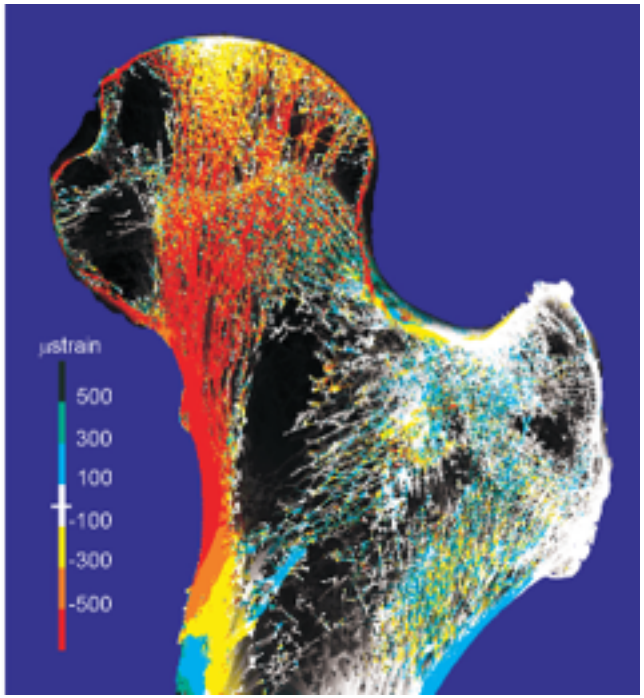
Bone structure and function

Biomechanical imaging

Finite element analysis

Healthy





Osteoporotic

Microstructural finite element analysis (µFE)

Van Rietbergen B *et al*, J Bone Miner Res 18(10):1781-8 (2003)

Bone structure and function

Biomechanical imaging

Finite element analysis

Problems of finite element analysis:
- Material properties unknown
- Exact boundary conditions unknown

=> **Finite element analysis requires validation**

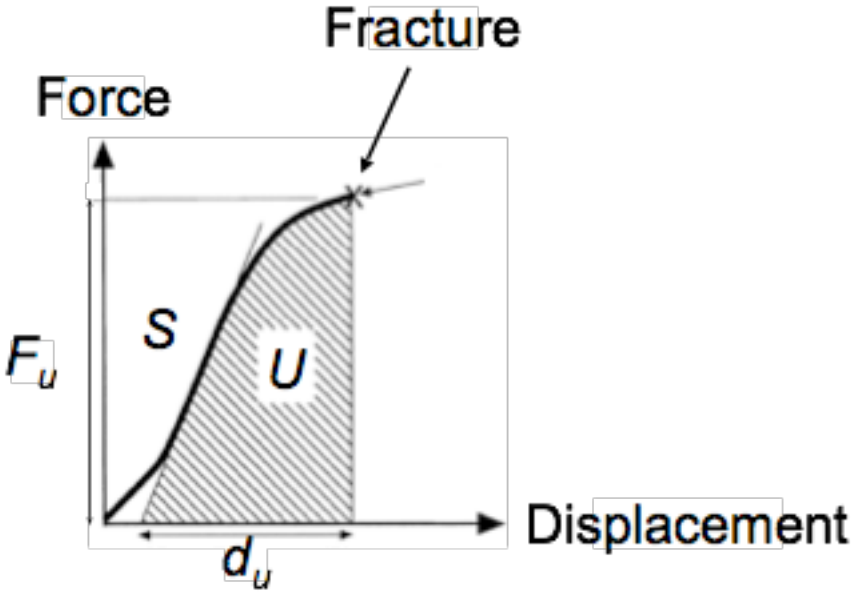
Further problems of finite element analysis:
- Often only linear (no prediction on plastic region, where fractures occur)
- Does often not reflect the hierarchical complexity of bone tissue

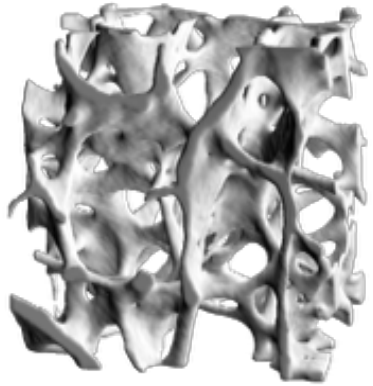
=> **Direct comparison with biomechanical testing is required**

Bone structure and function

Biomechanical imaging

Combining biomechanics and imaging





Biomechanics

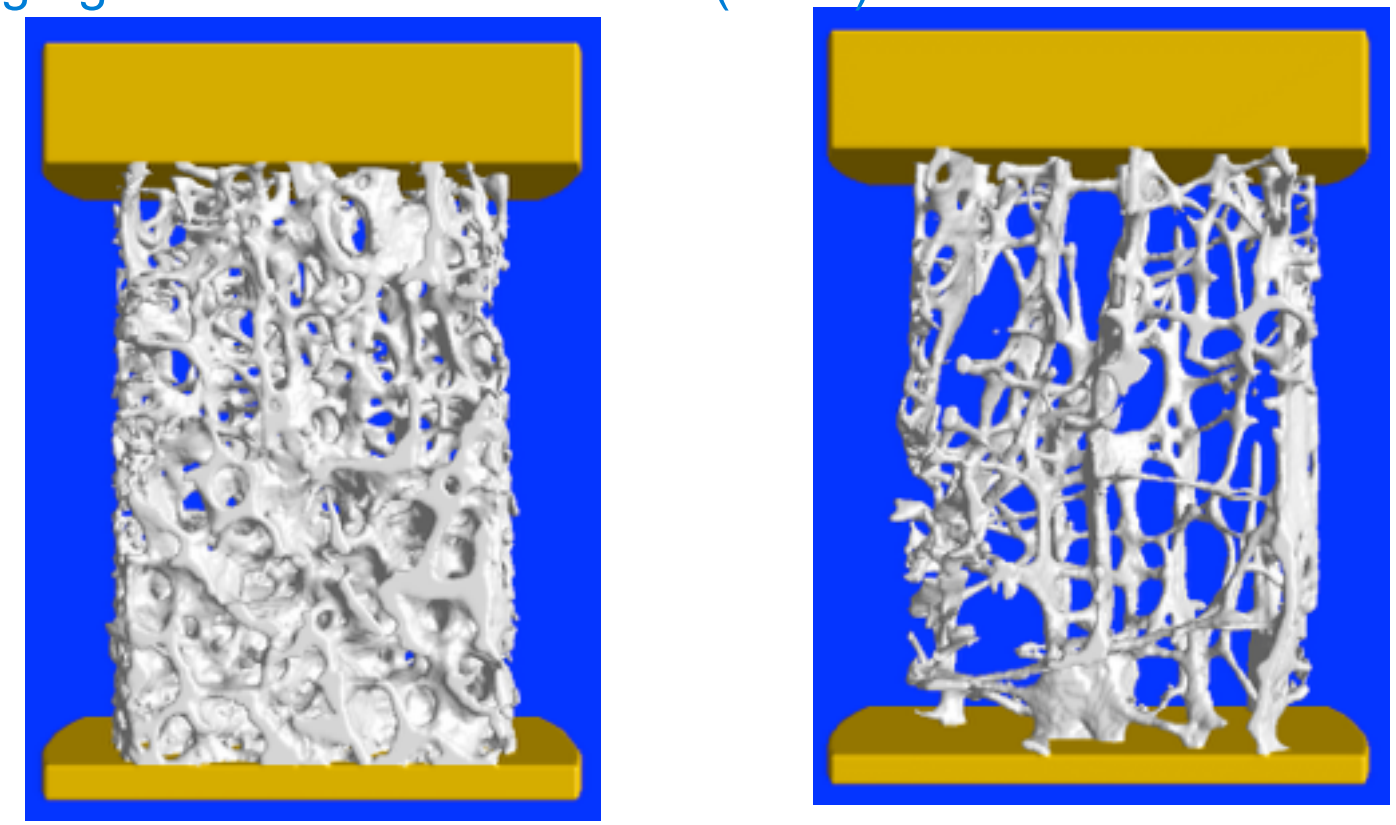
Imaging

Biomechanical imaging

Bone structure and function

Biomechanical imaging

Image-guided failure assessment (IGFA)

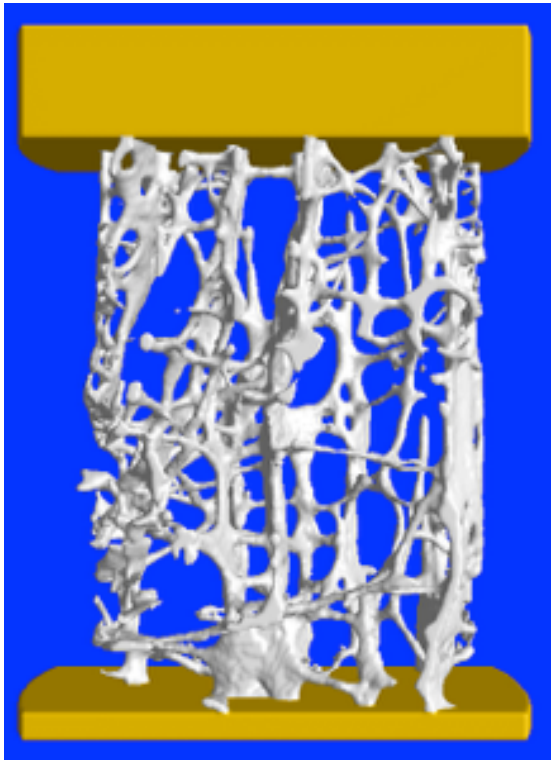


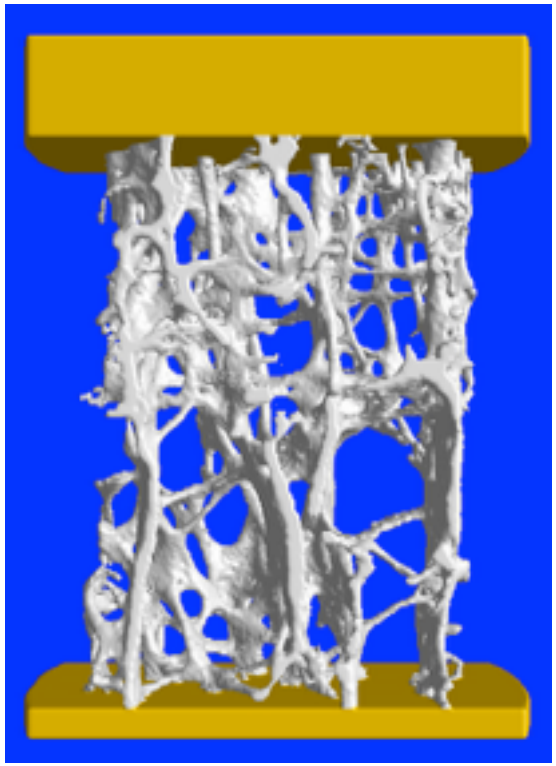
Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Image-guided failure assessment (IGFA)

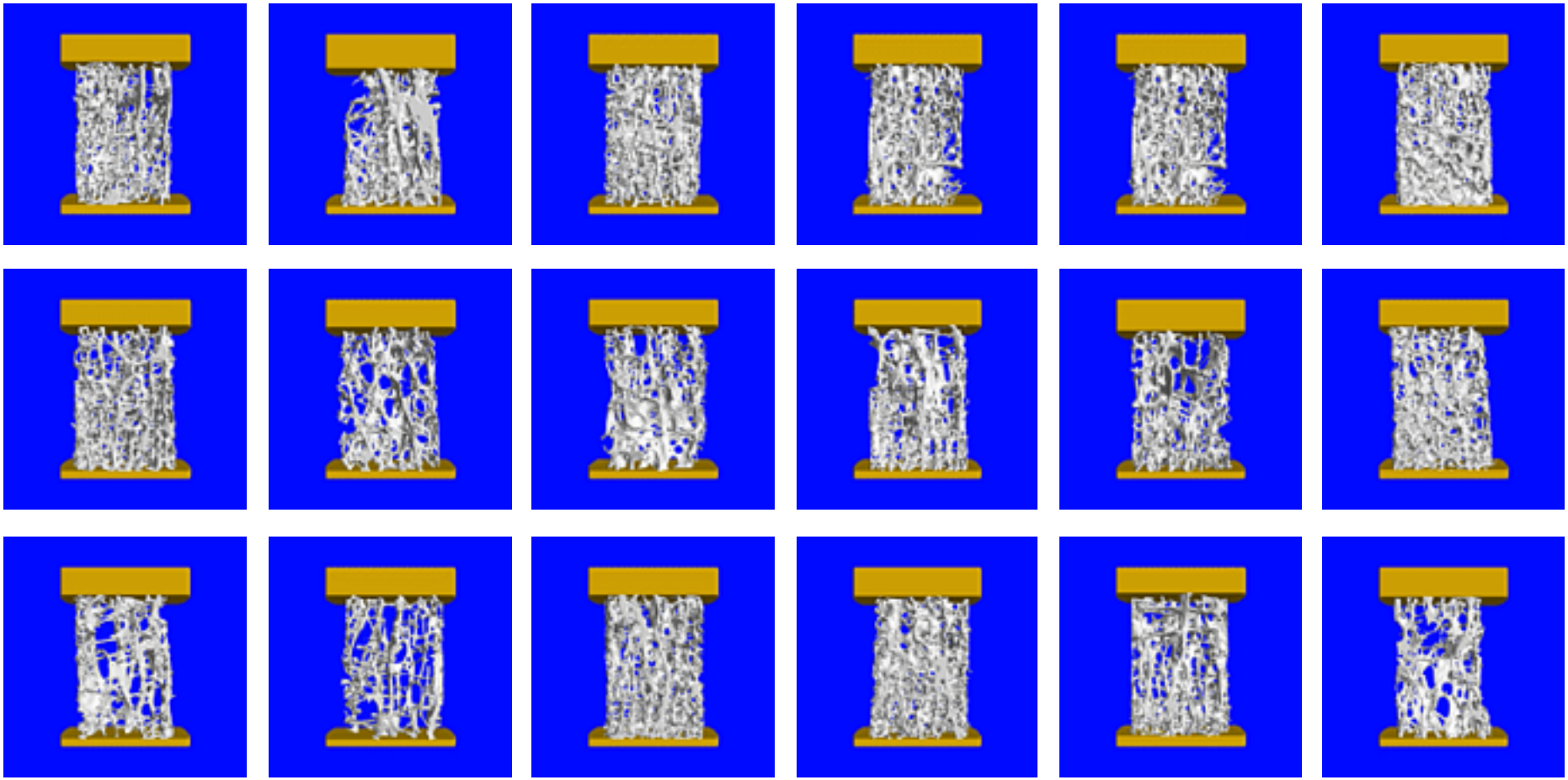




Human vertebral bone

Bone structure and function

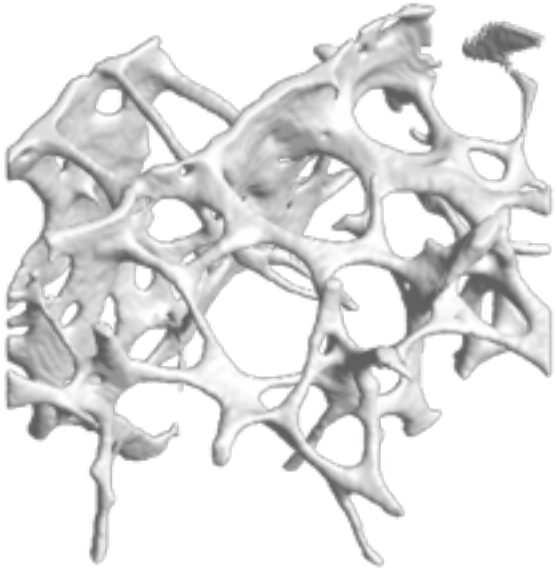
Biomechanical imaging



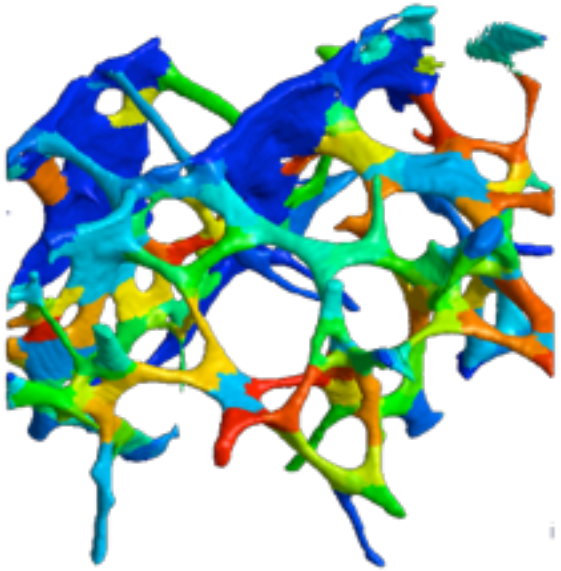
Bone structure and function

Biomechanical imaging

Element-based bone analysis



Volumetric
spatial decomposition

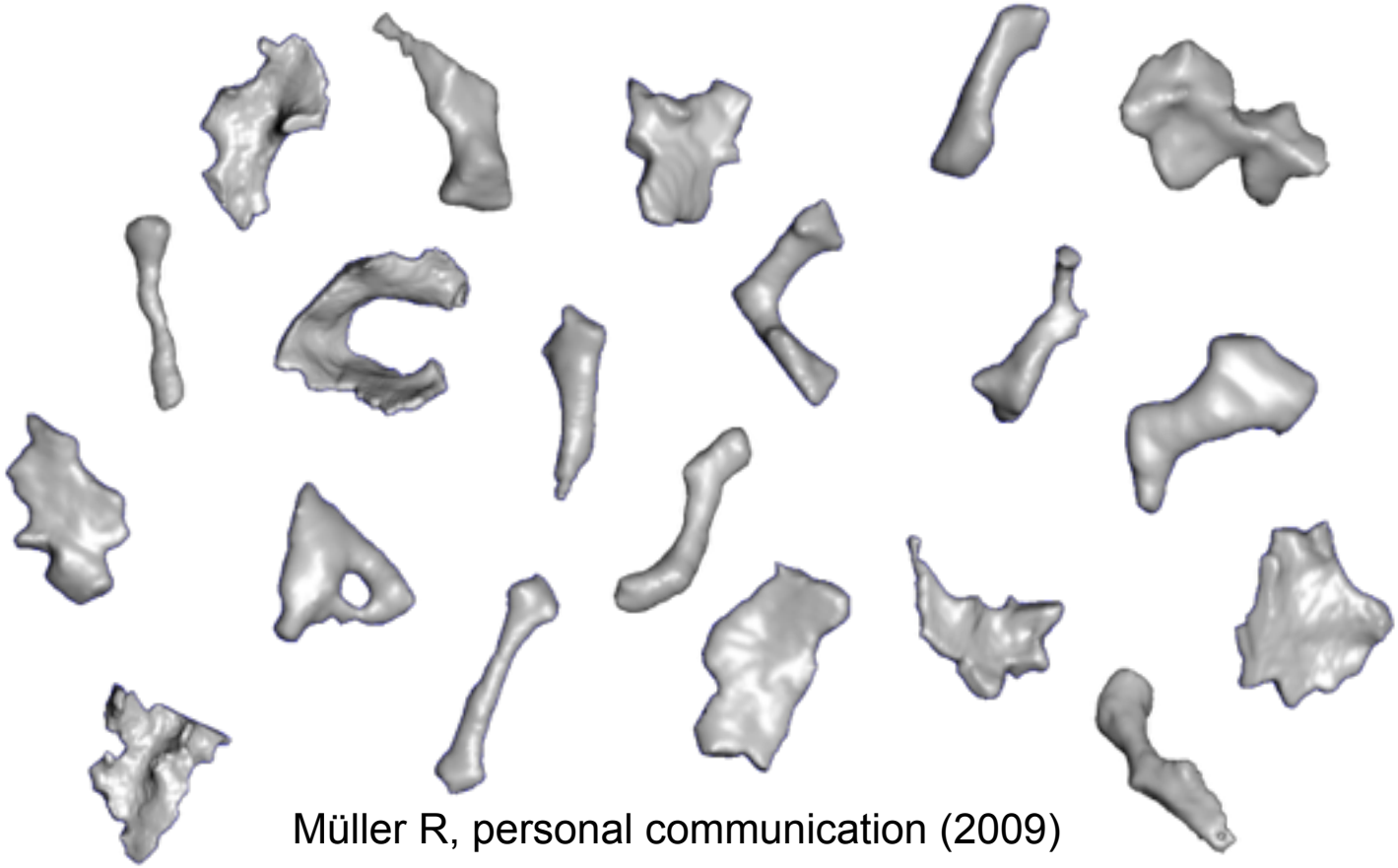


Stauber M and Müller R, Bone 38:475-484 (2006)

Bone structure and function

Biomechanical imaging

Element-based bone analysis

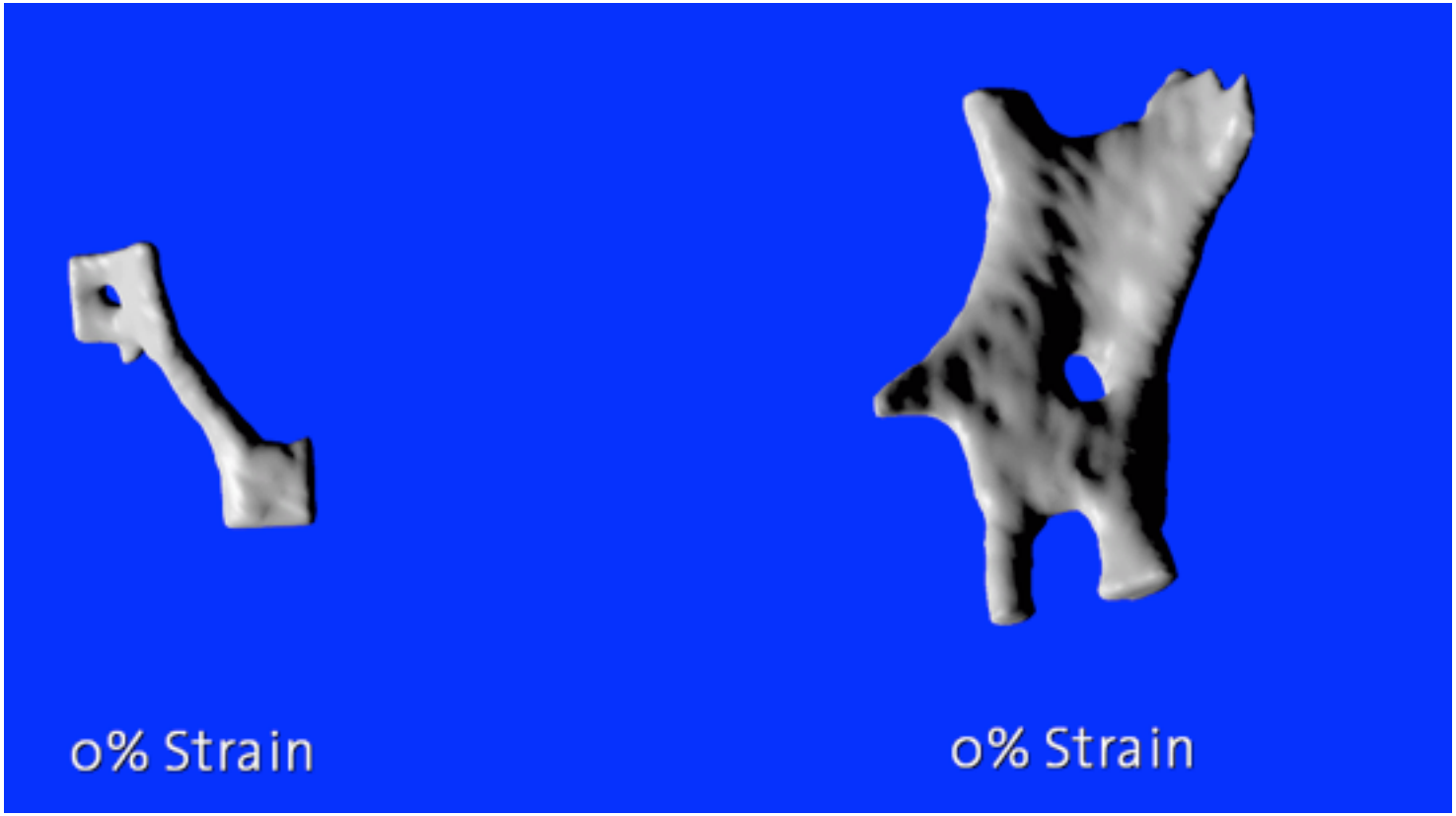


Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Element-based bone analysis

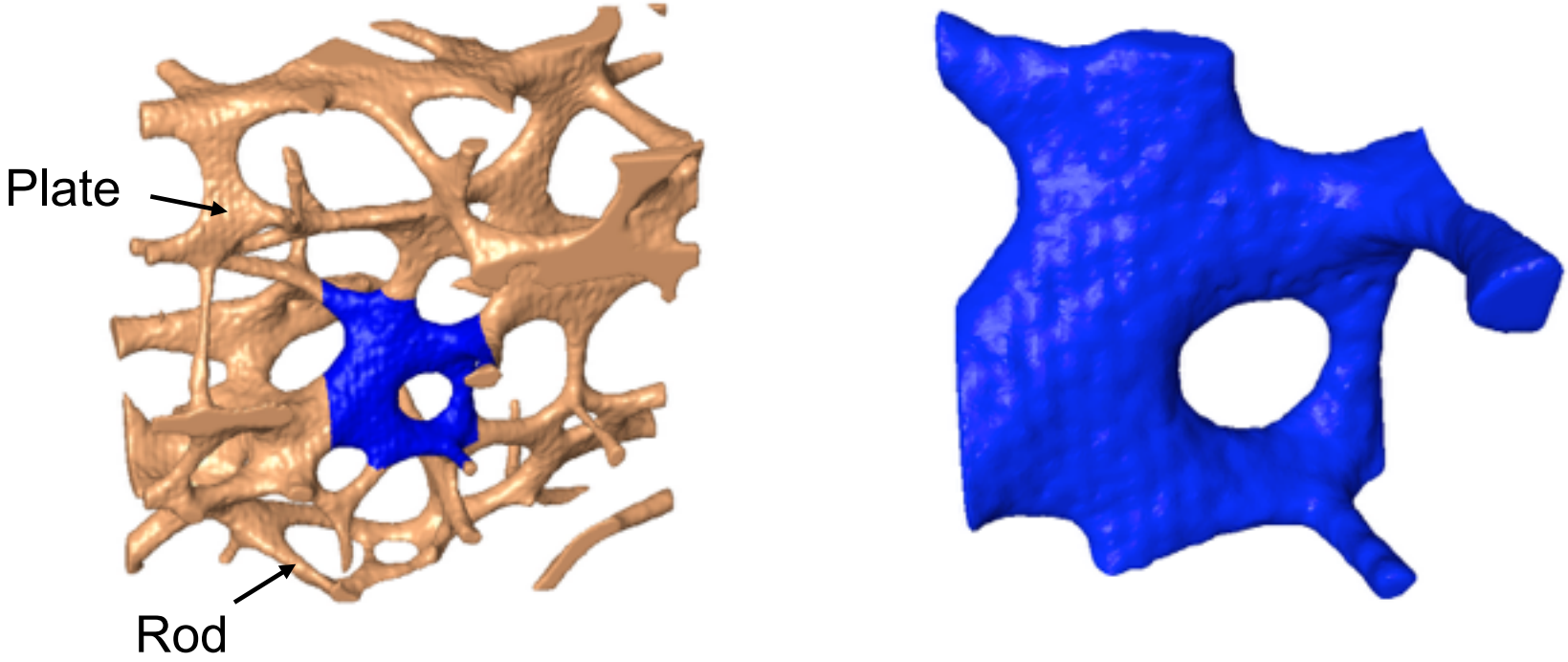


Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Element-based bone analysis

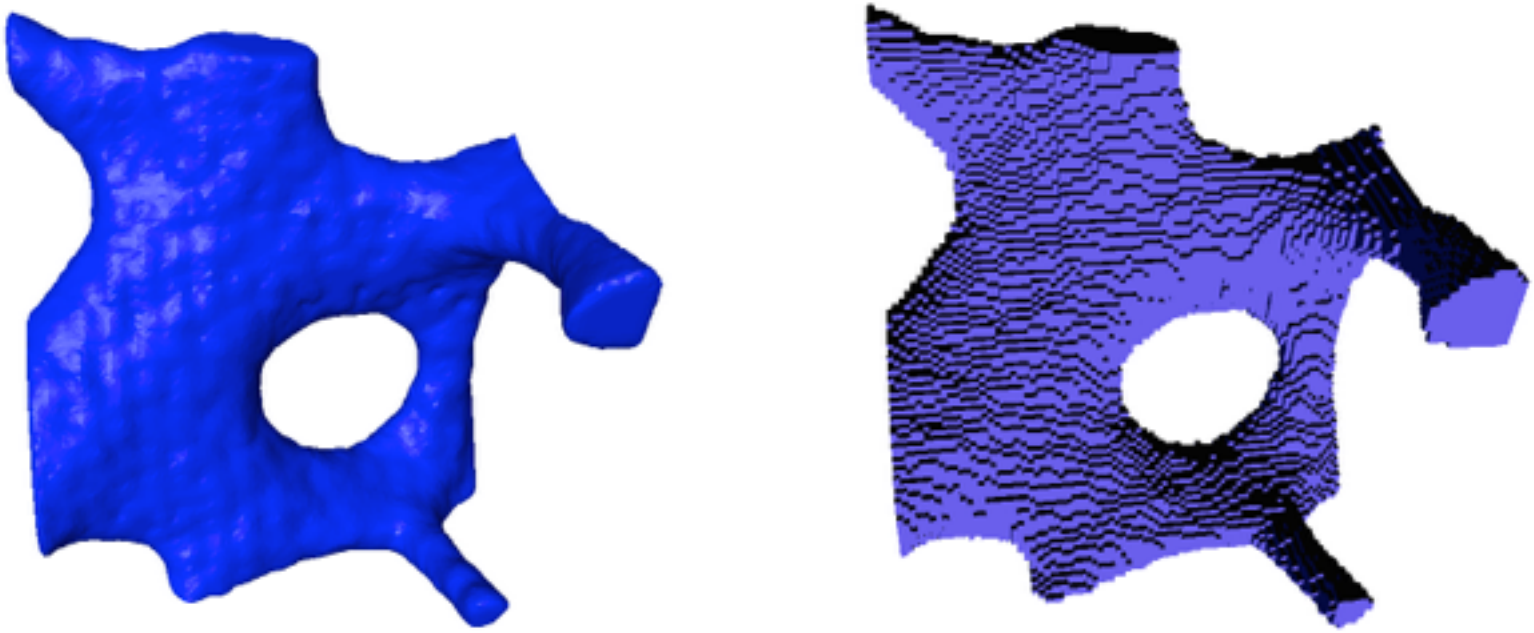


Stauber M and Müller R, Bone 38:475-484 (2006)

Bone structure and function

Biomechanical imaging

Element-based bone analysis

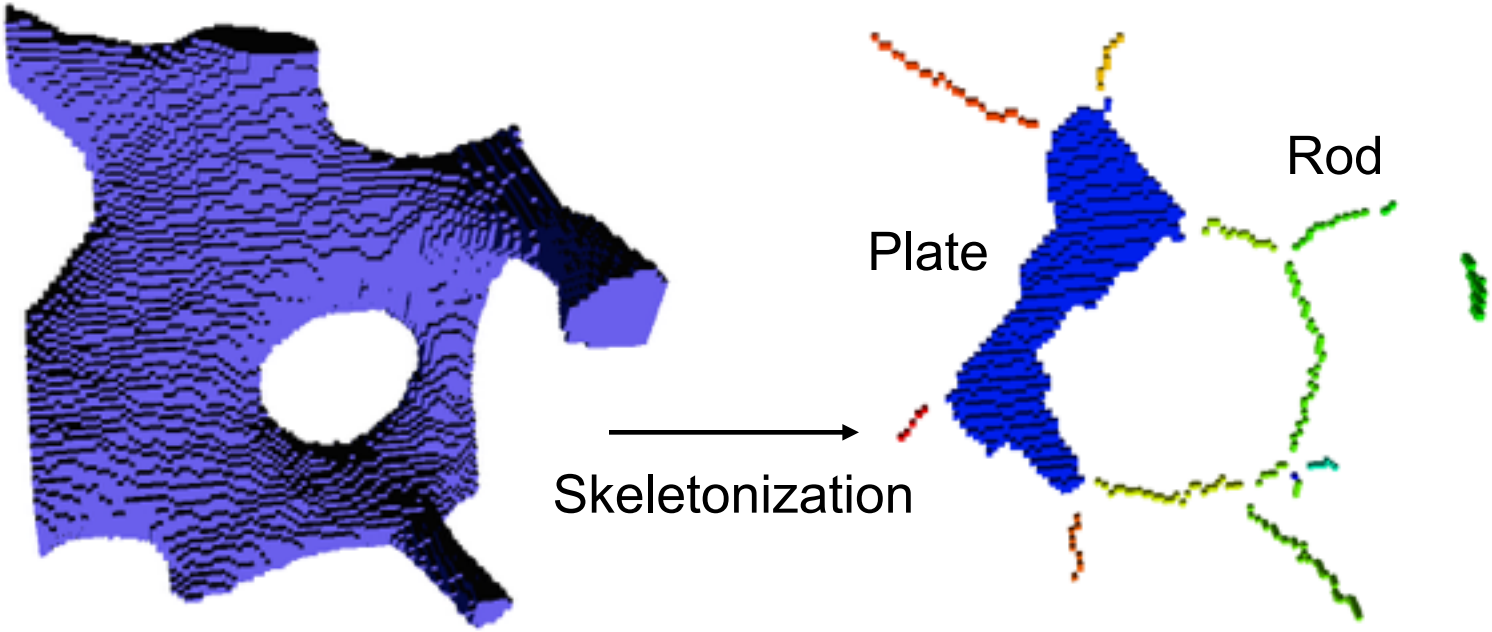


Stauber M and Müller R, Bone 38:475-484 (2006)

Bone structure and function

Biomechanical imaging

Element-based bone analysis

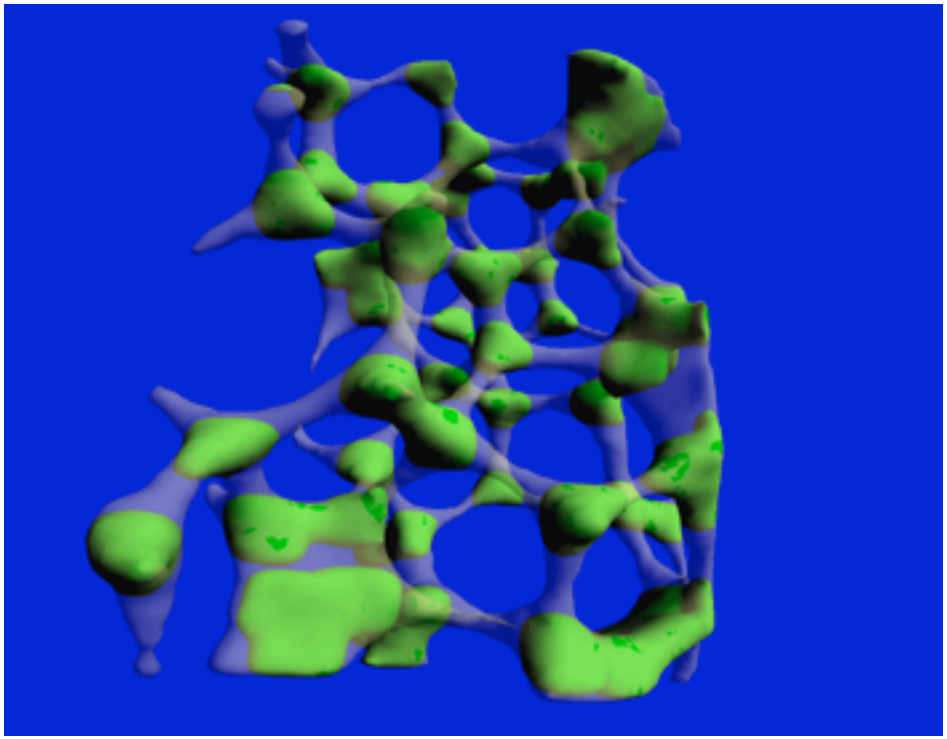


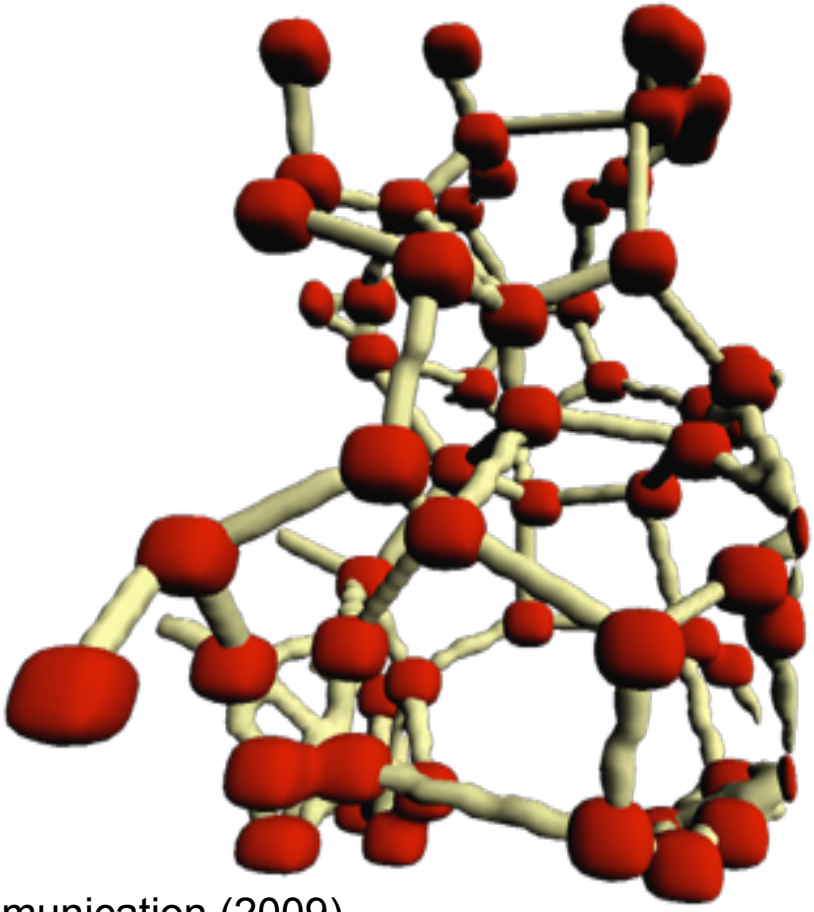
Stauber M and Müller R, Bone 38:475-484 (2006)

Bone structure and function

Biomechanical imaging

Local strain computation: introduction



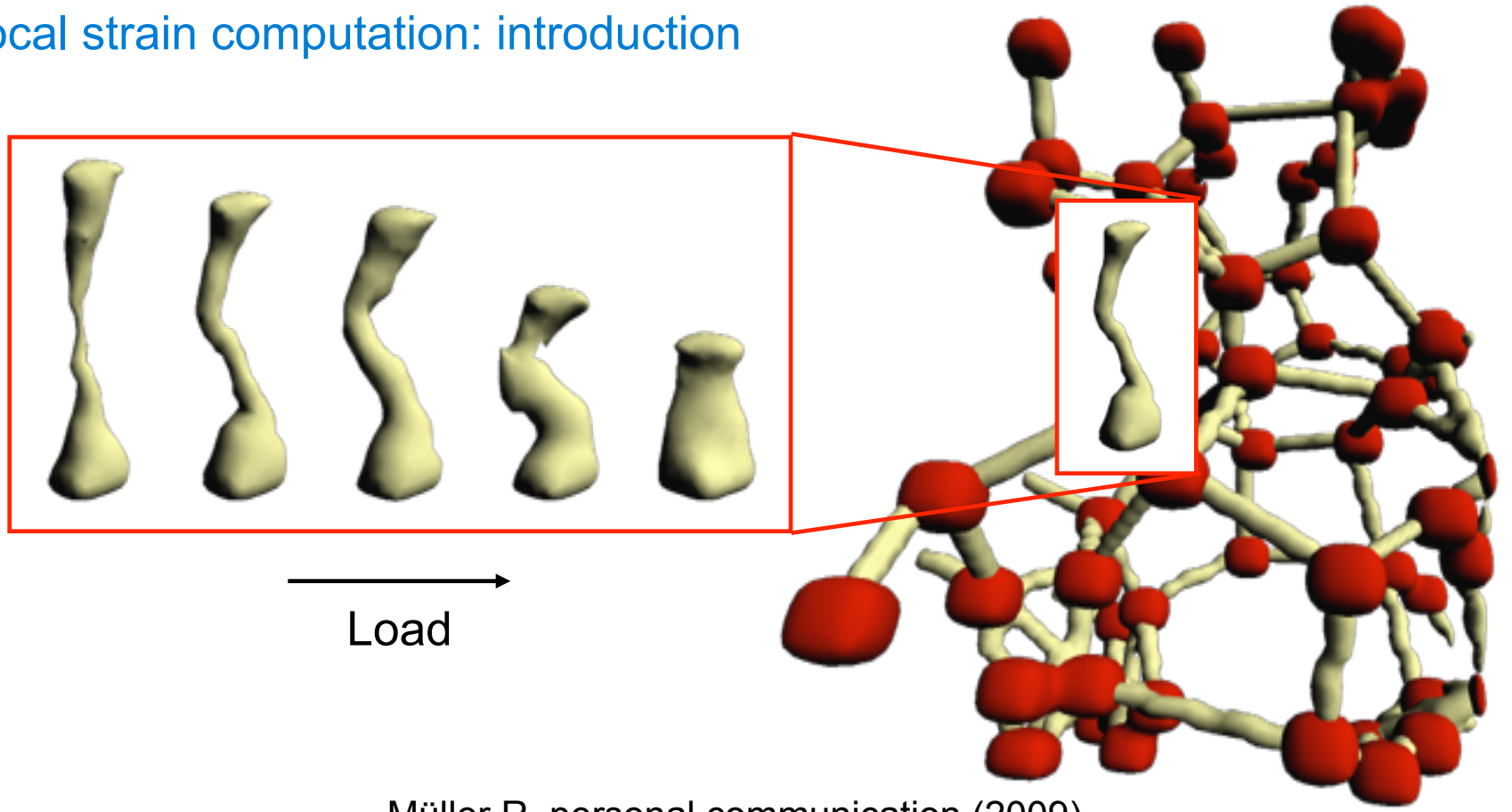


Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Local strain computation: introduction

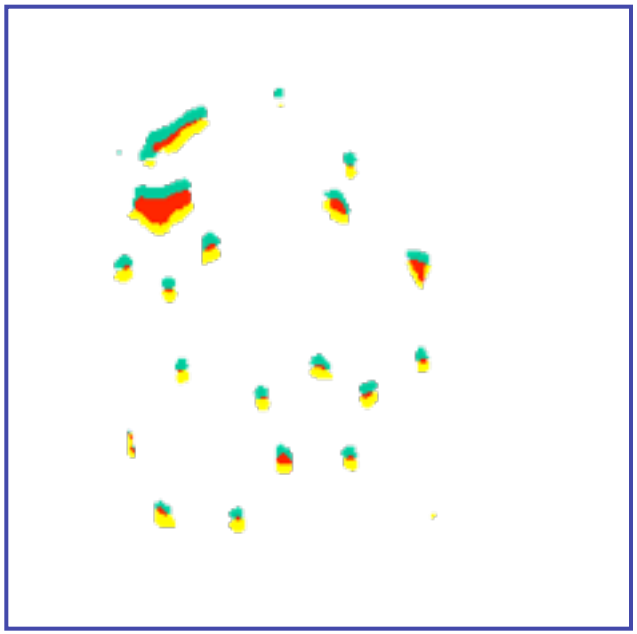


Müller R, personal communication (2009)

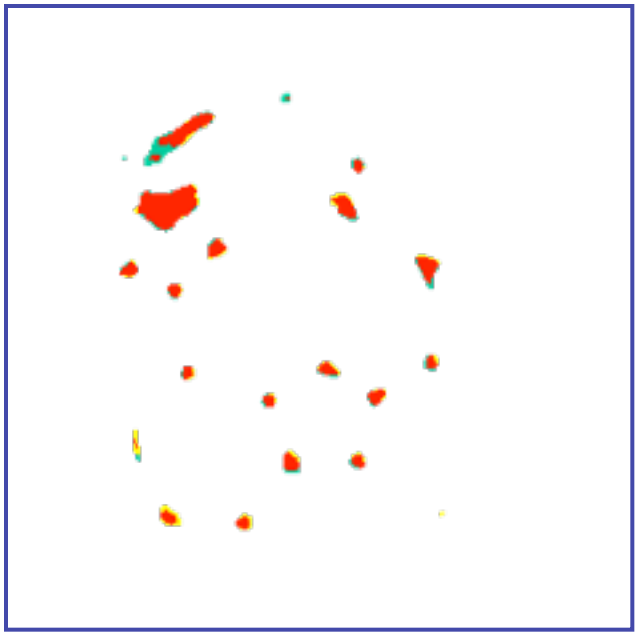
Bone structure and function

Biomechanical imaging

Local strain computation: image registration



Before registration



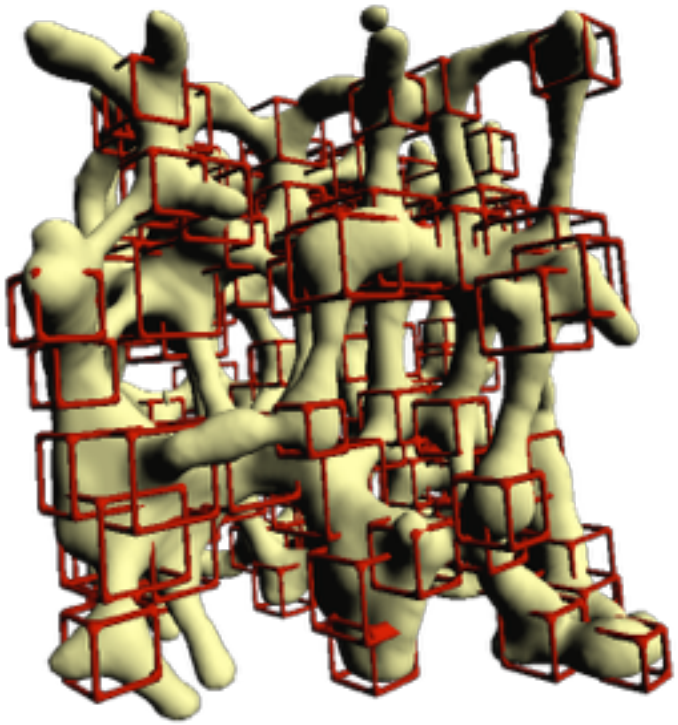
After registration

Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Local strain computation: identifying significant points



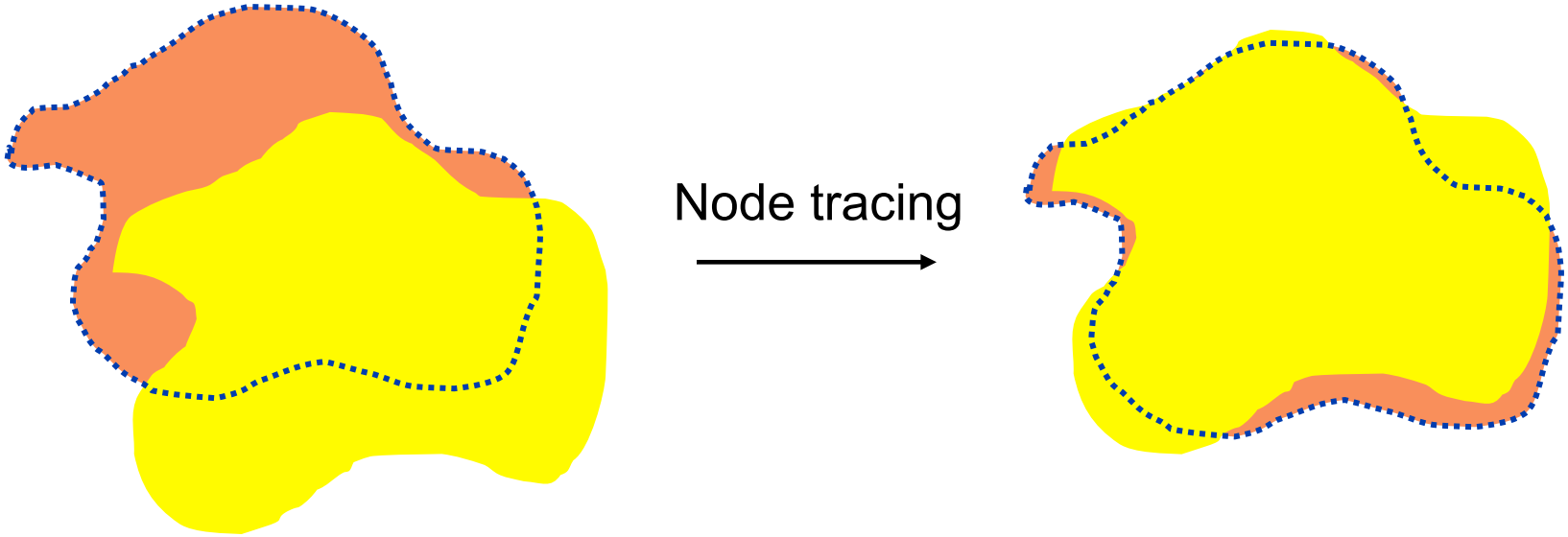
Nodes

Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Local strain computation: node tracing

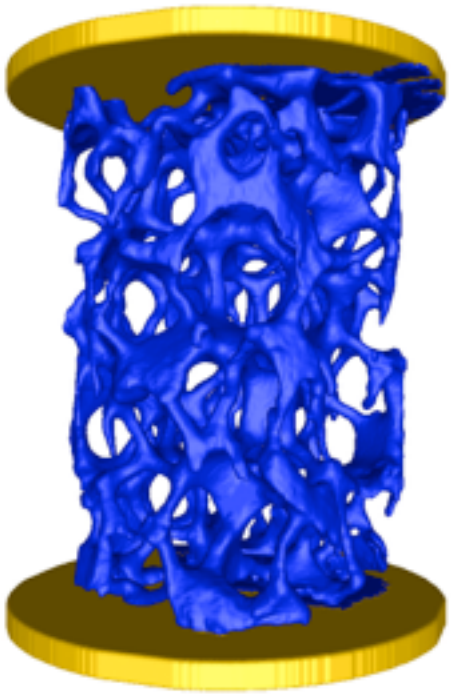


Müller R, personal communication (2009)

Bone structure and function

Biomechanical imaging

Image-guided failure assessment



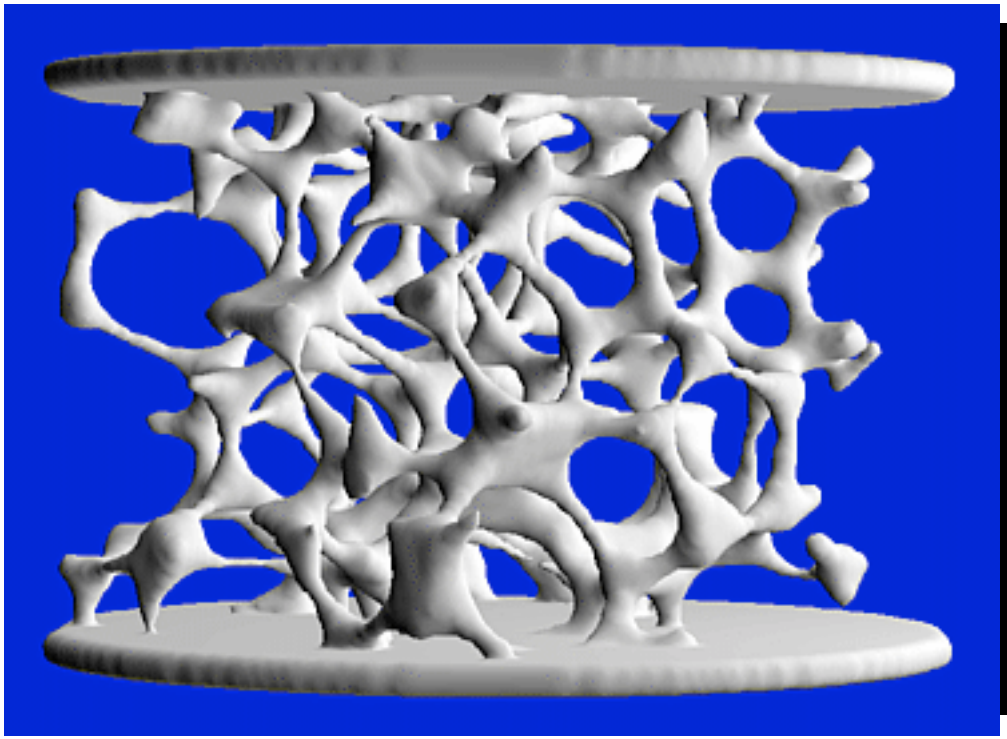
Christen D *et al*, J Biomech 41:S222 (2008)

Bone structure and function

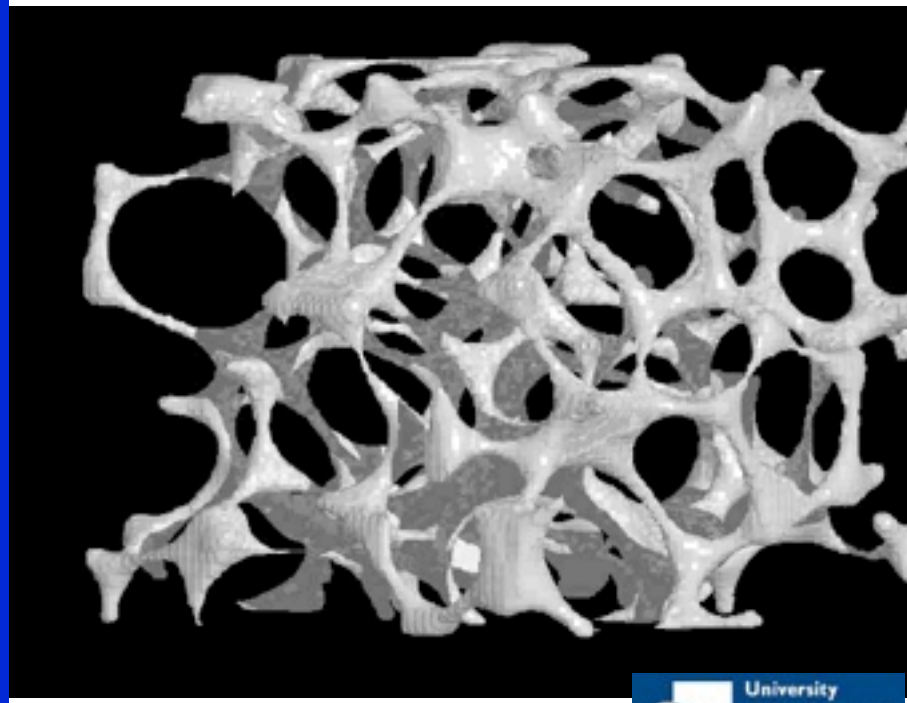
Biomechanical imaging

Image-guided failure assessment <-> finite element (FE) modeling

Image-guided failure assessment (experiment)



Finite element modeling (simulation)



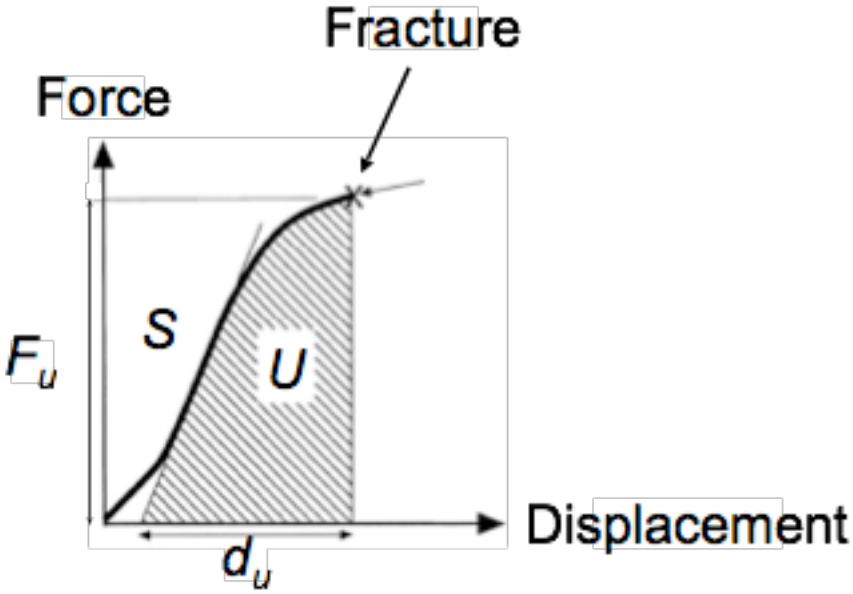
Müller R *et al*, Technol Health Care 6:433-44 (1998)

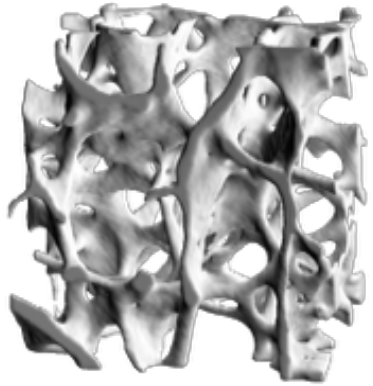
University of Southampton

Bone structure and function

Biomechanical imaging

Combining biomechanics and imaging





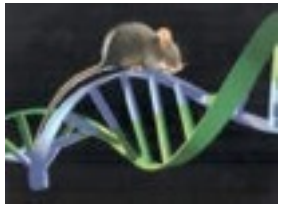
Biomechanics

Imaging

Biomechanical imaging

Bone structure and function

Biomechanical imaging



Mouse model

- High **homology** (~75%) between human and mouse genomes
- Given mouse strain -> unlimited number of **genetically identical twins**
- **Modulation of single genes** possible
- Other reasons: among the smallest mammals known, short generation time, very fertile, does not harm its young, docile, and easy to handle

Bone structure and function

Biomechanical imaging

Mouse model

B6-*lit/lit*

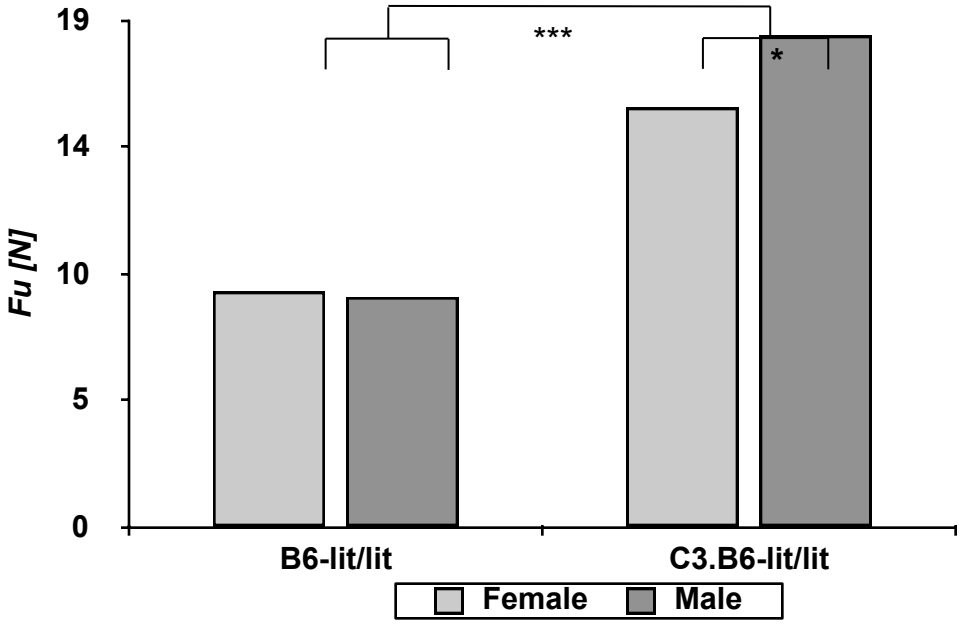
C3.B6-*lit/lit*

Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone mechanics



=> Bone strength is strain and gender specific

Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

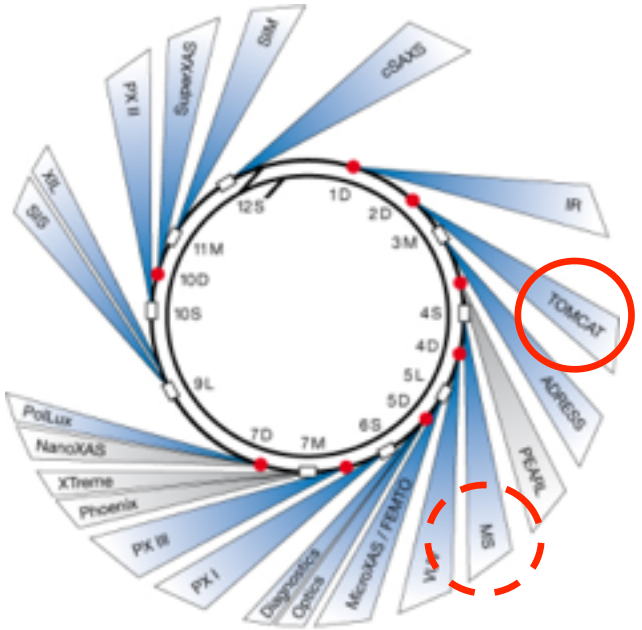
Bone structure and function

Biomechanical imaging

Bone mechanics

Swiss Light Source

TOMCAT beamline

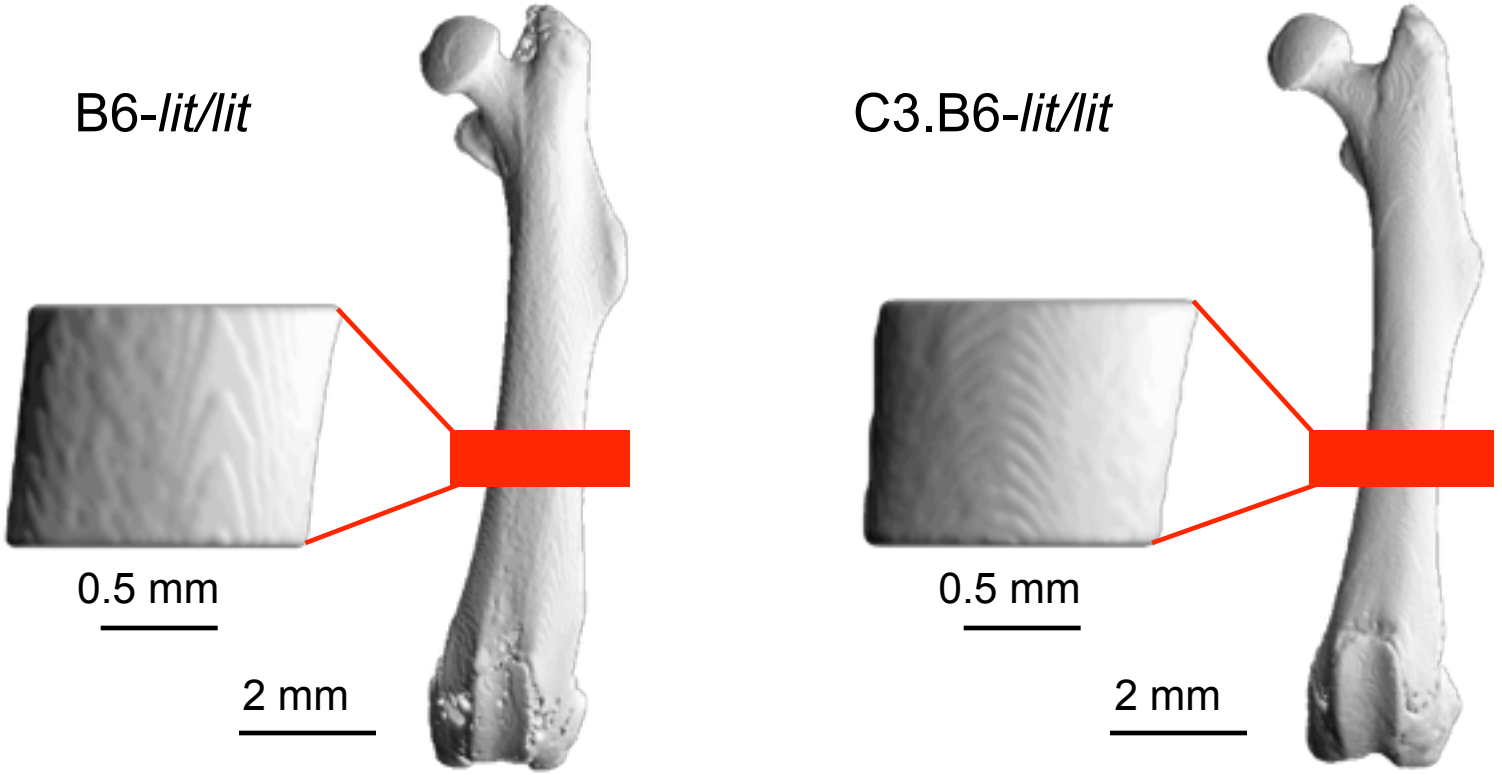


Collaboration with Prof. M. Stampanoni

Bone structure and function

Biomechanical imaging

Bone mechanics

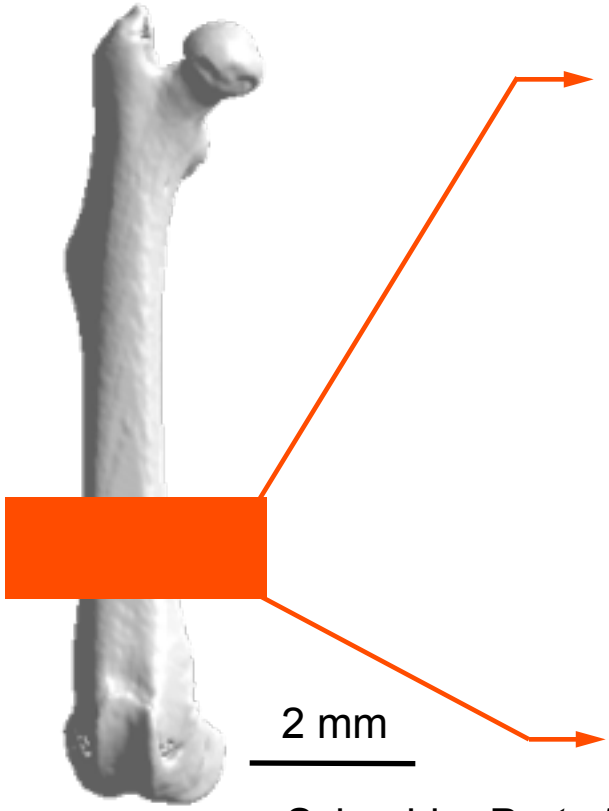


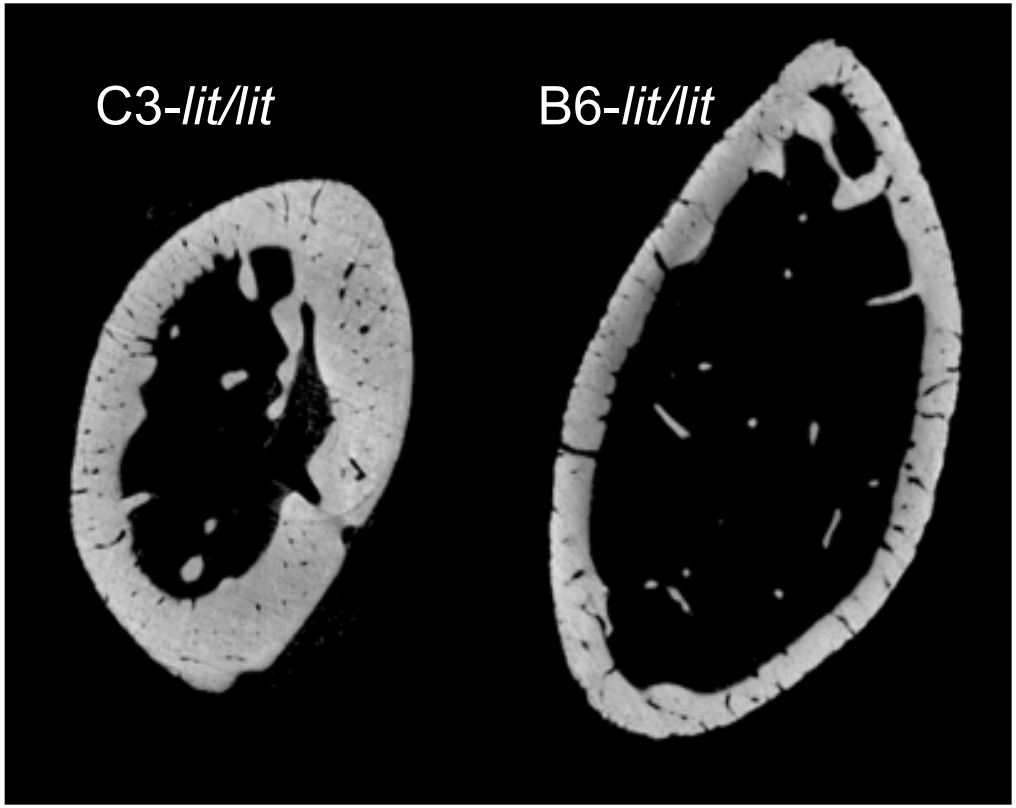
Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure



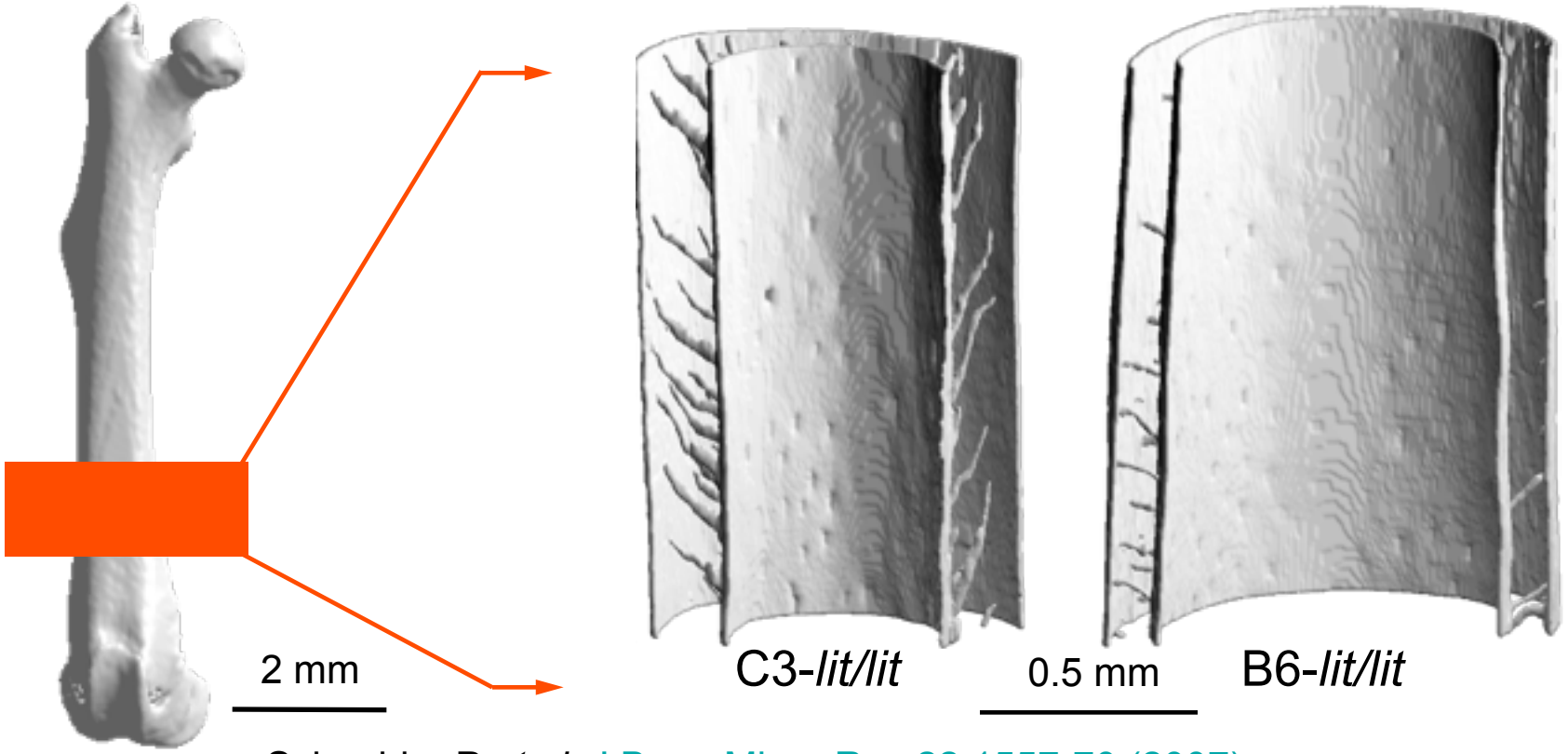


Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure

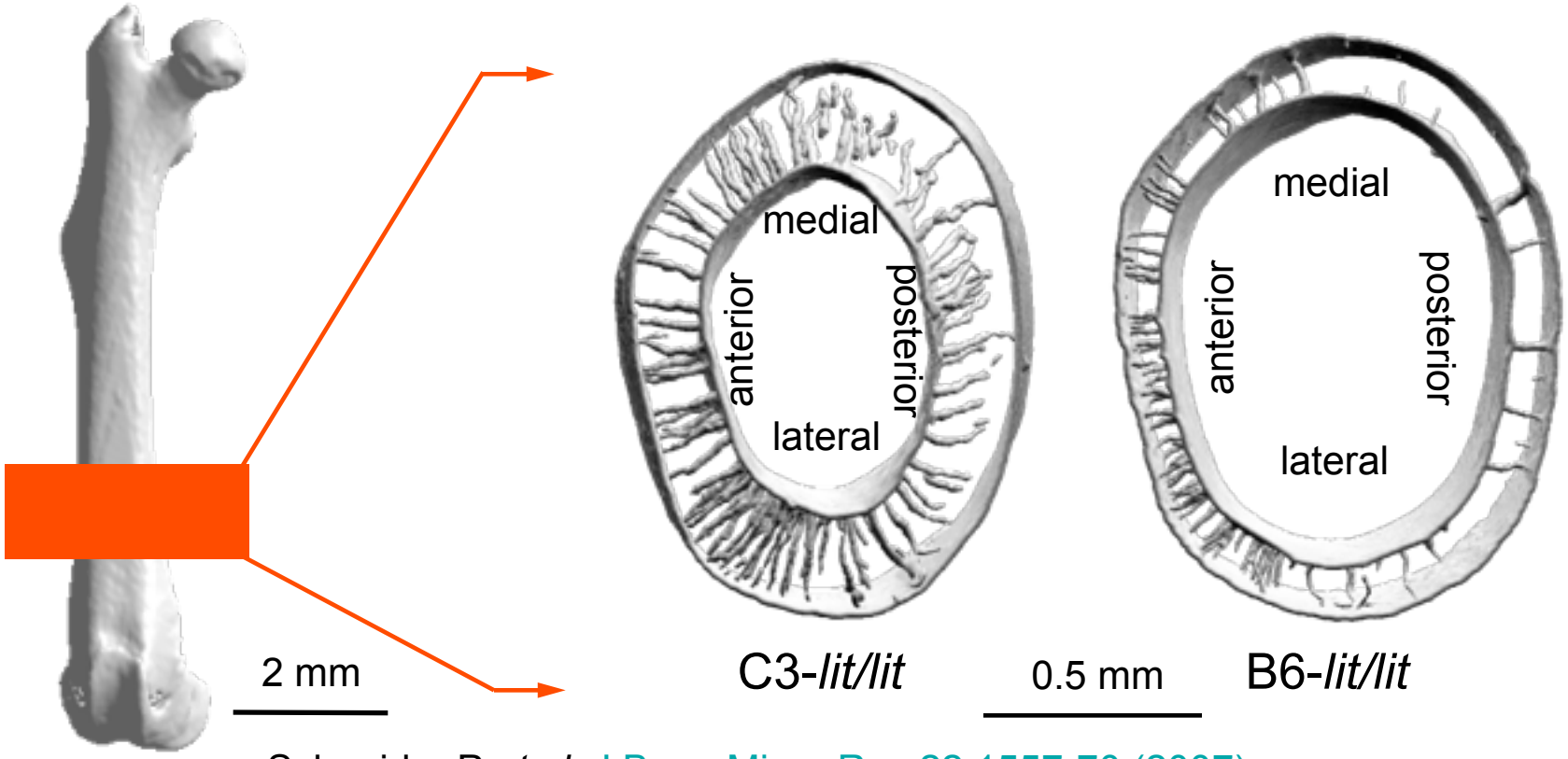


Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure

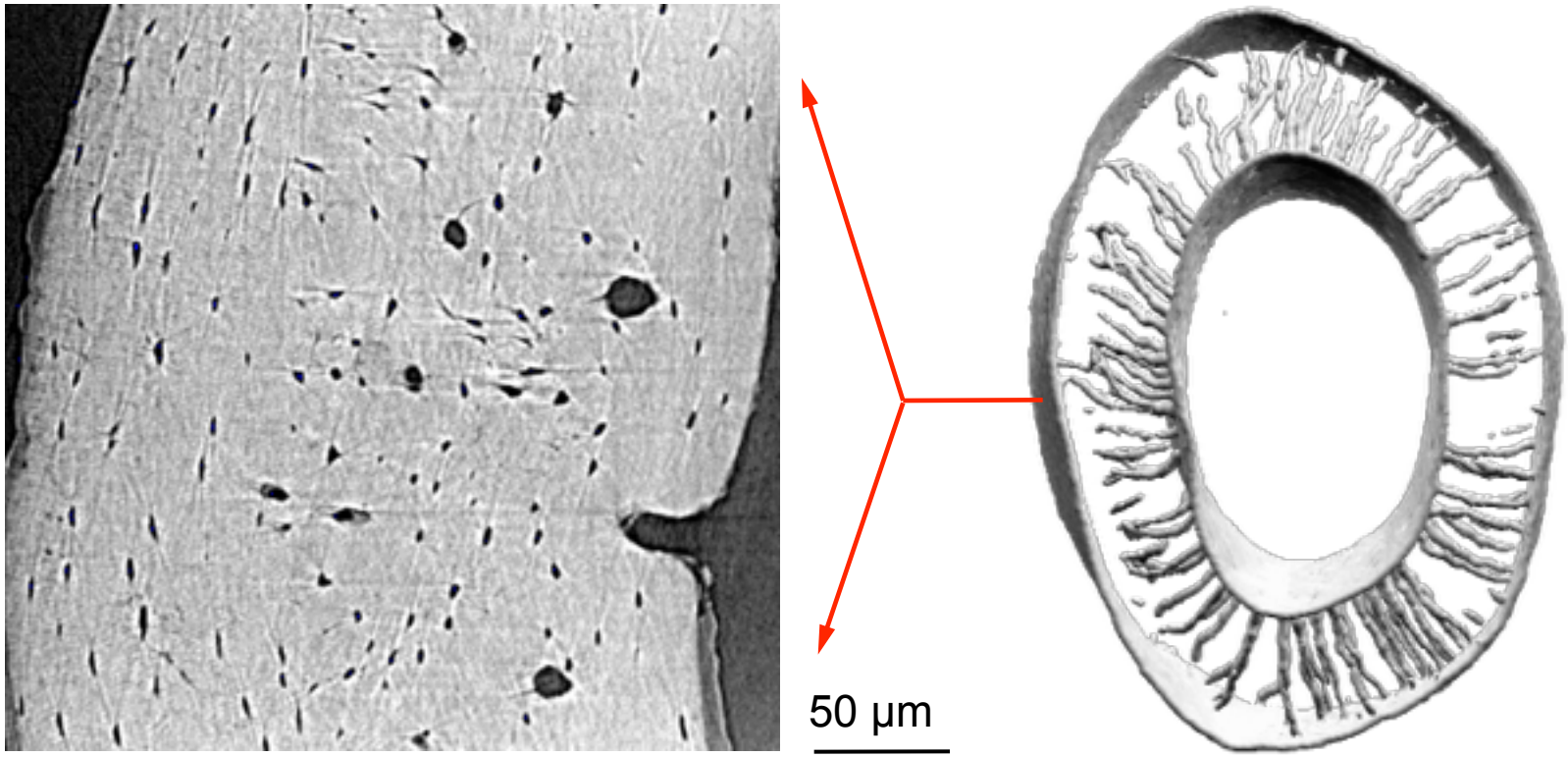


Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure

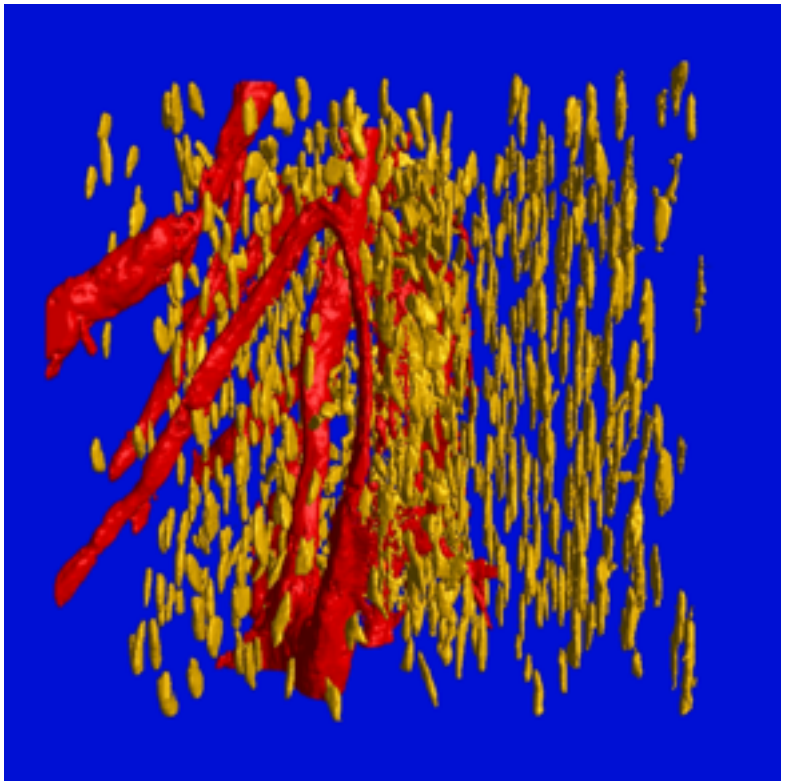


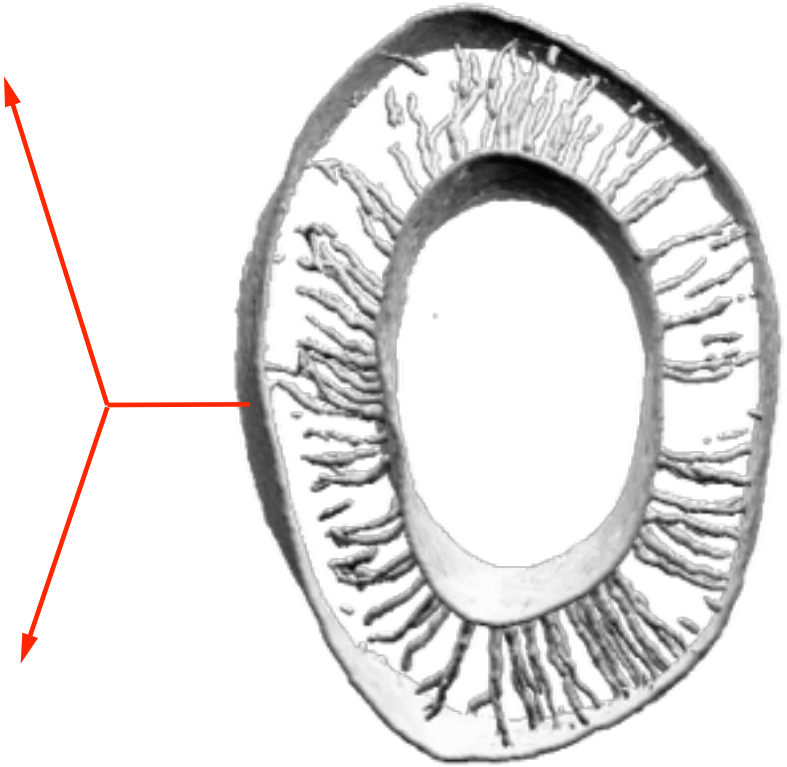
Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure



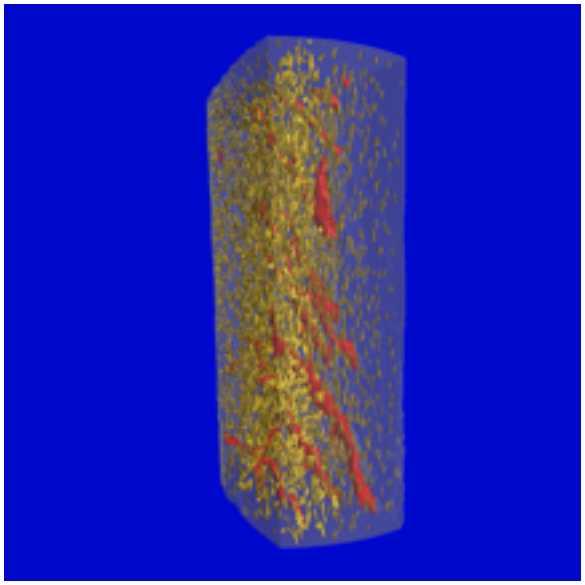


Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

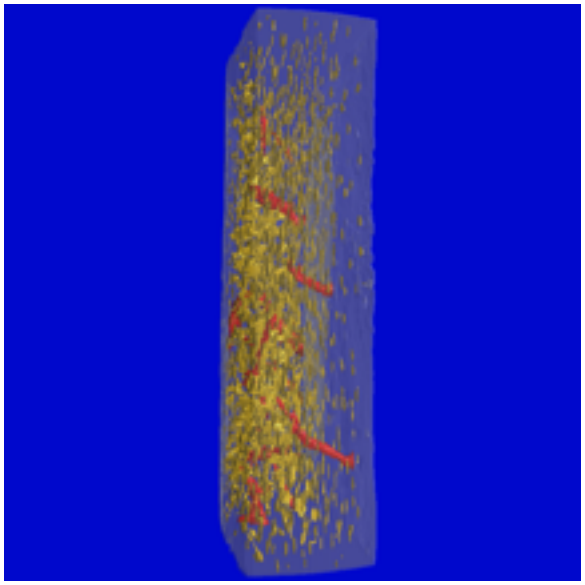
Bone structure and function

Biomechanical imaging

Bone microstructure



C3-*lit/lit* femur
posterior cortex



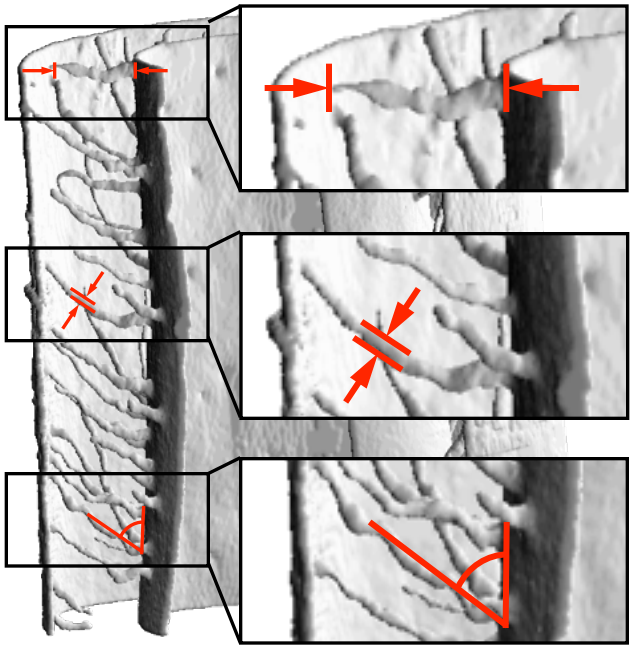
B6-*lit/lit* femur
posterior cortex

Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure



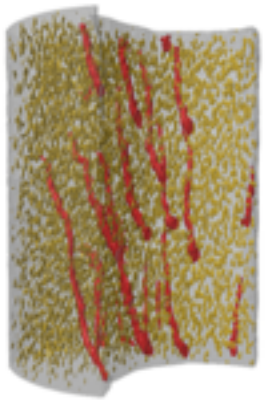
Schneider P *et al*, J Bone Miner Res 22:1557-70 (2007)

Bone structure and function

Biomechanical imaging

Bone microstructure

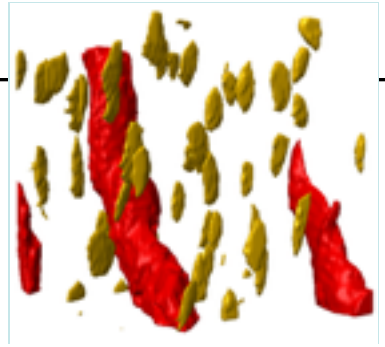
Morphometric index	B6	C3H	
Ca.V/Ct.V (%)	0.7 ± 0.5	1.4 ± 0.4	***p* < 0.05**
Ca.V/N.Ca ($10^3 \cdot \mu m^3$)	8 ± 8	55 ± 33	***p* < 0.01**
<Ca.Th> (μm)	8.0 ± 4.0	10.8 ± 3.8	*p* = 0.22
<Ca.Le> (μm)	197 ± 163	802 ± 703	*p* = 0.07



- Larger average canal volume and density in C3H
- Larger canal unit volume in C3H
- Trend for larger canal thickness in C3H

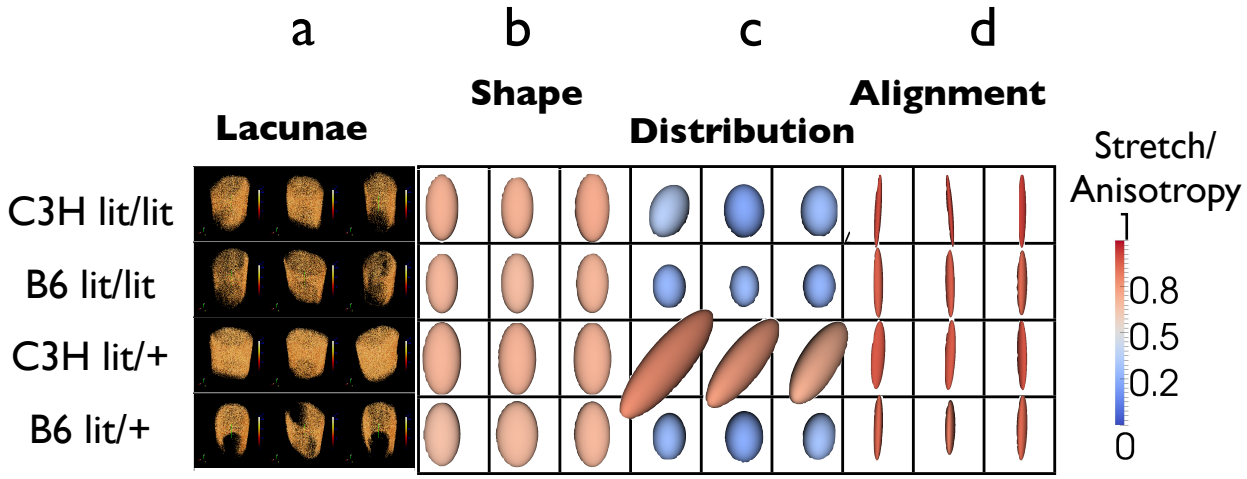
Voide R and Schneider P *et al*, Bone 49:1186-93 (2011)

Bone structure and function



Biomechanical imaging

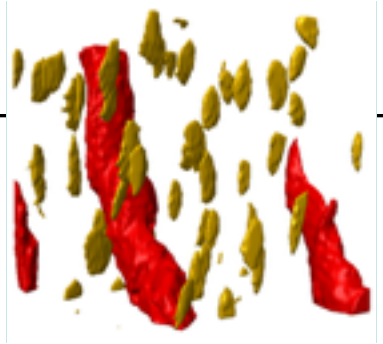
Bone microstructure



- Alignment is very consistent between samples
- Size and distribution of lacuna is different between B6 and C3H

Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1)

Bone structure and function



Biomechanical imaging

Bone microstructure

	B6lit/lit N=3	B6lit/+ N=3	C3Hlit/lit N=6	C3Hlit/+ N=5	p.lit/lit B6vC3H	p.lit/+ B6vsC3H	p.B6 lit v +	p.C3H lit v +
Lc.N (#)	26063 (4560)	32514 (2885)	33799 (13822)	53287 (3742)	0.59	0.02	0.78	0.01
Length (μm)	15.2 (0.09)	17.2 (0.59)	16.9 (0.92)	19.4 (0.25)	0.01	<0.01	0.01	<0.01
Width (μm)	8.29 (0.12)	9.96 (0.91)	8.92 (0.36)	10.1 (0.18)	0.24	0.97	<0.01	<0.01
Height (μm)	5.16 (0.19)	5.89 (0.14)	5.24 (0.16)	6.21 (0.12)	0.87	0.04	<0.01	<0.01
Lc.V (μm^3)	317 (16.2)	469 (45.6)	378 (41.8)	577 (15.4)	0.08	<0.01	<0.01	<0.01
Lc.St (%)	0.64 (0.01)	0.64 (0.01)	0.67 (0.01)	0.66 (0.01)	0.01	0.10	0.96	0.10
Lc.Ob	-0.31 (0.03)	-0.21 (0.13)	-0.33 (0.05)	-0.36 (0.02)	0.98	0.02	0.21	0.77
Lc.Te.V (μm^3)	22456 (2064)	26247 (3253)	28333 (3518)	25687 (1150)	0.04	0.99	0.31	0.36
Density (kLc/mm^3)	44.8 (4.15)	38.5 (4.53)	35.7 (4.22)	39.0 (1.77)	0.02	1.00	0.18	0.45
Lc.ND (μm)	19.4 (0.10)	20.4 (0.34)	20.7 (0.17)	21.2 (0.25)	<0.01	<0.01	<0.01	0.02
Lc.DN (#)	7.34 (0.06)	7.26 (0.08)	7.42 (0.04)	8.00 (0.08)	0.42	<0.01	0.37	<0.01
Lc.Gp	1.27 (0.03)	1.27 (0.03)	1.26 (0.04)	1.33 (0.01)	0.93	0.08	1.00	0.01
Lc.DtSt	0.29 (0.01)	0.29 (0.04)	0.26 (0.03)	0.74 (0.04)	0.81	<0.01	1.00	<0.01
Lc.DtOb	0.61 (0.09)	0.20 (0.30)	0.55 (0.11)	0.84 (0.02)	0.91	<0.01	0.01	0.02
Lc.Al (%)	0.87 (0.01)	0.84 (0.03)	0.91 (0.01)	0.88 (0.01)	<0.01	<0.01	0.13	0.01

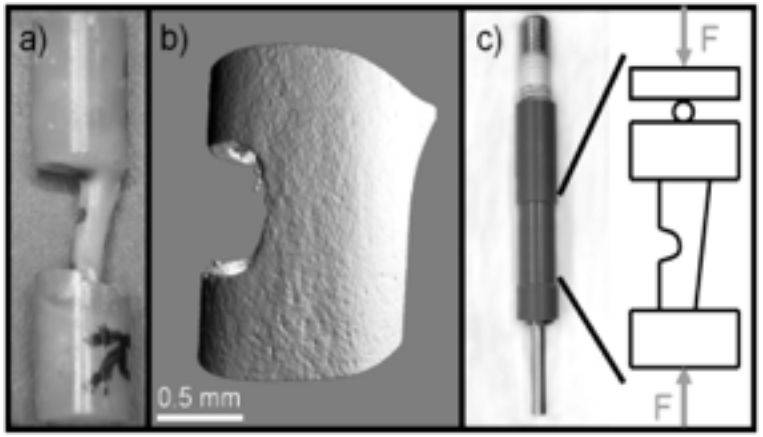
Size and distribution of lacuna is different between B6 and C3H

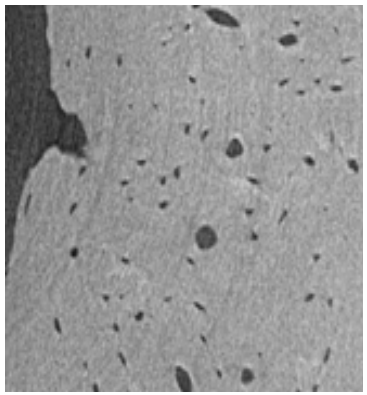
Grouping conserved despite density

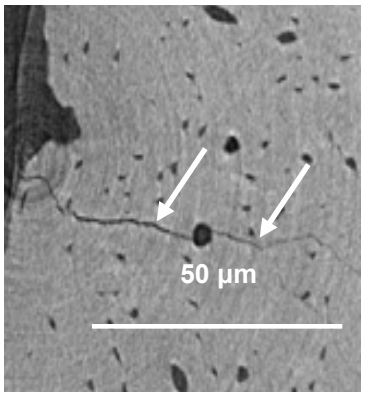
Mader, K. S., Schneider, P., Müller, R., & Stampanoni, M. (2013). A quantitative framework for the 3D characterization of the osteocyte lacunar system. Bone, 57(1)

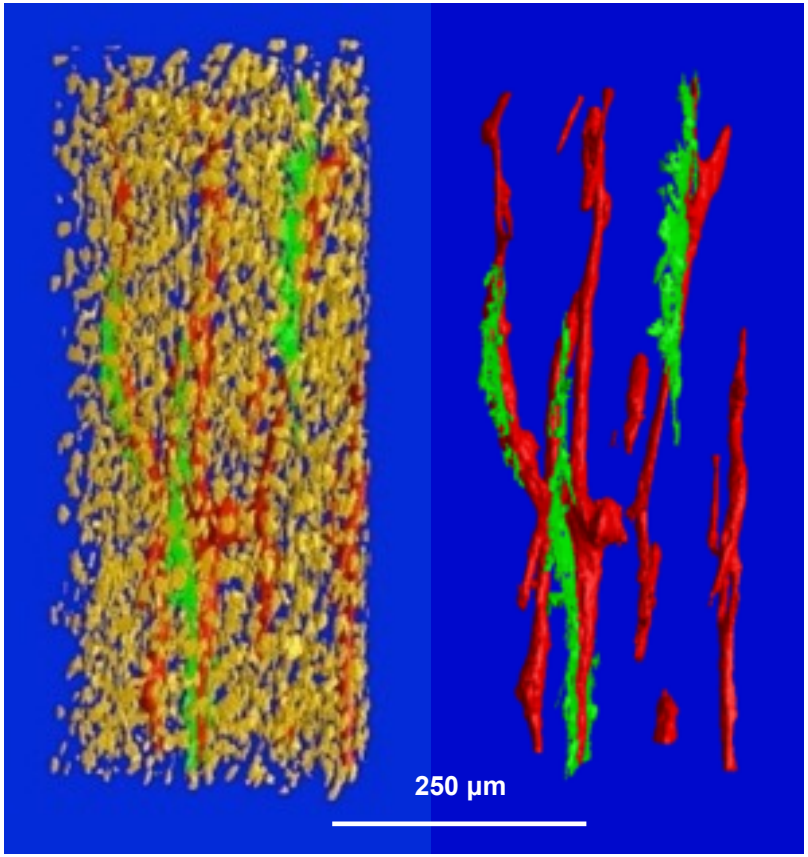
Bone structure and function

Biomechanical imaging







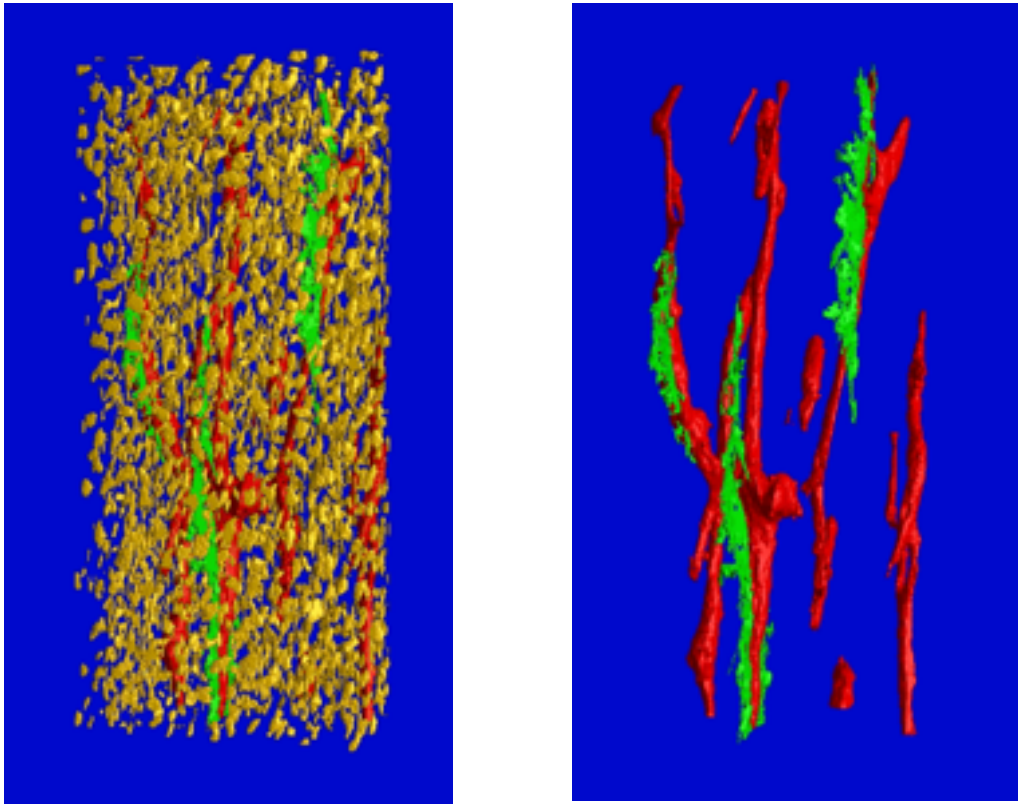


Voide R *et al*, Bone 45(2):164-73 (2009)

Bone structure and function

Biomechanical imaging

Microdamage



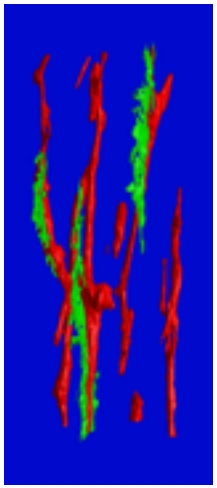
Voide R *et al*, Bone 45(2):164-73 (2009)

Bone structure and function

Biomechanical imaging

Microcracks

Morphometric	B6	C3H	
N.Cr/Ct.V (mm^{-3})	3.± 1	8 ± 3	***p* < 0.01**
Cr.V/Ct.V (%)	1.0 ± 0.5	0.7 ± 0.7	*p* = 0.29
<Cr.Th> (µm)	2.5 ± 1.0	2.6 ± 0.8	*p* = 0.87
<Cr.Le> (µm)	107± 48	293 ± 197	*p* = 0.07



- Larger average crack number and volume in C3H
- No difference in crack thickness between B6 and C3H
- Trend for larger crack length in C3H

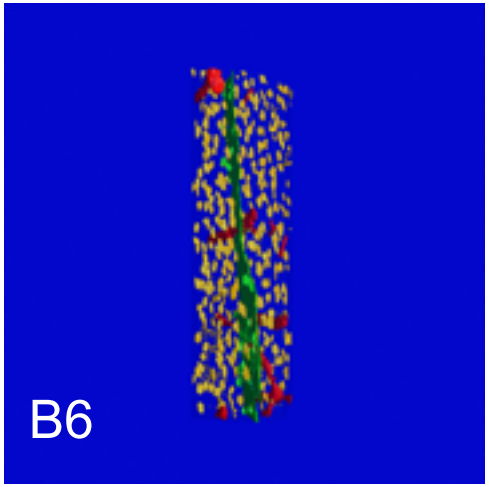
Voide R and Schneider P *et al*, Bone 49:1186-93 (2011)

Bone structure and function

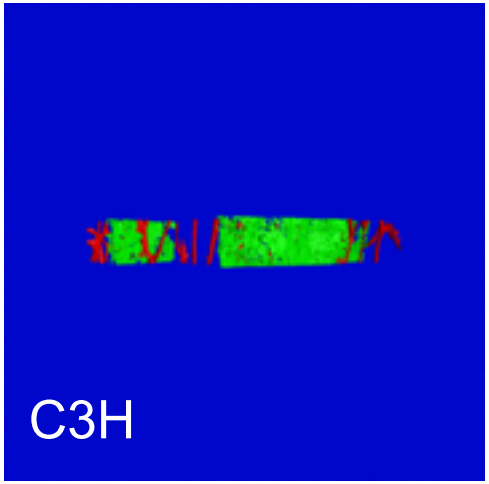
Biomechanical imaging

Microcracks

- In B6, 83% of all cracks go through canals:
  - 10% in line with canals
  - 90% across canals
- cracks go through lacunae



- In C3H, 100% of all cracks go through canals:
  - 96% in line with canals
  - 4% across canals
- cracks go through lacunae



Voide R and Schneider P *et al*, Bone 49:1186-93 (2011)

Bone structure and function

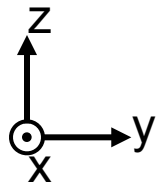
Biomechanical imaging

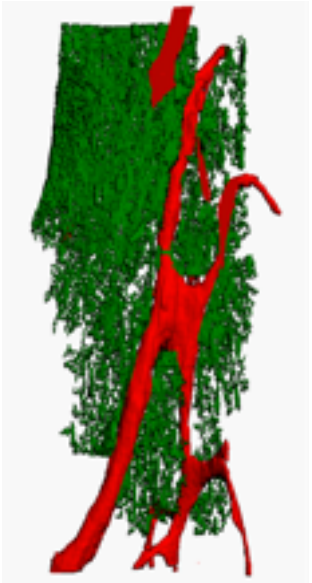
Bone microstructure <-> microcracks

- In B6, microcracks
  - initiate at bone surfaces
  - are guided by lacunae
  - run across the canals

- In C3H, microcrakcs
  - initiate at bone and canal surfaces
  - are guided by lacunae and canals
  - are in line with canals

100 µm





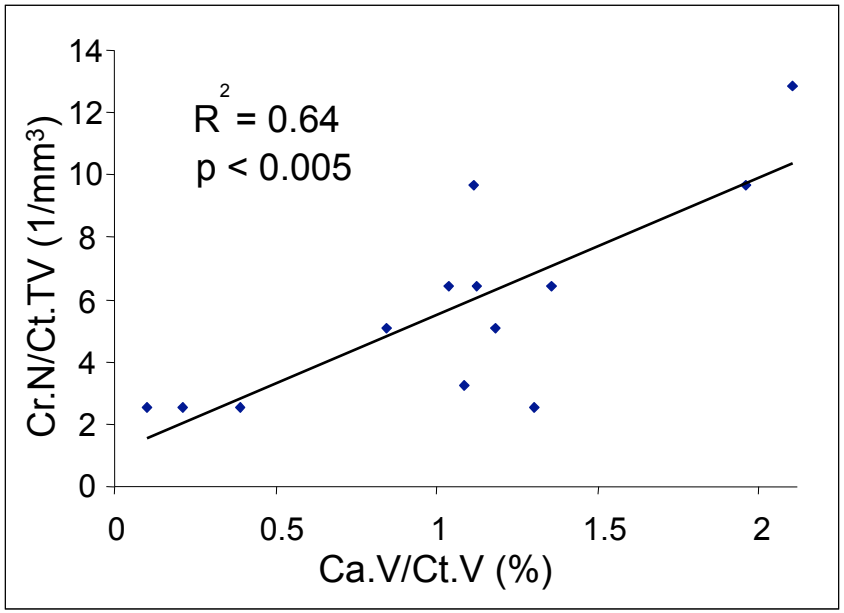
100 µm

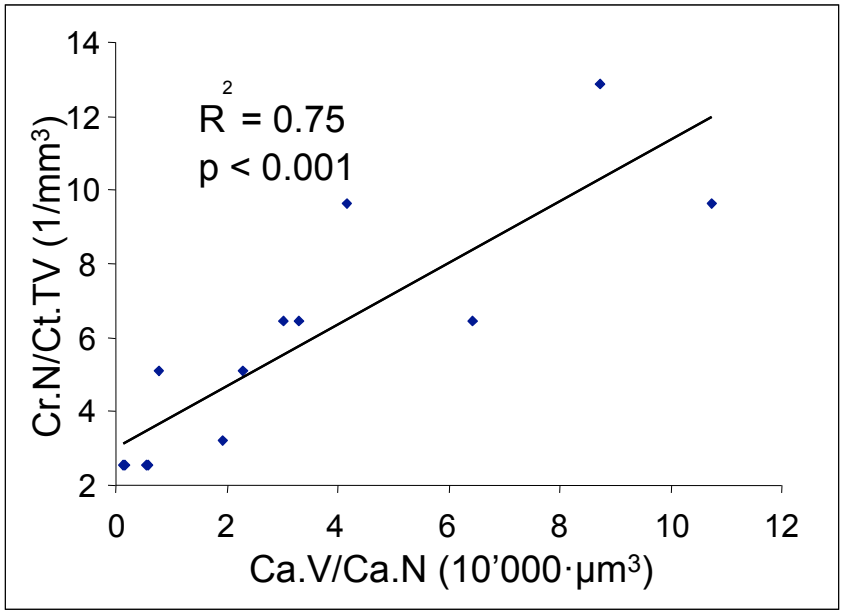
Voide R and Schneider P *et al*, Bone 49:1186-93 (2011)

Bone structure and function

Biomechanical imaging

Microdamage





=> Bone microstructure contributes to microcrack initiation and propagation

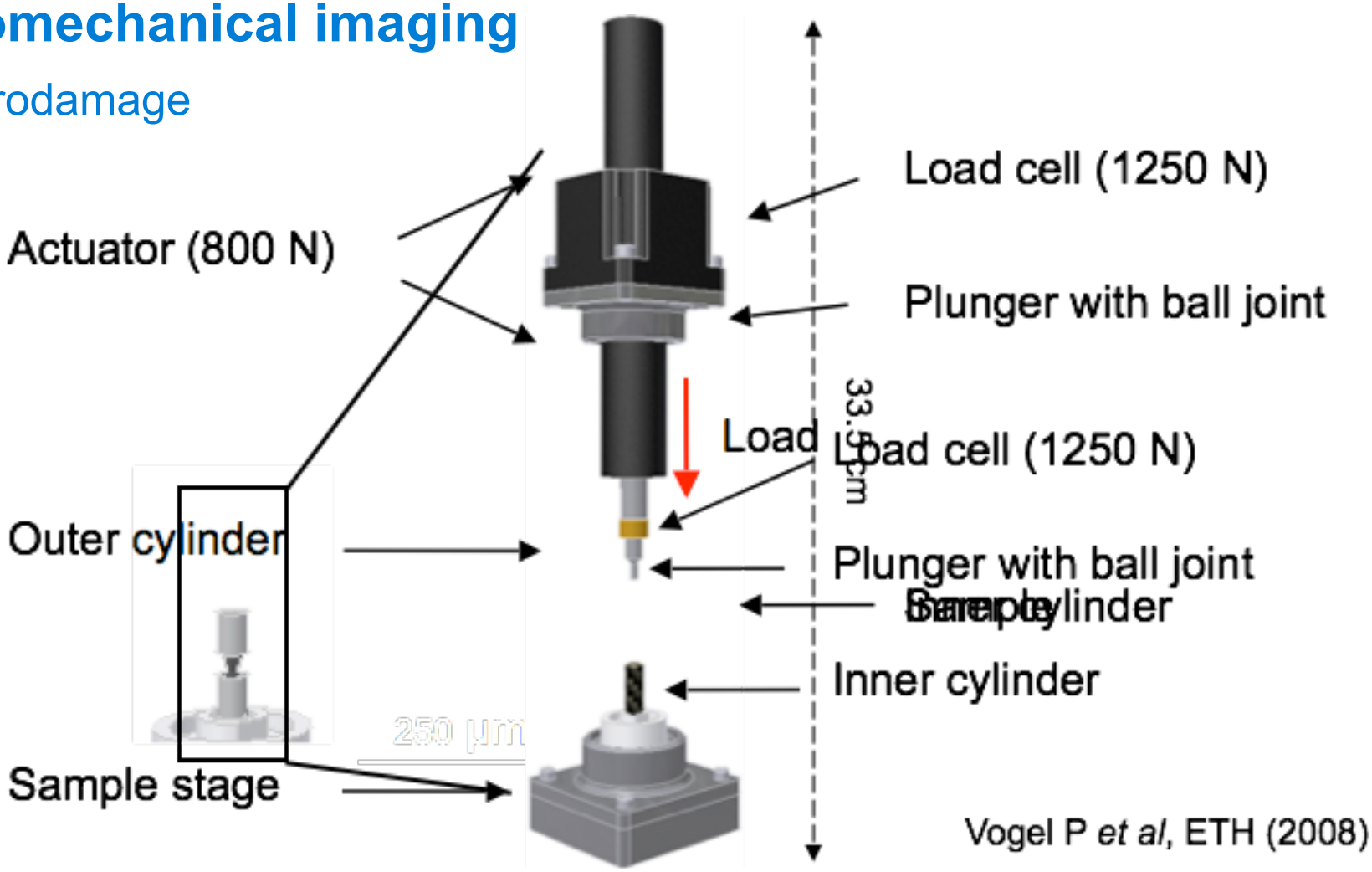
=> Microcrack morphometry can help explaining cortical bone failure

Voide R and Schneider P *et al*, Bone 49:1186-93 (2011)

Bone structure and function

Biomechanical imaging

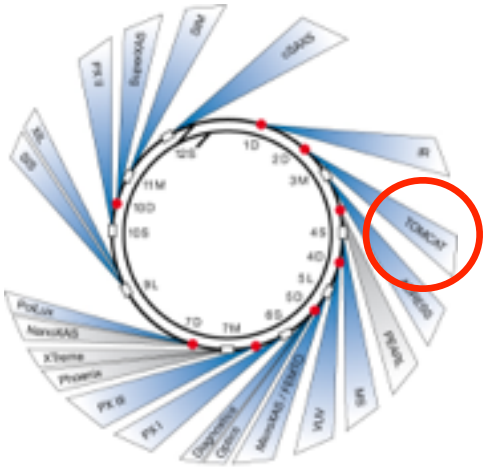
Microdamage

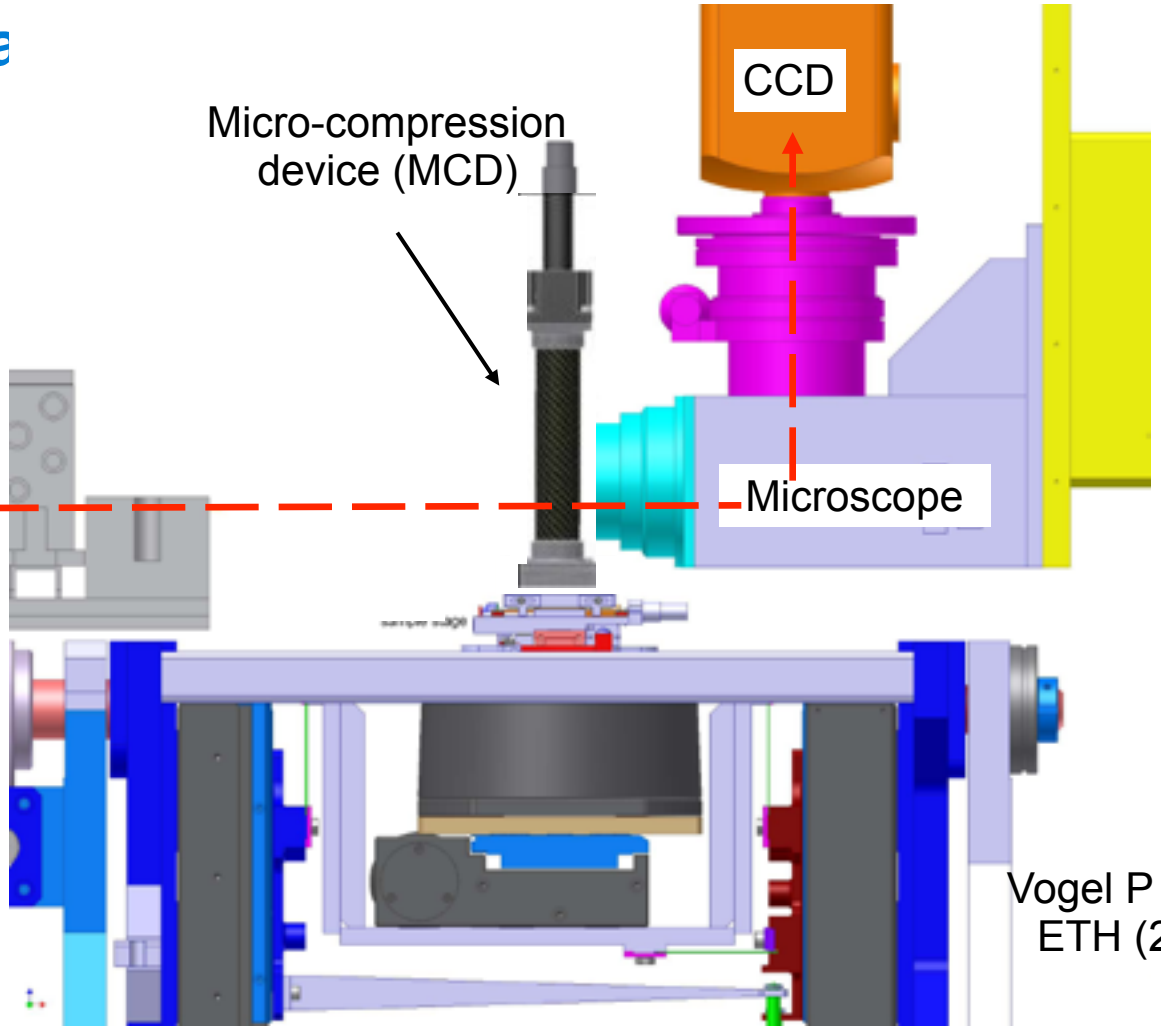


Vogel P *et al*, ETH (2008)

Bone structure and function

Biomechanical ima



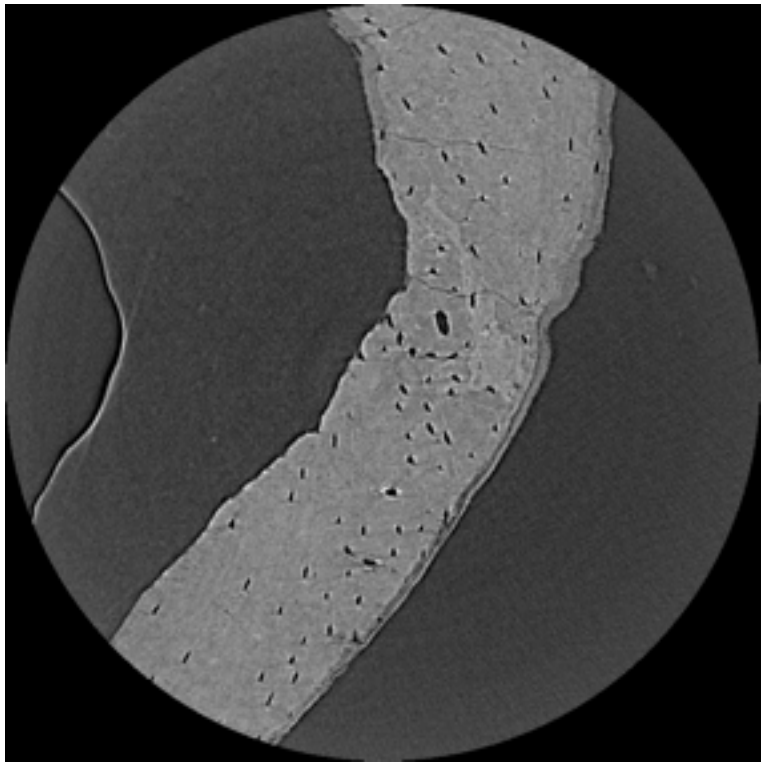


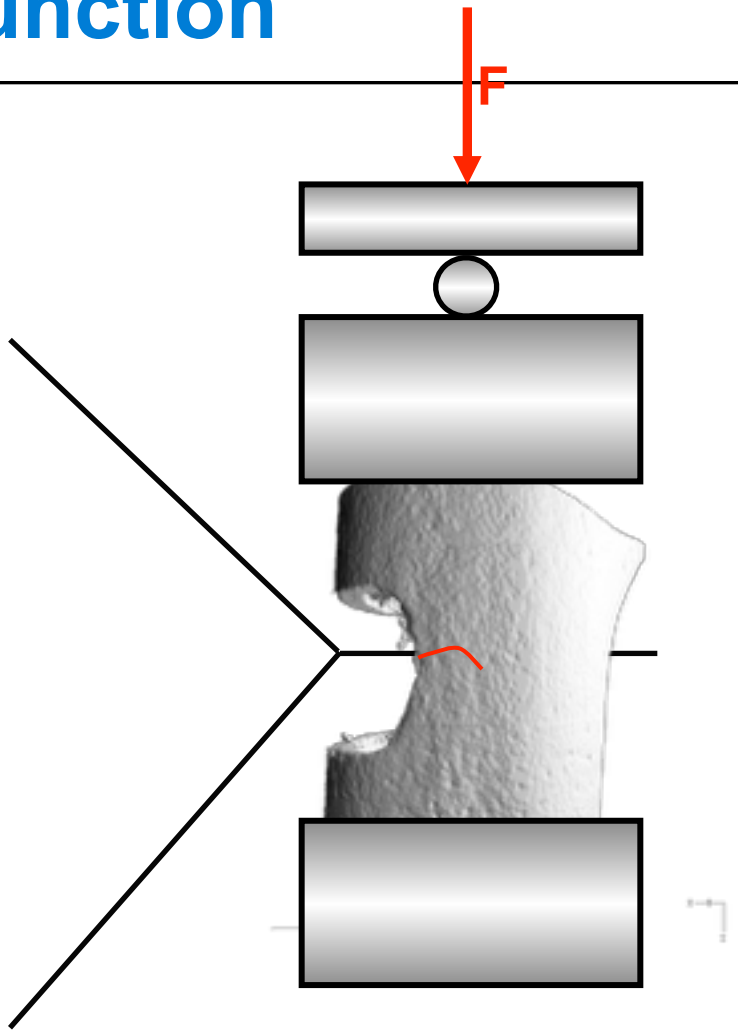
Vogel P *et al*, ETH (2008)

Bone structure and function

Biomechanical imaging

Microdamage





Meier M *et al*, J Biomech 41(Suppl 1):S76 (2008)

Bone structure and function

Biomechanical imaging

Microdamage

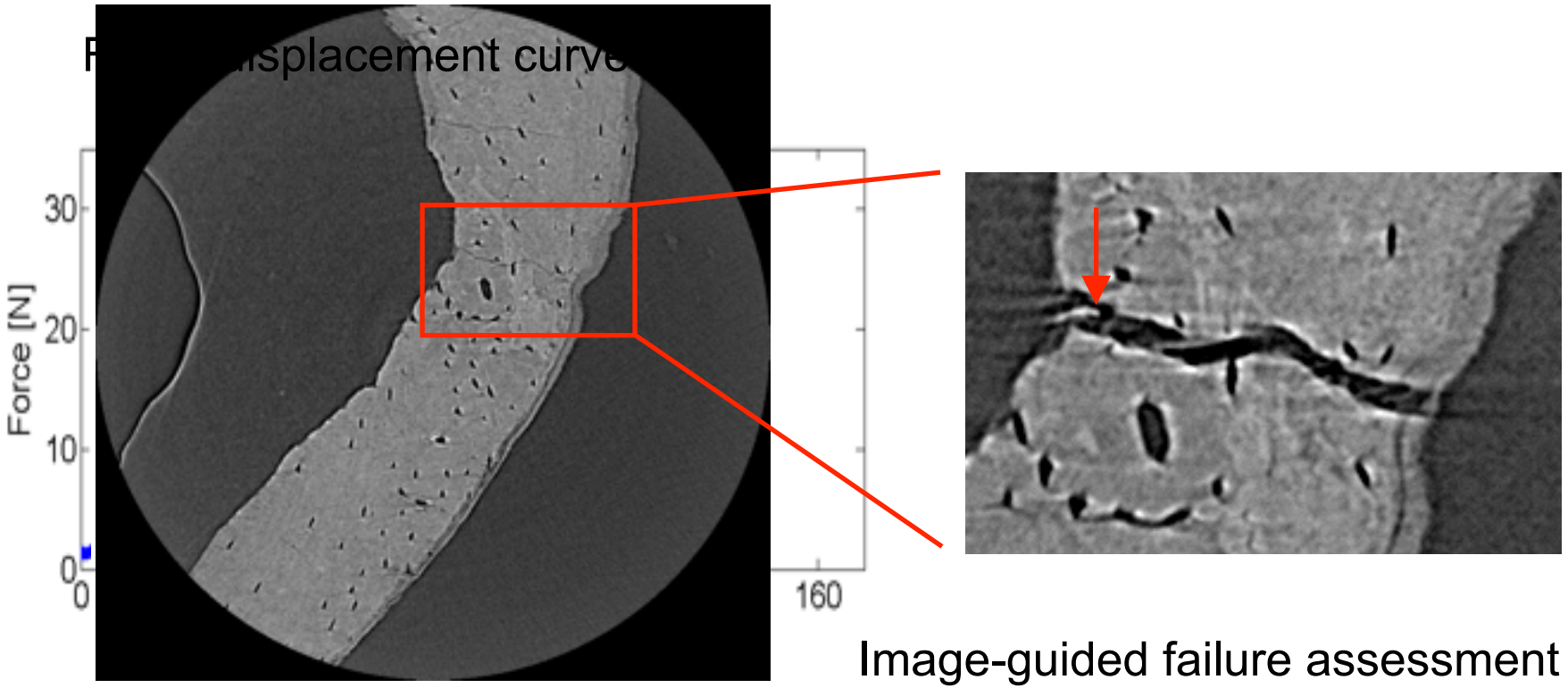


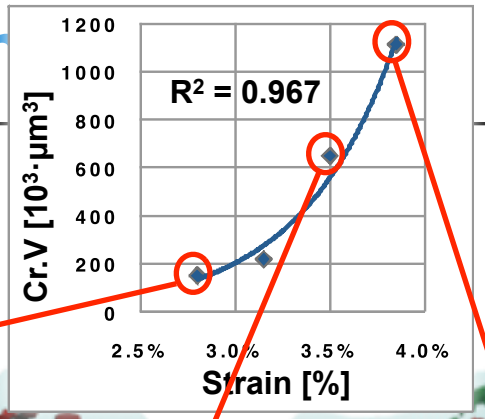
Image-guided failure assessment

Meier M *et al*, J Biomech 41(Suppl 1):S76 (2008)

Bone structure and functio

Biomechanical imaging

Microdamage



Unloaded

Microcrack initation

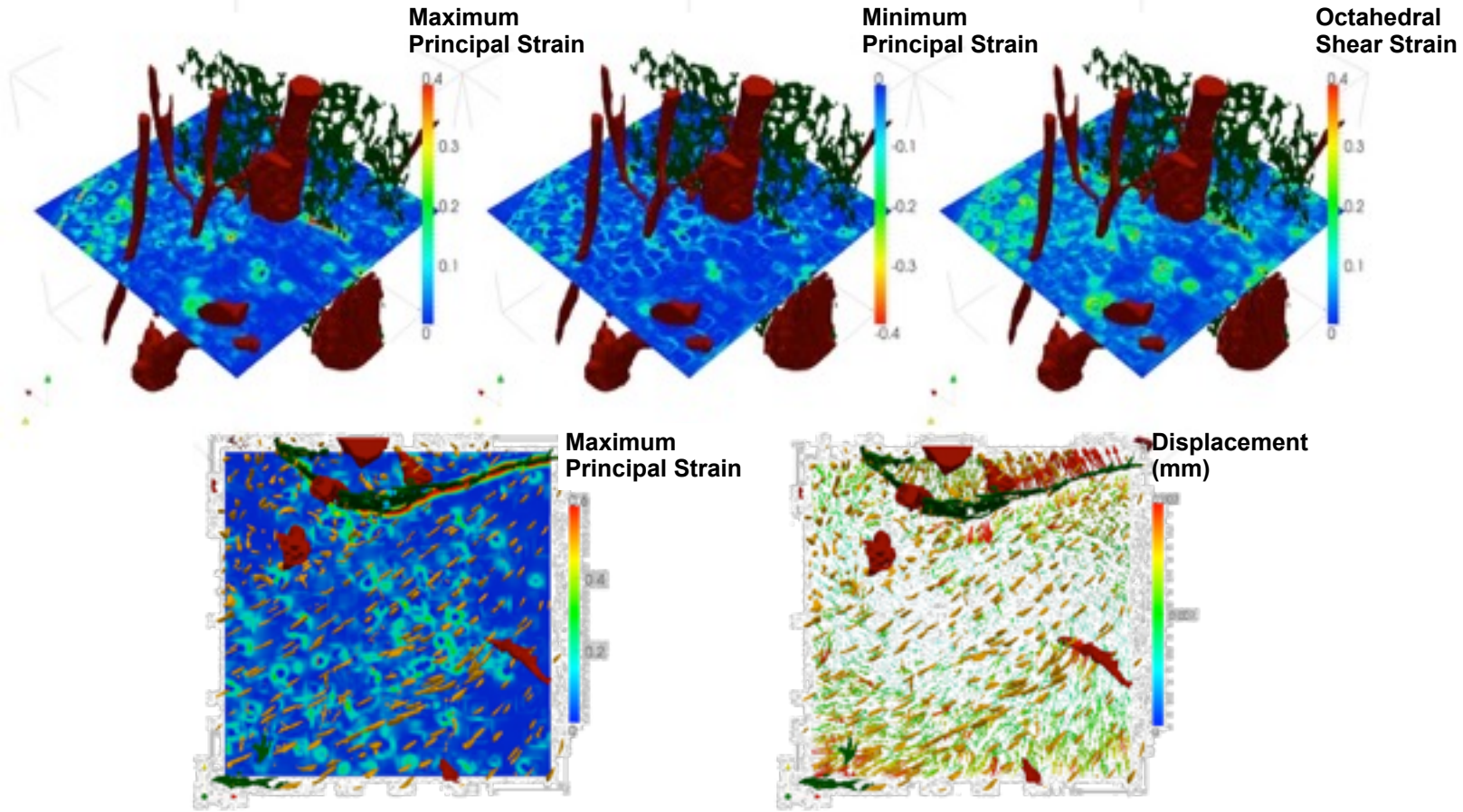
Microcrack propagation

Bone failure

Schneider P *et al*, Biomed Tech (Berl) 55:8-10 (2010)

Bone structure and function

Biomechanical imaging



Christen D *et al*, J Mech Behav Biomed Mater 8:184-93 (2012)

2. Quantitative imaging of brain vasculature

i. Alzheimer's disease (AD)
ii. Methods to study vasculature in AD
iii. Quantification of vascular networks

2. Quantitative imaging of brain vasculature

Literature

Heinzer S., “Hierarchical 3D imaging and quantification of brain microvasculature in a mouse model for Alzheimer’s disease”, Diss ETH 17’293 (2007)

2. Quantitative imaging of brain vasculature

Alzheimer's disease

Clinical picture

- 1907: Alois Alzheimer describes the case of Auguste D., Städtische Irrenanstalt Frankfurt am Main[1]
- Symptoms: continuous decline in memory function, apathy, aggression, anxiety, sleep disturbances
- Year 2000: 4.5 million Americans affected by AD[2]; direct and indirect costs for caring estimated to exceed $67 billion annually[3]

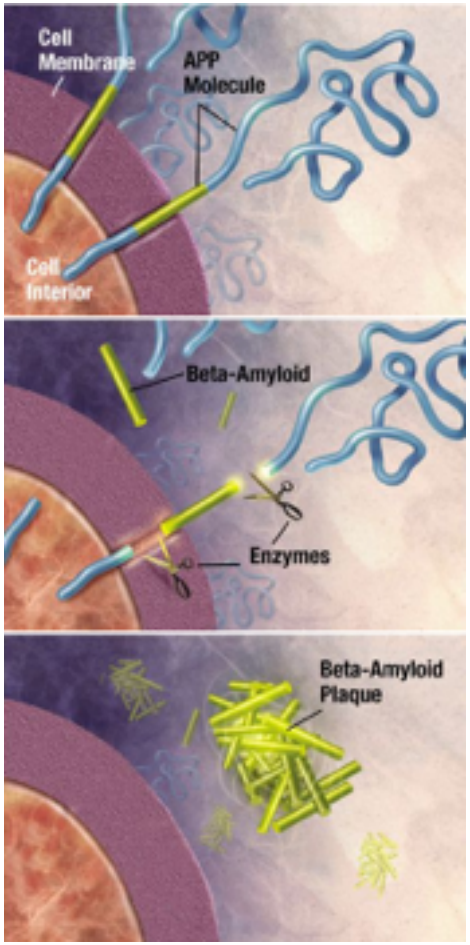
(1) Alzheimer A, Zentralblatt für Nervenkrankheiten 30:177-8 (1907)
(2) Hebert LE *et al*, Arch Neurol 60:1119-22 (2003)
(3) Ernst RL *et al*, Am J Public Health 84:1261-4 (1994)

2. Quantitative imaging of brain vasculature

Alzheimer's disease

Classic hallmarks

- Formation of Beta-amyloid (Aβ) plaques in the brain parenchyma due to enzymatic cleavage of *amyloid precursor protein* (APP)



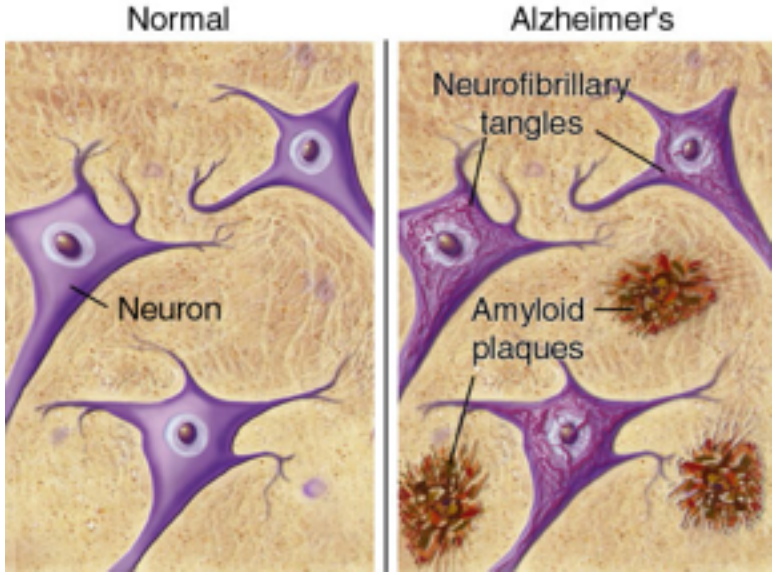
http://www.alzforum.org

2. Quantitative imaging of brain vasculature

Alzheimer's disease

Classic hallmarks

- Formation of Beta-amyloid (Aβ) plaques in the brain parenchyma due to enzymatic cleavage of *amyloid precursor protein* (APP)
- Neurofibrillary tangles (inside neurons)
- Inflammatory processes (microglia, astrocytes), neuron death



http://www.alzforum.org

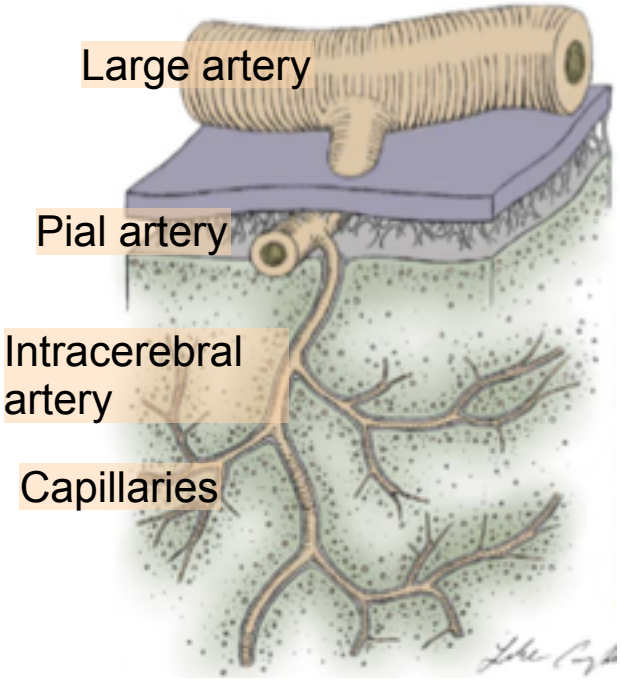
2. Quantitative imaging of brain vasculature

Alzheimer's disease

Vascular components

- Risk factors for vascular diseases are also involved in AD (hypertension, diabetes, and others)[1]
- Vascular alterations associated with AD are described on all levels of the vessel hierarchy[2]

Vessel hierarchy[3]



(1) Ott A *et al*, Diabetologia 39:1392-7 (1996)
(2) de la Torre JC, Stroke 33:1152-62 (2002)
(3) Zlokovic BV, Trends Neurosci 28:202-8 (2005)

2. Quantitative imaging of brain vasculature

Alzheimer's disease

Vascular components

- Risk factors for vascular diseases are also involved in AD (hypertension, diabetes, and others)[1]
- Vascular alterations associated with AD are described on all levels of the vessel hierarchy[2]

Vascular hypothesis[3]

- Faulty Aβ
- Aberrant angiogenesis
- Senescence of the vasculature

induce →

- Neurovascular uncoupling
- Vessel regression
- Brain hypoperfusion
- Neurovascular inflammation

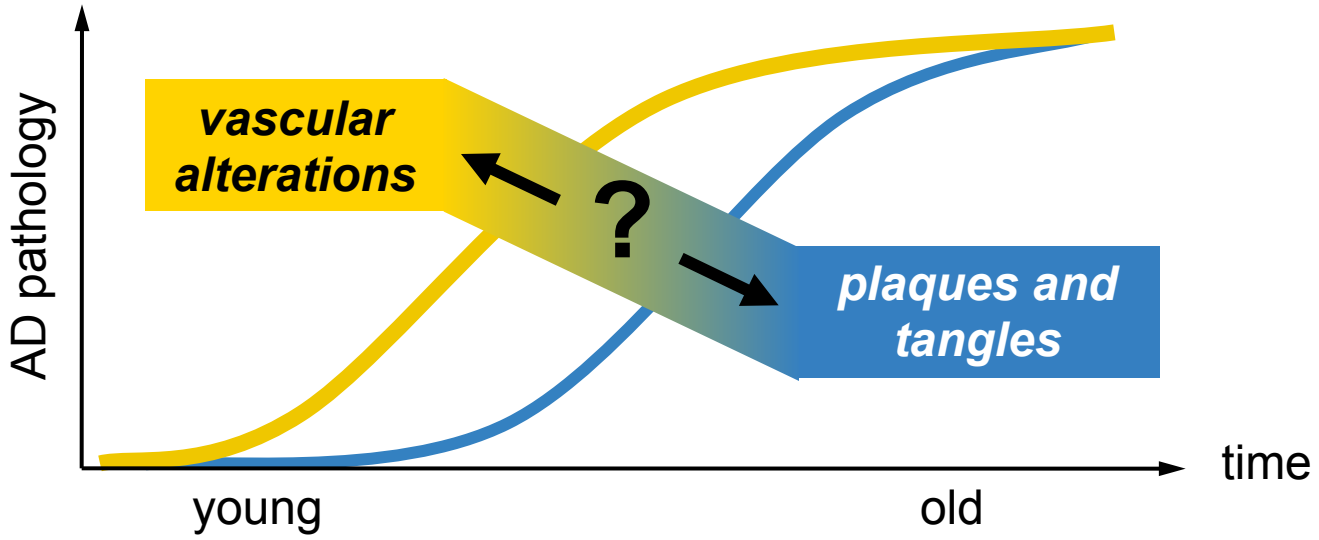
(1) Ott A *et al*, Diabetologia 39:1392-7 (1996)
(2) de la Torre JC, Stroke 33:1152-62 (2002)

2. Quantitative imaging of brain vasculature

Alzheimer's disease

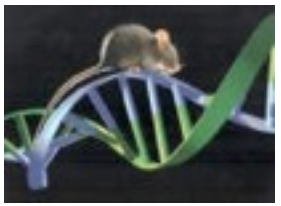
Rationale

- Cerebrovascular insufficiencies are critical factors initiating the disease
- Vascular pathology precedes parenchymal alterations ("plaques, tangles")



2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD



Animal disease model for AD

- APP23 transgenic (tg) mice[1]
- 7-fold overexpression of human amyloid precursor protein (APP) gene carrying the “swedish double mutation”
- Pathology reminiscent of AD: Aβ deposition (starting at 6 months of age), hyperphosphorylated tau, neuritic degeneration, inflammatory response

Animal disease model for AD[2]

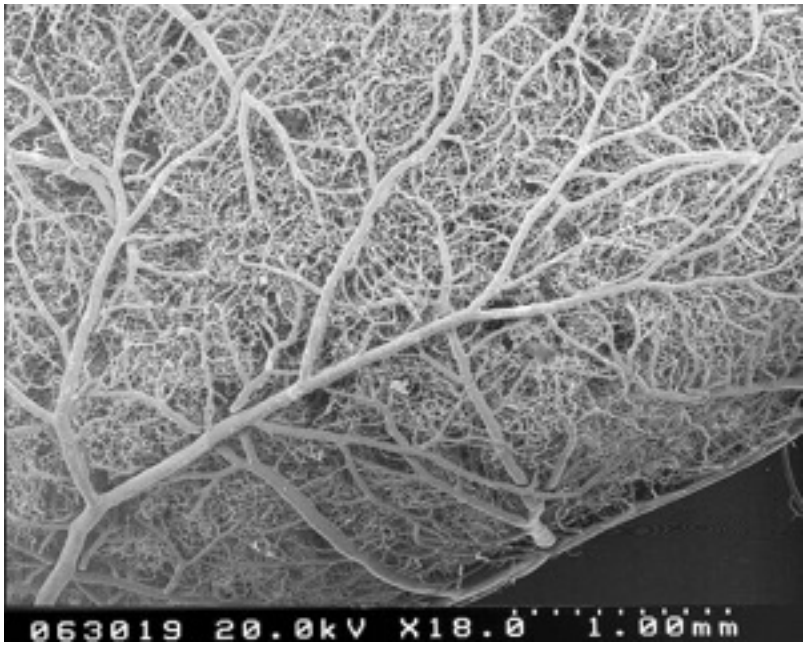
- Deeply anesthetized animal intracardially perfused with polymer resin
- Maceration: removal of soft tissue and bone

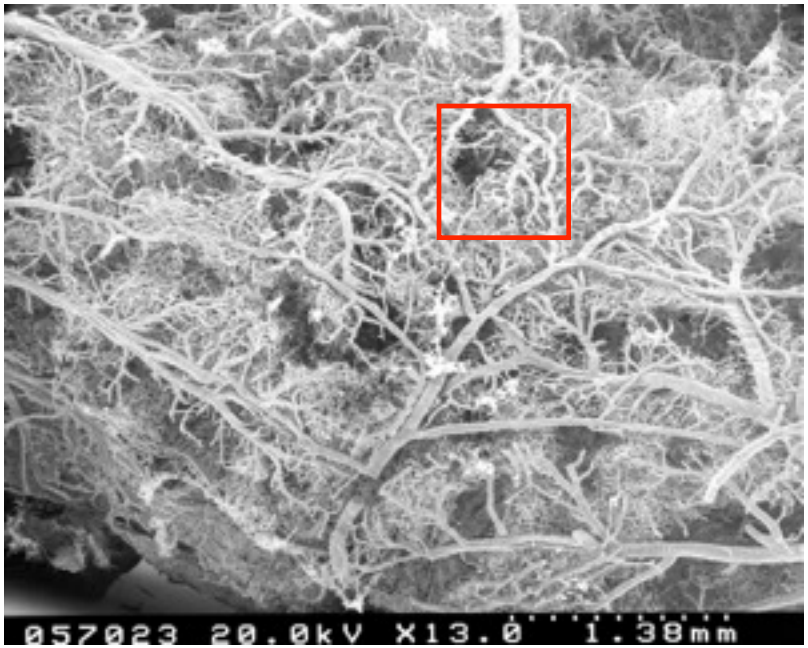
(1) Sturchler-Pierrat *et al*, Proc Natl Acad Sci USA 94:13287-92 (1997)
(2) Krucker T *et al*, Microsc Res Tech 69:138-47 (2006)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

SEM imaging of micro-infarcts



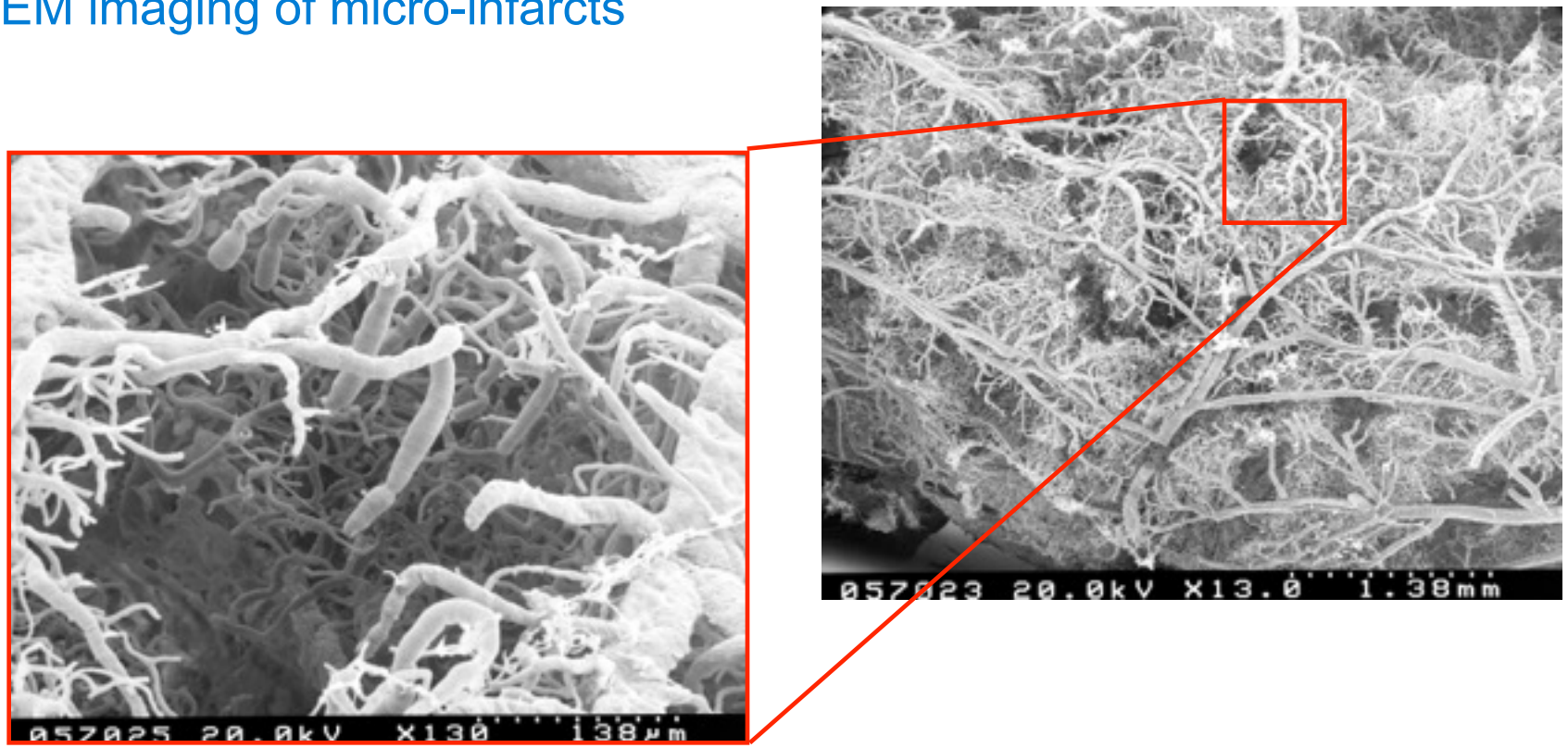


Schuler A, diploma thesis, Institute of Zoology, University of Zurich, Zurich (2002)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

SEM imaging of micro-infarcts



Schuler A, diploma thesis, Institute of Zoology, University of Zurich, Zurich (2002)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Computer-based vessel analysis

DB web interface

- Project management
- Animal, sample and measurement data manipulation

ROI Picker software

- ROI selection
- Visual analysis

Software

Imag/Eval Software

- Image processing
- Morphometry
- Network analysis

WWW

Database (MySQL)

Automation (SR µCT)

Hardware

Evaluation Cluster

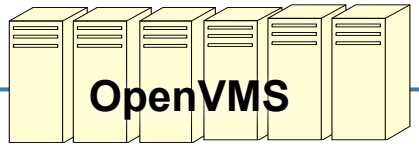


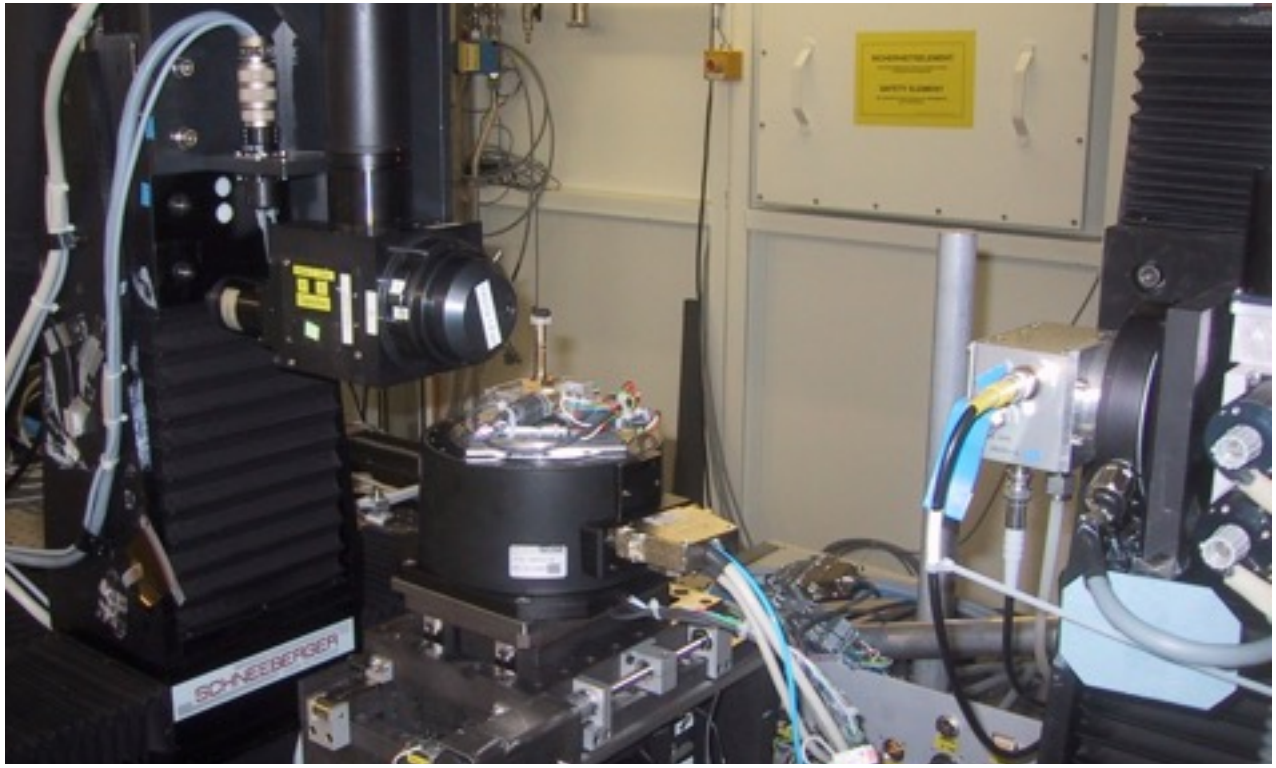
Image File Server (6 TB)

Scanners

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Synchrotron radiation-based computed tomography (SR CT)

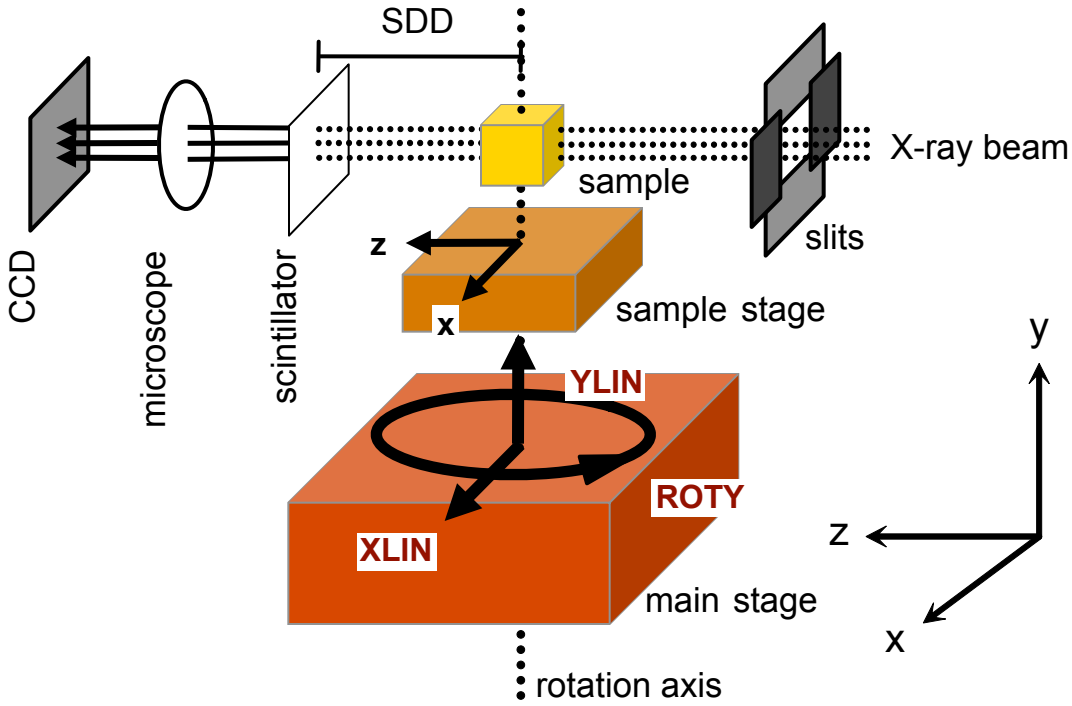


X-ray Tomographic Microscopy (XTM) station, Materials Science (MS) beamline, Swiss Light Source (SLS)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Local tomography at the Swiss Light Source (SLS)

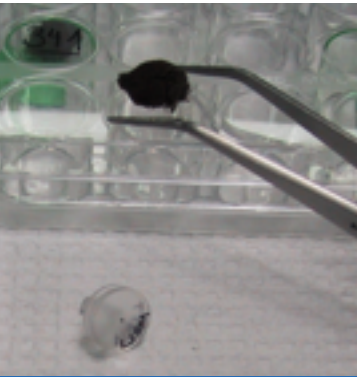
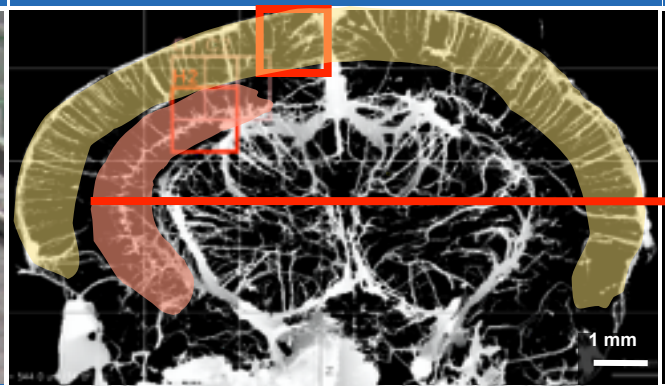
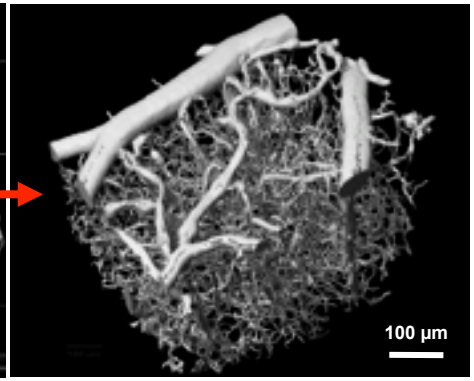
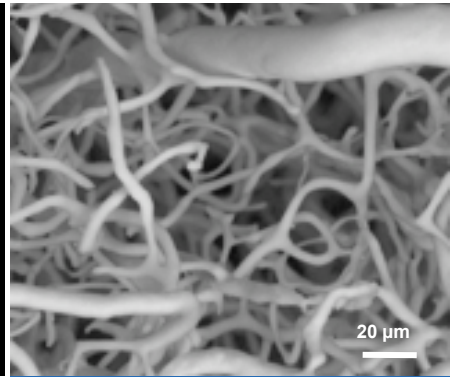


Heinzer S *et al*, Neuroimage 32:626-36 (2006)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Hierarchical imaging

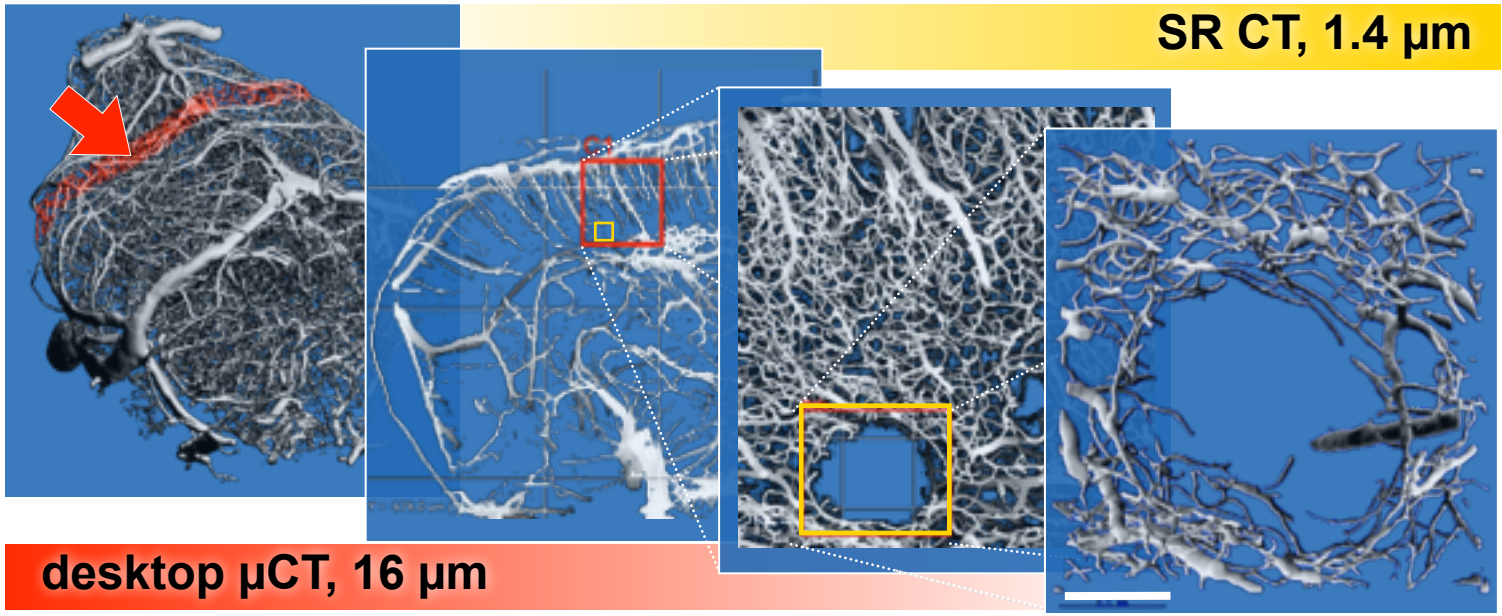
Corrosion cast	Desktop µCT (16 µm)	SRµCT (1.4 µm)	SEM (140 nm)
			
	Anatomical regions (full brain) → ROI selection	Visualizes all micro-vasculature	2D, surface only, but very high resolution

Heinzer S *et al*, Neuroimage 32:626-36 (2006)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Hierarchical analysis

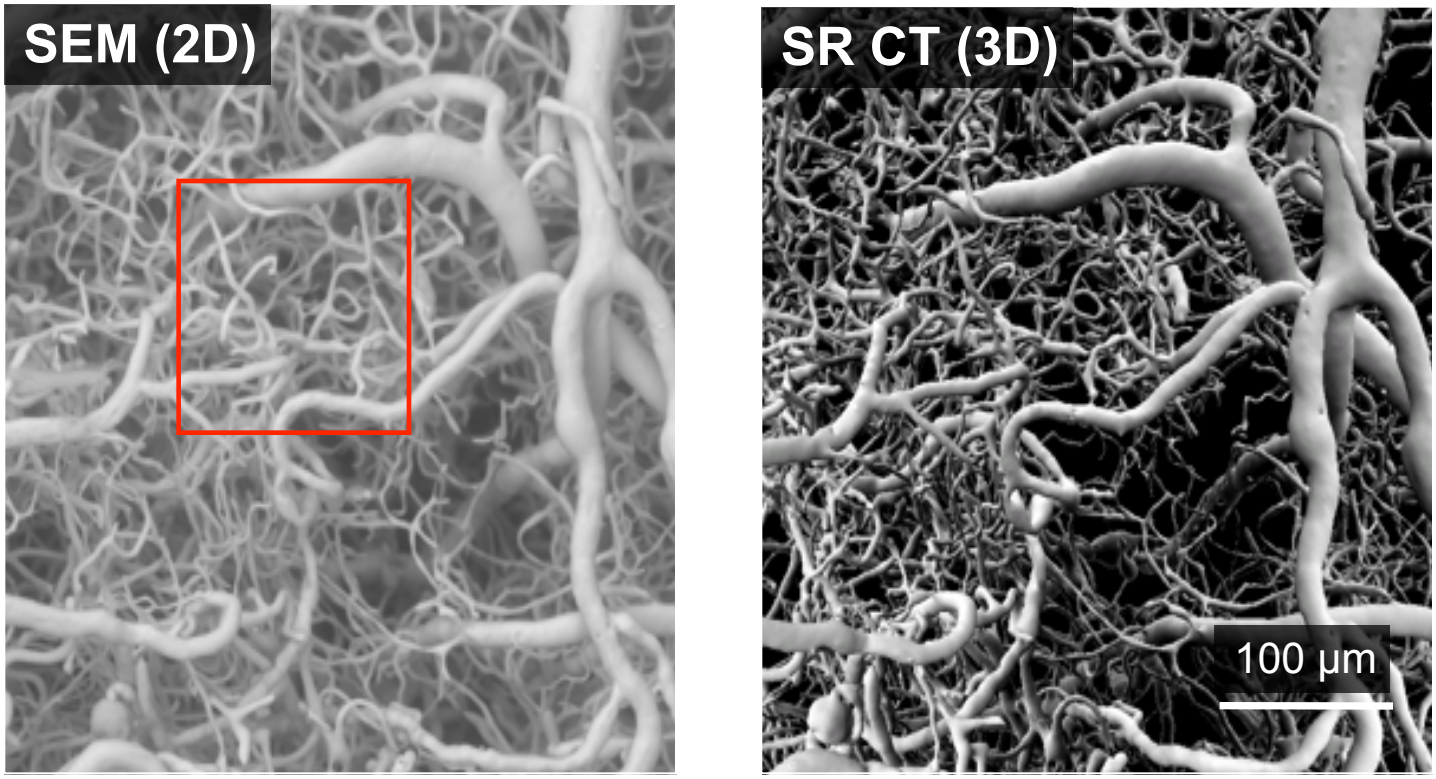


Heinzer S *et al*, Neuroimage 32:626-36 (2006)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Non-destructive microimaging

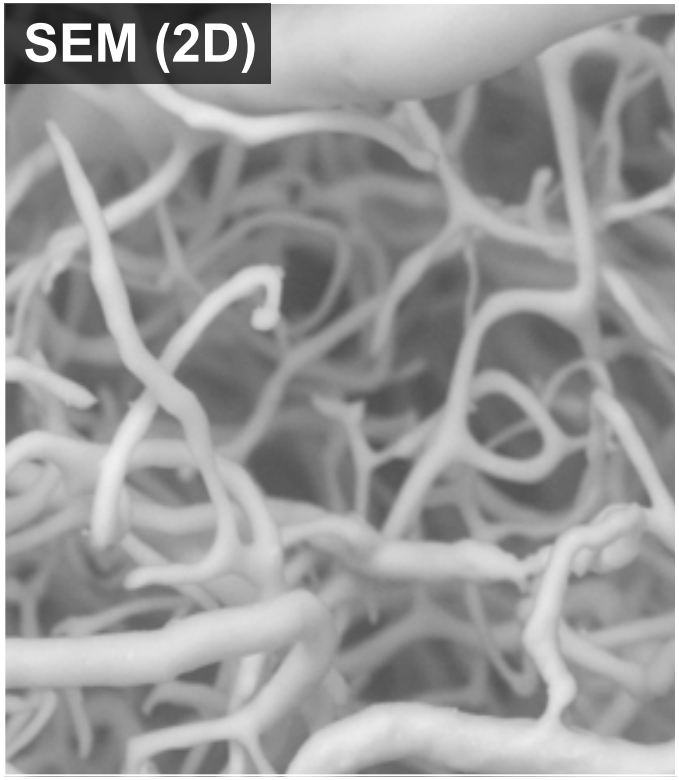


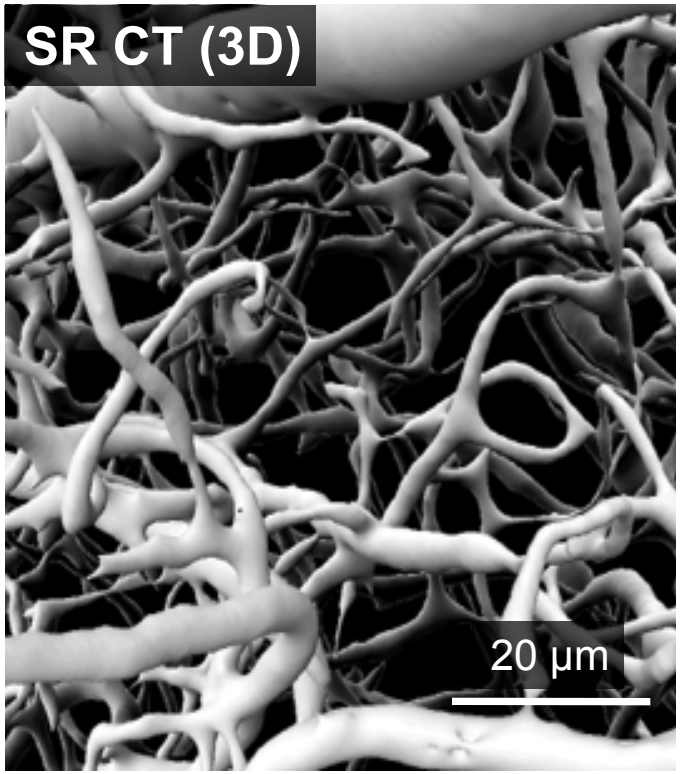
Heinzer S *et al*, Neuroimage 32:626-36 (2006)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Non-destructive microimaging

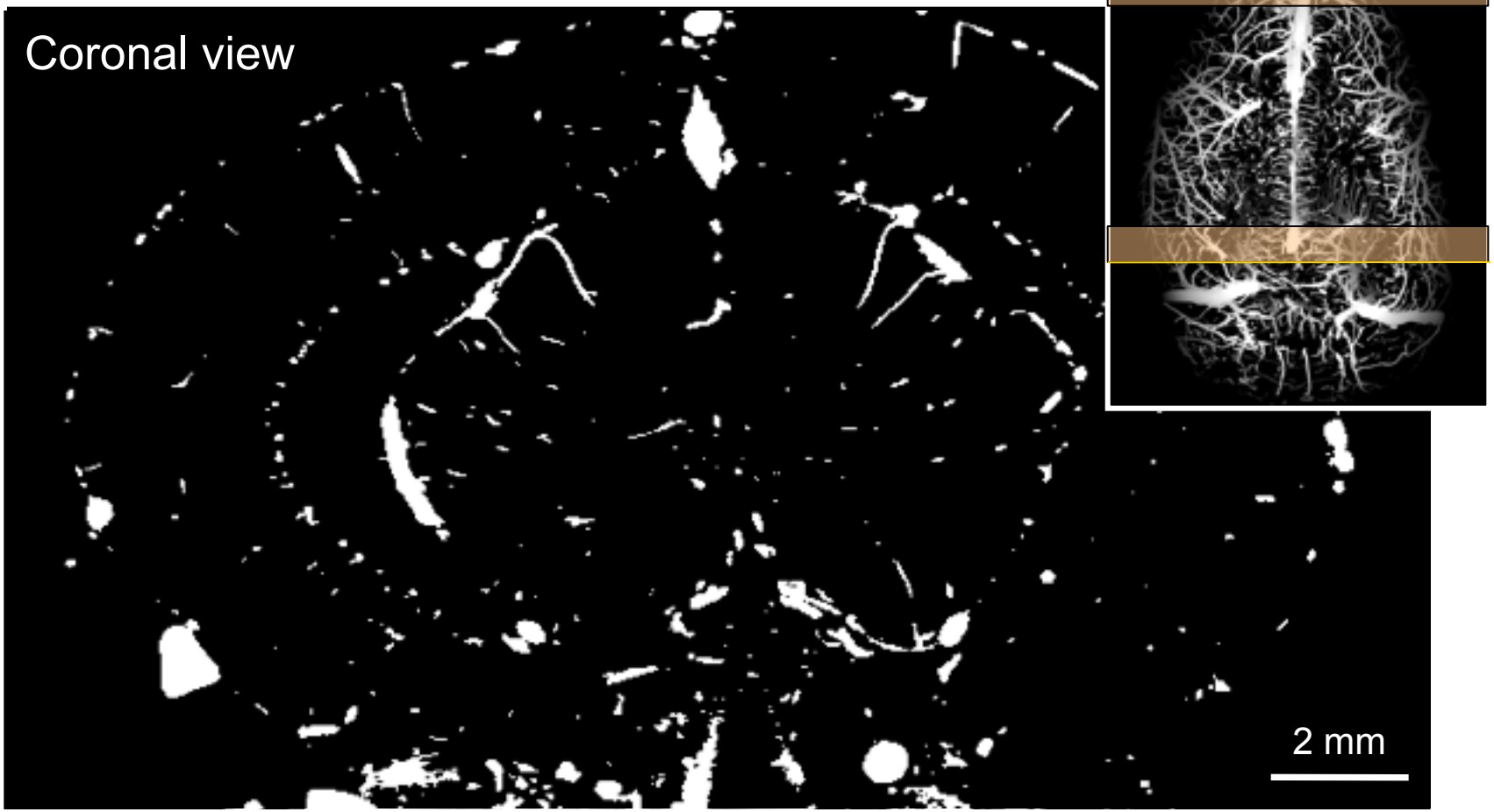




Heinzer S *et al*, Neuroimage 32:626-36 (2006)

2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

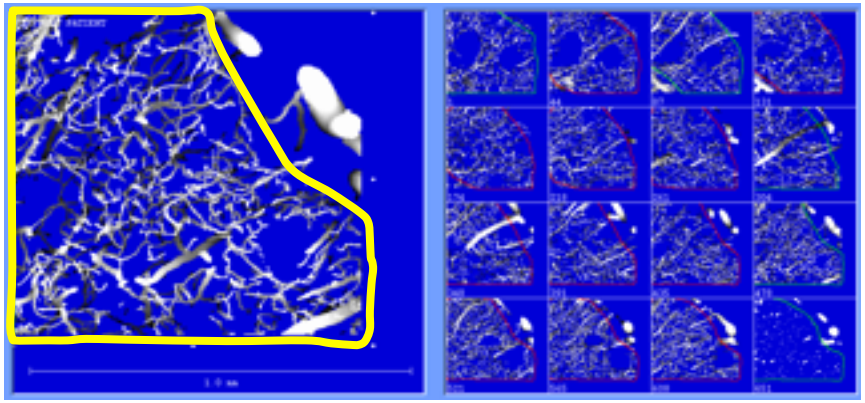


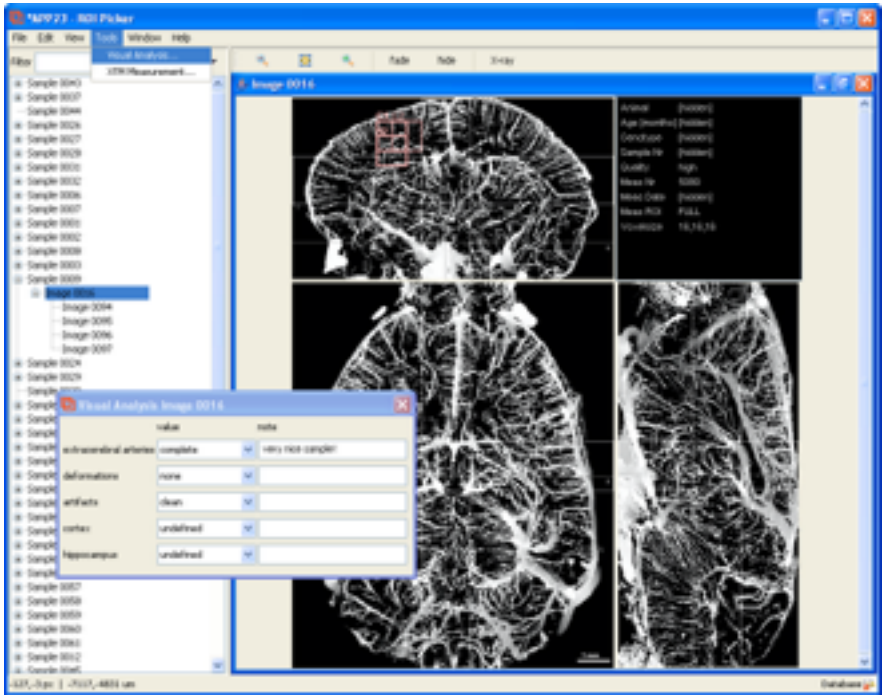
2. Quantitative imaging of brain vasculature

Methods to study vasculature in AD

Browsing of large image data

- Region of interest (ROI) selection
- Systematic visual analysis
- Pathology contouring for selective evaluation



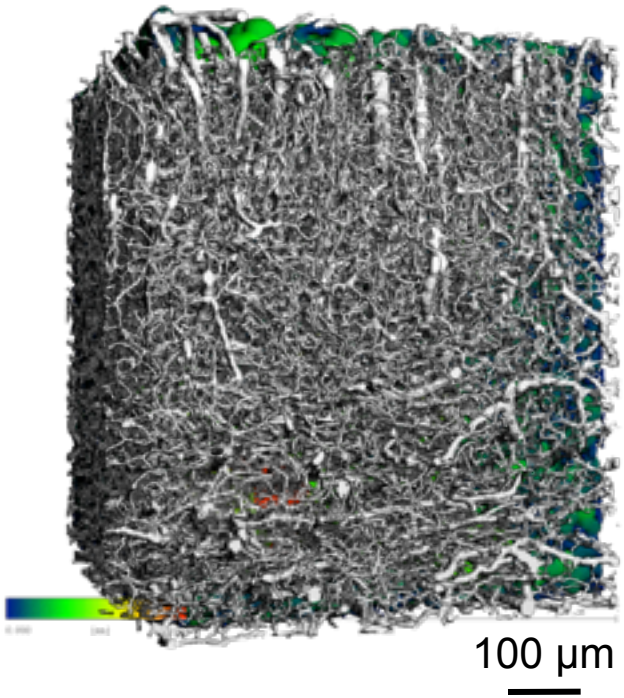


2. Quantitative imaging of brain vasculature

Quantification of vascular networks

Morphometry

- Example of vessel separation (V.Sp):
  - Software fits spheres between vessels
  - V.Sp := average sphere diameter

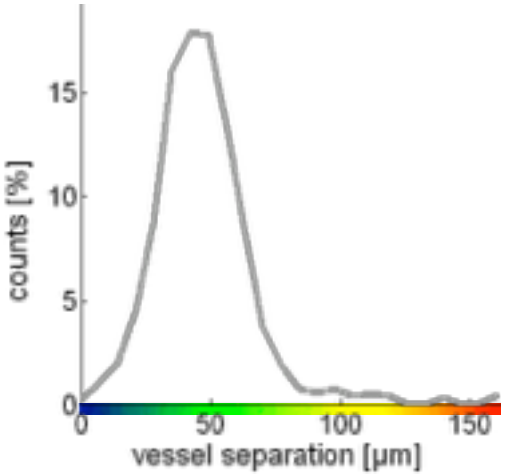


2. Quantitative imaging of brain vasculature

Quantification of vascular networks

Morphometry

- Example of vessel separation (V.Sp):
  - Software fits spheres between vessels
  - V.Sp := average sphere diameter
  - V.Sp distribution -> histogram

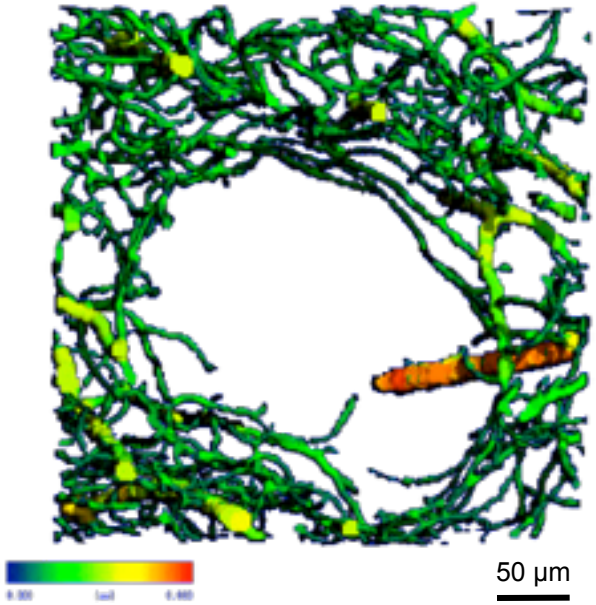


2. Quantitative imaging of brain vasculature

Quantification of vascular networks

Morphometry

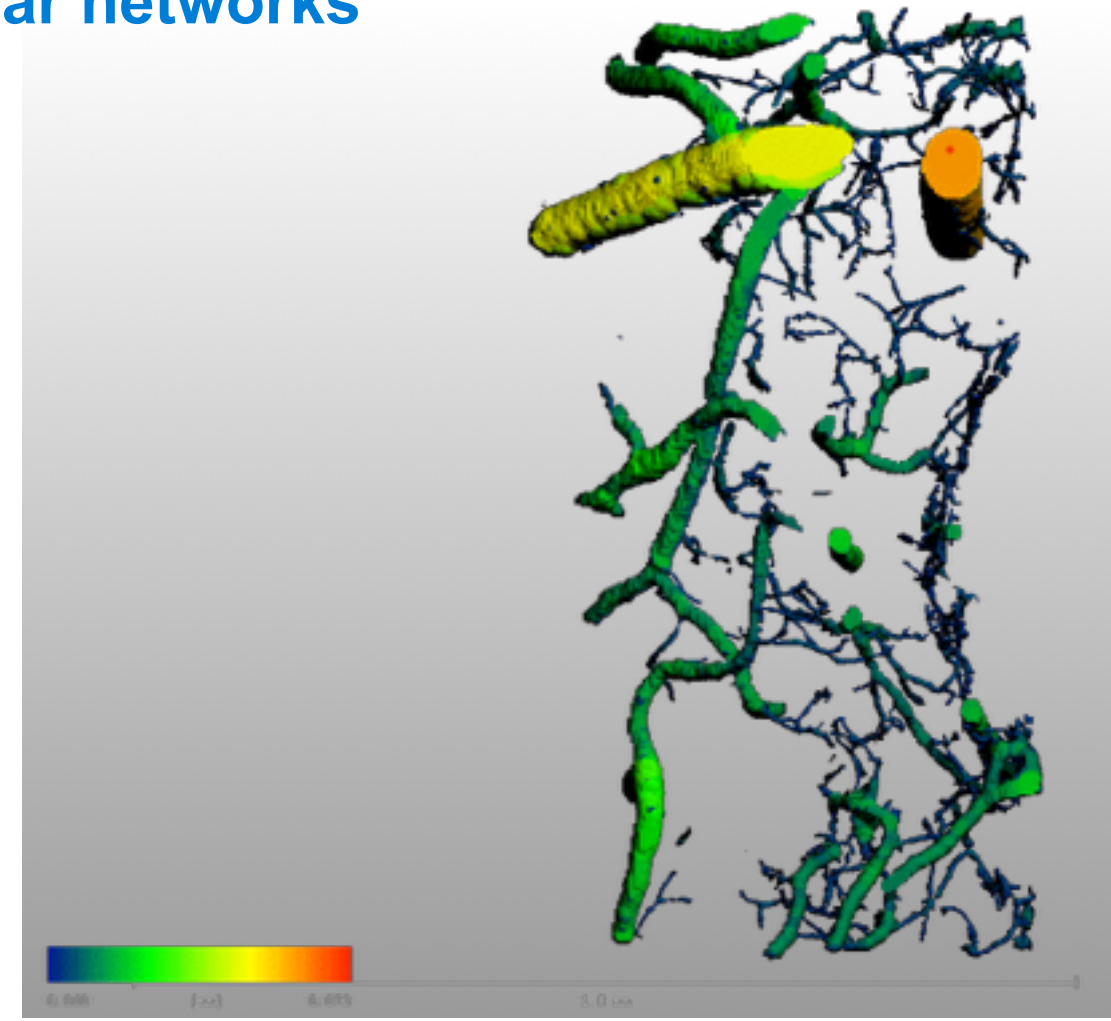
- Example of vessel separation (V.Sp):
  - Software fits spheres between vessels
  - V.Sp := average sphere diameter
  - V.Sp distribution -> histogram
- Other morphometric indices:
  - Vessel thickness (V.Th)
  - Vessel volume (V.V)
  - Vessel volume density (V.TV)



2. Quantitative imaging of brain vasculature

Quantification of vascular networks

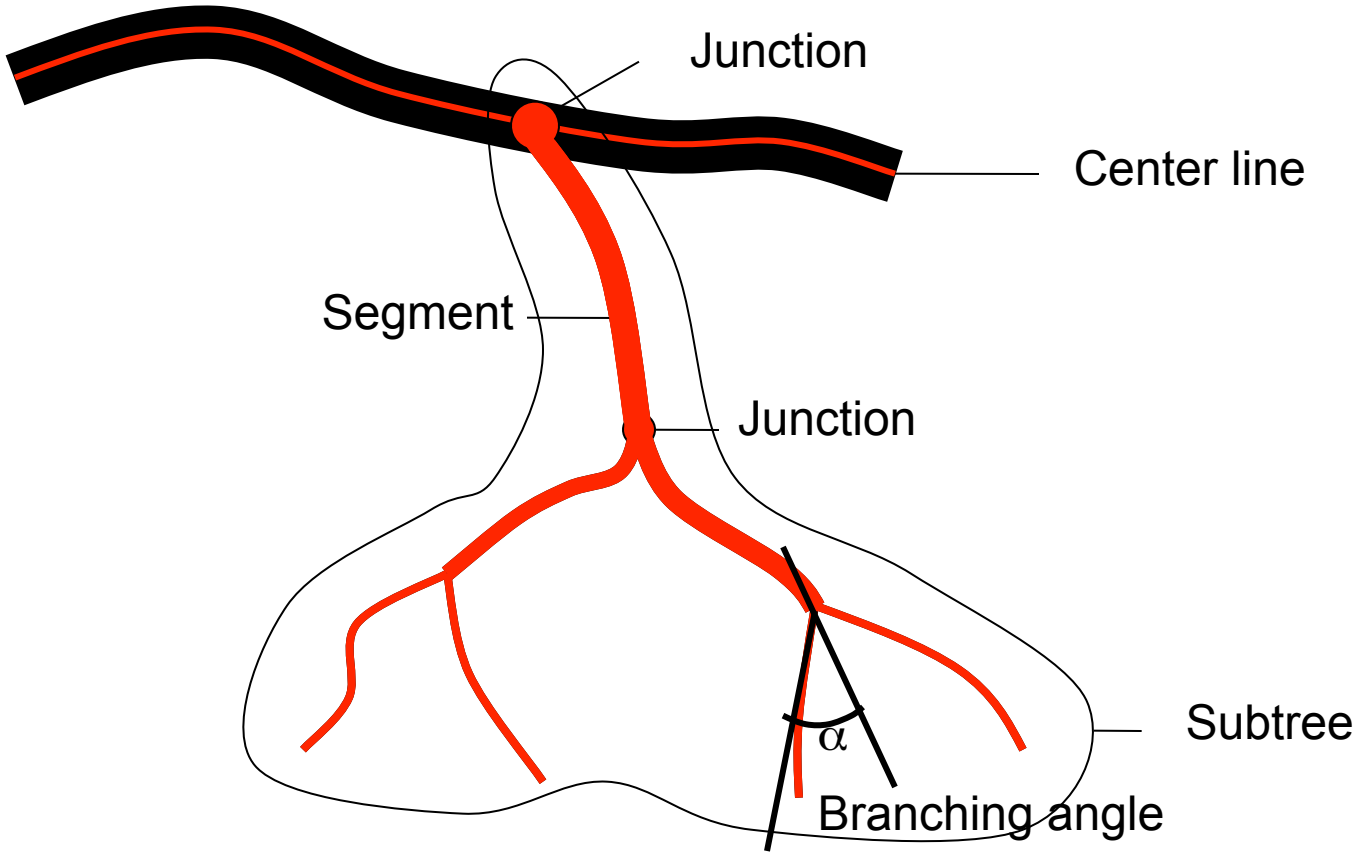
Morphometry



2. Quantitative imaging of brain vasculature

Quantification of vascular networks

Vascular network analysis

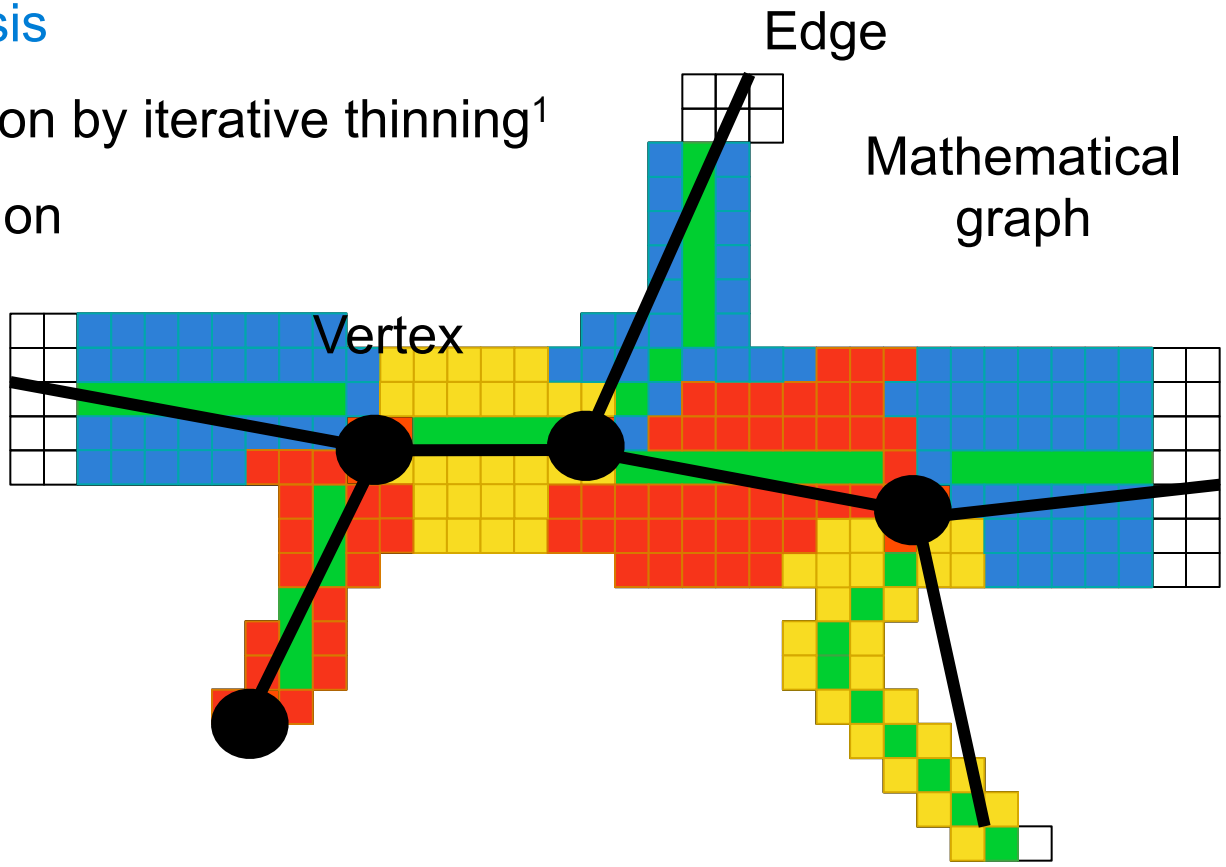


2. Quantitative imaging of brain vasculature

Quantification of vascular networks

Vascular network analysis

1. Center line extraction by iterative thinning[1]
2. Junction identification ($N(v)=1$ | $N(v)>2$)
3. Segment extraction
4. Dilation
5. Quantification



(1) Palagyi K and Kuba A, Pattern Recognit Lett 19:613-27 (1998)

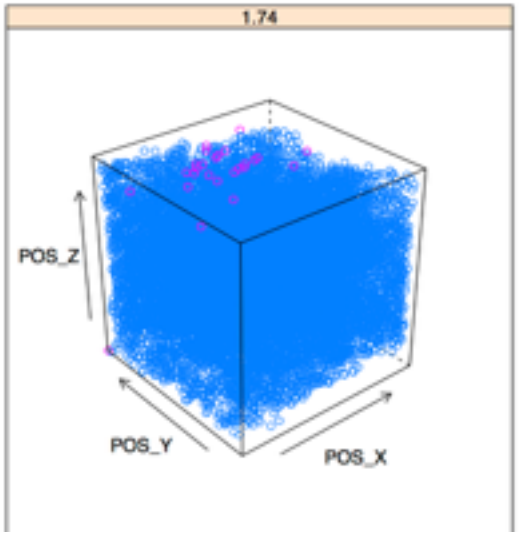
3. Visualizing Data

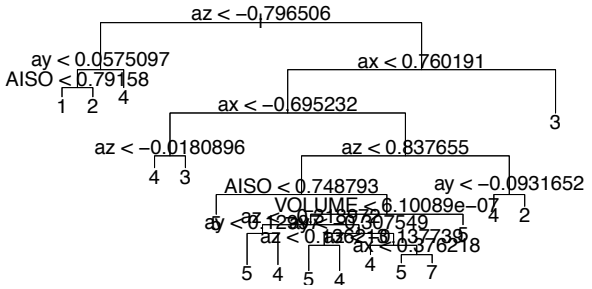
http://circos.ca/documentation/course/visualizing-genomic-data.pdf

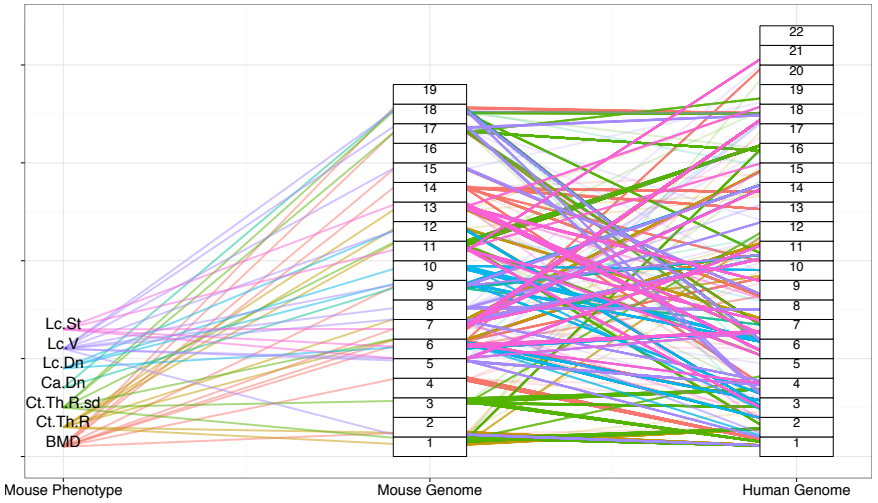
- Imaging and in particular 3D experiments can produce horrendous amounts of data
- Creating easy visual representations is crucial for making sense of complex systems

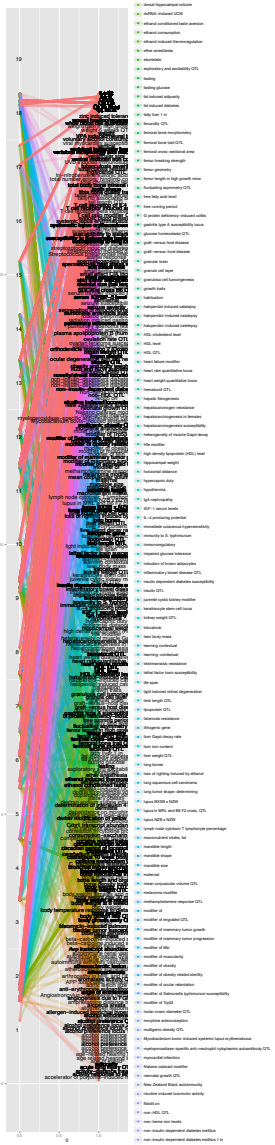
Bad Graphics

- There are enough graphs that say ‘my data is very complicated’ / ‘I know how to use __ toolbox in Matlab/R/Mathematica’



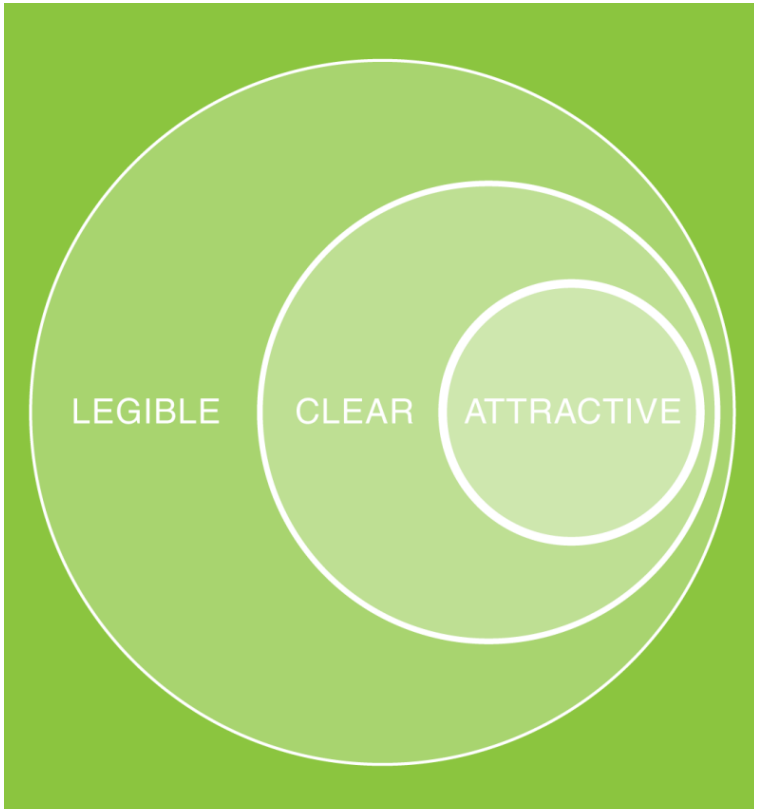






Key Questions

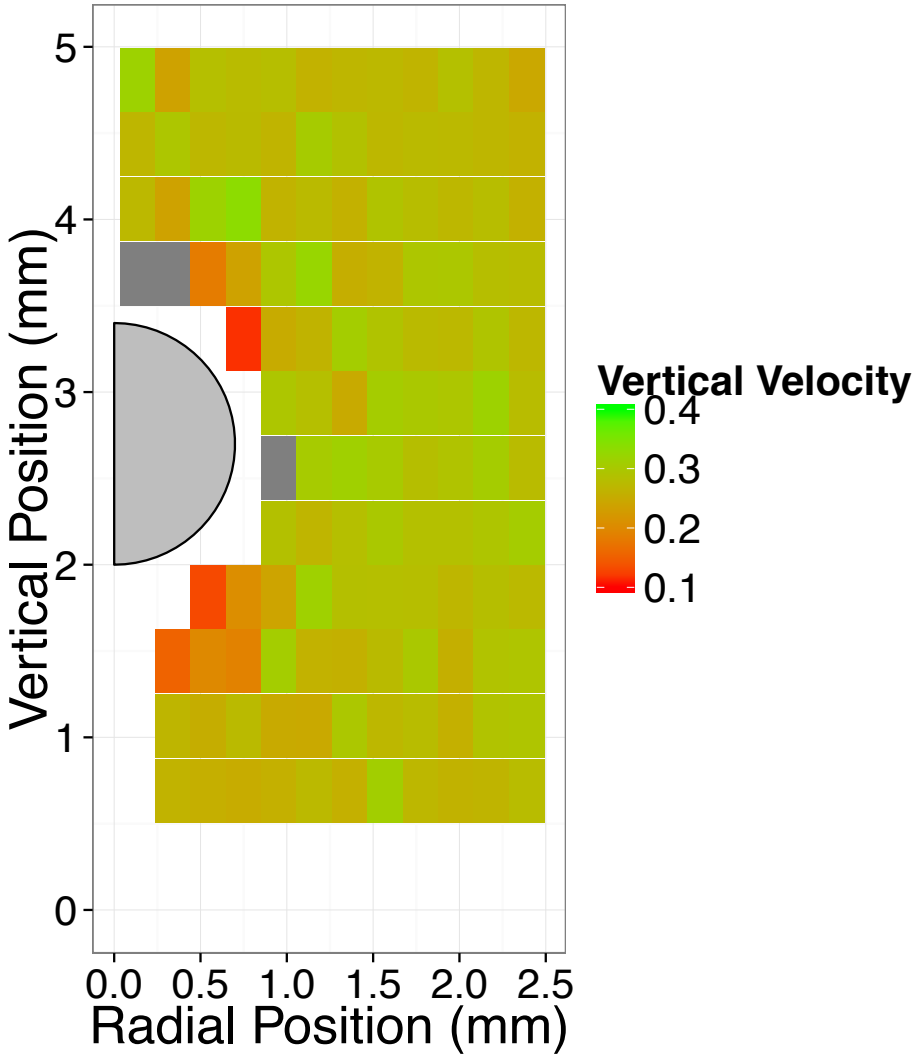
- What is my message?
- Does the graphic communicate it clearly?
- Is a graphic representation really necessary?
- Does every line / color serve a purpose?



http://circos.ca/documentation/course/visualizing-genomic-data.pdf

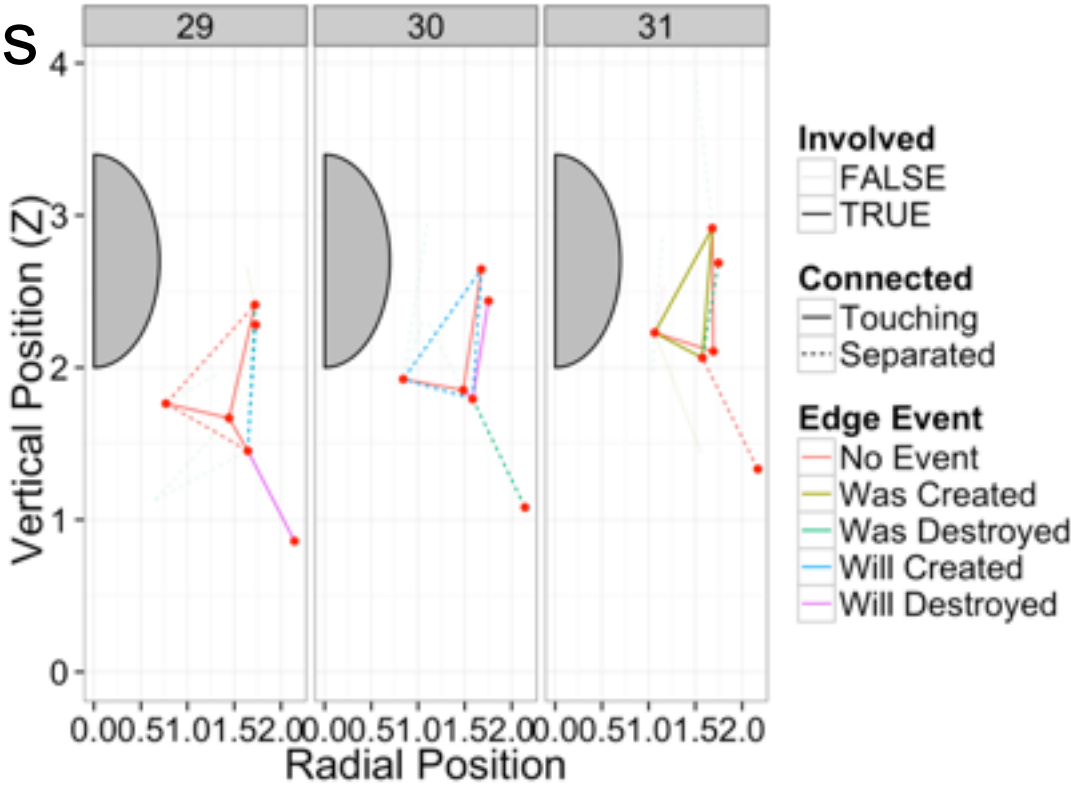
What is my message?

- Dynamic experiments are particularly difficult to analyze
- Instead of trying to show everything reduce the data to single metrics
- From here is everything to average velocity



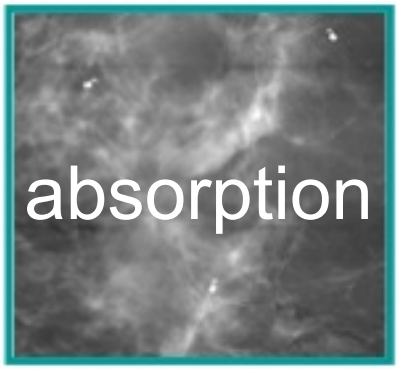
Does my graphic communicate it clearly

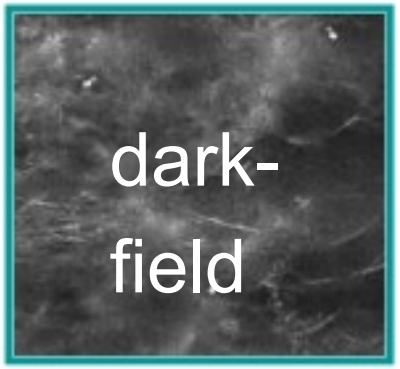
- Too much data makes it very difficult to derive a clear message
- Filter and reduce information until it is extremely simple



Filter and Reduce

- 3 images of a breast sample from a DPC setup (absorption, phase, and dark field)
- Difficult to interpret parallel images and visually correlate features

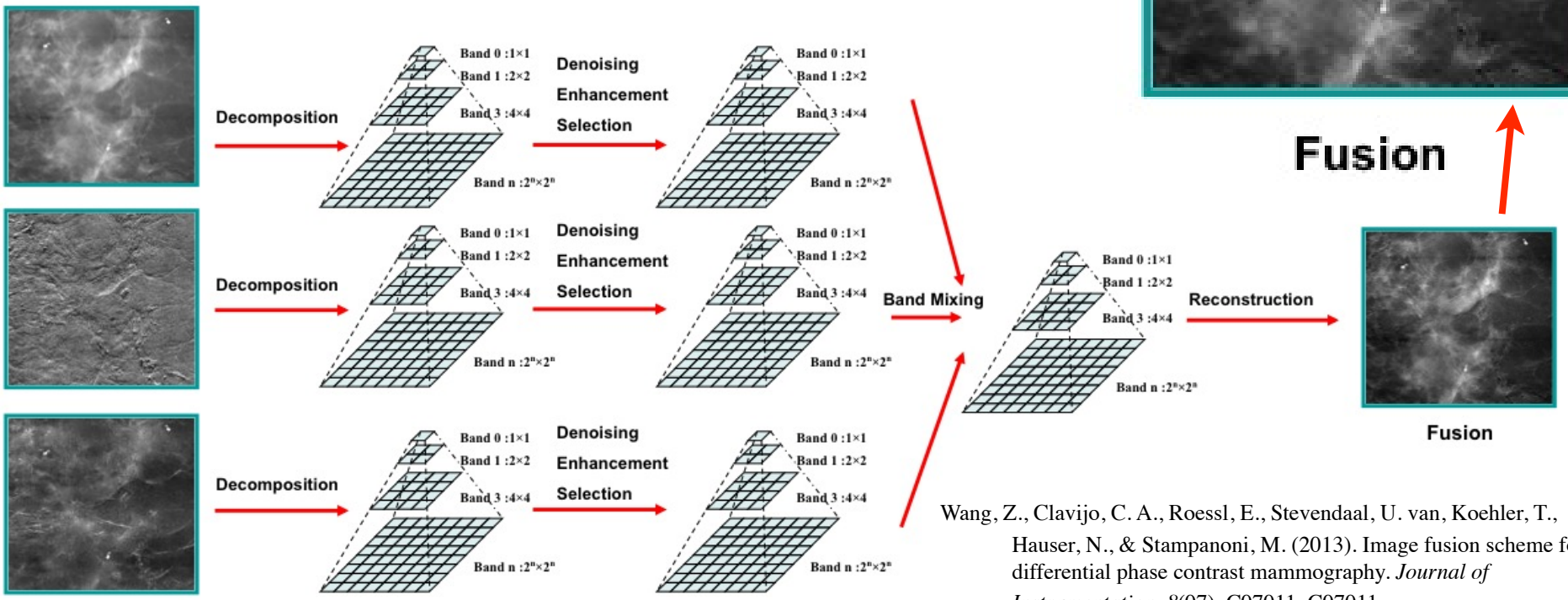




Wang, Z., Clavijo, C. A., Roessl, E., Stevendaal, U. van, Koehler, T., Hauser, N., & Stampanoni, M. (2013). Image fusion scheme for differential phase contrast mammography. *Journal of Instrumentation*, *8*(07), C07011–C07011

Image Fusion

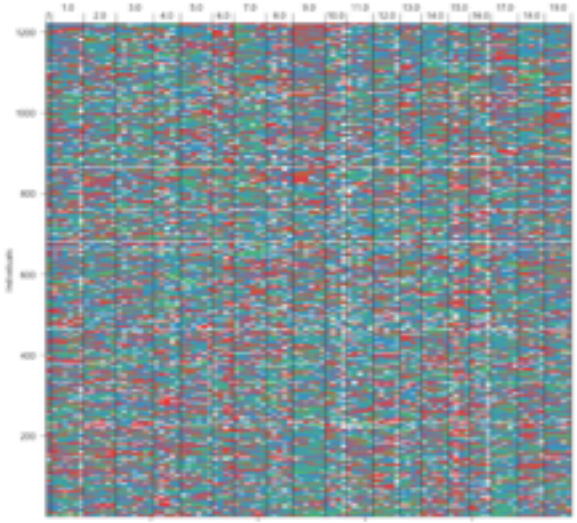
- Take the best parts of each image and combine them together



Wang, Z., Clavijo, C. A., Roessl, E., Stevendaal, U. van, Koehler, T., Hauser, N., & Stampanoni, M. (2013). Image fusion scheme for differential phase contrast mammography. *Journal of Instrumentation*, *8*(07), C07011–C07011

Is it necessary?

- This graph shows 2000 mice and 98 tags
- There is no pattern, some samples are missing data, ...
- This graph is absolutely useless



Each F2 mouse was genotyped by tagging its genome in 98 locations distributed across the 19 chromosomes

The tag is either B6 indicating homozygous **B6**, **C3H** indicating homozygous C3H, **heterozygous** or

Does every line / color serve a purpose?

- Why is it circular?
- The gray areas and lines inside them communicate nothing
- Markers are unreadably small