E. P. Paschalis

VIBRATIONAL SPECTROSCOPIC METHODS: FTIRI & RAMAN.

AUVA
Wiener Gebietskrankenkasse WGKK
hanusch krankenhaus
Ludwig Boltzmann Gesellschaft

HOW DOES VIBRATIONAL SPECTROSCOPY RECOGNIZE BONE?

FRACTURES vs COMMON WISDOM

SHOWCASE THE INFORMATION THAT VIBRATIONAL SPECTROSCOPY CAN PROVIDE IN RELATION TO BONE

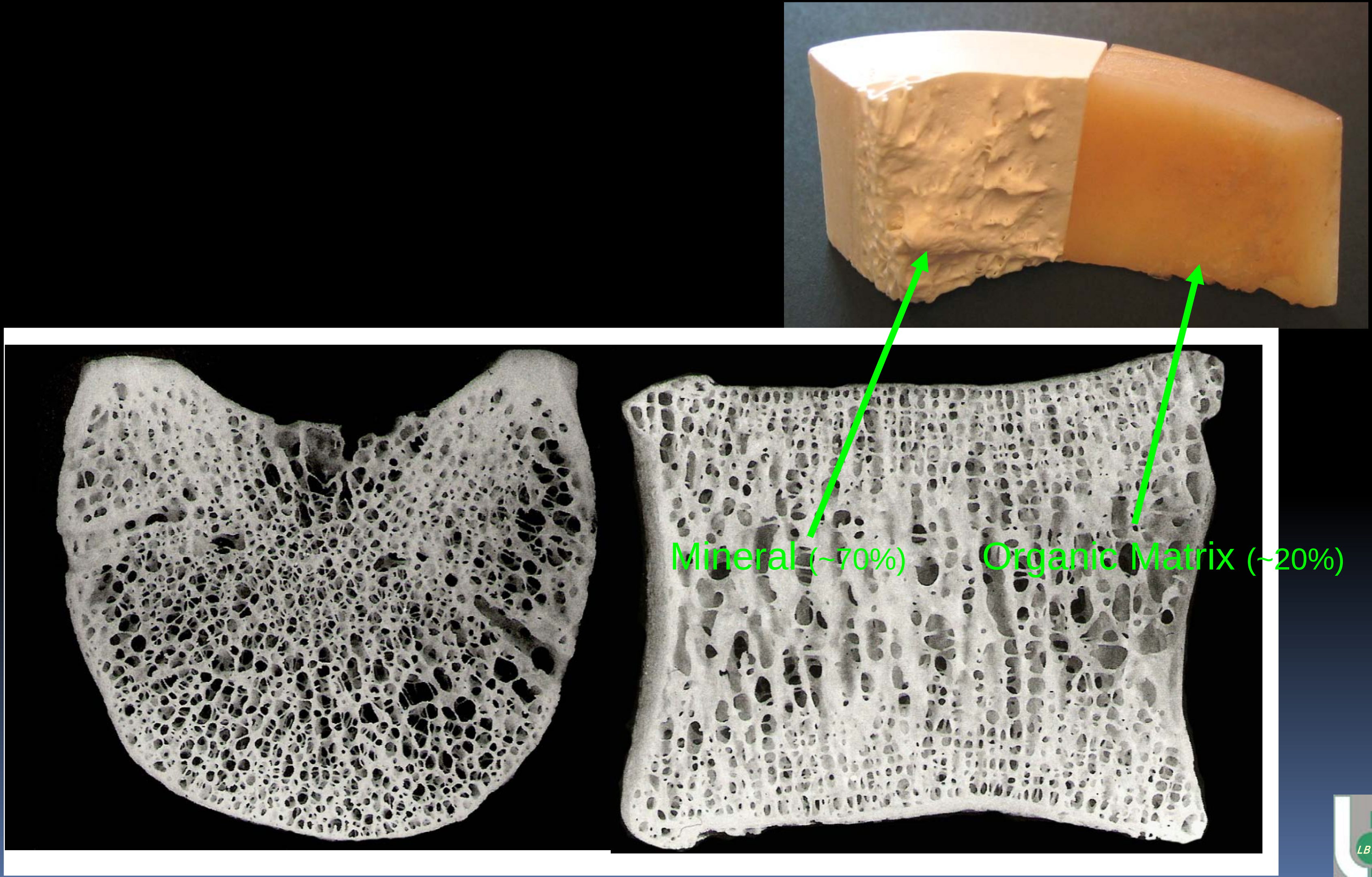
HOW DOES THIS INFORMATION AGREE/DISAGREE WITH OSTEOPOROSIS MANTRA OF BMD AND TURNOVER

HOW CAN IT HELP US IMPROVE EXISTING DEFINITIONS

WHAT IS ITS PLACE IN THE BIGGER PICTURE?

HOW DOES VIBRATIONAL SPECTROSCOPY RECOGNIZE BONE?

Bone = Collagen/Mineral Nano-Composite



FRACTURES vs COMMON WISDOM

Contribution of Post-Treatment BMD Increases to Vertebral Fracture Risk Reduction over 3 Years

Author	Treatment	Analysis Method*	% Fx Reduction Explained by BMD
Cummings	Alendronate **(FIT)**	IPD	16%
Sarkar	Raloxifene **(MORE)**	IPD	4%
Watts	Risedronate **(VERT and HIP)**	IPD	18%

*** Analysis of individual patient data (IPD)**

Cummings et al, Am. J. Med. 2002
Sarkar et al, JBMR 2002
Watts et al, J Clin Densitometry 2004

FRACTURES vs COMMON WISDOM

OSTEOPOROSIS: WE ARE SHOOTING BUT ARE WE AIMING?

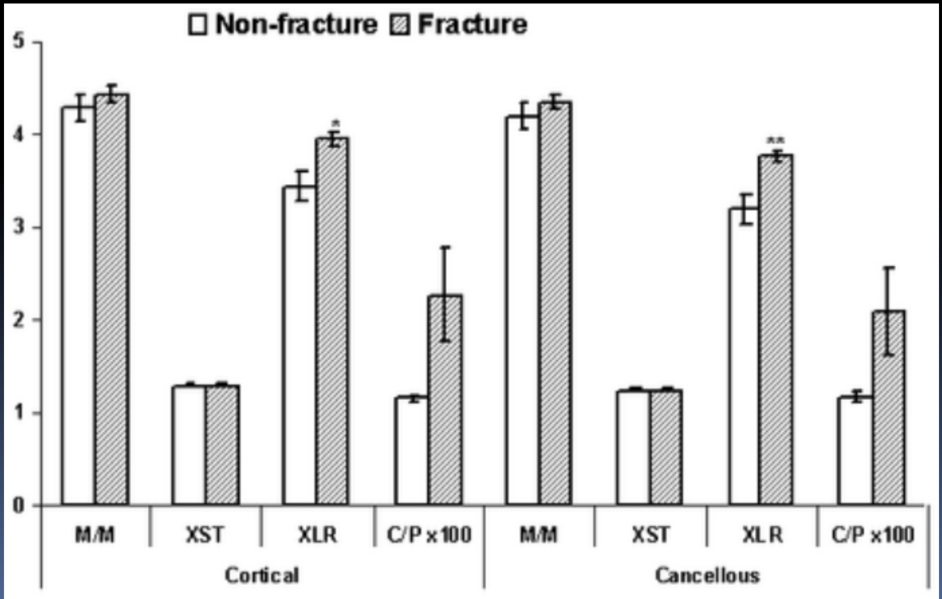
Use of FTIR Spectroscopic Imaging to Identify Parameters Associated with Fragility Fracture

Samuel Gourion-Arsiquaud[1,2], Dan Faibish[1,2], Elizabeth Myers[2,] Lyudmila Spevak[2], Juliet Compston[3],, Anthony Hodsman[4], Elizabeth Shane[5], Robert R. Recker[6], Elizabeth R. Boskey [7], Adele L. Boskey[2,8]

Cortical and cancellous bone were independently evaluated in ***iliac crest biopsies*** *from 54 women (32 with fractures, 22 without) who had significantly different spine but not hip BMDs.*

The ONLY "universal" correlation between fracture and bone properties was collagen cross-link ratio

	Fracture (N=32)	Non-fracture (N=22)	P value
Age at biopsy	59 ±17	56±5	0.31
BMD Spine (gm/cm^2)	0.74+0.2	0.99±0.16	<0.005
BMD-T score Spine	-2.7±1	0.12±2	<0.005
BMD Hip (gm/cm^2)	0. 70±0.06	0.83±0.26	0.15
BMD-T score Hip	-2.5± 0.6	-1.0+2	0.16



JBMR in press

FRACTURES vs COMMON WISDOM

Bone strength & quality

Structural Properties:

- Geometry
  - Size
  - Shape
- Microarchitecture
  - Trabecular
  - Cortical / porosity

Material Properties:

- Mineral
  - *Mineral : Matrix*
  - *Crystallite maturity/size*
- Collagen
  - Type
  - ***Cross-links***
- Microdamage

Bone Turnover

P. Chem

Lathyrogens

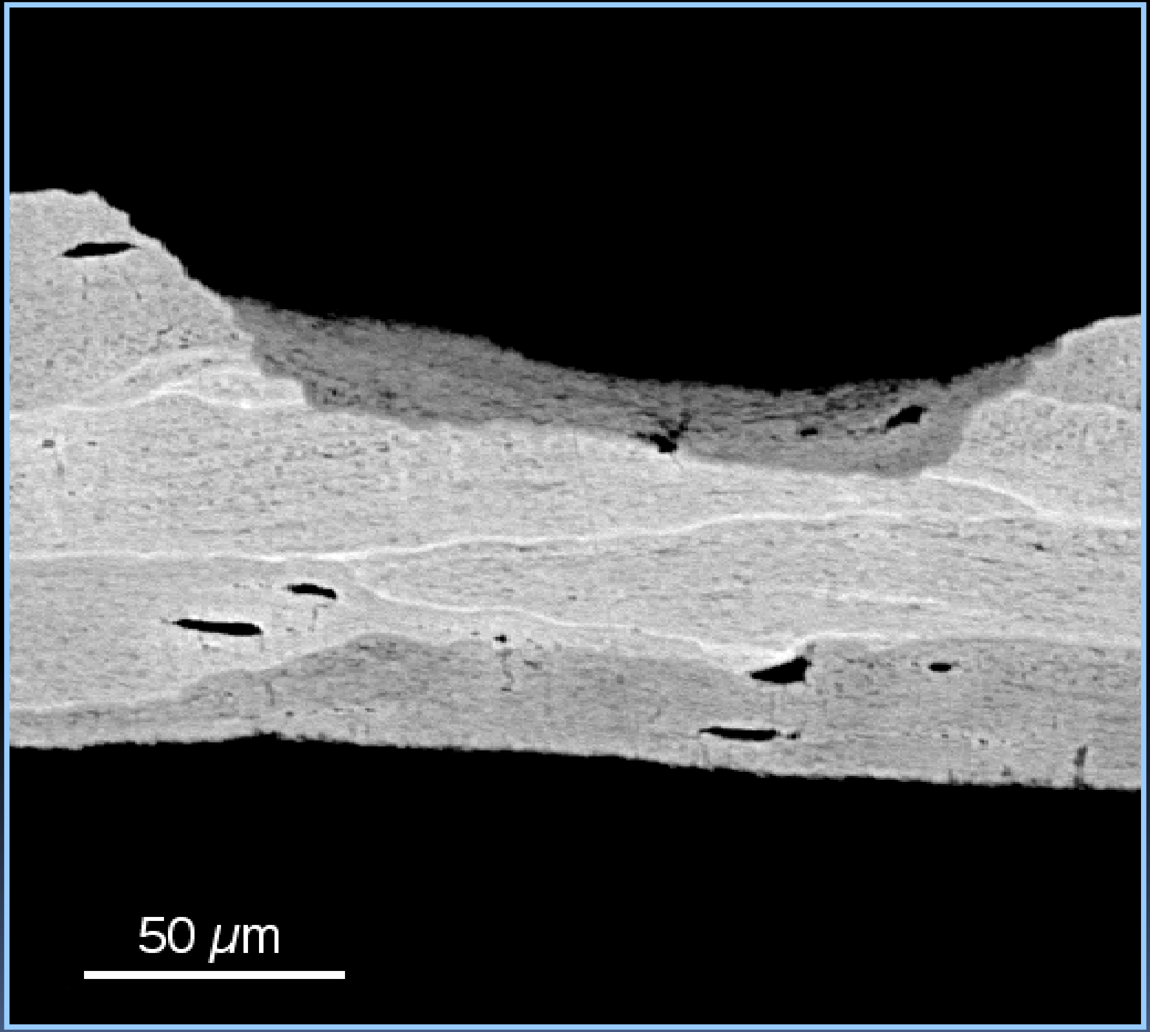
F
BPs
Sr

Features of Bone Tissue That Reduce Strength and Fracture Toughness

Feature	Modulus	Ultimate strain	Ultimate stress	Toughness	Cause/Example
Poorly mineralized bone	⇓	⇑	⇓	⇓	Osteomalacia
Hypermineralized bone	⇑	⇓	⇑	⇓	Reduced turnover Increased mean tissue age
Increased crystallinity: Δ morphology of apatite crystal	⇑	⇓	?	⇓	Reduced turnover Increased mean tissue age Fluoride accumulation
Denaturation of collagen molecule	⇓	⇓	⇓	⇓	Unclear
Debonding of mineral collagen	⇓	⇑	⇓	⇓	Fluoride accumulation

Burr DB and Turner CH Biomechanical Measurements in Age-Related Bone Loss. In: The Aging Skeleton. CJ Rosen, J Glowacki and JP Bilezikian (eds). San Diego: Academic Press 1999; Chapter 26, pp. 301-311

TISSUE AGE



Mineral Crystallites

Biological Apatites

Derived from Greek word meaning "to deceive"

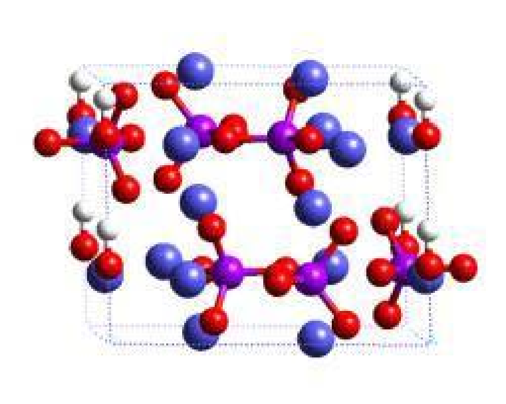
Crystallite chemical composition is changing

Maturity

phase purity, stoichiometry, solubility

Concomitantly/as a result, crystallite size changes

Crystallinity



white -- Hydrogen , Red -- Oxygen, purple - Phosphorous , Blue - calcium

http://www.ecs.syr.edu/faculty/schwarz/project1.htm

Ca_x $(PO_4)_y$ OH_z

Na^{+1} CO_3^{-3} Cl^{-1}

Mg^{+2} HPO_4^{-2} F^{-1}

Sr^{+2} CO_3^{-3}

CRYSTAL LATTICE VACANCIES

Mineral Crystallinity matters

Theoretical Considerations

Study of biological composites at the nano level reveals that optimal mechanical properties are associated with a definite plateau in mineral crystallinity. Crystllite sizes larger that the plateau value result in inferior mechanical performance

Gao H, et al 2003 Proc Natl Acad Sci U S A 100(10): 5597-600, 2003.

Animal model data

Plenty of animal models with altered mineral crystallinity & bone mechanical properties

Compromised Mechanical Properties

Ovariectomized animals	larger
Osteogenesis Imperfecta	smaller
Hyp mice	smaller
Osteopontin KO mice	larger
Osteocalcin KO mice	smaller

Human Clinical data

Fluoride treatment resulted in increased BMD & bone fragility. Upon analysis, fluoride-treated bone had higher crystallinity compared to normal

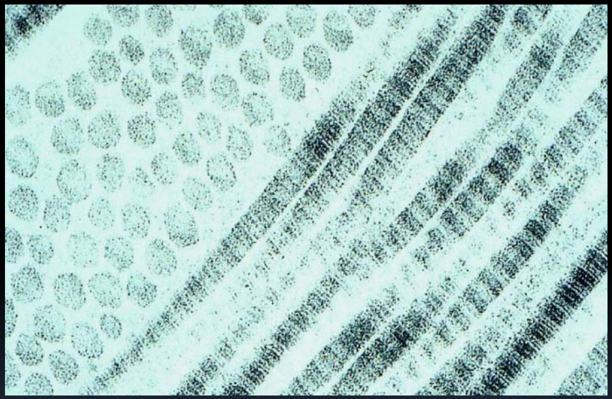
Animals & Humans

- X-ray powder diffraction
- Fourier transform infrared
- Small angle x-ray scattering
- Fourier transform infrared imaging

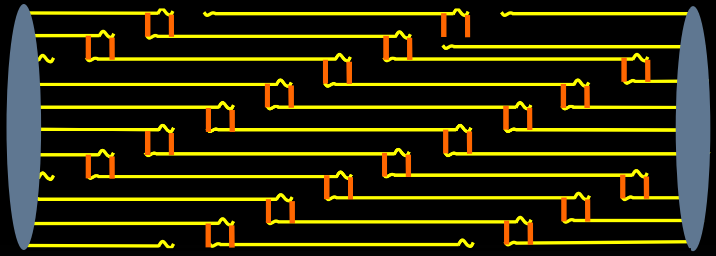
COLLAGEN

- SCAFFOLD
- MECHANICAL PROPERTIES
- INITIATION OF MINERALIZATION?
- Cross-links are tissue- rather than collagen type-specific

How Do Collagen Molecules Form a Functional/Stable Fibril?



Covalent Intermolecular Cross-linking



Importance of collagen cross-links

Theoretical Considerations

The matrix is proposed to play an important role in alleviating impact damage to mineral crystallites, and to matrix/ mineral interfaces.

Animal model data

Animal models with altered collagen cross-links & bone mechanical properties

Osteogenesis Imperfecta	mouse
Lathyrism	rat
Vitamin B6 deficiency	chicken

Human Clinical data

Osteogenesis Imperfecta

???

Mineral & Collagen — Tissue Variability

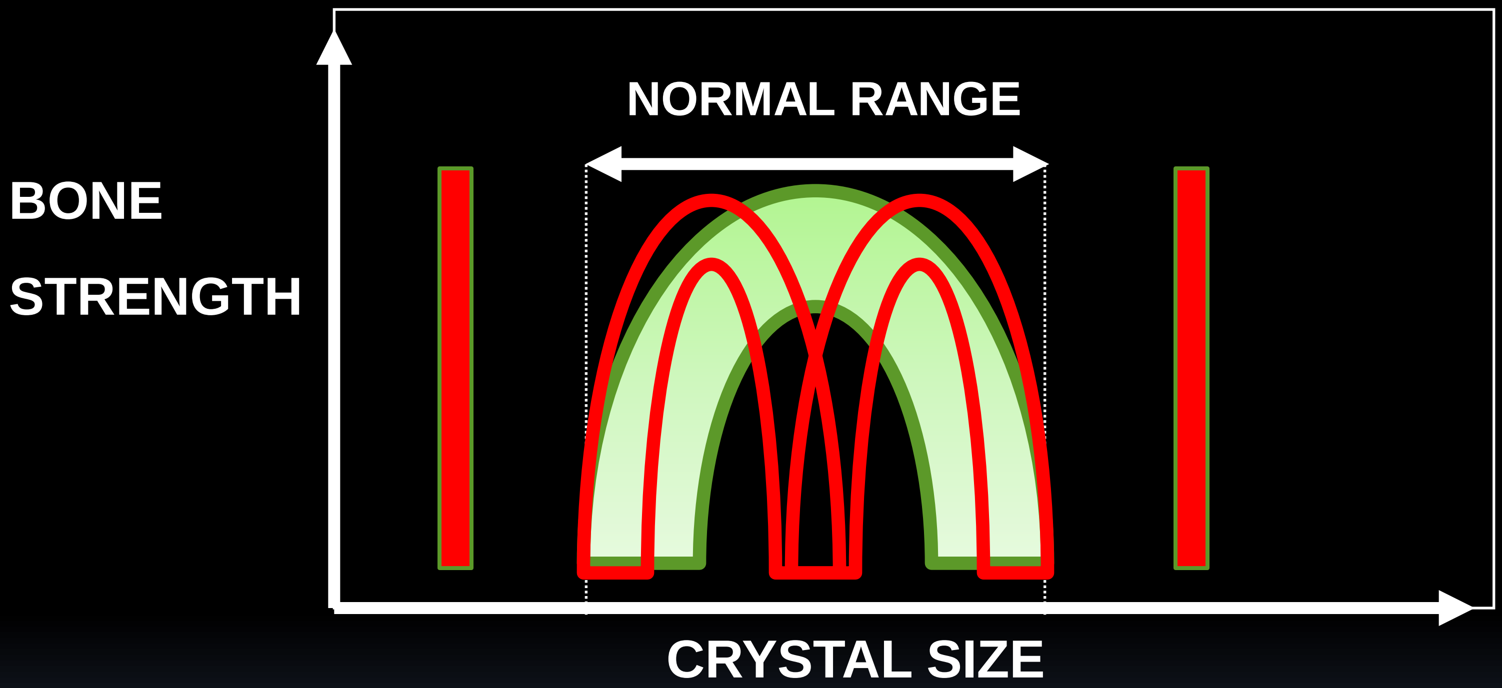
Tissue "Age"

Bone mineral crystallites are capable of sustained growth when bathed in aqueous media, even in the absence of cellular activity.

Rey C., Glimcher M. et al; Cells & Mat. 5, 345-356, 1995

Post-translational modifications may take place in biological fluids, even in the absence of cellular and/or enzymatic activity.

Yamauchi M 1996 Collagen: The major matrix molecule in mineralized tissues. In: Anderson JJB, Garner SC (eds.) Calcium and Phosphorus in Health and Disease. CRC Press, New York, pp 127-141.



COLLAGEN x-links

- OI
- Hyp
- Osteocalcin KO

- *OI*
- *Lathyrism*
- *Vitamin B6-deficiency*

- Fluoride
- Ovx
- Osteopontin KO

INFRARED SPECTROSCOPY

BASIS

TWISTING - BENDING - ROTATION - VIBRATION

ATOMS IN A MOLECULE

ABSORPTION AT PARTICULAR WAVELENGTHS

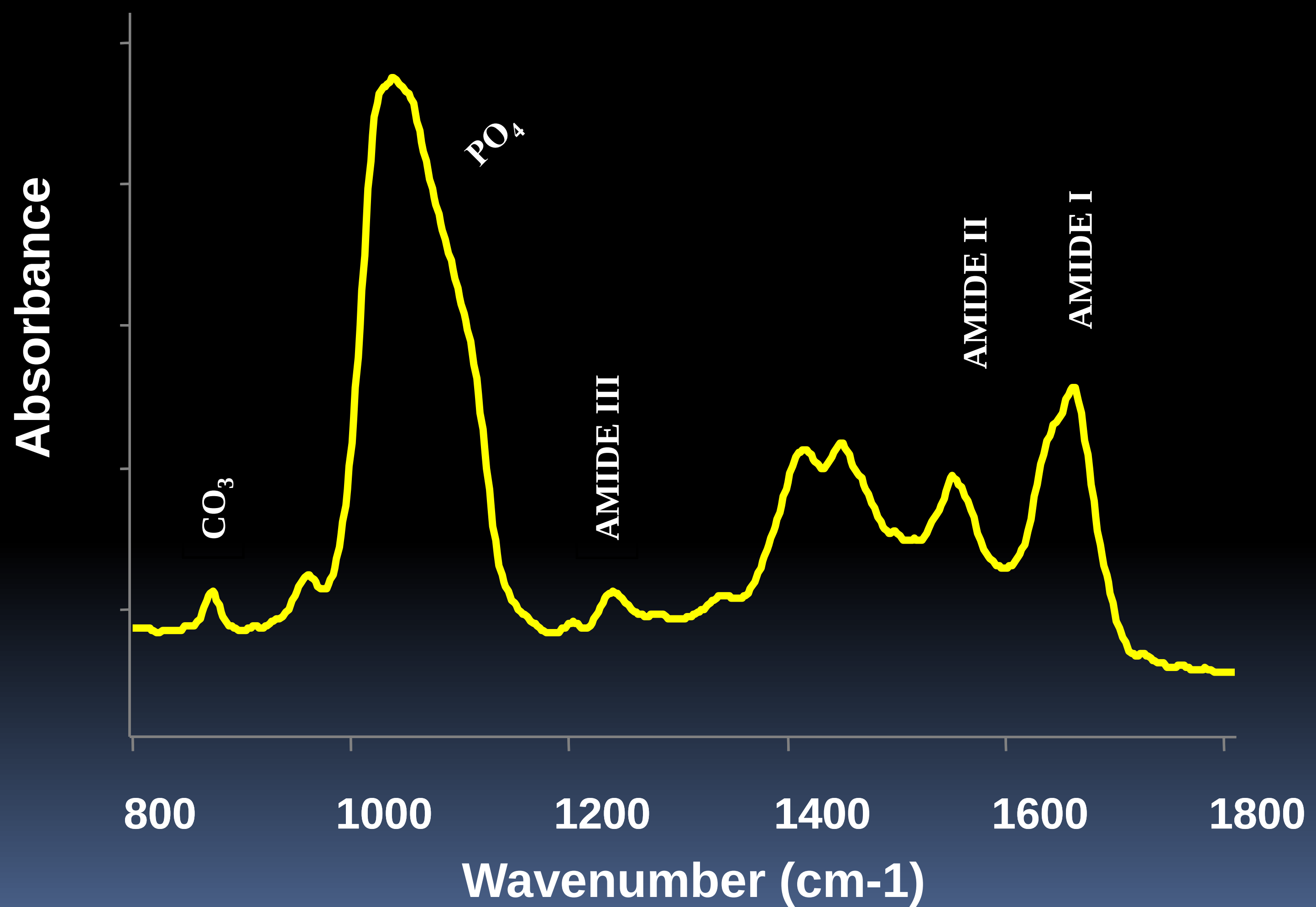
CHARACTERISTIC OF FUNCTIONAL GROUPS

OVERALL CONFIGURATION OF ATOMS

SUBTLE INTERACTIONS WITH SURROUNDING ATOMS OF MOLECULE

STAMP OF INDIVIDUALITY ON THE SPECTRUM OF EACH COMPOUND

TYPICAL FTIRM SPECTRUM OF BONE

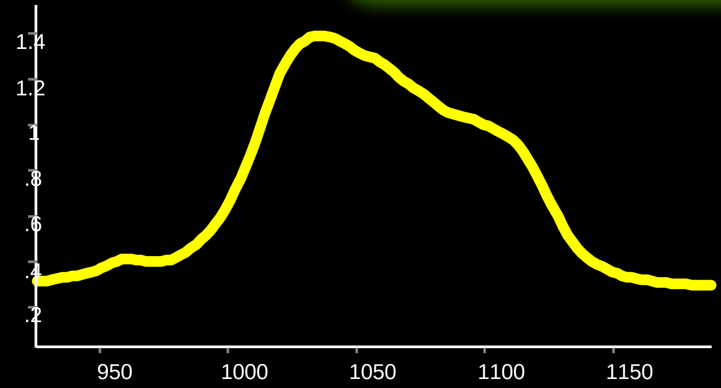


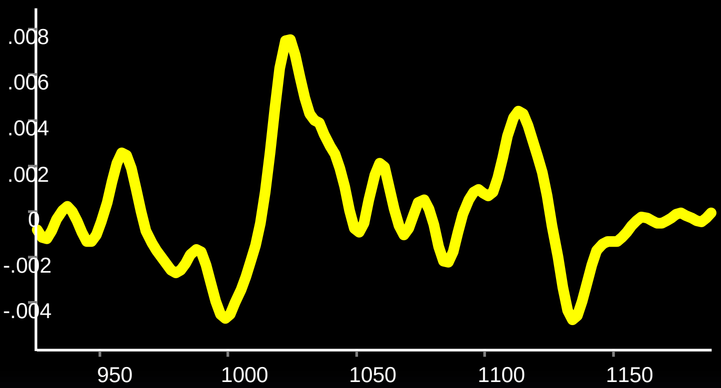
Fourier Transform Infrared Spectroscopy

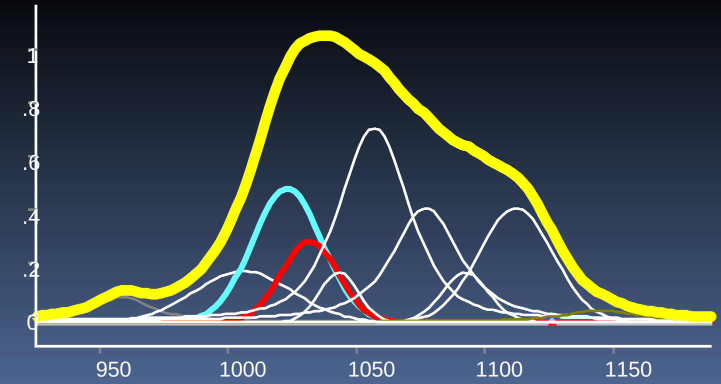
Major Bone Relevant Outcomes

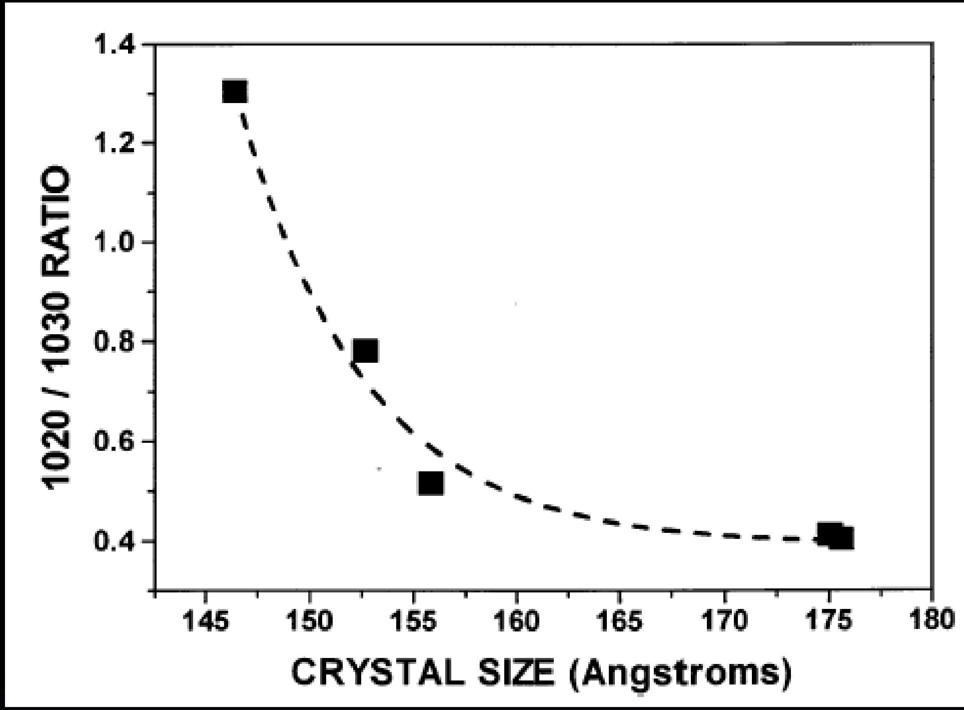
MINERAL DENSITY (mineral : matrix)	May or may not agree with BMD or BMDD as it expresses mineral with respect to volume AND matrix
CARBONATE CONTENT & TYPE	Important for crystal lattice considerations and solubility
MINERAL MATURITY	Direct measure is chemistry. Crystallinity may be extrapolated, but caution should be exercised when interpreting results
COLLAGEN CROSS-LINKS	UNIQUE capability to describe them as a function of tissue age AND surface metabolic activity

RELEVANCE OF 1020 / 1030 RATIO





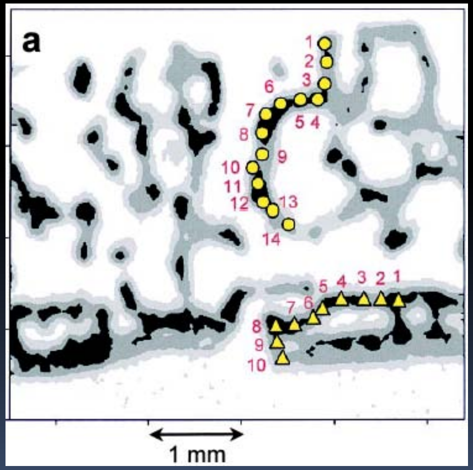


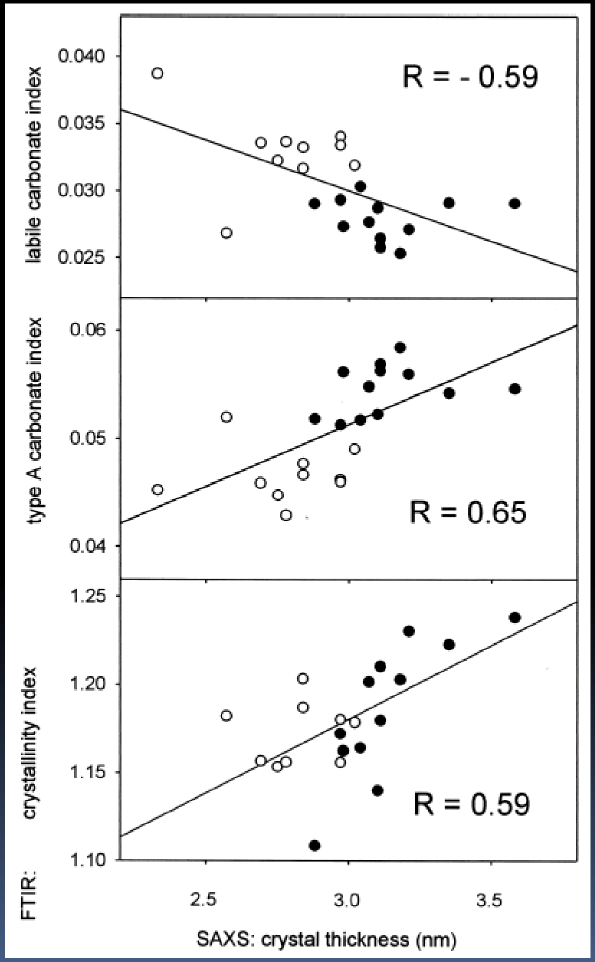


CTI, 59:480-487, 1996

FTIRM parameter validation against SAXS
Chemistry vs Structure

Human, L4 vertebrae





Statistically significant correlation of the FT-IRM parameters with the SAXS parameter of crystal thickness.

Open and full symbols represent data from cortical and cancellous bone, respectively.

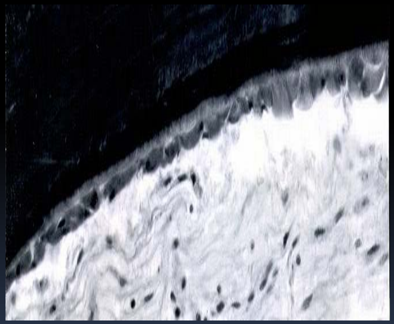
Bone 25(3) 287-293, 1999

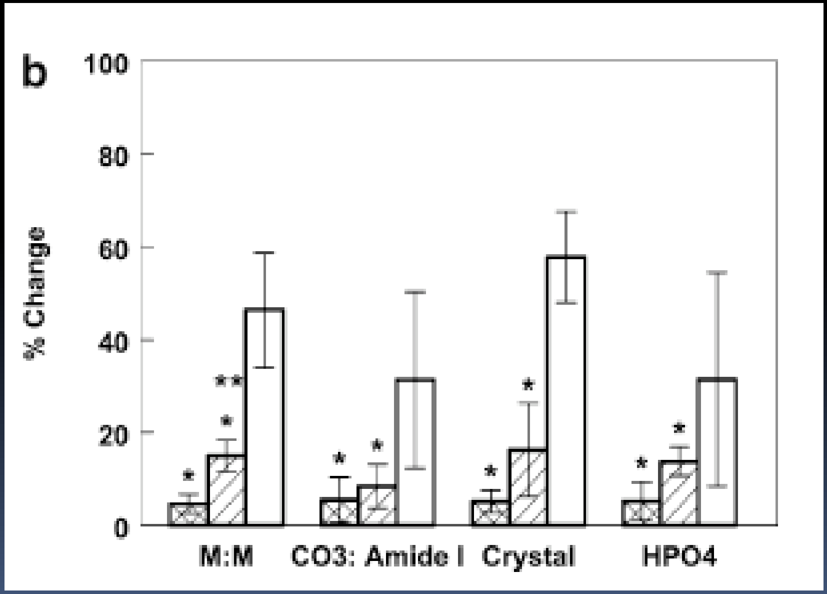
MINERAL MATURITY / CRYSTALLINITY CHANGES IN OSTEOPOROSIS

Table 1. Global averages of FTIR parameters

Parameter	Normal ($n = 10$)	SD	High Turnover ($n = 6$)	SD	Low Turnover ($n = 6$)	SD
Mineral: matrix	3.95	0.52	2.91	0.35**	3.67	0.27
Carbonate: phosphate	0.0097	0.005	0.0105	0.0019	0.0094	0.0017
Carbonate: amide I	0.034	0.018	0.0300	0.006	0.0350	0.0005
1030:1020 peak area ratio	1.069	0.091	1.585	0.18**	1.313	0.270*
1112:960 peak area ratio	3.82	1.70	4.94	1.6	3.32	1.20

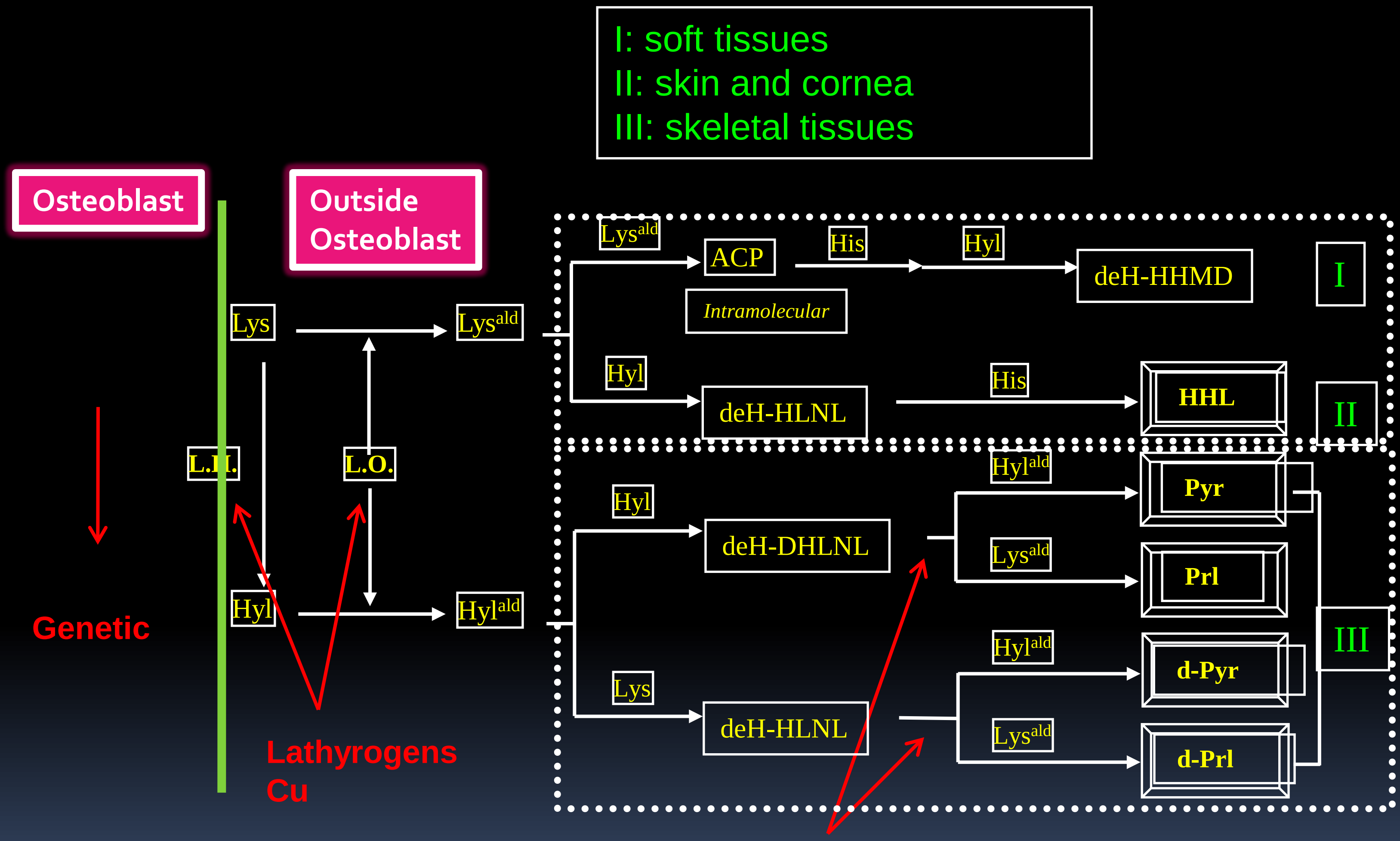
% CHANGES IN MINERAL QUALITY WITHIN THE FIRST 60 um AT BONE FORMING SURFACES





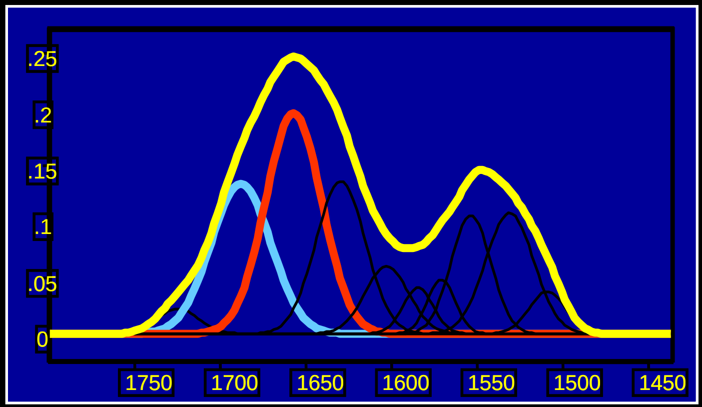
Osteoporos Int (2005) 16: 2031–20

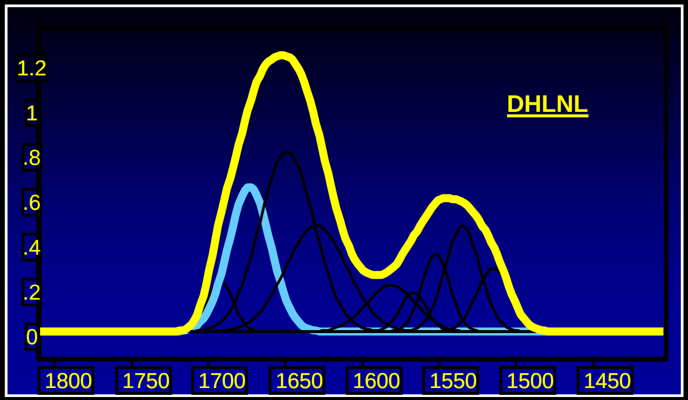
How Do Collagen Molecules Form a Functional / Stable Fibril?

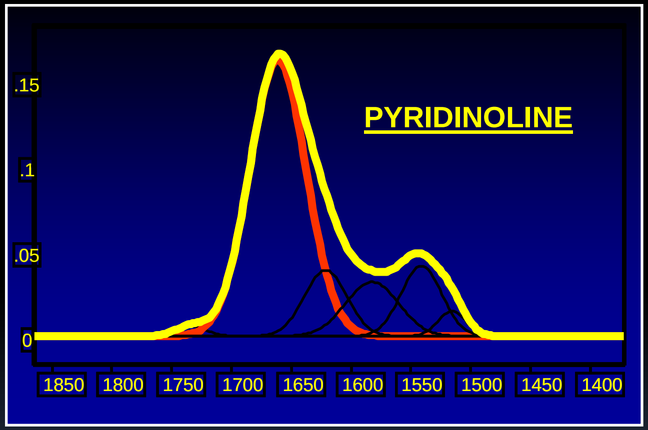


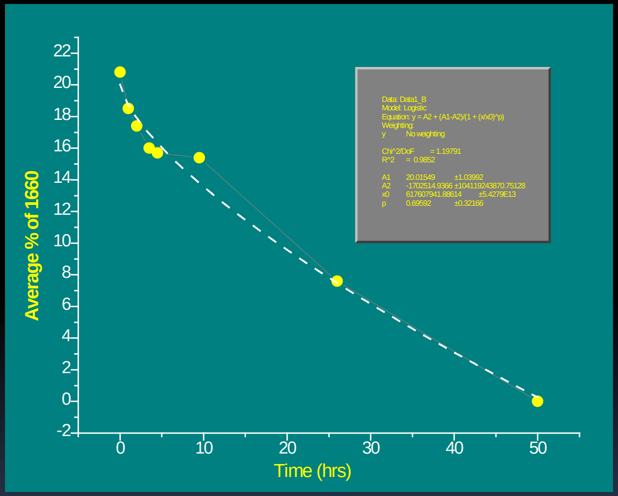
1. dehydro-dihydroxylysinonorleucine (deH-DHLNL),
2. dehydro-hydroxylysinonorleucine (deH-HLNL),
3. dehydro-histidinohydroxymerodesmosine (deH-HHMD),
4. pyridinoline (Pyr),
5. deoxypyridinoline (lysyl analog of Pyr, d-Pyr),
6. histidinohydroxylysinonorleucine (HHL).
7. pyrrole

COLLAGEN





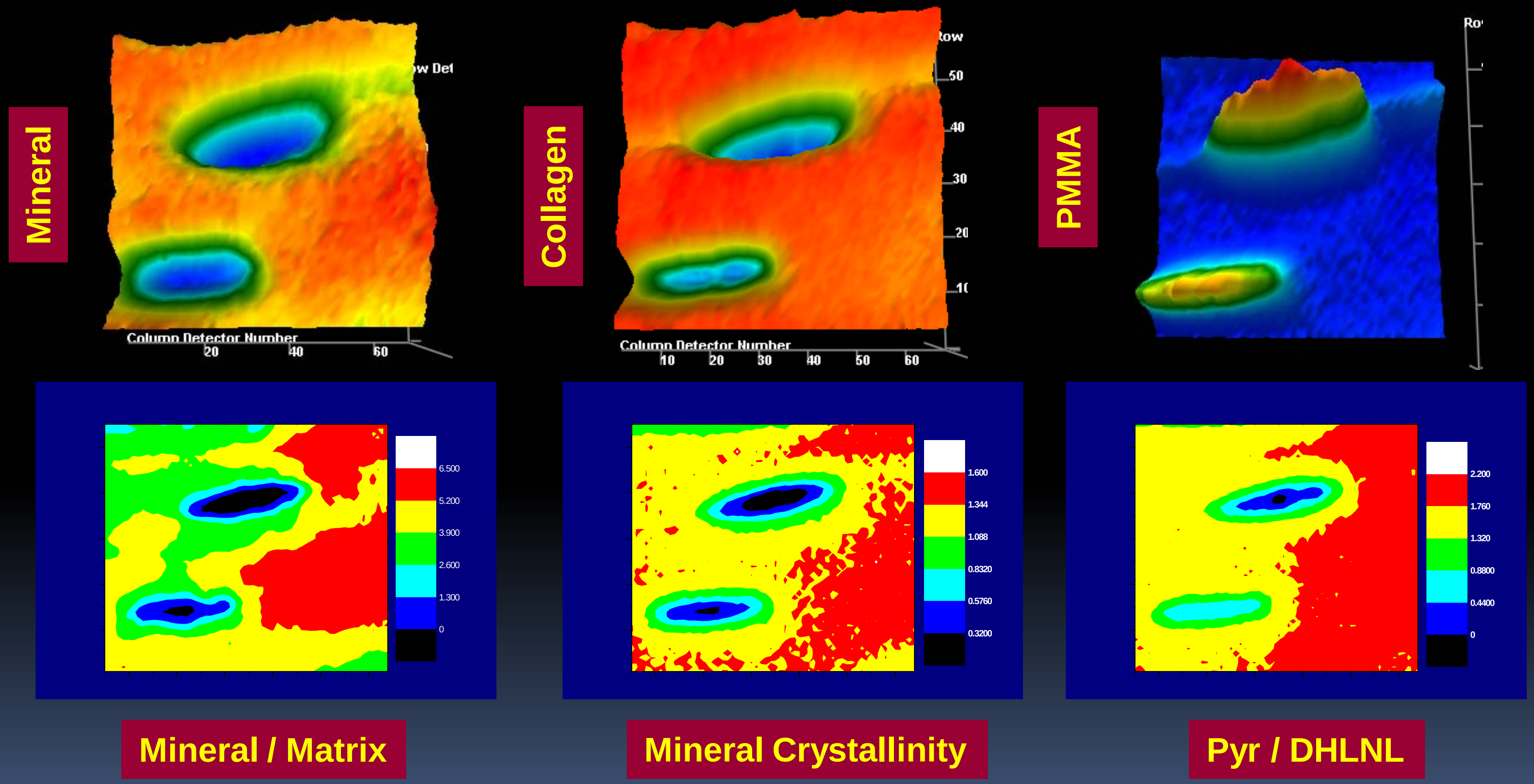




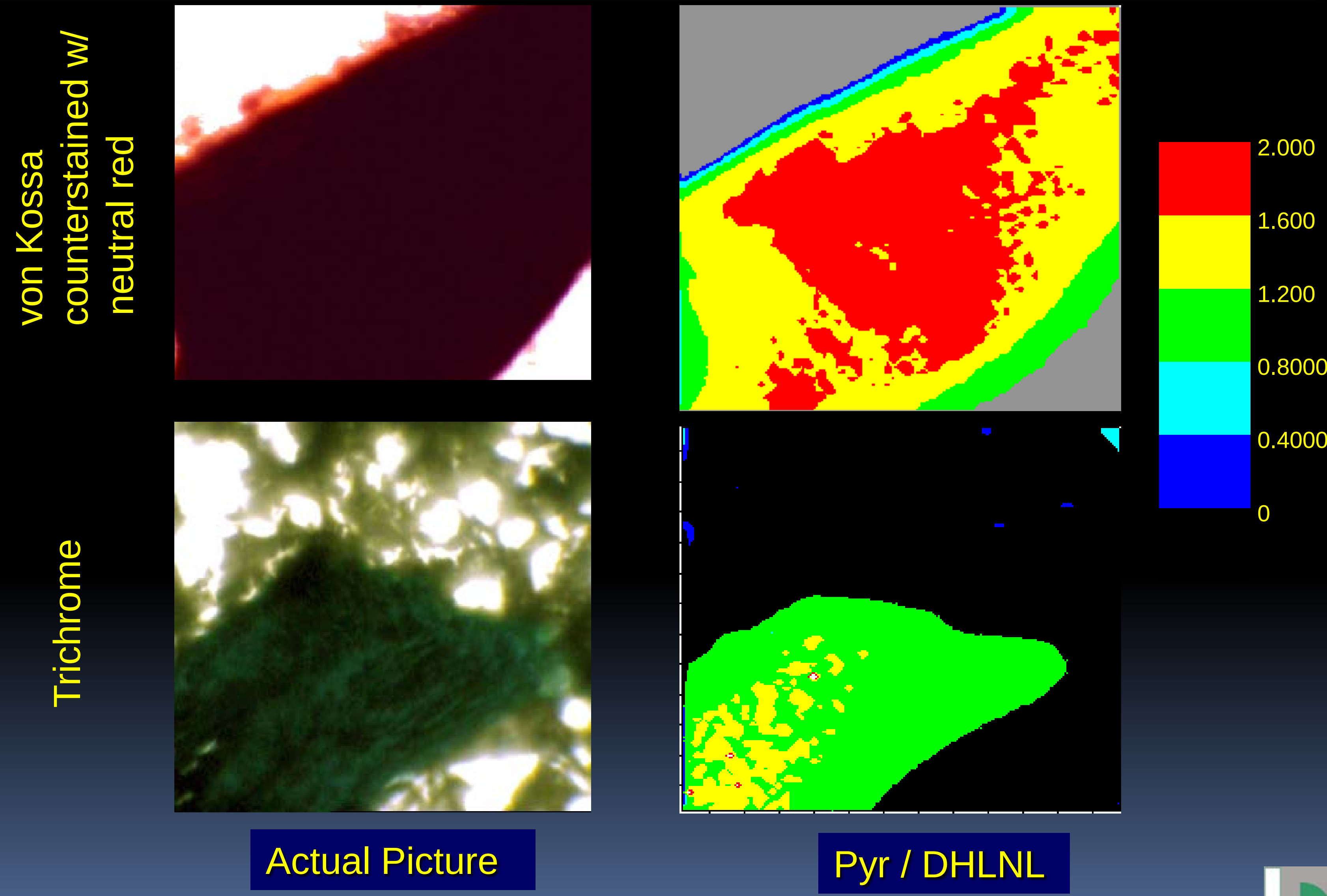
J of Bone and Mineral Research, 16(10), 1821-8, 2001.

INFRARED IMAGING

FTIR IMAGING DATA

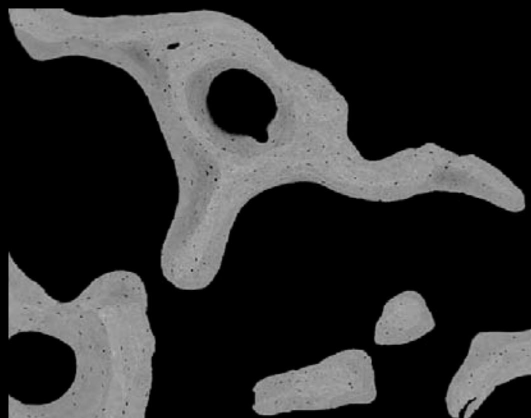


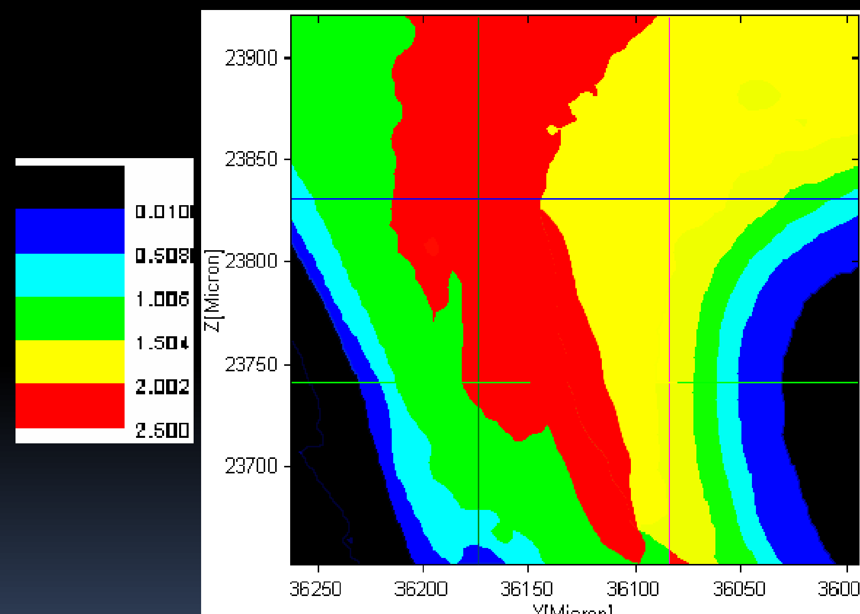
In case of collagen analysis, histologically stained sections may be employed



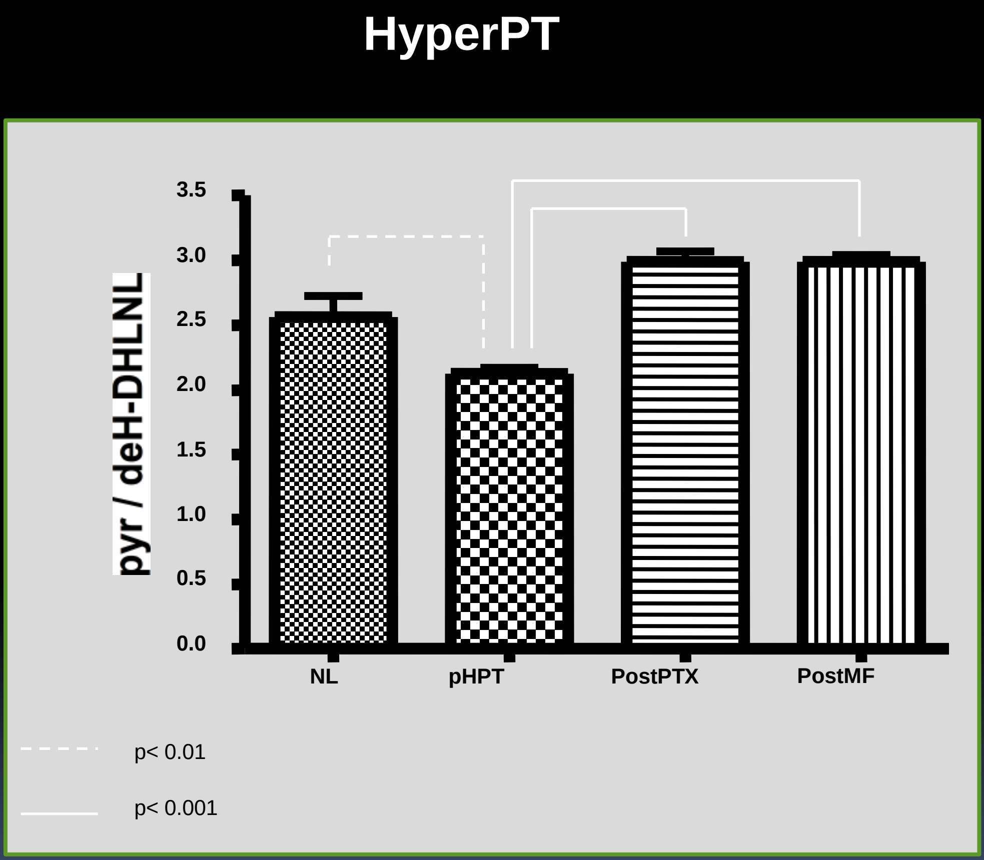
J of Bone and Mineral Research, 16(10), 1821-8, 2001

Match spatial distribution of collagen cross-links with mineralization





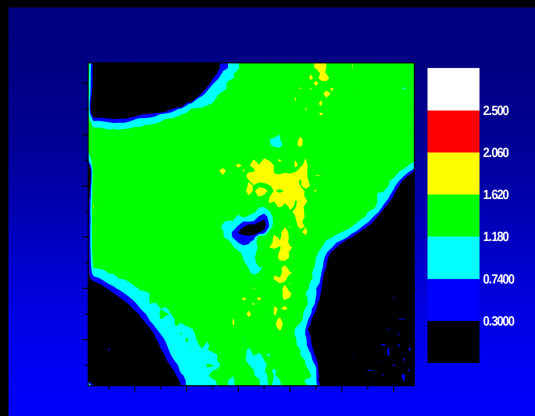
Collagen cross-links & bone turnover
Mild Primary Hyperparathyroidism

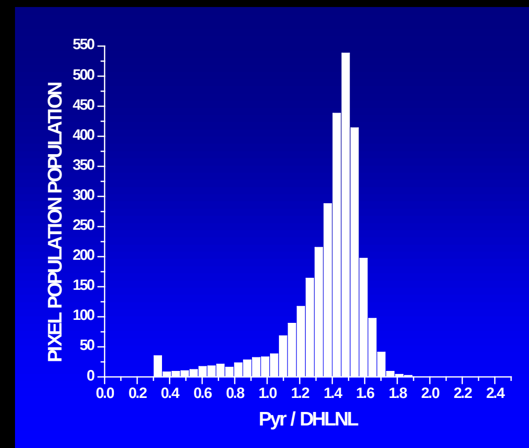


J Clin Endocrinol Metab. 2008 Sep;93(9):3484-9.

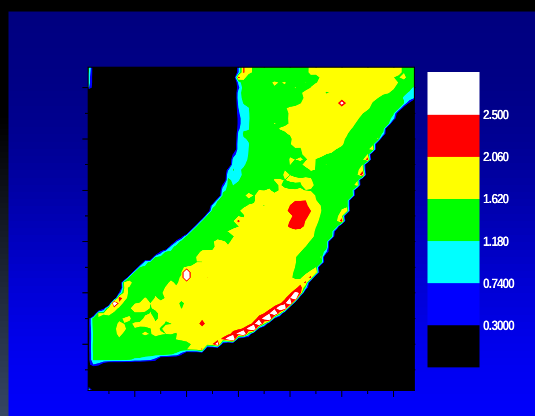
HOW IS COLLAGEN QUALITY IN OSTEOPOROSIS?

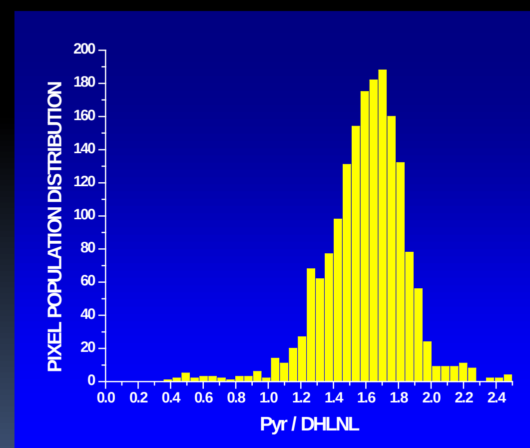
NORMAL



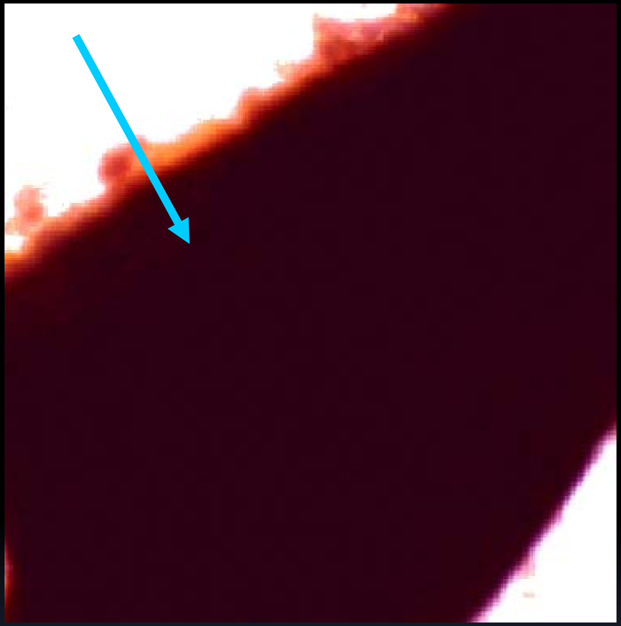


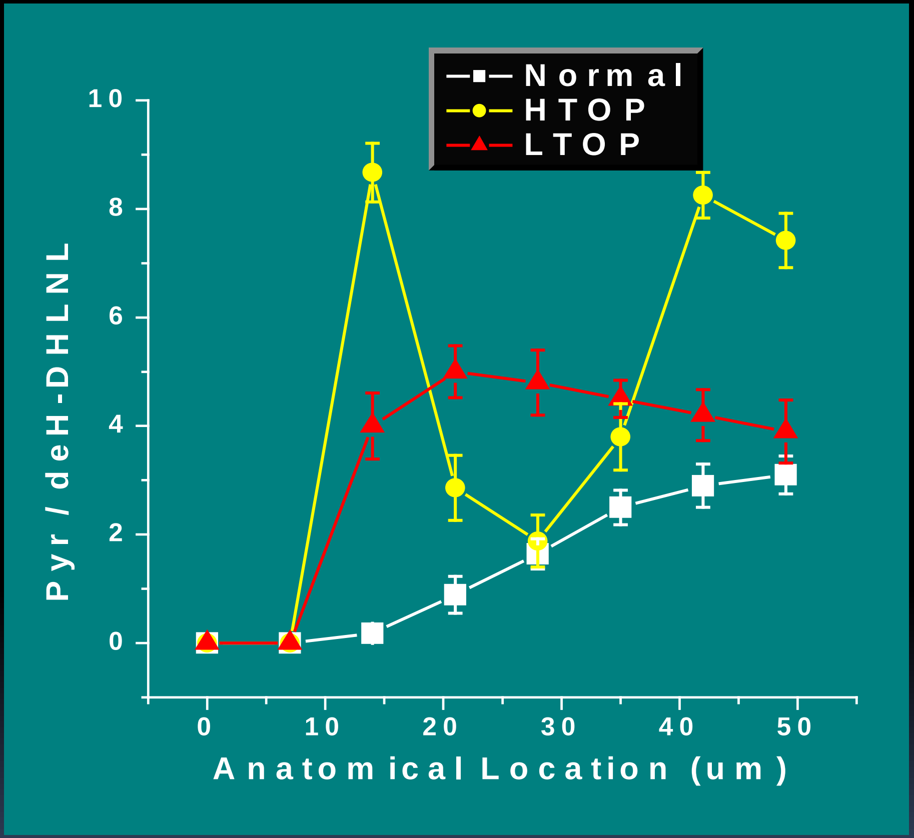
OP





HOW IS COLLAGEN QUALITY IN OSTEOPOROSIS?

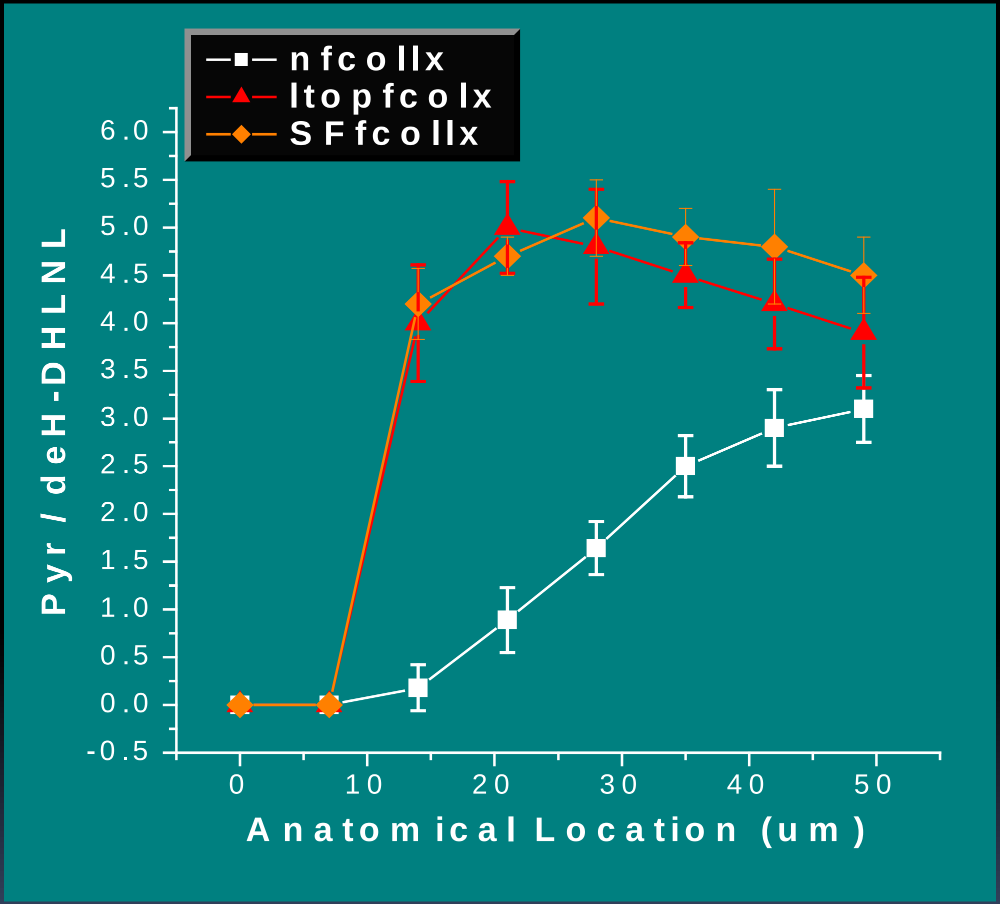




Normal N = 10
Low-turnover (LTOP) = 10
High-turnover (HTOP) = 10

JBMR 18(11): 1942-1946 2003
JBMR (2004)

HOW IS COLLAGEN QUALITY IN FRAGILE BONE?



JBMR (2004)

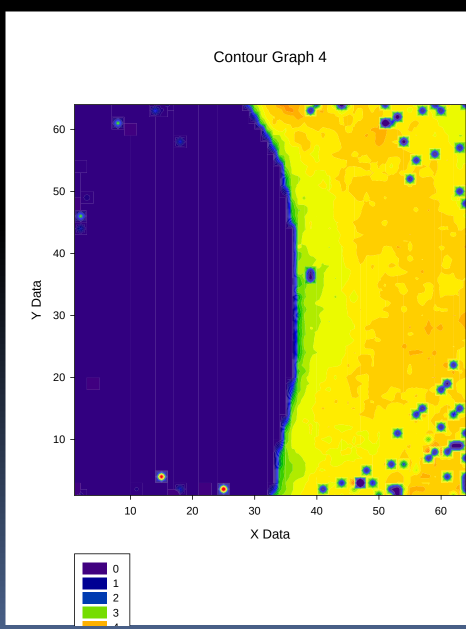
What is the clinical evidence / suggestions?

- *J Bone Miner Res. 2007 May;22(5):747-56.*
- *Bone. 2007 Mar;40(3):730-6.*
- *J Bone Miner Res. 2007 Jan;22(1):127-34*
- *Calcif Tissue Int. 2006 Sep;79(3):160-8.*
- *Am J Med. 2005 Nov;118(11):1250-5*
- *JAMA. 2005 Mar 2;293(9):1121-2*
- *N Engl J Med. 2004 May 13;350(20):2042-9.*
- *N Engl J Med. 2004 May 13;350(20):2033-41*

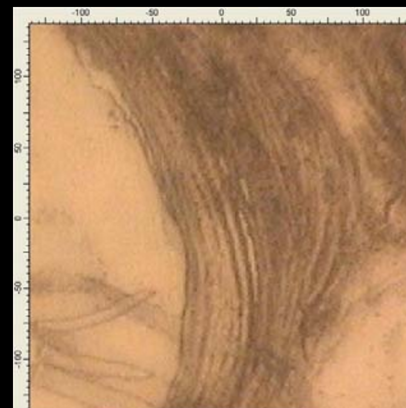
HOMOCYSTEINE & FRACTURE

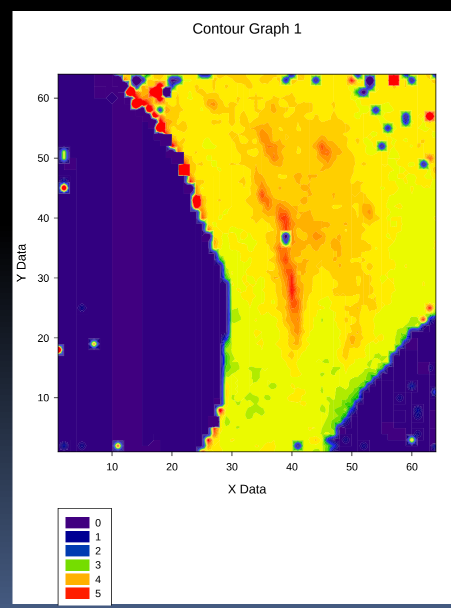
PLASMA HOMOCYSTEINE LEVELS & COLLAGEN CROSS-LINKS

Hcys = 6.0 uM/L



Hcys = 43.99 uM/L





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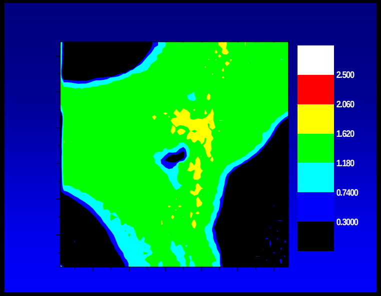
NORMAL



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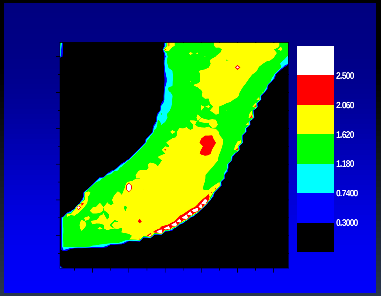
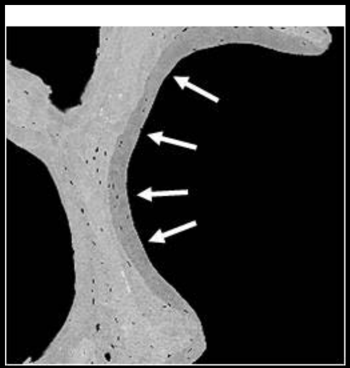


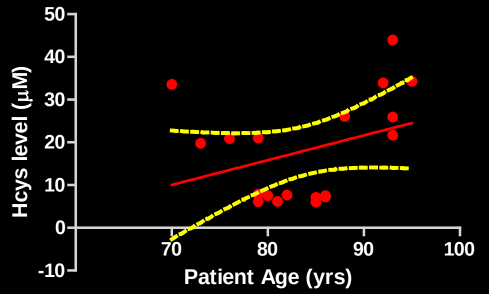
Bone. 2009 May;44(5):959-64.

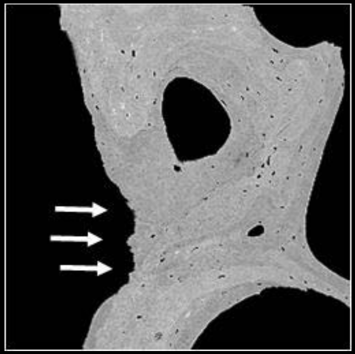
PLASMA HOMOCYSTEINE LEVELS & COLLAGEN CROSS-LINKS



Spearman r	0.3715
95% confidence interval	-0.1139 to 0.7138
P value (two-tailed)	0.1173
P value summary	ns

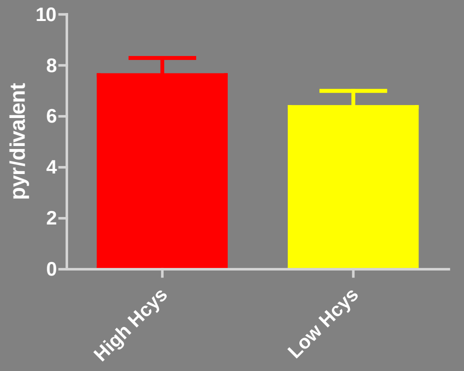
Patient data





Unpaired t test	
P value	0.0007
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4.129 df=17

collx form



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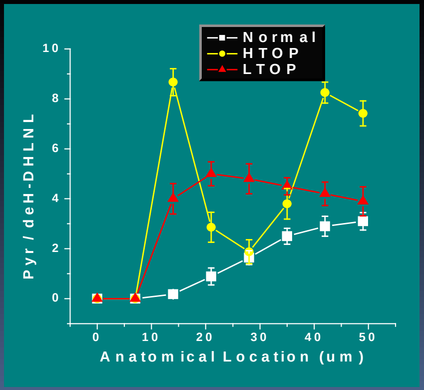
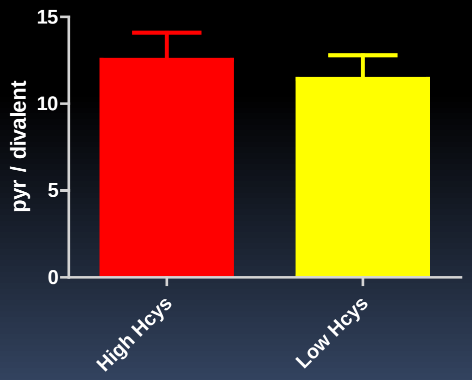


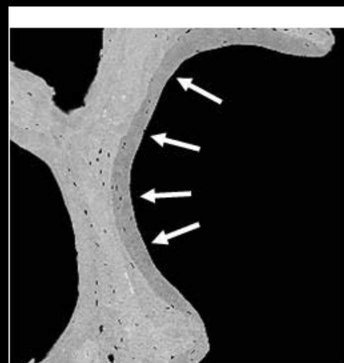
Unpaired t test	
P value	0.1197
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.638 df=17

collx res

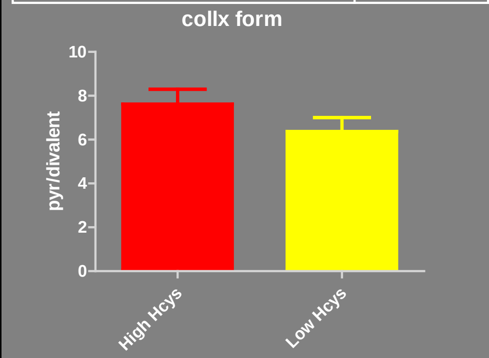


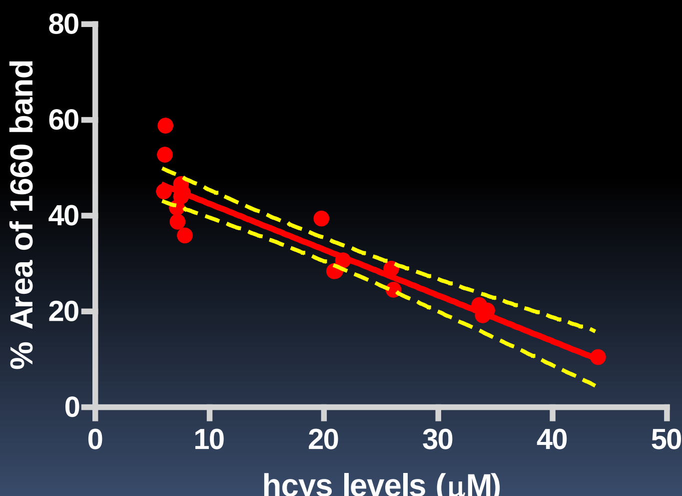
Bone. 2009 May;44(5):959-64.

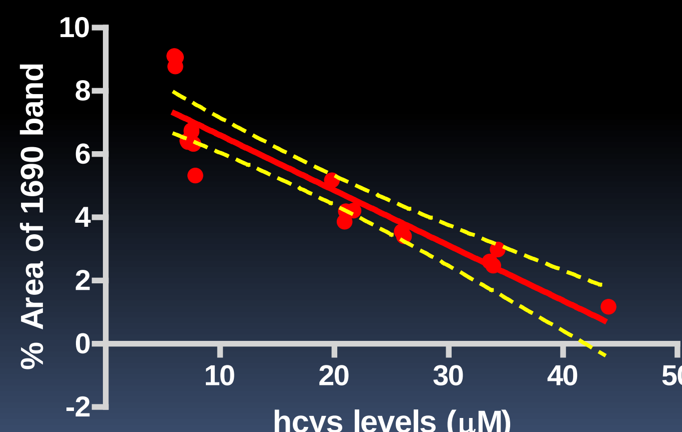
PLASMA HOMOCYSTEINE LEVELS & COLLAGEN CROSS-LINKS



Unpaired t test	
P value	0.0007
P value summary	***
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4.129 df=17







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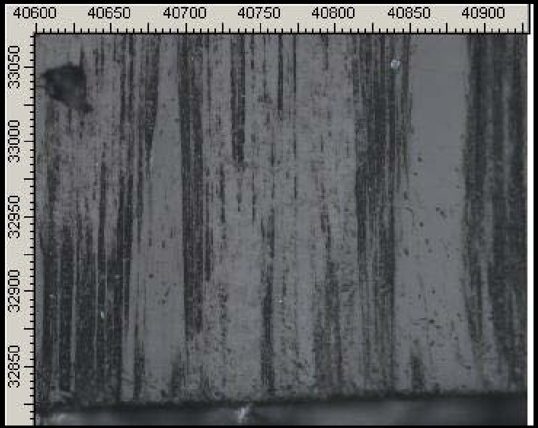
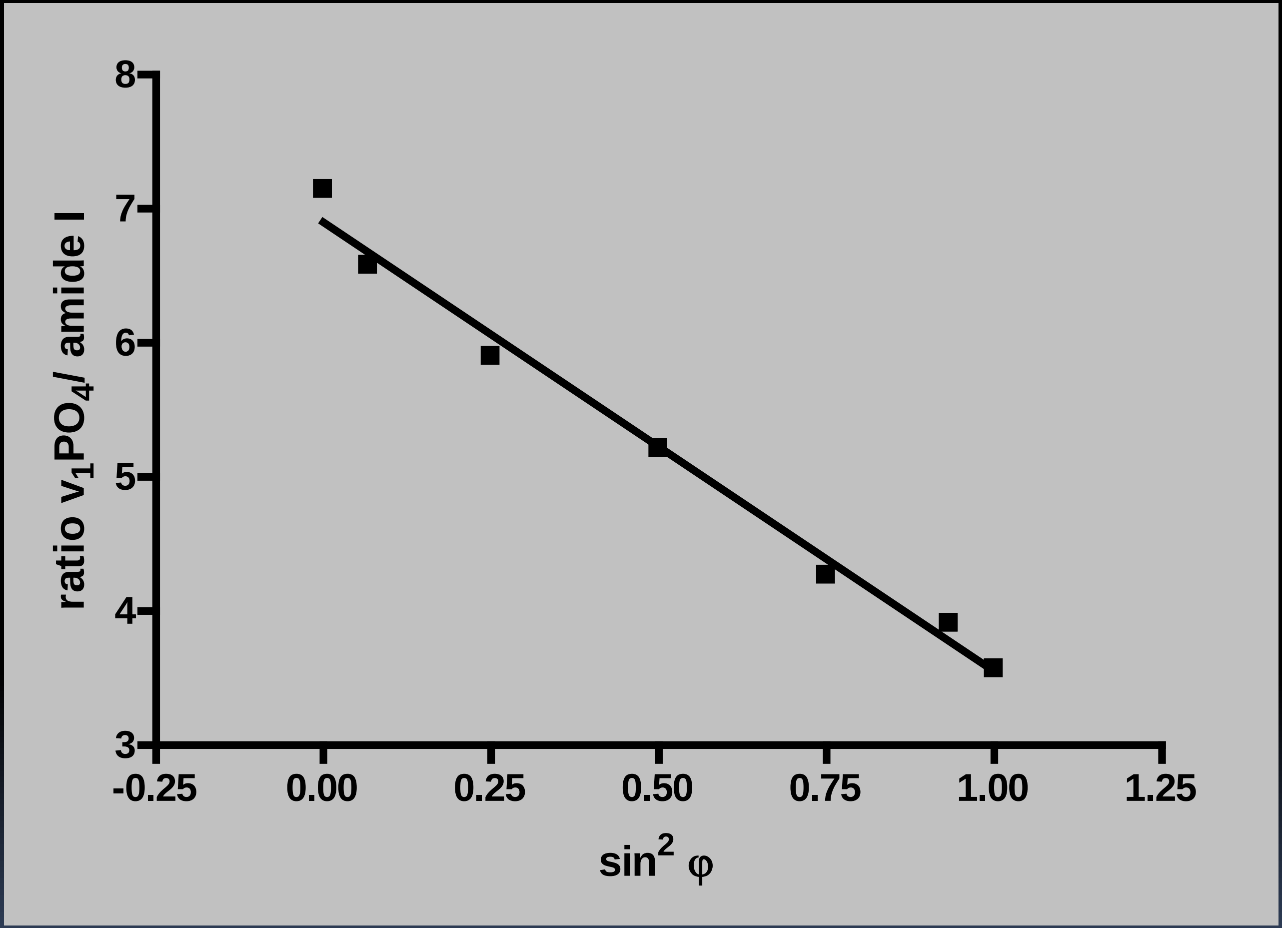
Vibrational Spectroscopy vs Vibrational Spectroscopy

	FTIRI	RAMAN
Spatial resolution	7 um	0.6 um
Tissue preparation	Dehydration & embedding	None
Background literature	Solid	Chaotic
Acquisition times	5 minutes	Seconds – 2 minutes
Signal to noise	Superior	Inferior
Mineral information	A lot of information	Some
Carbonate information	A lot of calculations involved	Straightforward
Collagen	A lot of information	Some
Orientation	Natively none	A lot of information

RAMAN & ORIENTATION DEPENDENCE : *turkey leg tendon*

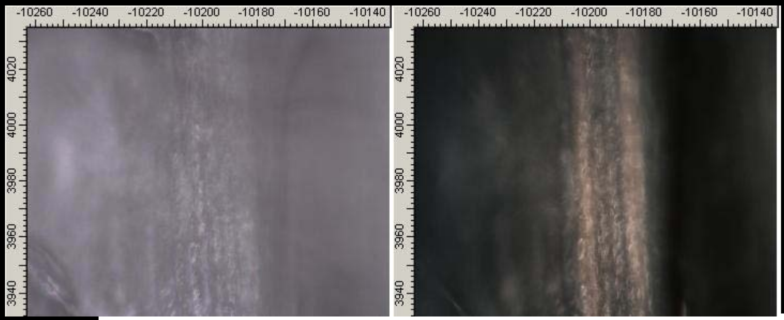


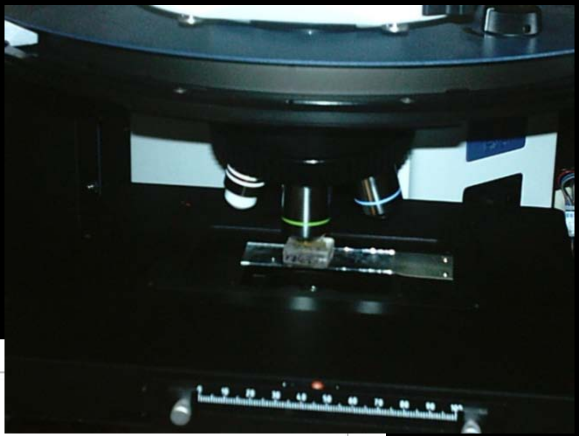


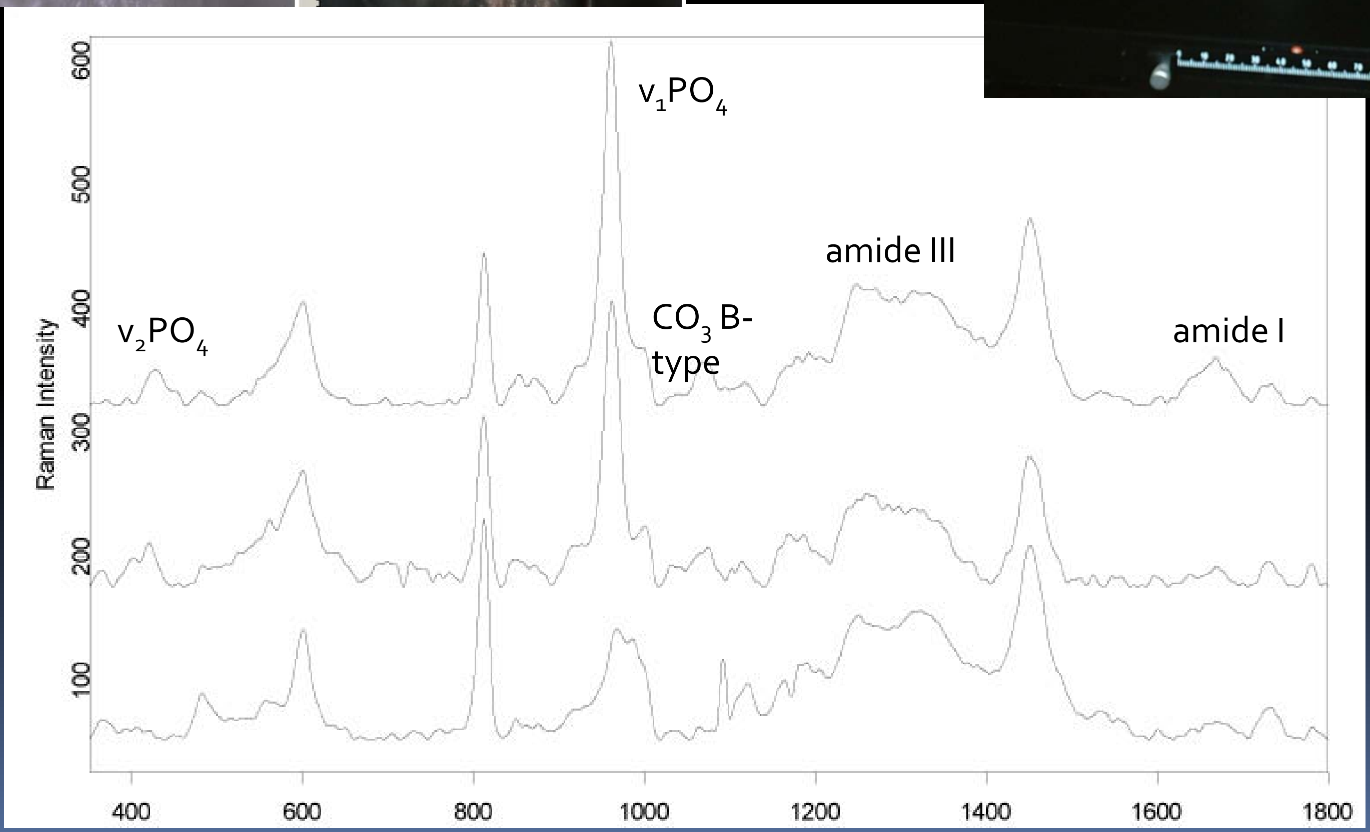
Amer, MS (2009) Raman Spectroscopy for Soft Matter Applications, Chapter 9, Raman Applications in Bone Imaging (S. Gamsjäger, M. Kazanci, E.P. Paschalis, and P. Fratzl) Wiley

P045 CORTICAL BONE ORIENTATION AND COMPOSITION IN A MOUSE MODEL AS A FUNCTION OF TISSUE AGE VS ANIMAL AGE S. Gamsjaeger, P. Roschger, K. Klaushofer, E. P. Paschalis, P. Fratzl

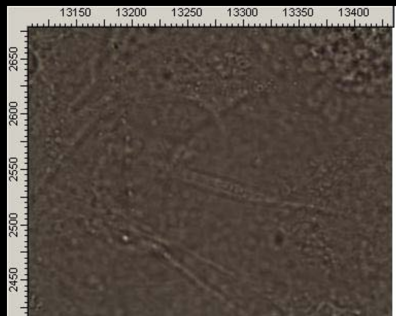
RAMAN & TISSUE AGE

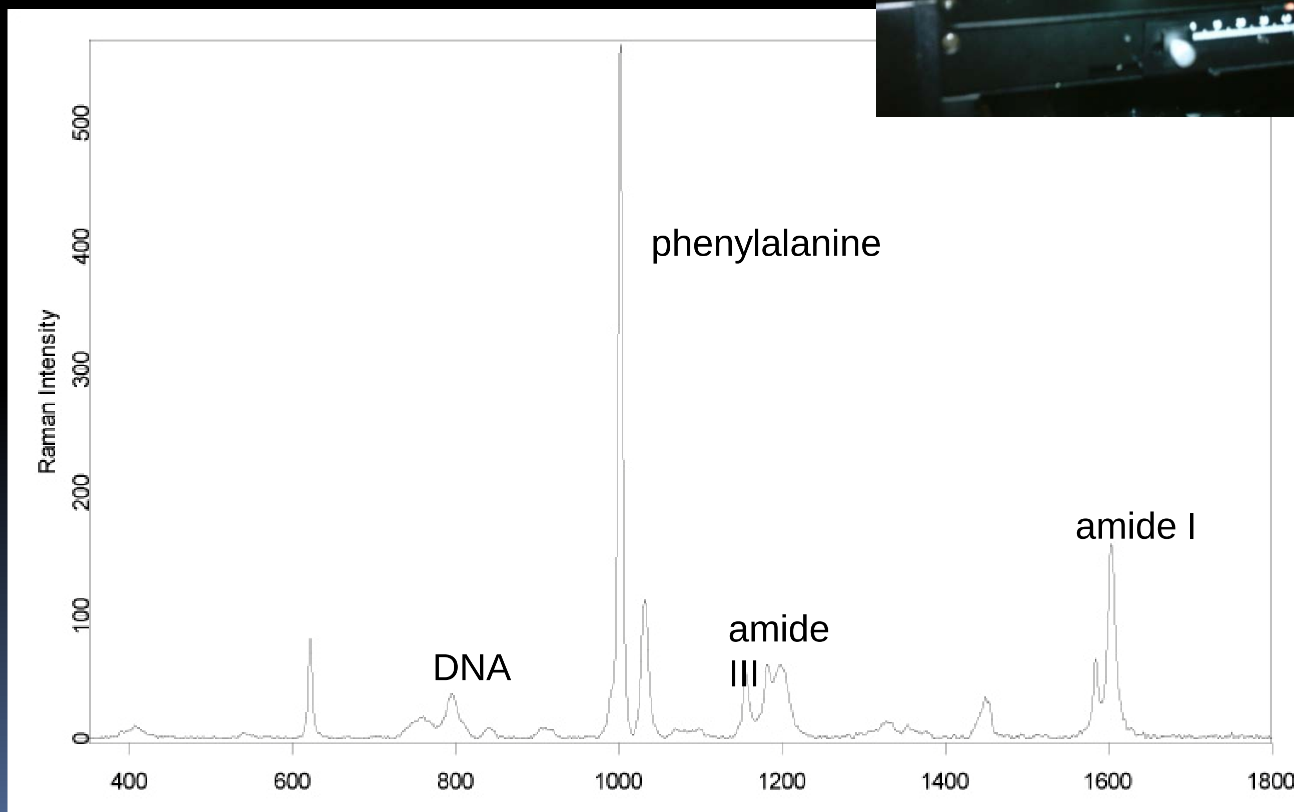






RAMAN & CELL CULTURES





SUMMARY

FTIRI & Raman vibrational spectroscopic techniques are capable of providing information on material properties

Information on all tissue components at the same time

No probe molecule required. Non-destructive

<u>Employed parameters have been / are validated against "golden standard" techniques such as XRD, and Biochemical analyses</u>

To date, mineral maturity & the ratio of two of the major mineralizing collagen cross-links, may be deduced in thin tissue sections with optimal spatial resolution of ~ 0.6-6.3 um. Moreover, structure vs orientation effects may be discerned

Its major strength is that it may provide answer to the question: <u>what is the difference between normal and diseased/treated tissue at EQUIVALENT tissue age anatomical locations?</u>

As a result, effects due to alterations in turnover and/or other factors may be readily discerned

SUMMARY
Clinical Value

Information provided in spatially resolved manner thus can compare material properties as a function of tissue age and bone surface activity

Not likely to be used in everyday clinical practice as biopsy is required

Excellent tool in cases where BMD does not fully account for fracture risk (SF)

Excellent tool in animal model experiments

Excellent tool for determining material properties (clinical trials)

Excellent tool for establishing effect of therapies on bone material properties.