Imaging Chemical Changes in Disease: From Bones to Brains

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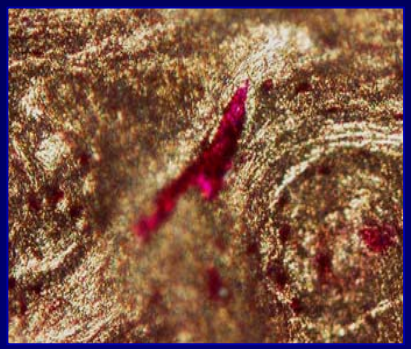
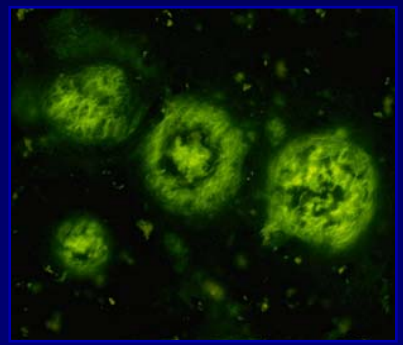
Office of Basic Energy Sciences

Outline

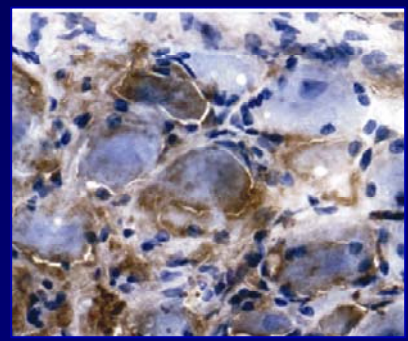
- Infrared microspectroscopy and imaging of biological tissue
- Advantages of a synchrotron source
- Current applications: From bones to brains



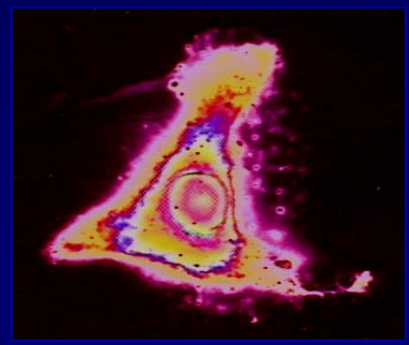
Osteoporosis & treatments



Alzheimer's disease

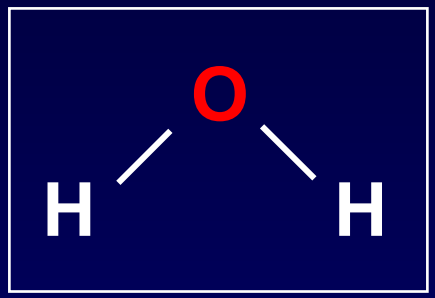


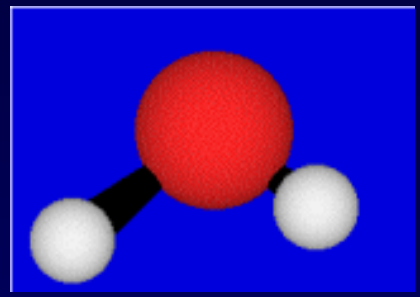
Prion diseases

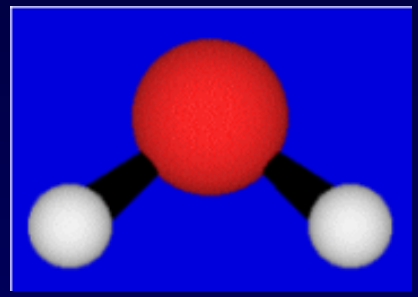


Skin cancer & therapy

Infrared Light Probes Molecular Vibrations



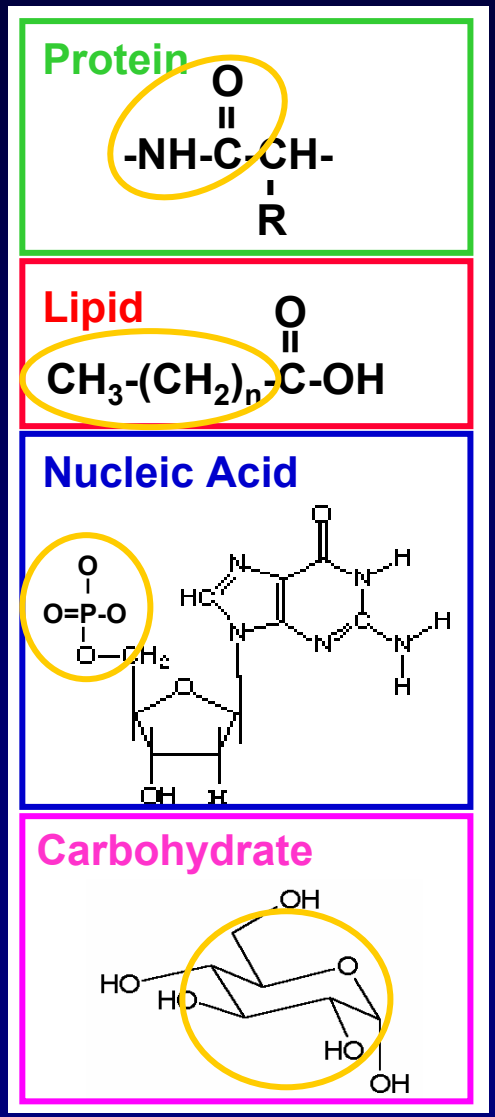


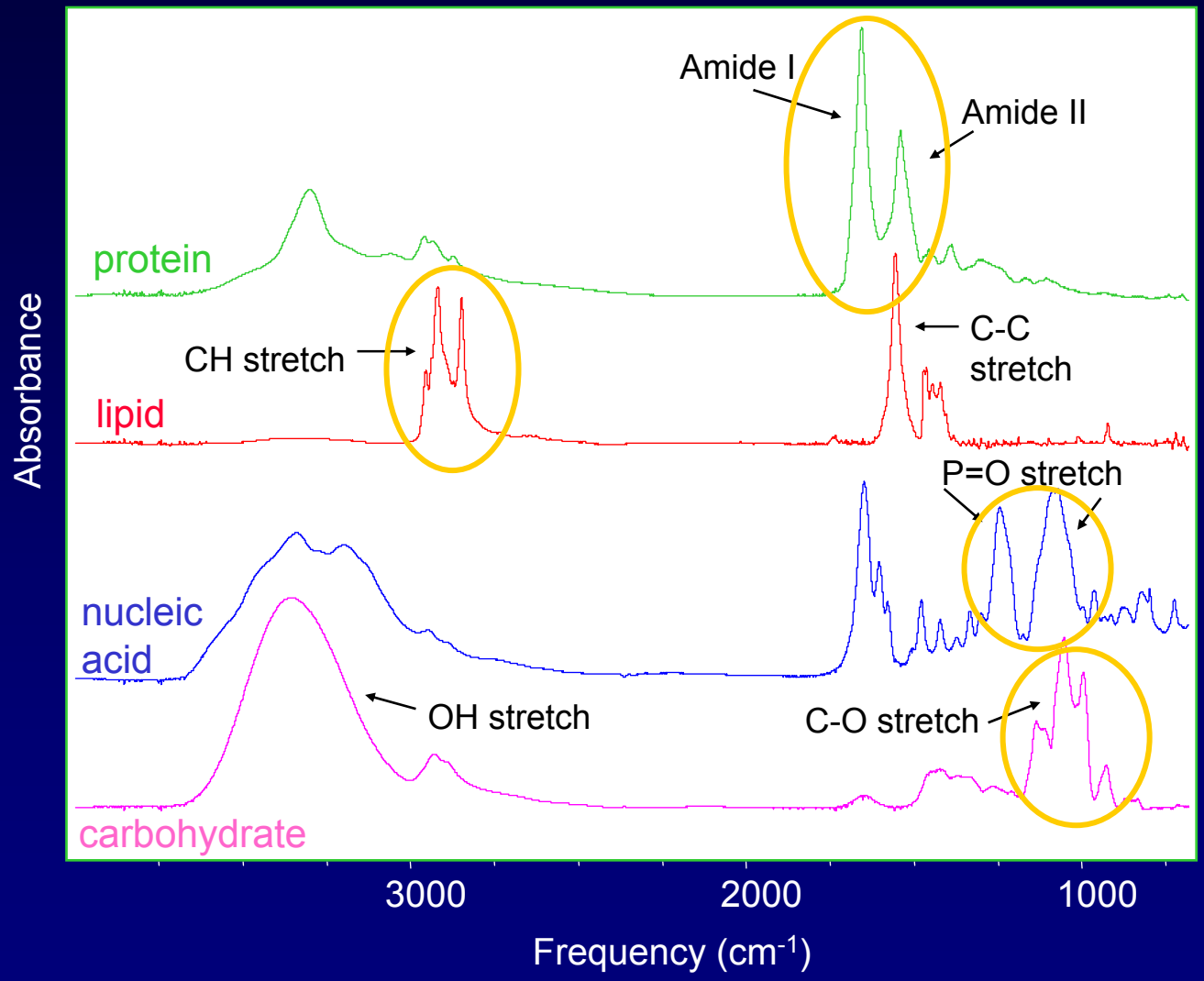


Atom Type, Size, Environment, and Bond Strength Influence Vibrational Frequencies

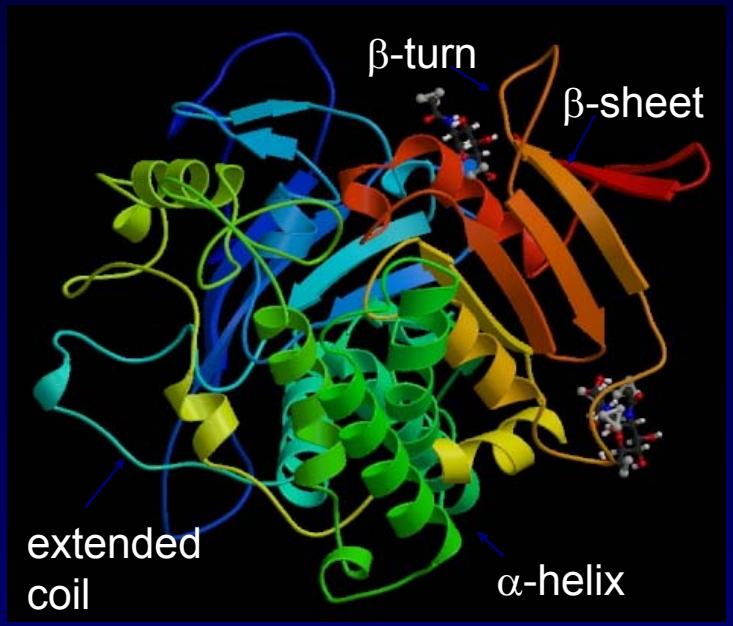
-C-H	2850-2960 cm^{-1}	-C=O	1640-1750 cm^{-1}
=C-H	3020-3100 cm^{-1}	-C-O	1050-1150 cm^{-1}
-C=C-	1650-1670 cm^{-1}		
		-O-H	3400-3640 cm^{-1}
-C-Cl	600-800 cm^{-1}	-N-H	3310-3500 cm^{-1}
-C-Br	500-600 cm^{-1}		

Chemical Features of Biological Components

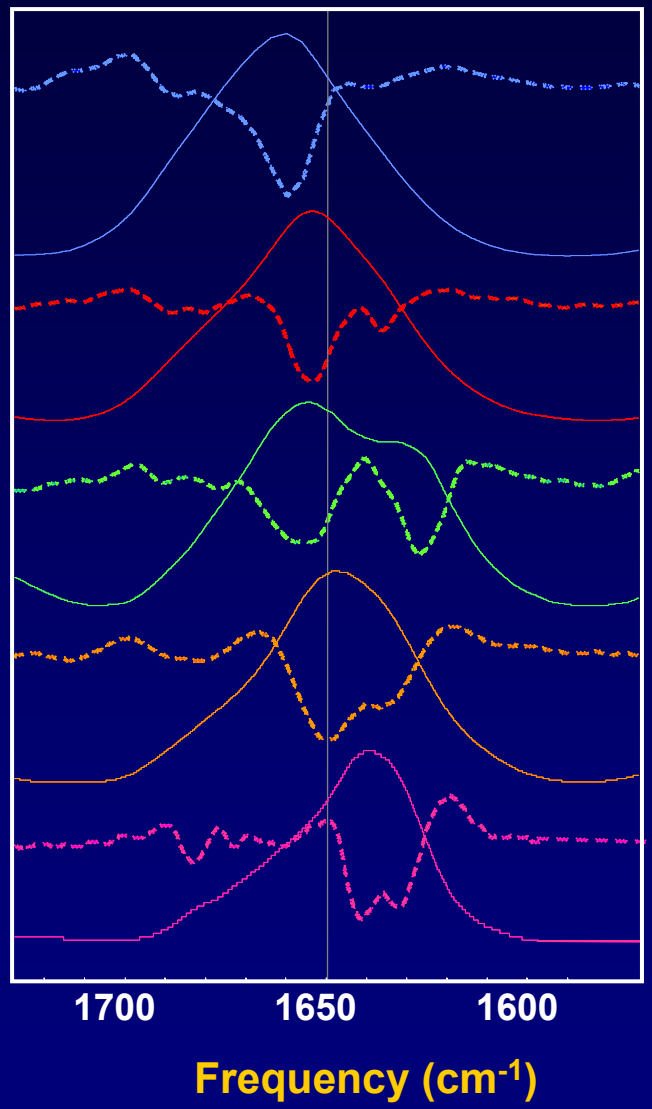




Protein Structure Determination with FTIR



Secondary Structure Assignments:	
1620 – 1640	β-sheet
1645	extended coil (D_2O)
1652 – 1657	α-helix
1660	triple helix
1670 – 1695	β-turn



Collagen (triple helix)

Myoglobin (α-helix)

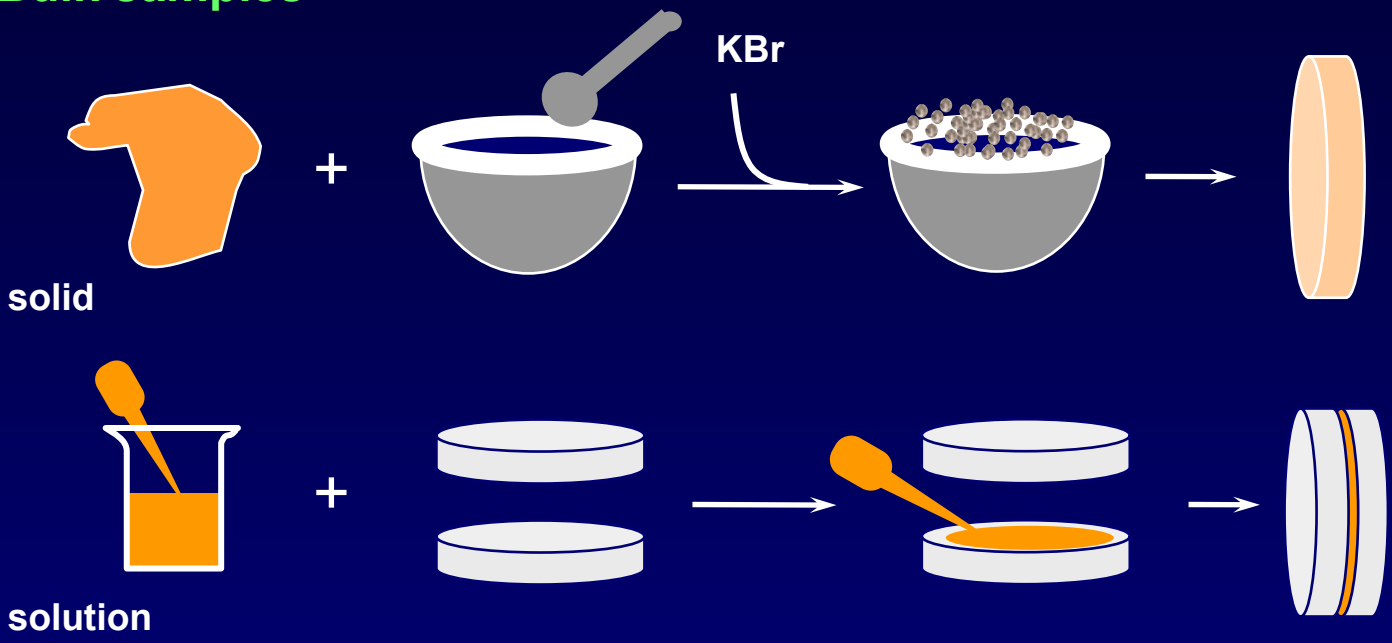
Abeta Amyloid (α-helix + β-sheet)

Cytochrome c (α-helix + coil)

STI (β-sheet + coil)

IR Spectroscopy vs. Micro-Spectroscopy

Bulk samples

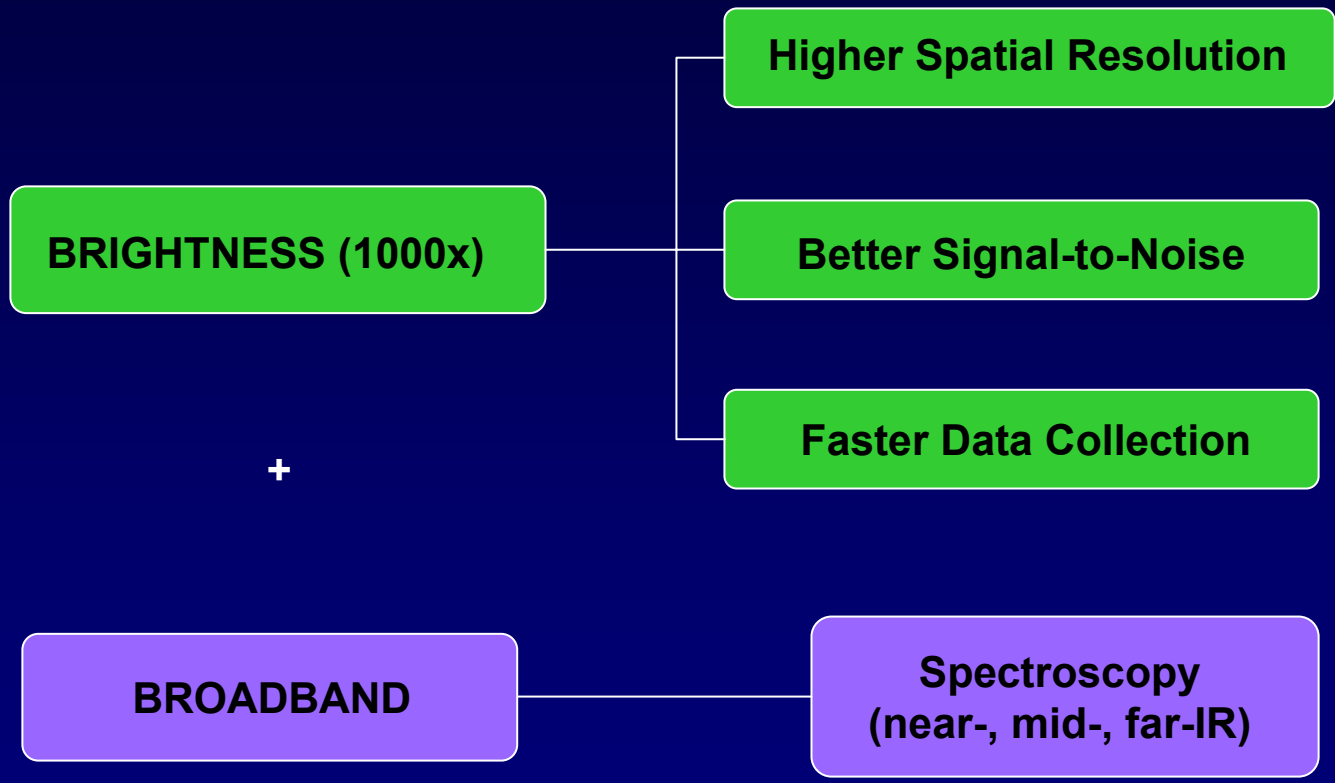


Spatial Resolution:
~1-3 mm

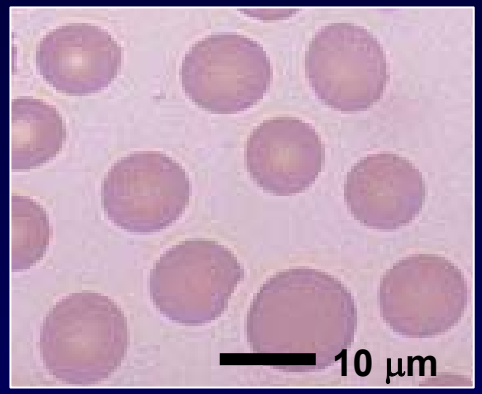
Microscopic Heterogeneity

Spatial Resolution:
~ 25 - 30 μm (globar)
~ 3 - 10 μm (synchrotron)

Advantages of a Synchrotron IR Source

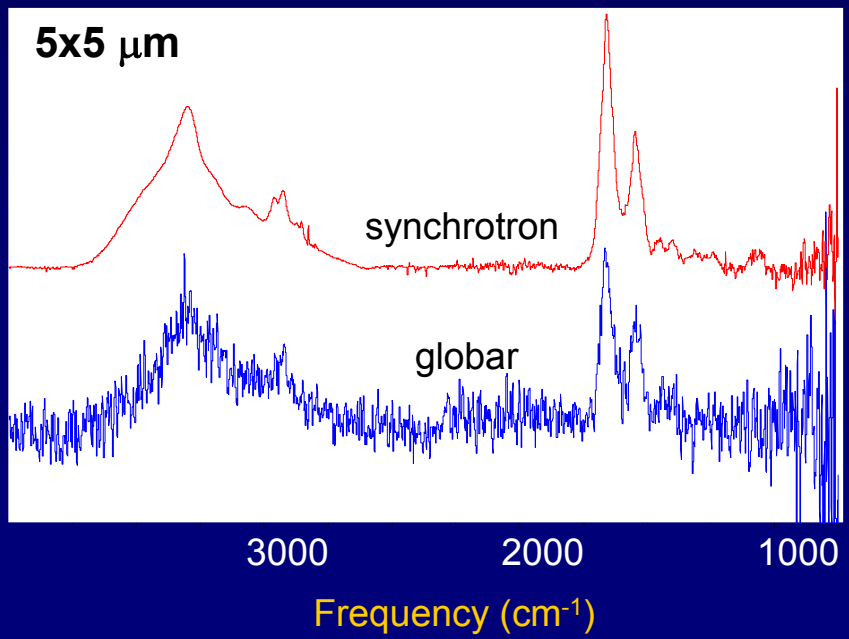


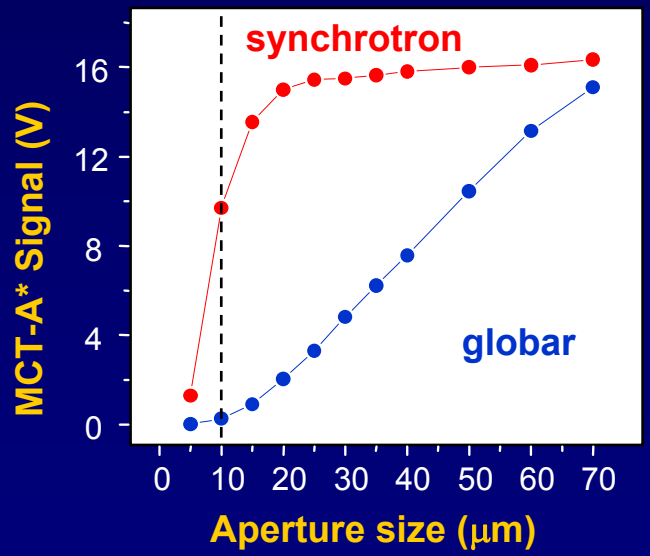
Microspectroscopy: Globar vs. Synchrotron Source



Diffraction Limit	
4000 cm^{-1}	2.5 μm
2950 cm^{-1}	3.4 μm
1650 cm^{-1}	6.1 μm
1200 cm^{-1}	8.3 μm
1000 cm^{-1}	10 μm
600 cm^{-1}	17 μm
200 cm^{-1}	50 μm

- Synchrotron IRMS is diffraction-limited
- Conventional IRMS is throughput-limited





Beamline U10B

Synchrotron infrared beam pipe

Nicolet Magna 860 FTIR

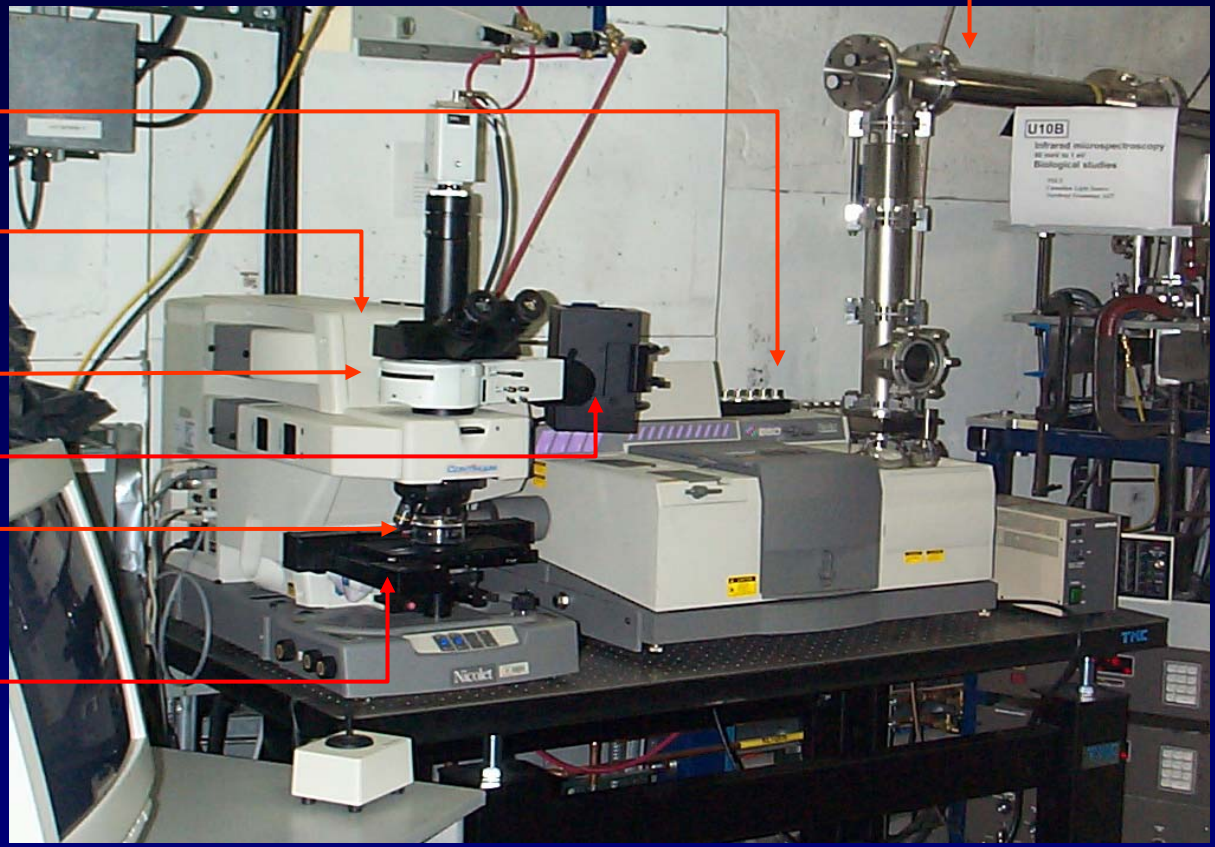
Nicolet Continuμm IR microscope

Filter cube turret

UV light source

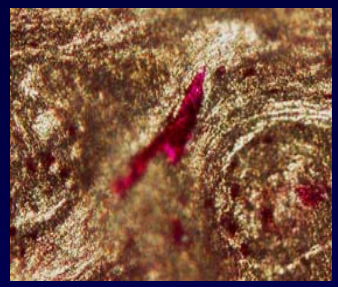
Schwarzchild 32x IR objective

Automated mapping stage



* 4 synchrotron infrared microscopes at the NSLS

Current Applications



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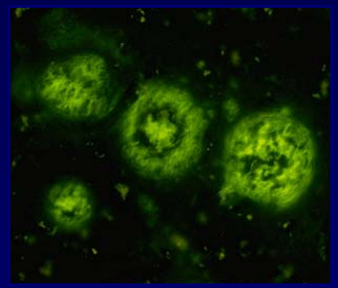

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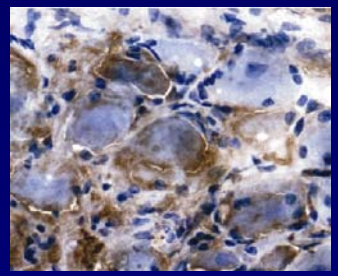

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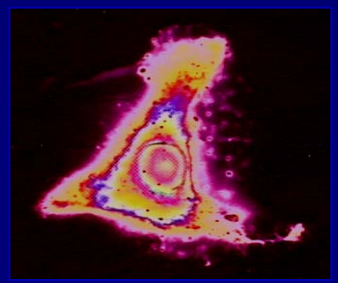

Skin Cancer and Therapies:

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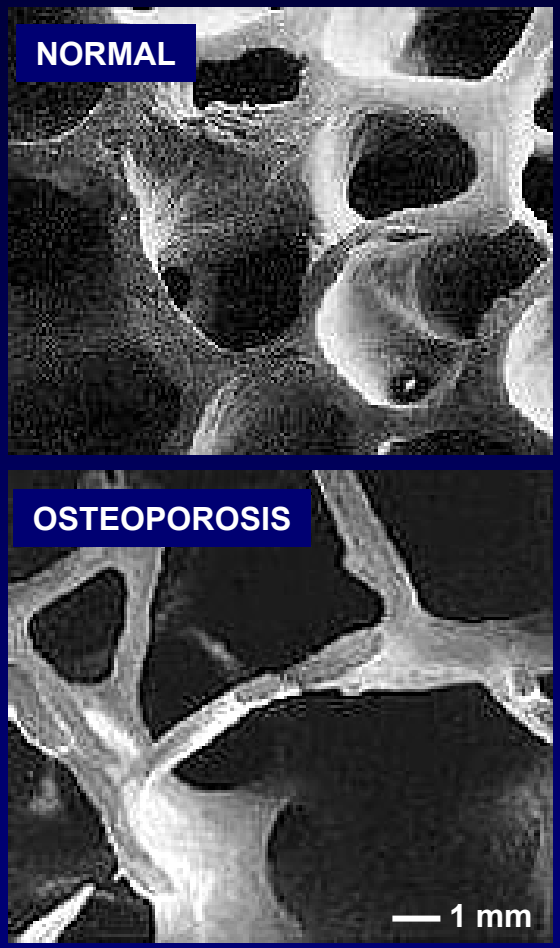
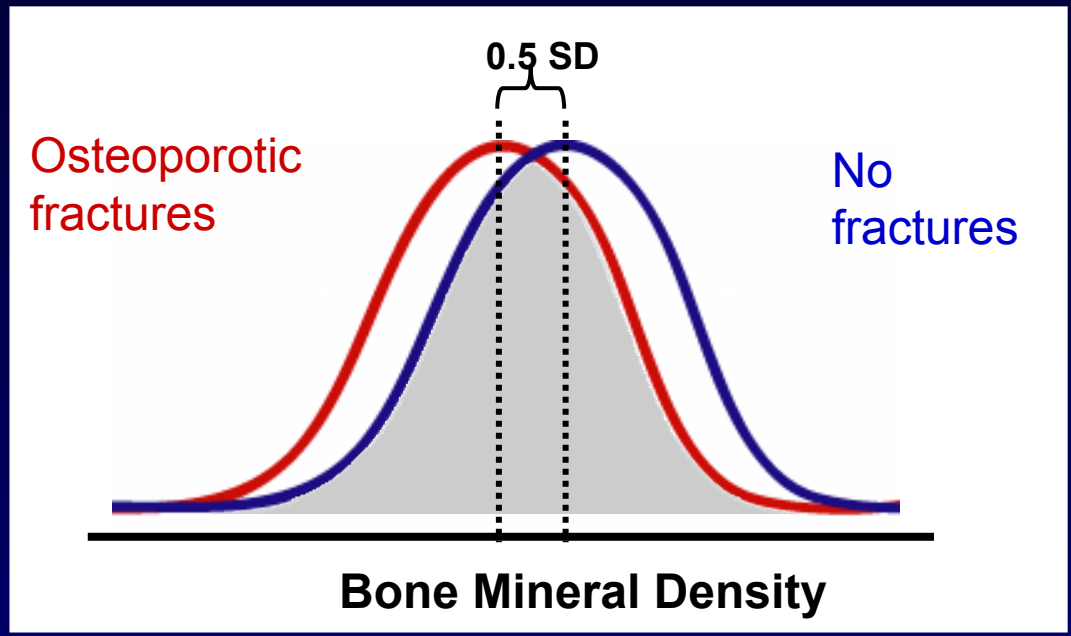
Osteoporosis

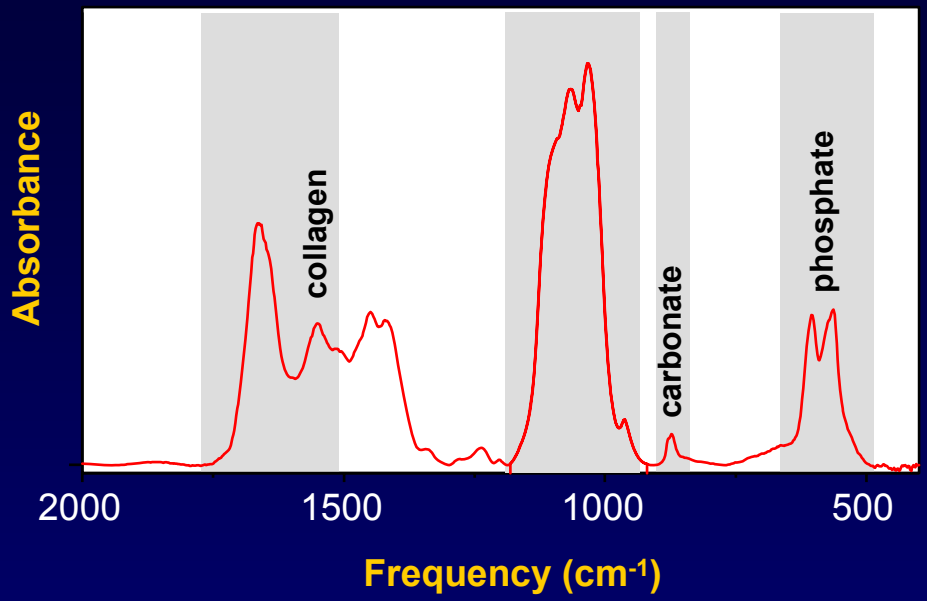


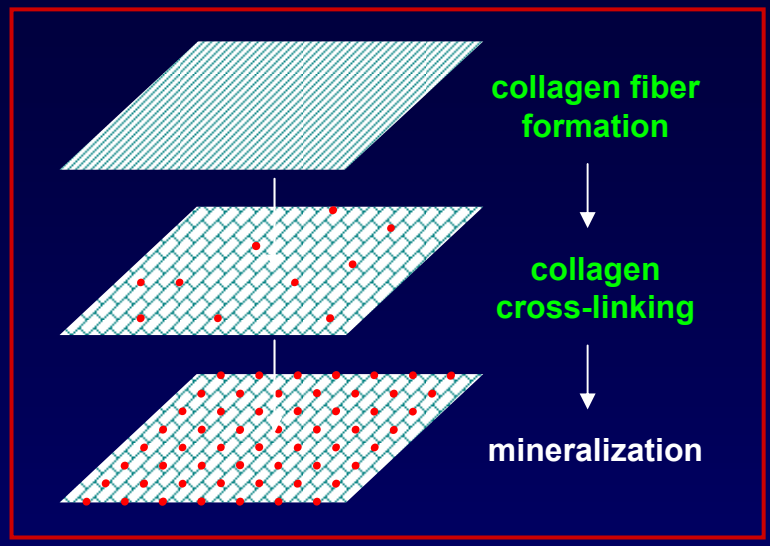


- Bone mineral density is lower in women with osteoporotic fractures
- However, there is a large overlap between the populations

1. Is bone composition different in osteoporosis?
2. Do current osteoporosis treatments affect bone composition?

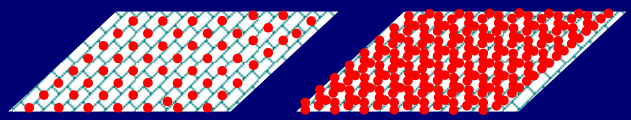
Infrared Spectroscopy of Bone





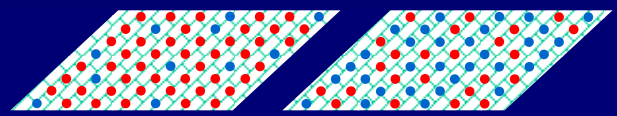
Parameters:

Mineralization (phosphate/protein)



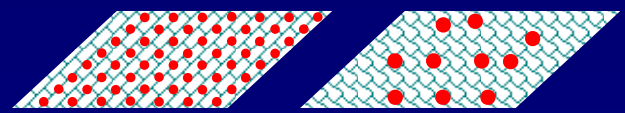
- ↑ increases brittleness
- ↓ increases flexibility
- ↓ decreases strength

Carbonate content (carbonate/protein)



- ~8% carbonate in bone
- content increases with bone age
- ? affect on material properties

Collagen structure

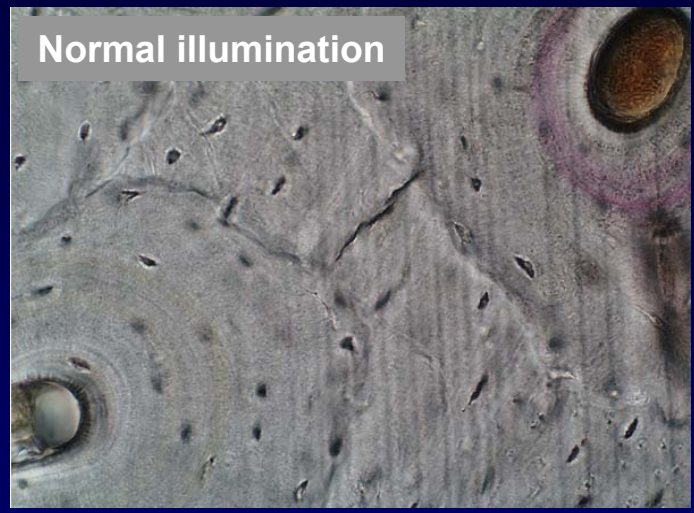


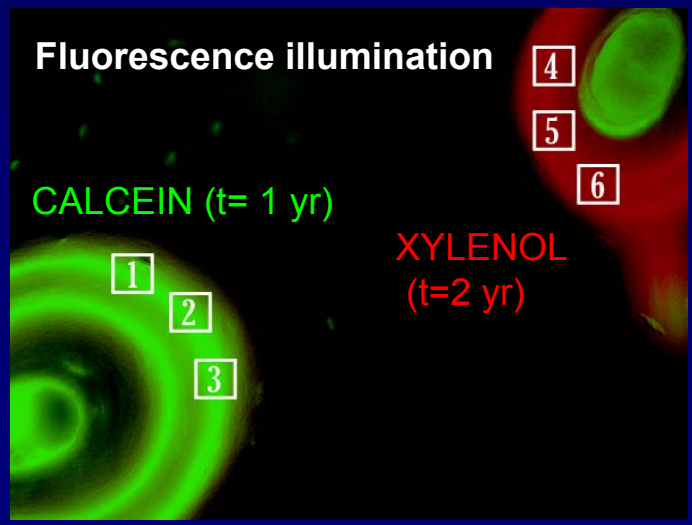
- cross-linking affects mineralization

Does the Chemical Composition of Bone Change in Osteoporosis?

In this study:

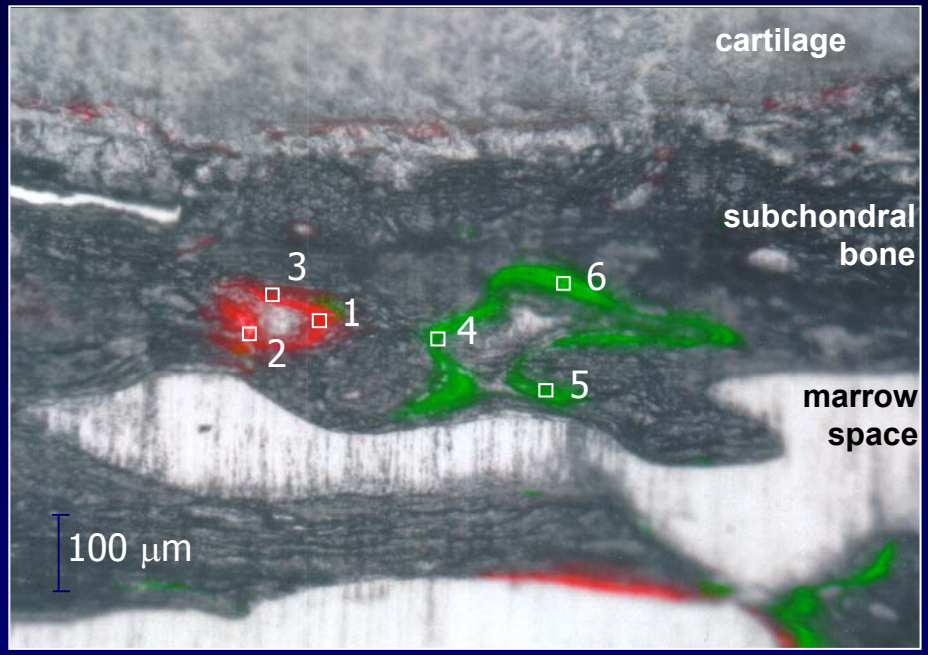
- To "induce" osteoporosis, female monkeys have their ovaries removed (Ovx)
  - 2 colonies: Ovx and Sham-Ovx
- Osteoporosis is treated with nandrolone decanoate
  - 2 colonies: Nan and Control
- Fluorescent drugs are administered and taken up into newly remodeled bone
  - t = 0 yr. post-surgery: tetracycline
  - t = 1 yr. post surgery: calcein
  - t = 2 yr. post surgery: xylenol orange

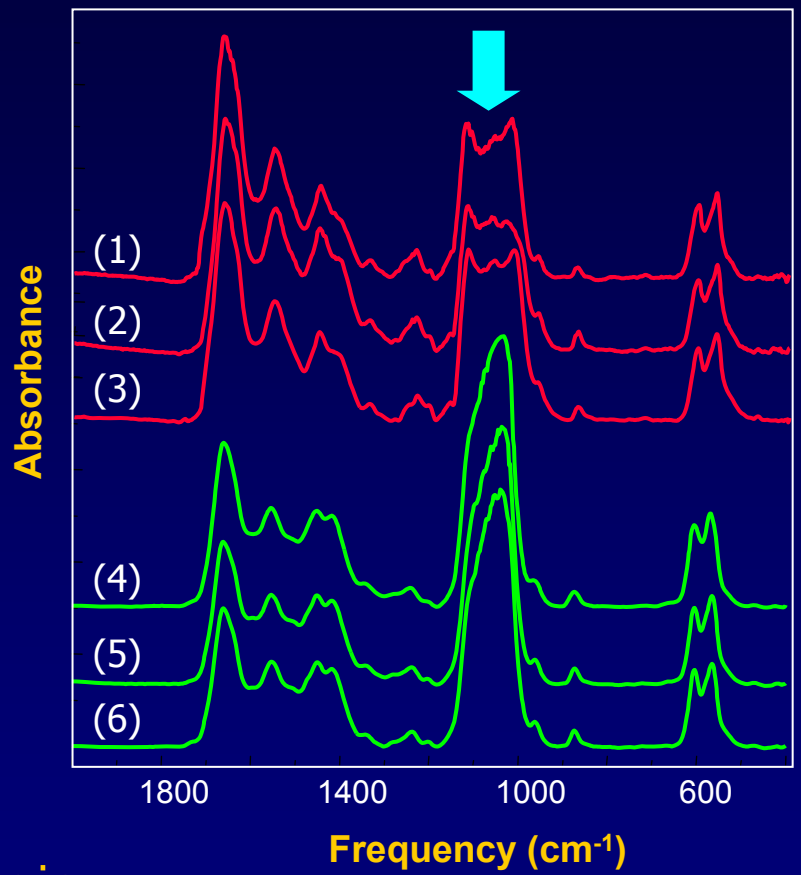




T. Tague, L.M. Miller. *Microscopy Today*, 2: 26-32 (2000).

Bone Mineralization is Slowed in Osteoporosis



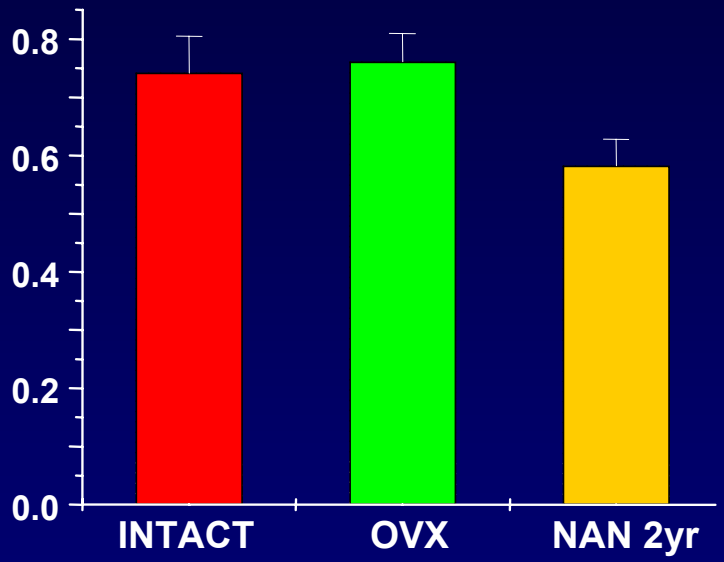


- Rate of bone mineral formation is slower in osteoporosis, i.e. bone quality is reduced

L.M. Miller, J. Tibrewala, C.S. Carlson. *Cellular and Molecular Biology*, 46:1035-44 (2000).

Nandrolone Treatment Affects Carbonate Substitution

Type A / Type B Carbonate Ratio



Hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$

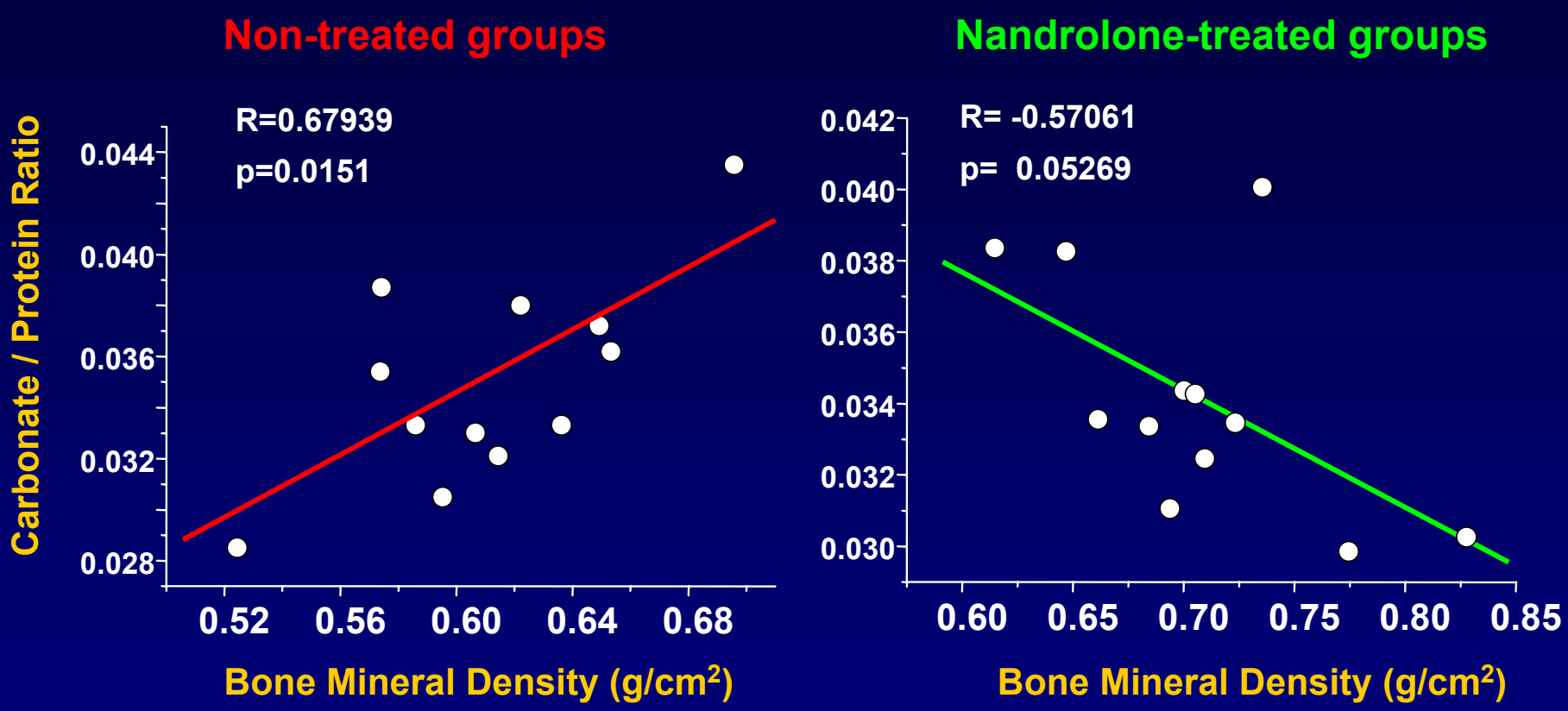
Type B Carbonate
- + CO_3^{2-}, - PO_4^{3-}
- decreases Ca^{2+} content

Type A Carbonate
- + CO_3^{2-}, - OH^-
- increases Ca^{2+} content

- Nandrolone decanoate is an anabolic steroid proposed to increase bone mineral density (BMD)
- Carbonate composition in bone changes with nandrolone treatment
- Changes in carbonate can affect calcium content in bone

R. Huang, L.M. Miller, C.S. Carlson, M.R. Chance. *Bone* 30: 492-97 (2002).

Carbonate Content and Bone Mineral Density



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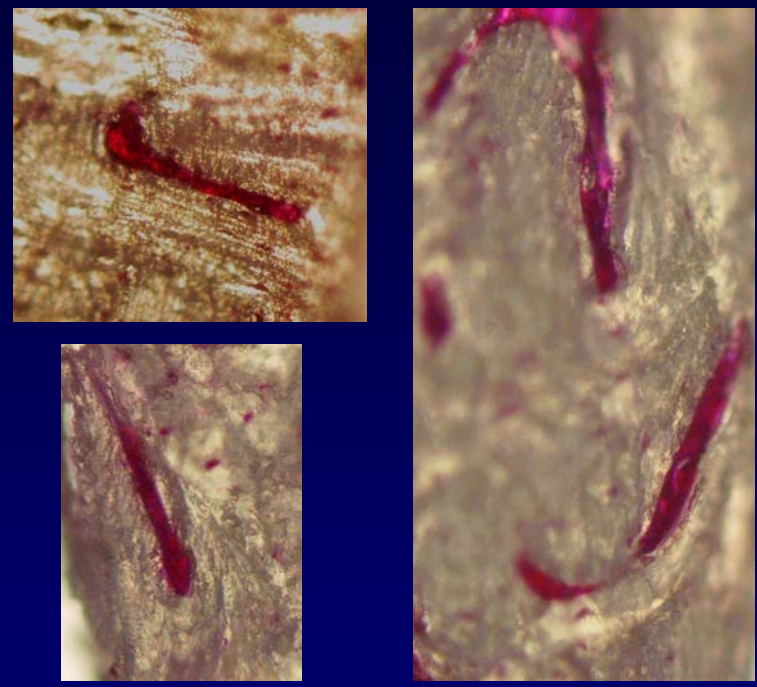
Bisphosphonate Treatment and Microdamage in Bone

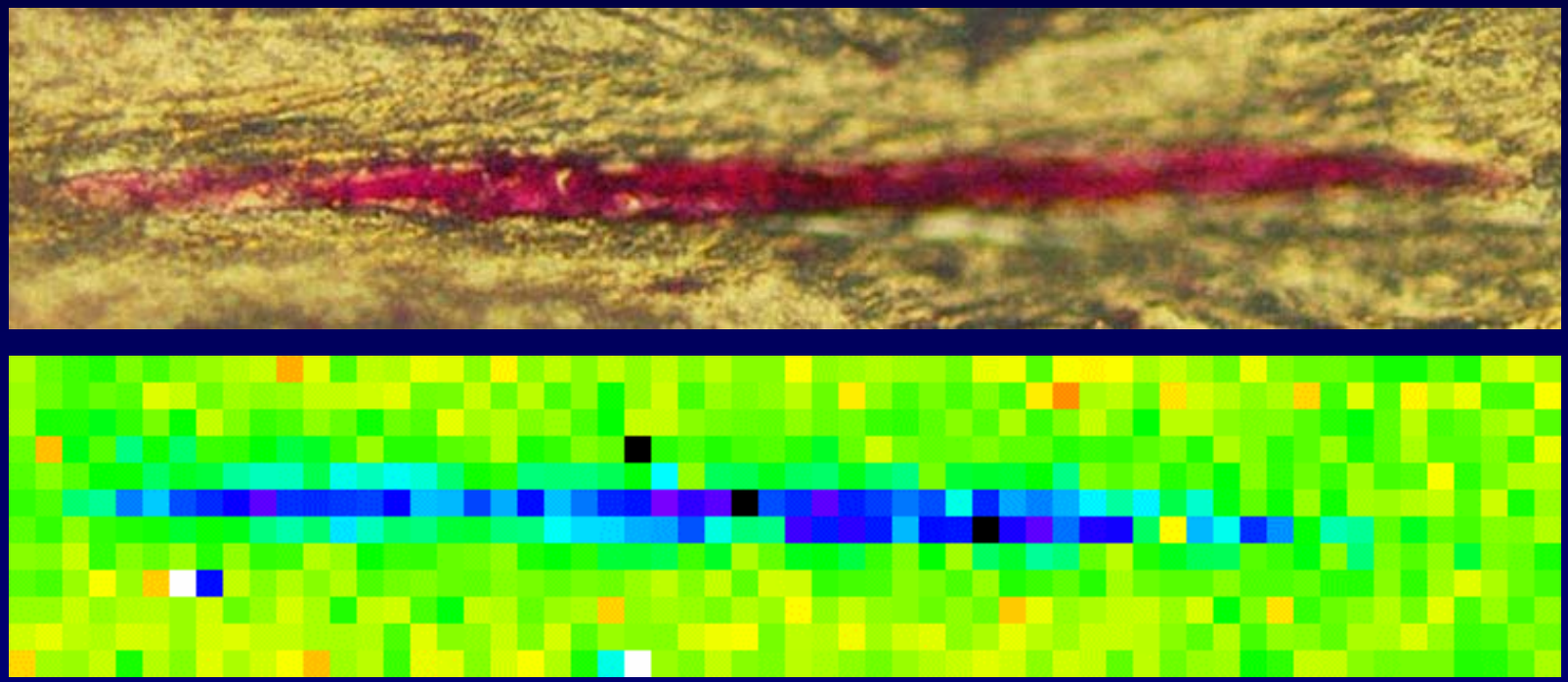
- Through normal activity, microdamage occurs in bone, which is naturally repaired by the bone remodeling process.
- With reduced remodeling, microdamage accumulation can occur.
- In animals with normal bone density, high doses of bisphosphonates increase the occurrence of microdamage.



Why does damage accumulation occur?

- **reduced repair of microcracks**
- **rapid accumulation of microcracks (changes in bone composition)**

Bone Composition Differs in Microcrack Areas



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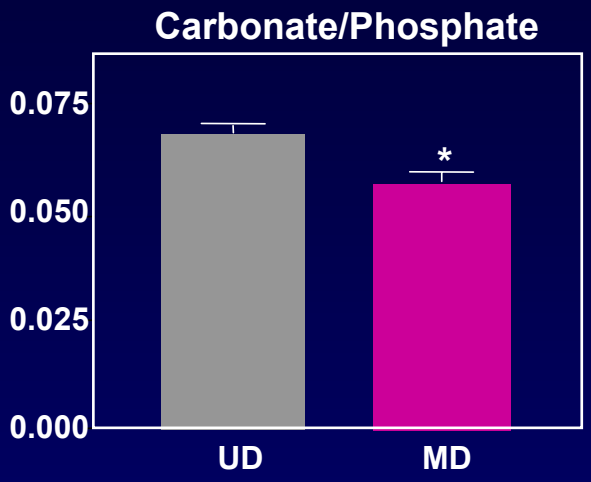
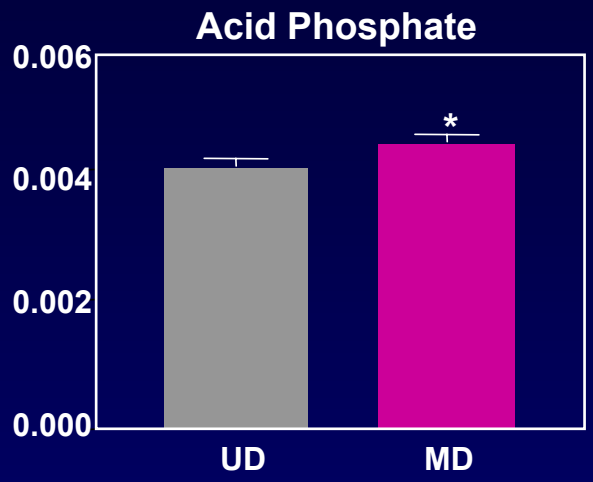
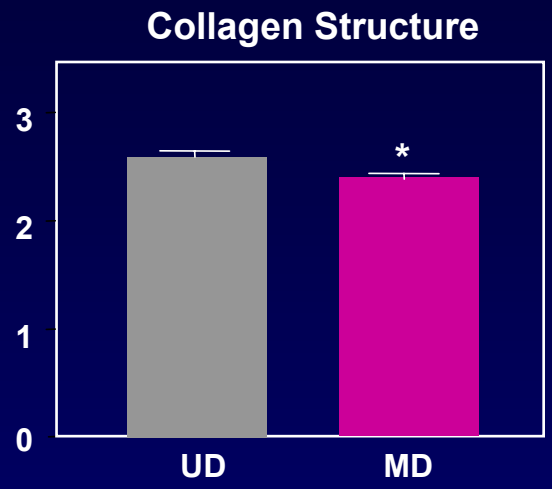
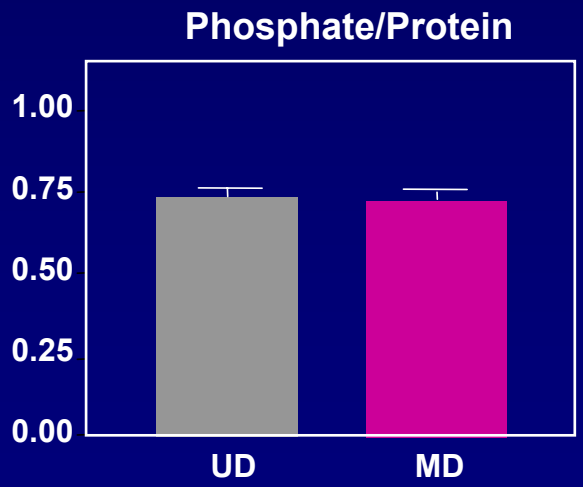




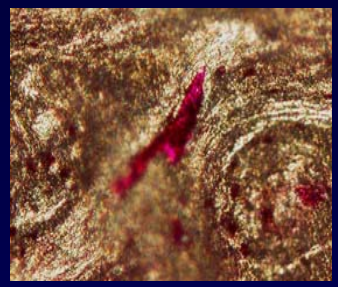




In microdamaged bone:

- Carbonate accumulation in bone is lower
- Acid phosphate content is higher
- Collagen cross-linking is disturbed, consistent with broken/reduced cross-links
- Mineralization level is unchanged, i.e. bone is not more brittle

Current Applications



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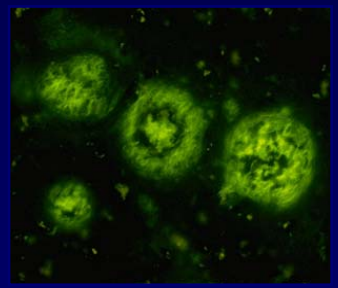

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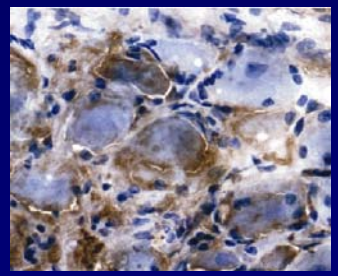

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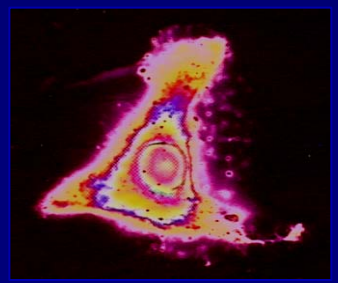

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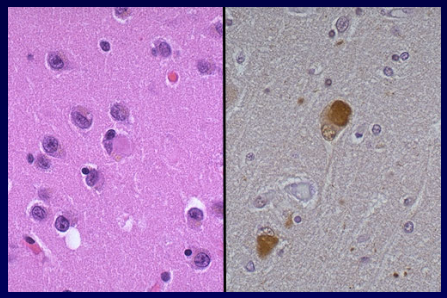
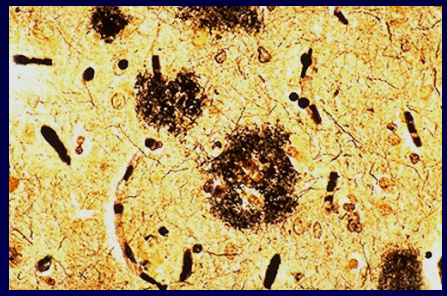
Protein-Folding Diseases



Parkinson's disease



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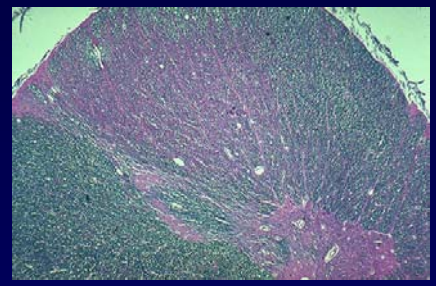
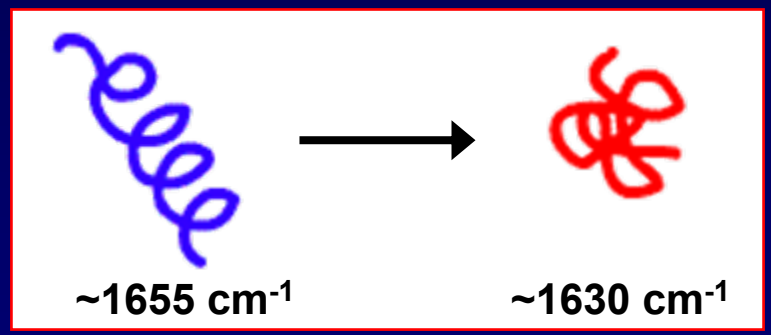
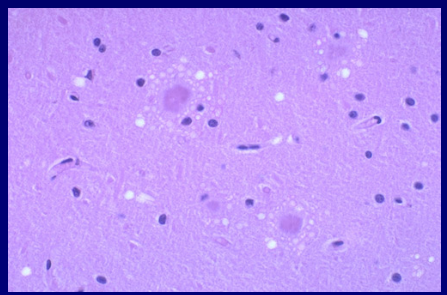
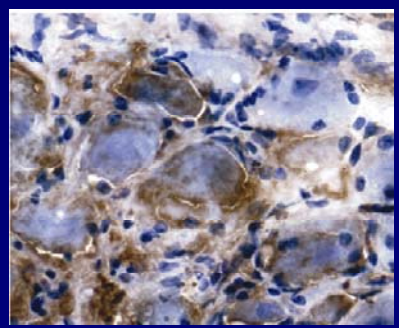


Lou Gehrig's disease

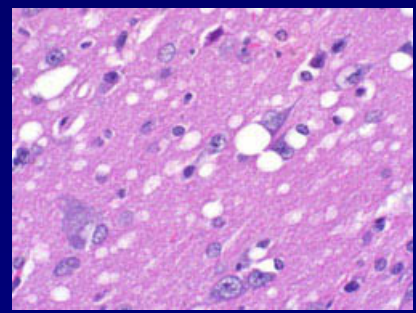




Creutzfeldt Jakob disease

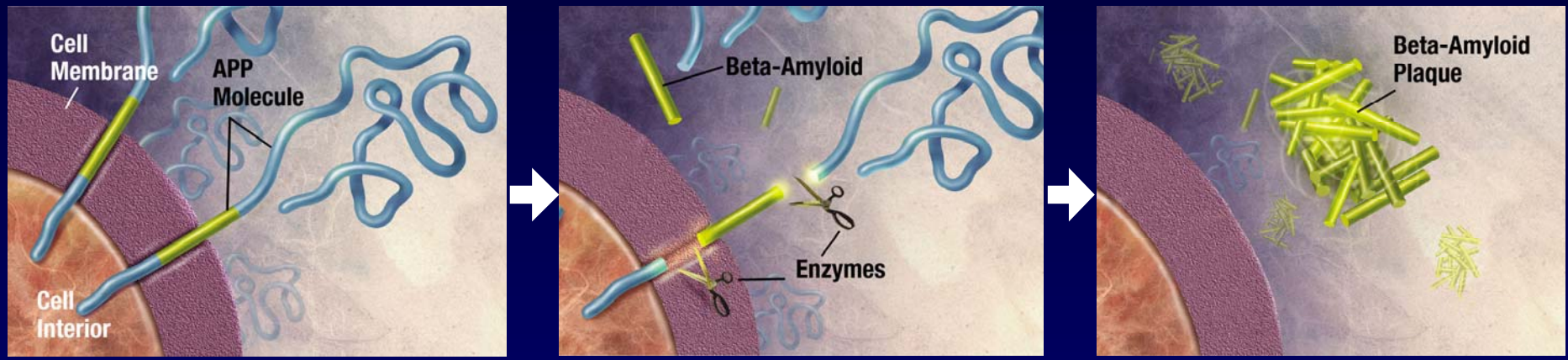


scrapie



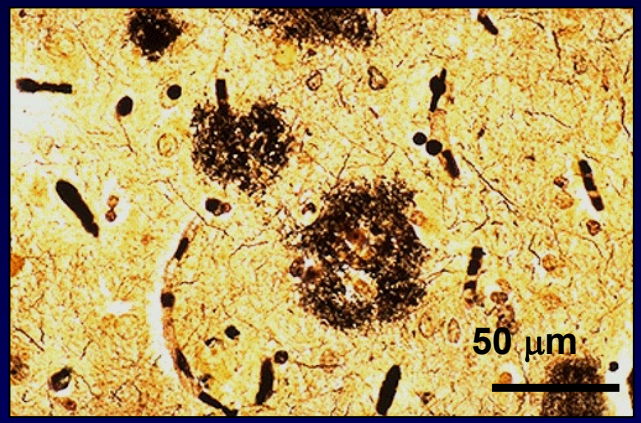
mad cow disease

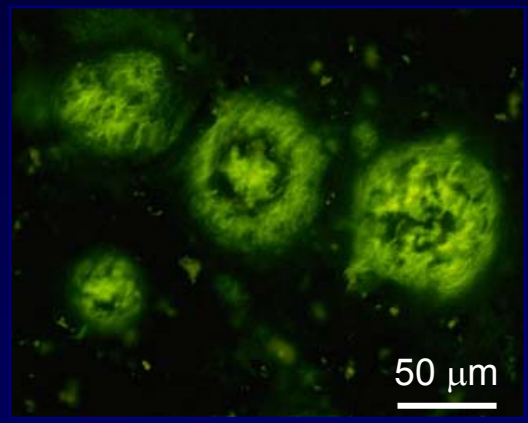
Plaque Formation in Alzheimer's Disease



- ABeta = Amyloid beta (40,42,43 amino acids)
  - Normal Abeta = 40 amino acids
  - Abeta that forms plaques = 42,43 amino acids
- Fragment of larger protein APP (Amyloid Precursor Protein)
- Cut by α-secretase, β-secretase and γ-secretase

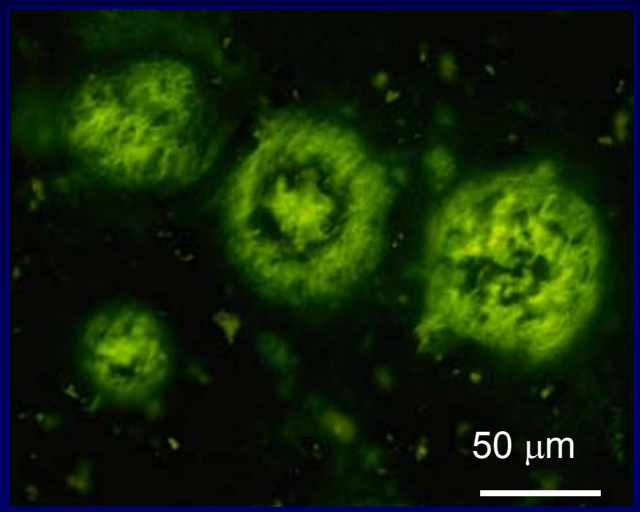
Pathology of Alzheimer's Disease





- Alzheimer's plaques are misfolded Abeta protein. They are 20 – 100 microns in size.
- Plaques are thought to disrupt communication between neurons
- Plaques may also be toxic to neurons, resulting in their death.
- Plaques have recently been identified in other organs: liver, pancreas, ovary, testis, and thyroid.

Unanswered Questions in Alzheimer's Disease



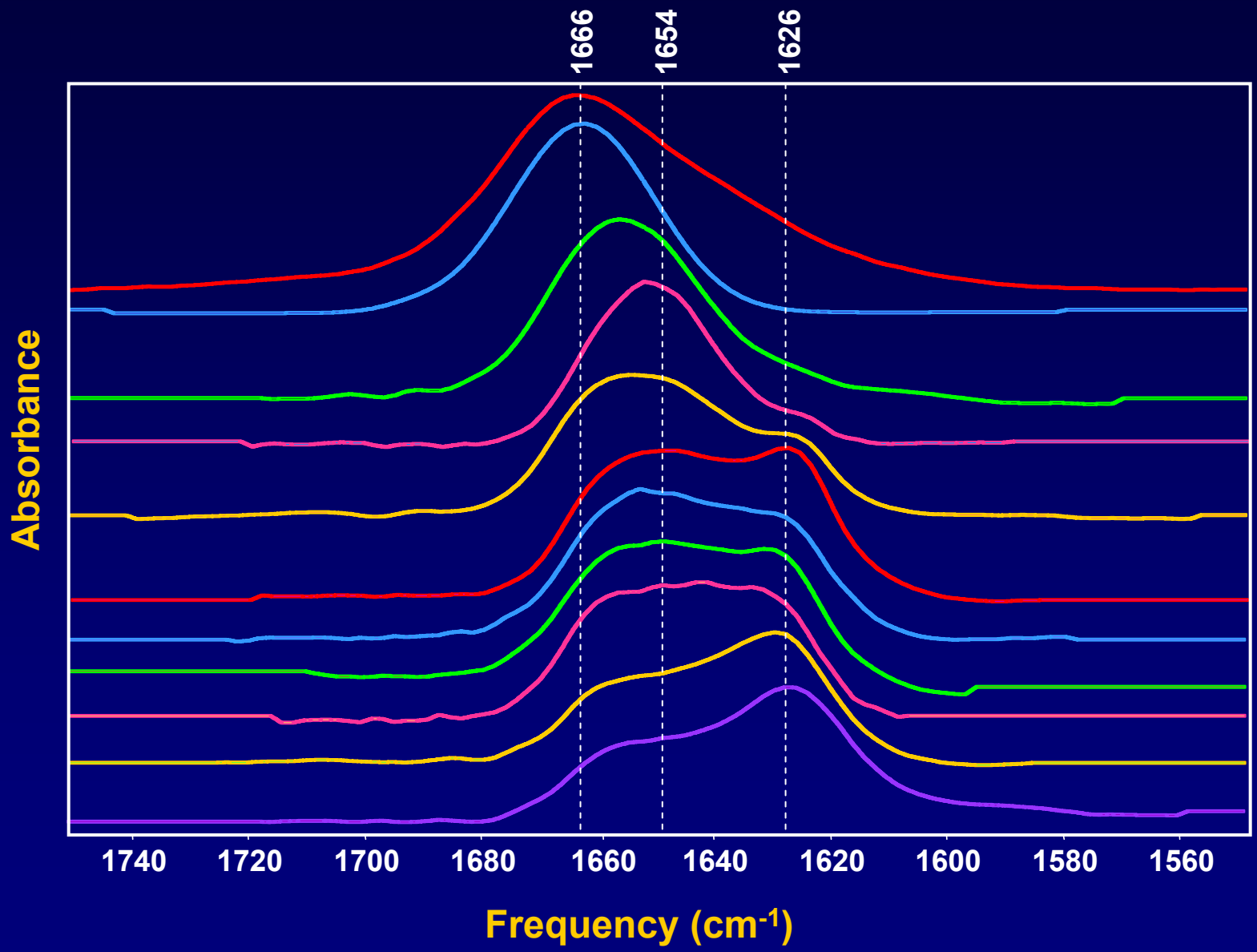
Abeta structural studies in AD have primarily been studied either (1) in vitro, or (2) histologically in tissue:

- Abeta structure in vitro is dependent upon concentration, pH, solvent, and temperature.
- Histological staining for amyloid plaques in other tissues can be non-specific.

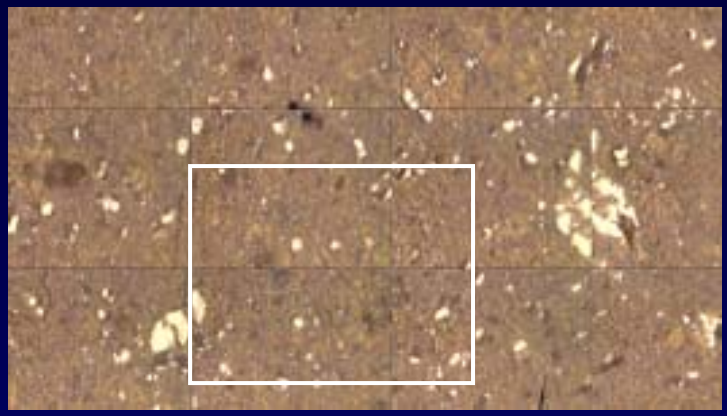
On the microscopic level in tissue, we want to understand:

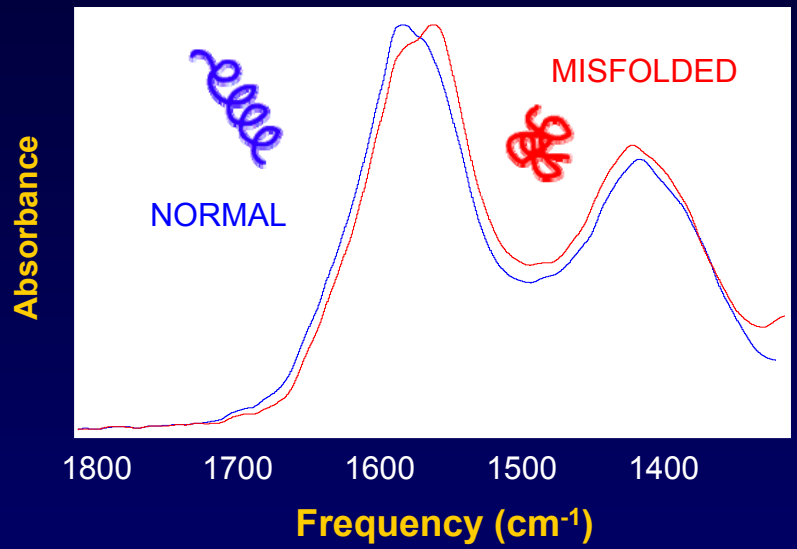
1. What is the structure of Abeta in AD plaques?
2. Are the plaques and tangles identified in other tissues analogous to those found in the brain?
3. Is Alzheimer's disease a systemic disorder, i.e can fibrillary changes observed in other organs aid in the early diagnosis of AD?

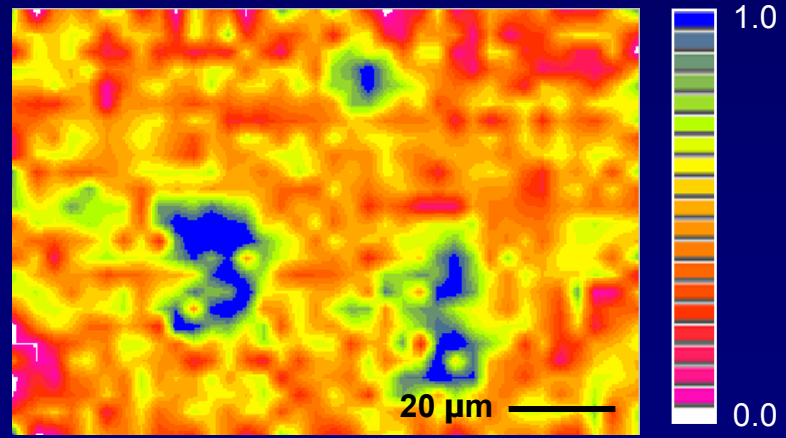
Structure of Abeta$_{1-42}$ in Solution



Protein Aggregation in Alzheimer's Plaques



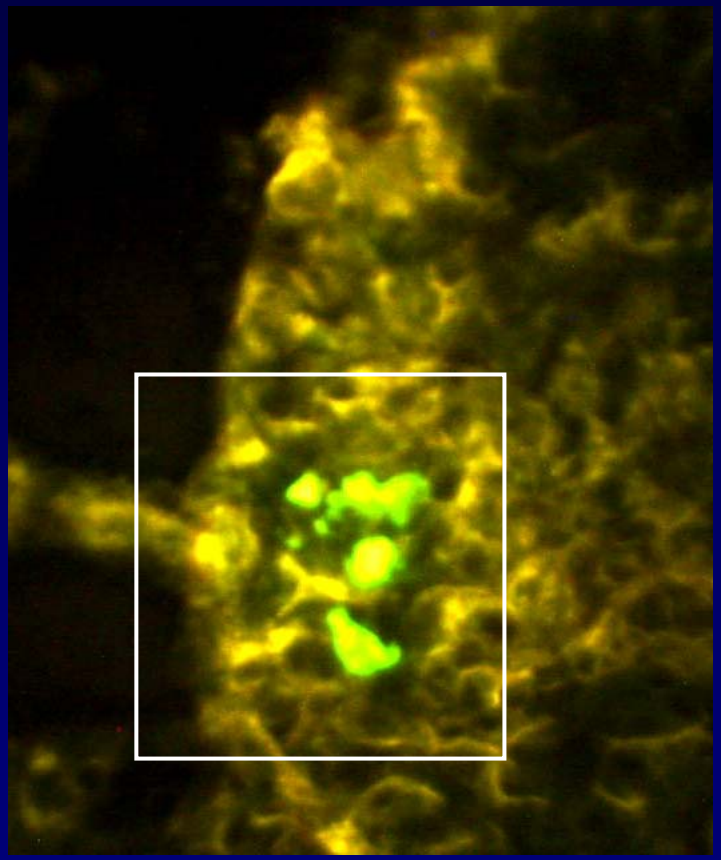


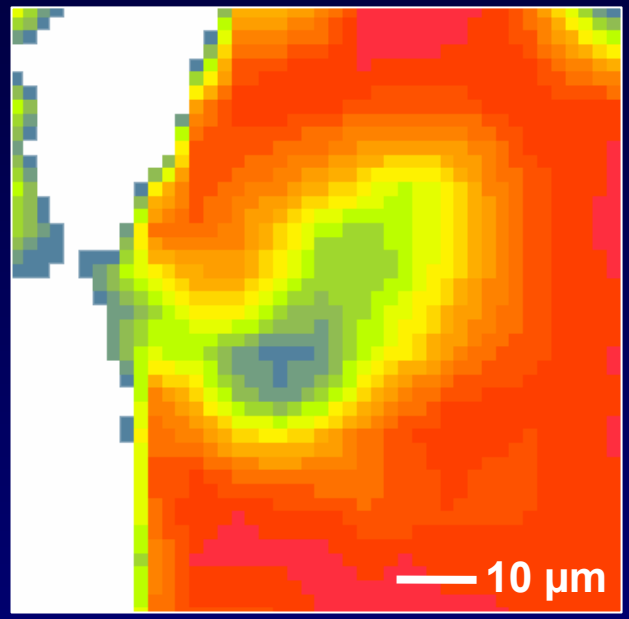


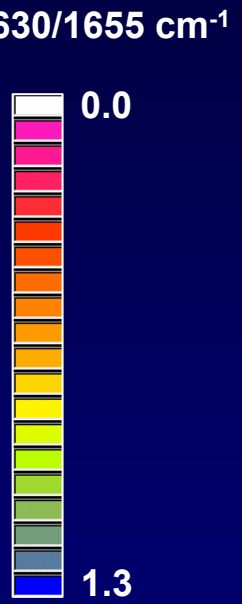
- misfolded, aggregated protein is associated with Abeta plaques

L.M. Miller, P. Dumas, N. Jamin, J.-L. Teillaud, J. Miklossy, L. Forro. *Rev. Sci. Instr.*, 73: 1357-60 (2002).

Plaque Structure in the Pancreas





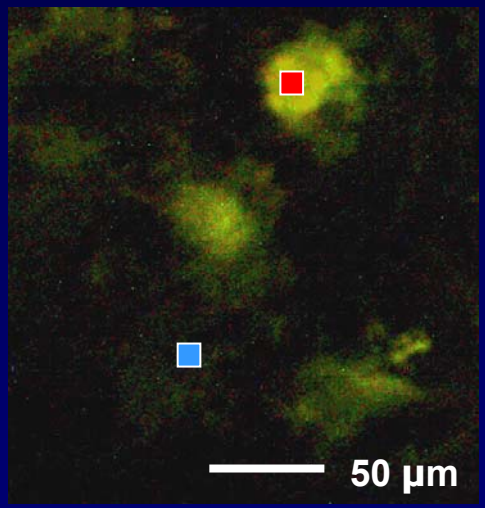


Evidence of amyloid formation in pancreas:

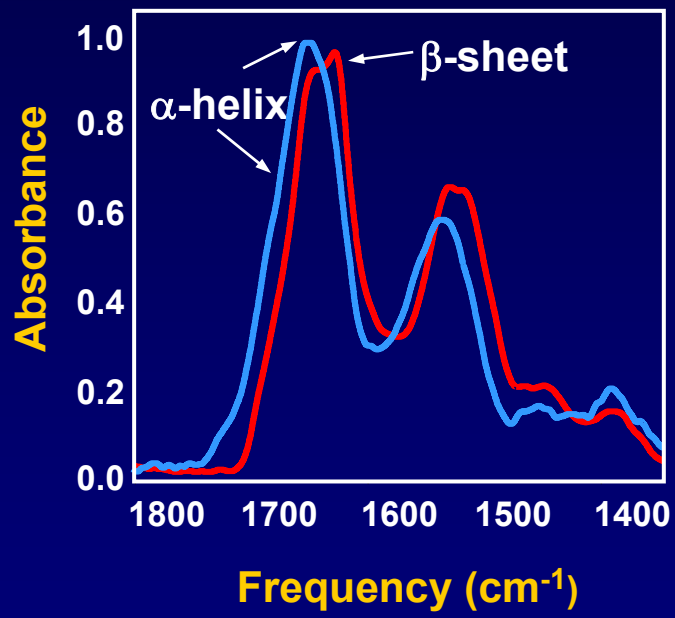
- Thioflavin-S fluorescence
- misfolded protein aggregates by IRMS

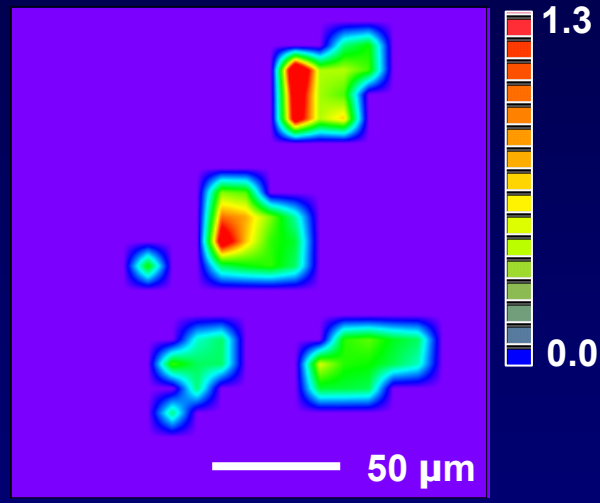
Other Directions in AD Research

- Can bacteria initiate amyloid plaque formation?



Neuronal cells infected with spirochetes for 6 weeks

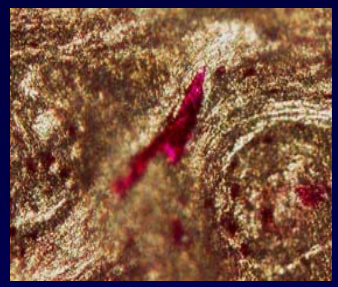




Infrared image: 1630 / 1655 cm^{-1}

J. Miklossy, A. Kis, A. Radenovic, L.M. Miller, L. Forro, et al. *Neurobiology of Aging*, in press (2005).

Current Applications



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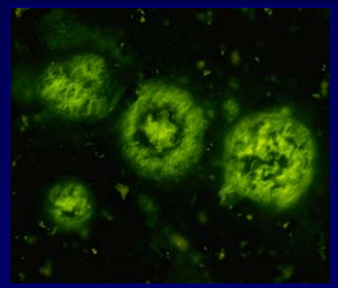

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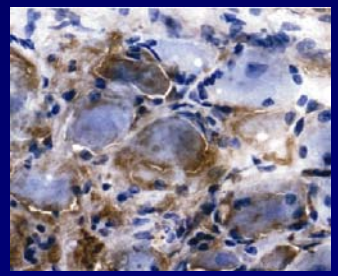

Prion Diseases:

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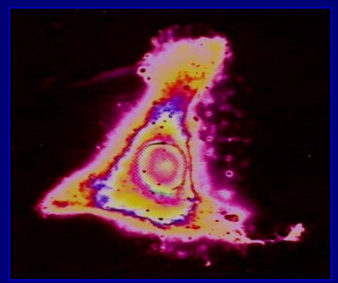
- Michael Beekes (Robert Koch Inst)



Skin Cancer and Therapies:

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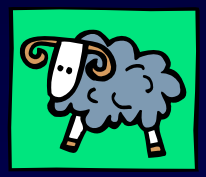
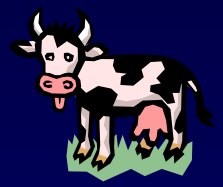
- Abdel Mamoon (Egyptian Atomic Energy Authority)

Prion Protein Diseases



Scrapie (1732)



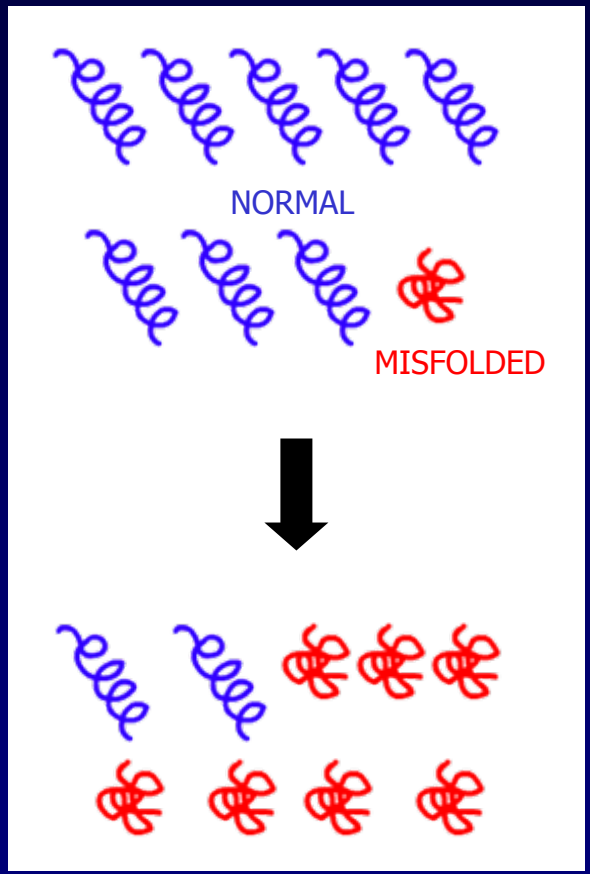
Mad Cow Disease (1987)

Creutzfeldt-Jakob Disease (1920/21)
Kuru (1957)
New-variant Creutzfeldt-Jakob Disease (vCJD) (1996)

Chronic Wasting Disease, Feline Spongiform Encephalopathy, Transmissible Mink Encephalopathy

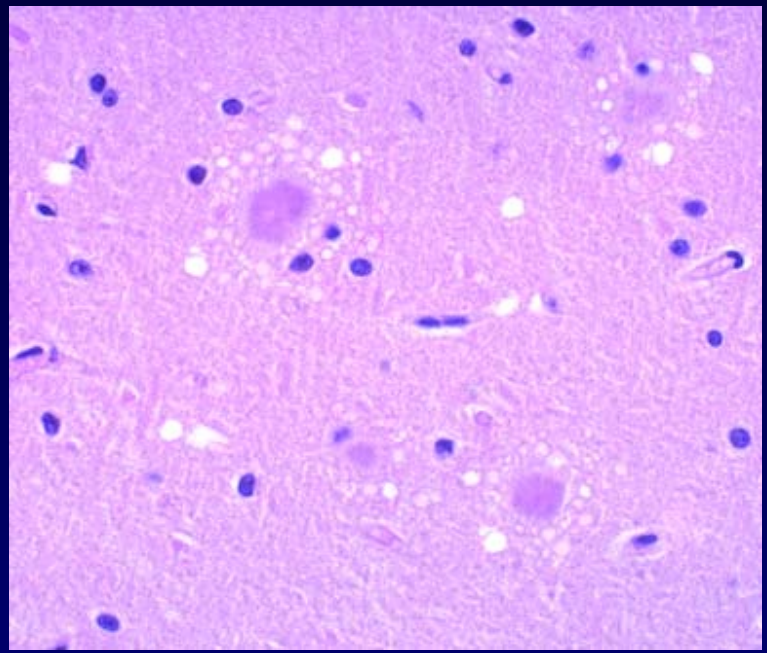
263K Scrapie-hamster

“Infectivity” of Misfolded Prion Proteins



- Prion proteins naturally occur in the nervous tissue.
- Their functions is unclear, but it is thought that they may have antioxidant function.
- Prion proteins can misfold and become “infectious”, causing more normal protein to misfold.
- Misfolded prion proteins form aggregates. They are small and diffuse, about 5 – 30 microns in size.
- Plaques are thought to disrupt communication between neurons
- Plaques may also be toxic to neurons, resulting in their death.

Unanswered Questions in Prion Diseases



Variant Creutzfeldt Jakob disease

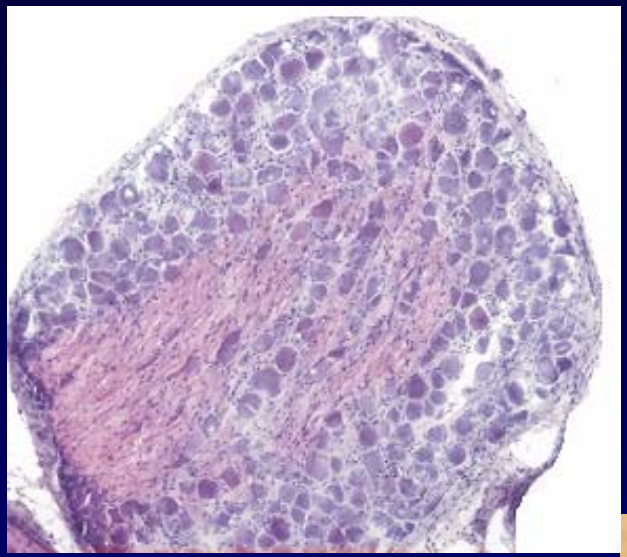
Prion protein structural studies have primarily been studied either (1) in vitro, or (2) with immunohistochemistry:

- PrP^{Sc}-folding in vitro is dependent upon concentration, pH, solvent, and temperature.
- Antibodies to the prion protein are not structure-specific, and only show where the protein is located in high concentration.

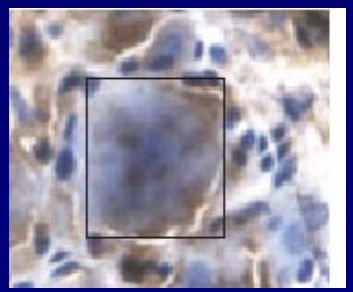
On the microscopic level in tissue, we want to understand:

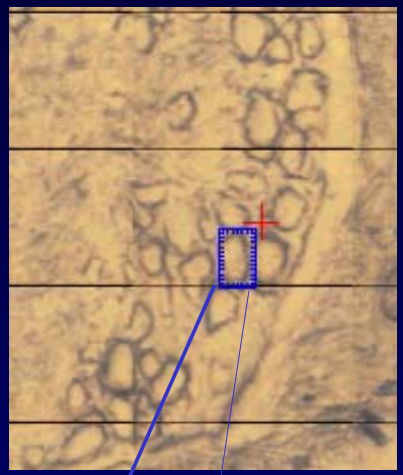
1. How and why do prion proteins misfold?
2. How is the misfolded prion protein infectious, i.e. how does the misfolded protein "catalyze" further misfolding?
3. What is the structure of the misfolded prion protein within the tissue?

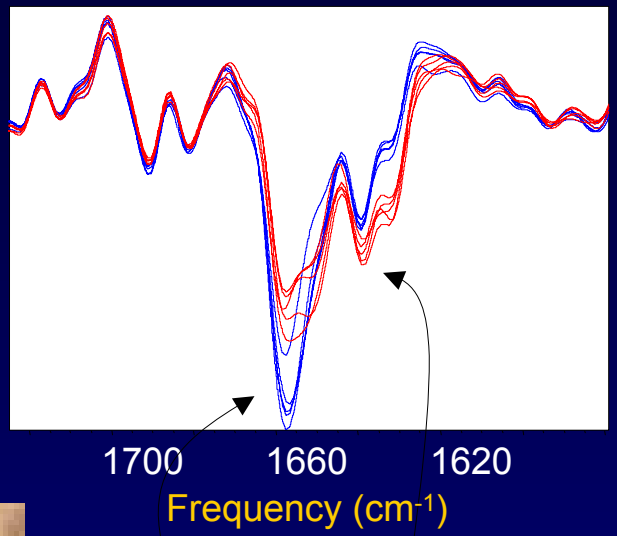
Prion Protein Structure and Location

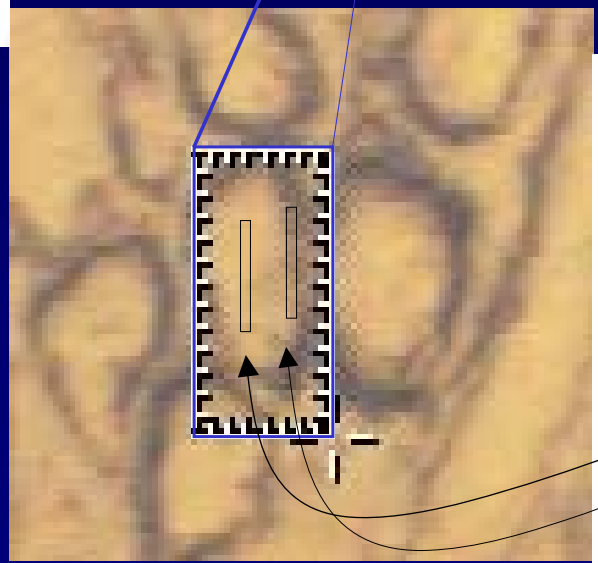


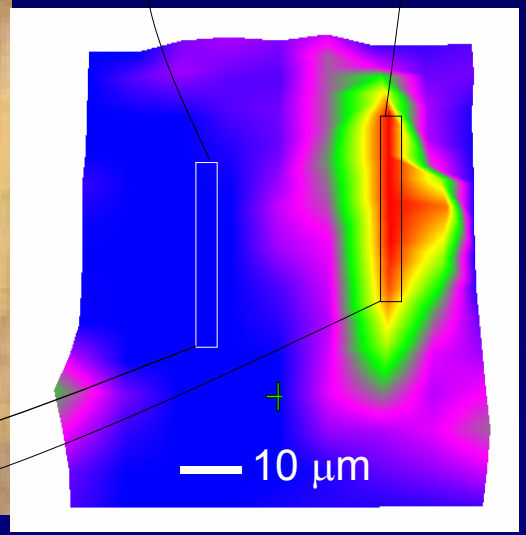
- Hamster scrapie 263K
- Dorsal root ganglia
- Terminally infected





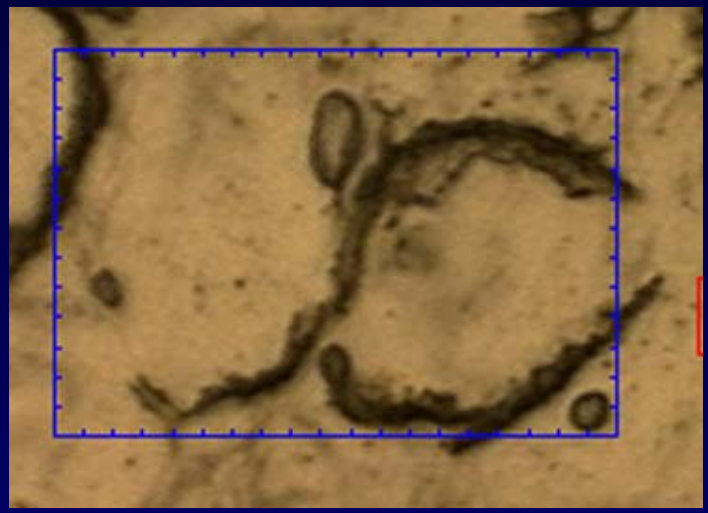


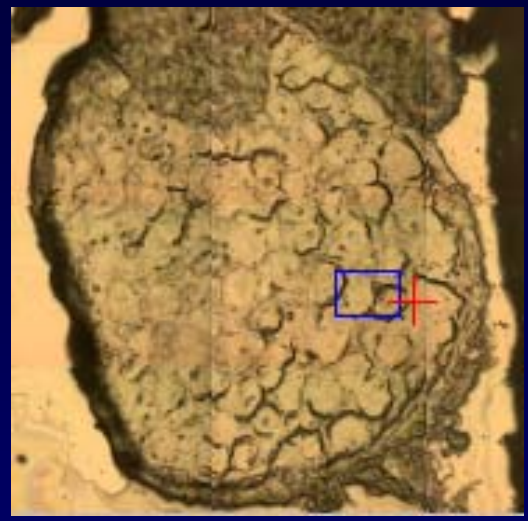


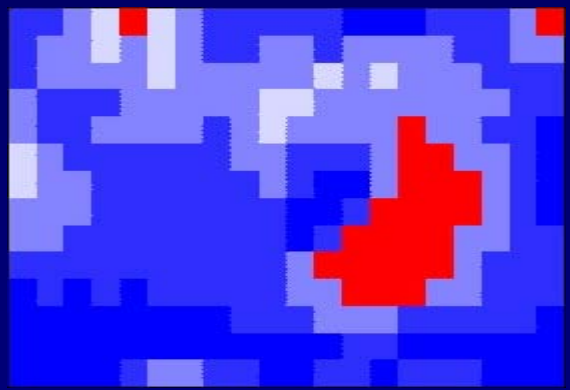


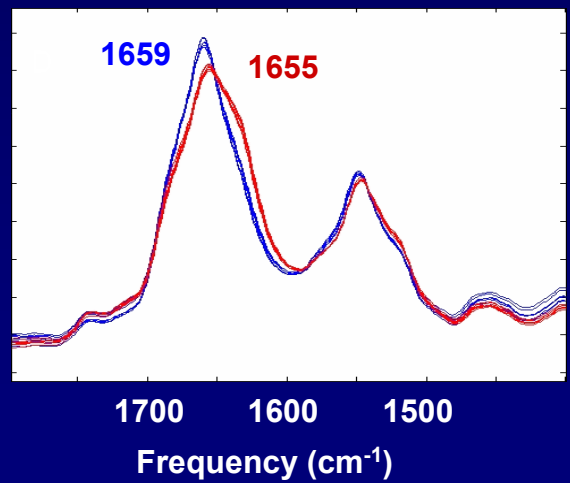
J. Kneipp, L.M. Miller, M. Kittel, M. Beekes, D. Naumann. *BBA, 1639: 152-58 (2003).*

Not All Cells are Affected



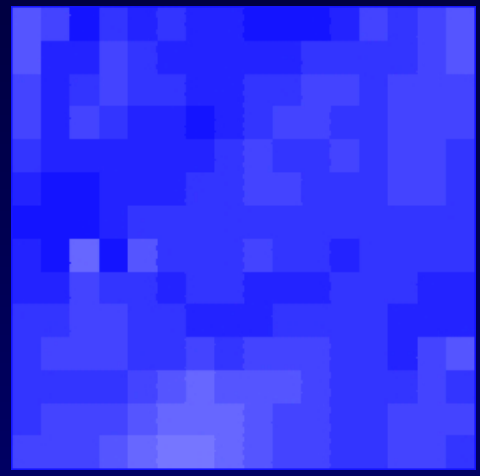


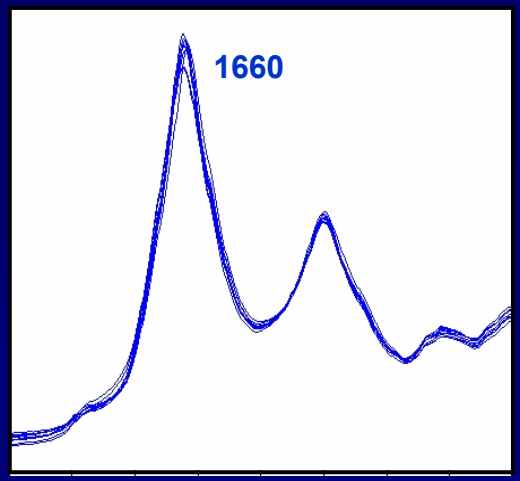


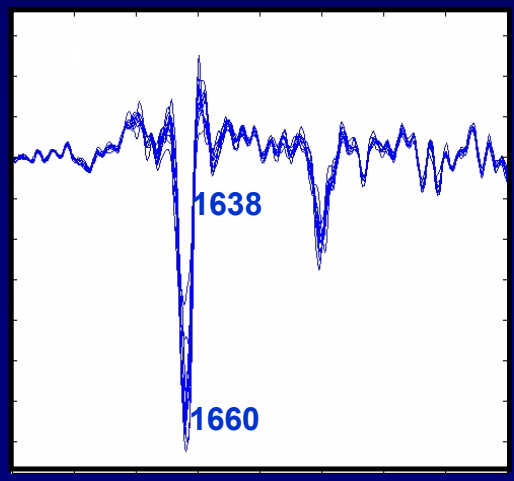


Q. Wang, A. Kretlow, M. Beekes, D. Naumann, L.M. Miller. *Vibr. Spectr.,* in press *(2005).*

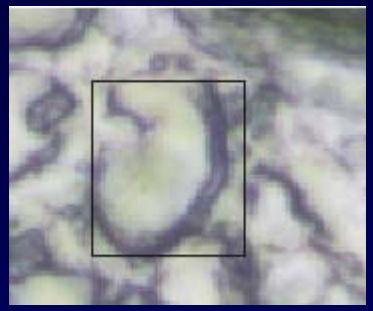
Control Animals Do Not Exhibit Misfolded Prions



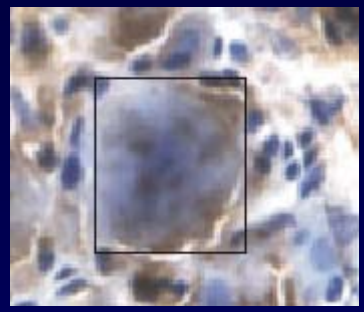




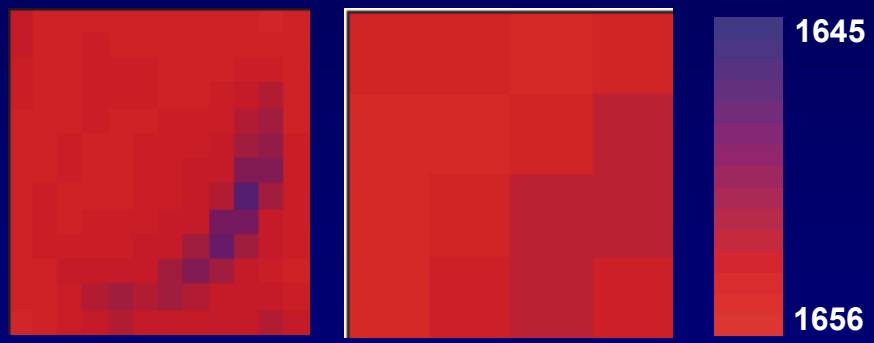
Importance of a Synchrotron IR Source



unstained cell

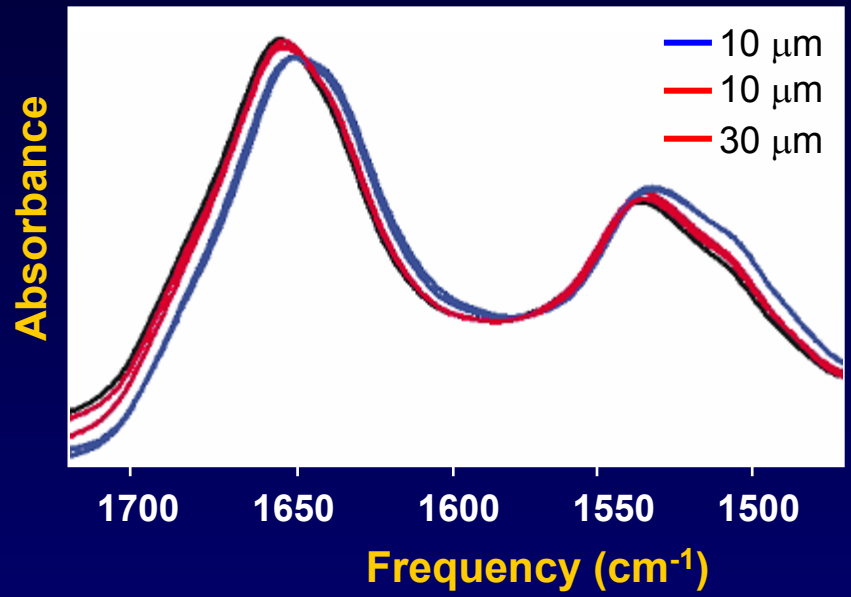


stained cell



10 μm aperture

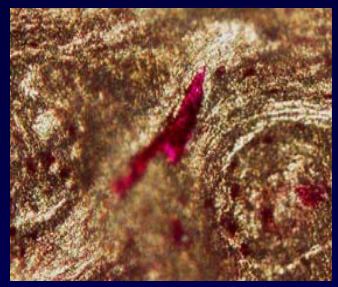
30 μm aperture



- Misfolded prion protein within tissue is β-sheet structure
- Proteins are generally located near (or in) the cell membrane
- Not all neurons are affected equally

J. Kneipp, L.M. Miller, M. Kittel, M. Beekes, D. Naumann. *BBA, 1639: 152-58 (2003).*

Current Applications



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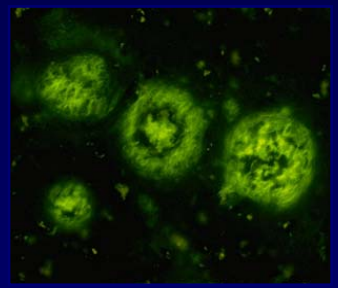

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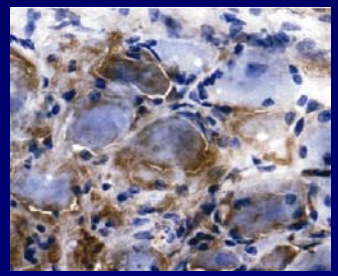

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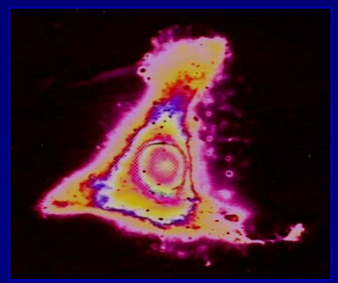

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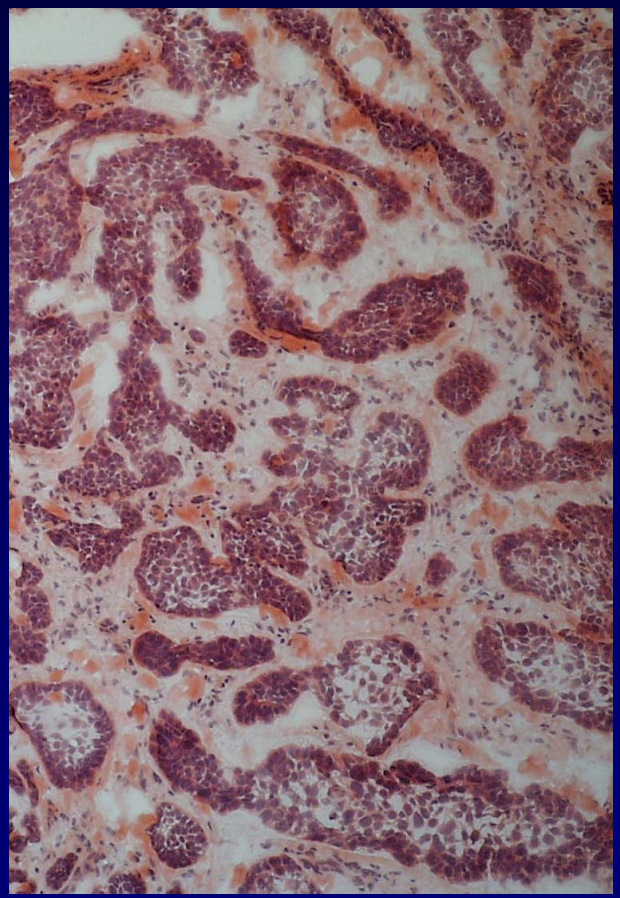
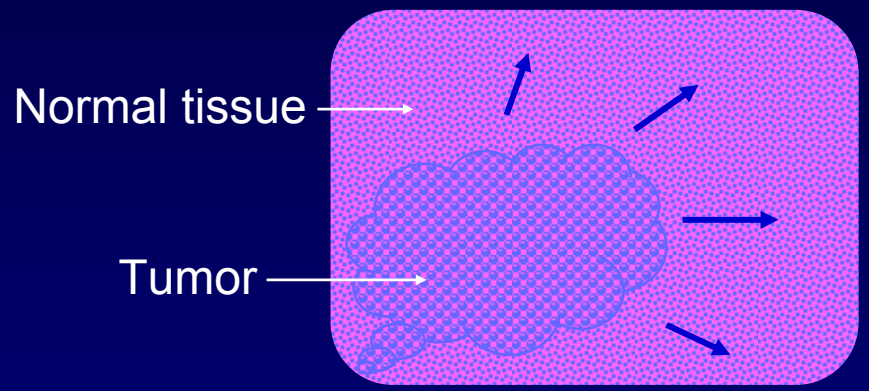
Gelatinase B is Implicated in Tumor Growth

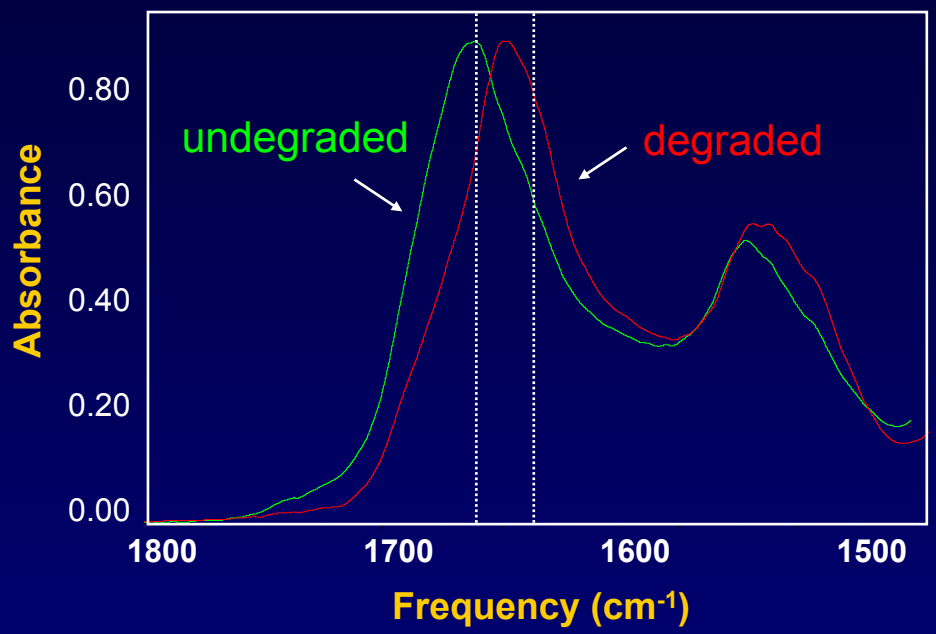


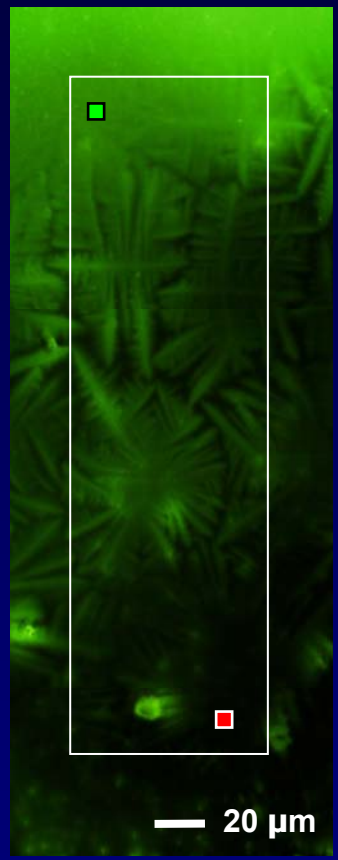
- Tissue is made of cells and extracellular matrix (ECM)
- For tumor to grow, these must be degraded

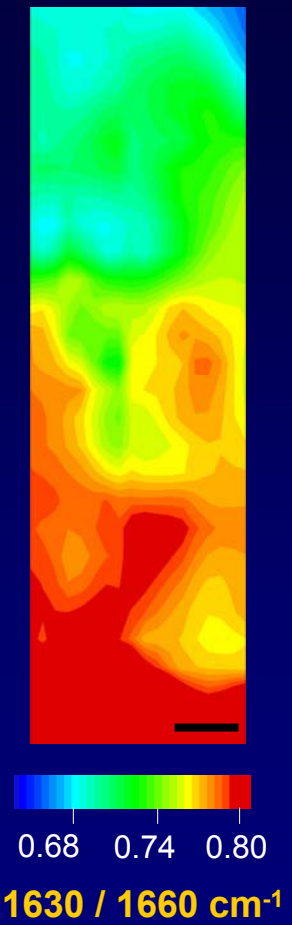


- Gelatinase enzyme is implicated in degradation of ECM

Green Gelatin Degradation by Gelatinase B (MMP-9)



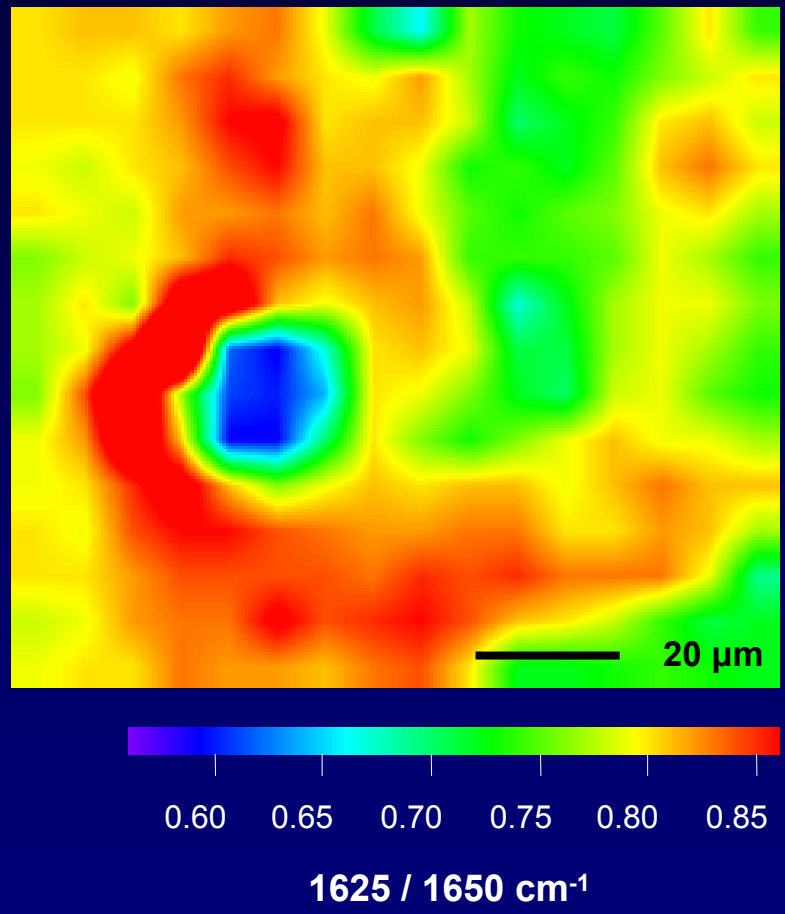




- Green gelatin can be used to visibly identify degradation of gelatin films by gelatinase B
- The structure of degraded gelatin can be determined with IR micro-spectroscopy

S. Federman, L.M. Miller, I. Sagi. *Matrix Biology*, 21: 567-77 (2002).

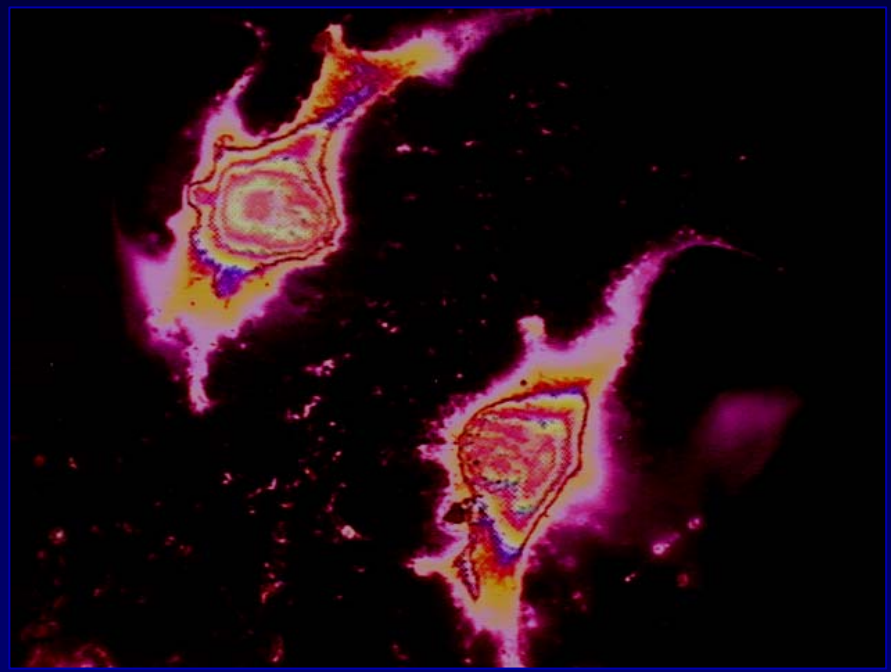
Gelatin Degradation by HT-1080 Tumor Cells



- IR micro-spectroscopy can be used to identify gelatin degradation by HT-1080 tumor cells

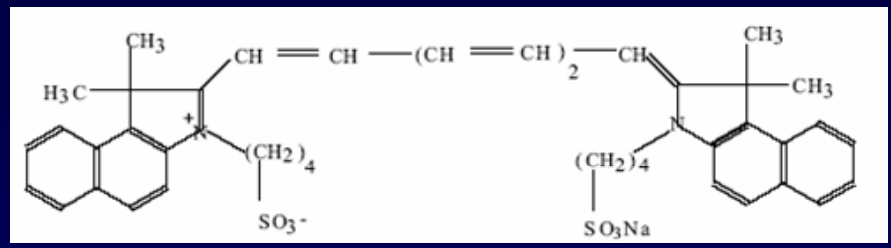
S. Federman, L.M. Miller, I. Sagi. *Matrix Biology*, 21 567-77 (2002).

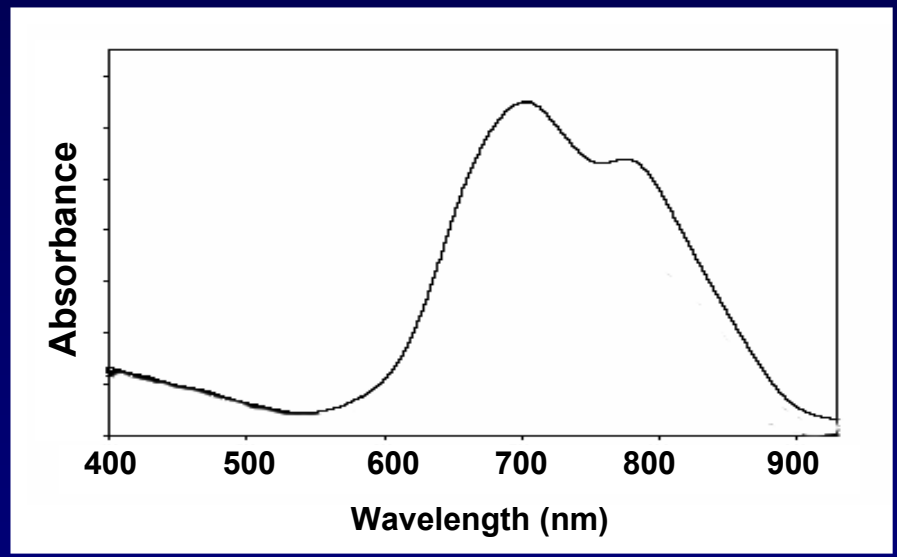
Human Skin Melanoma and Photodynamic Therapy



- Human melanoma cells
- 100 μM indocyanine green
- Ti-Sapphire laser (780 nm)
  - 50 – 150 mW / cm^2
  - 10 – 30 min irradiation
- Re-incubate 48 hours

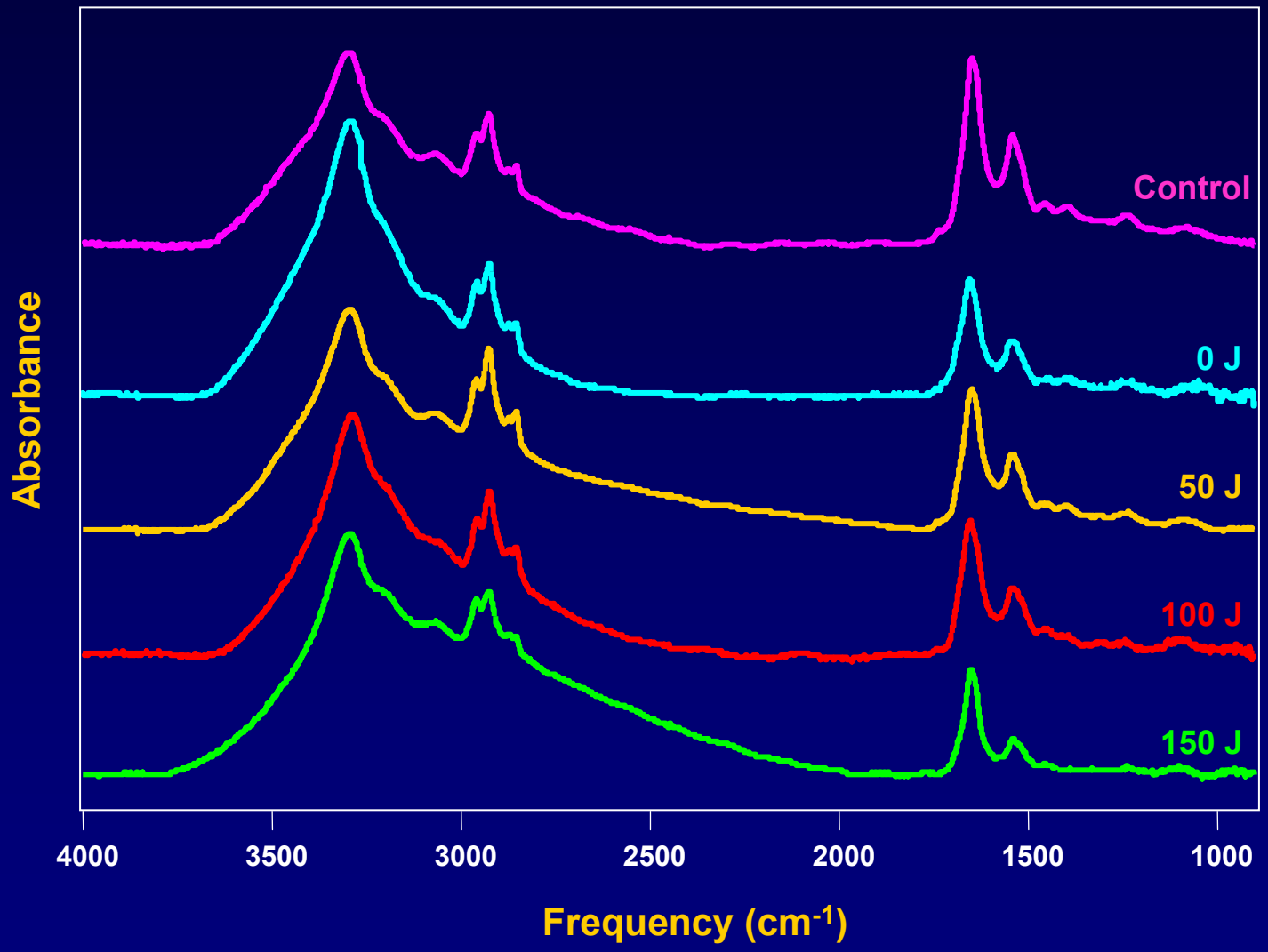
Indocyanine Green





- **Assess cell death with trypan blue staining**
- **Probe chemical composition with FTIR microscope (~50 cells per time point)**

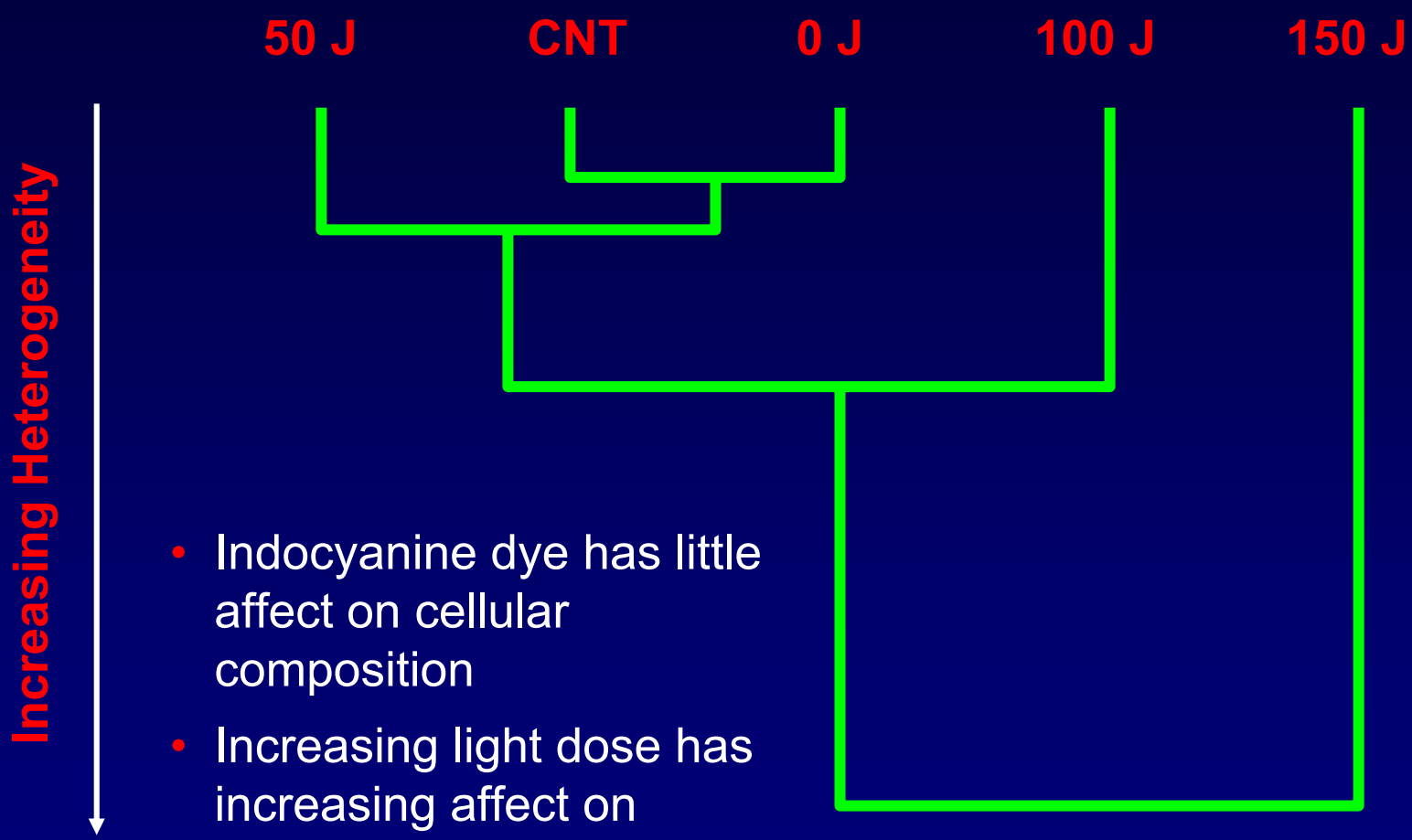
Affect of PDT on Melanoma Cell Composition



% Survival
(of control)

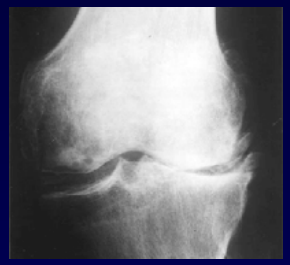
Control	100 %
0 J	100 %
50 J	95 %
100 J	85 %
150 J	80 %

Cluster Analysis of PDT Therapy

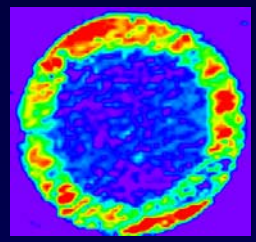


- Indocyanine dye has little affect on cellular composition
- Increasing light dose has increasing affect on composition
  - **Largest affect in protein and nucleic acid structure**

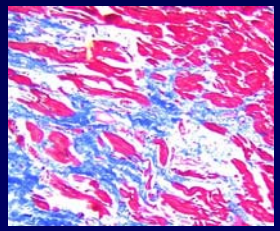
Other Applications in the Biosciences



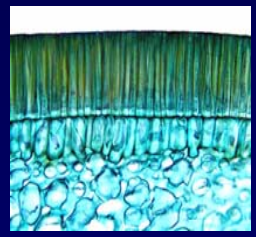
Osteoarthritis:
- Cathy Carlson (Univ. of Minnesota)
- David Hamerman (AECOM)



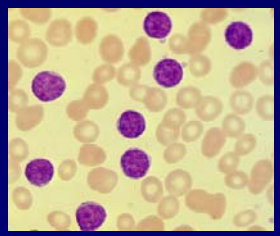
Biopolymerization:
- Richard Gross (Polytechnic Univ)
- Bo Chen (Polytechnic Univ)



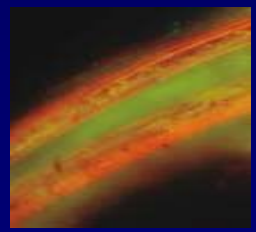
Dilated Cardiomyopathy:
- Wasiem Sanad (Charite Hospital, Berlin)



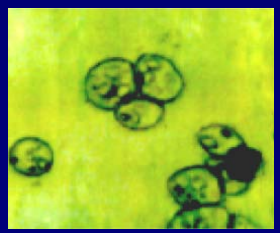
Agricultural Science:
- Peiqiang Yu (Univ. Saskatchewan)
- David Wetzel (Kansas State Univ.)



Chronic Lymphocytic Leukemia:
- Nicholas Chiorazzi (North Shore-LIJ, NY)



Bioremediation:
- U. Ghosh (Stanford Univ.)
- M. Fuhrmann (BNL Environ. Sci.)



Apoptosis:
- Jean-Luc Teillaud (Inst Curie)
- Paul Dumas (LURE)
- Nadege Jamin (LURE)

Cosmetics:
- Paul Dumas (LURE)
- Stefania Nuzzo (L'oreal)

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