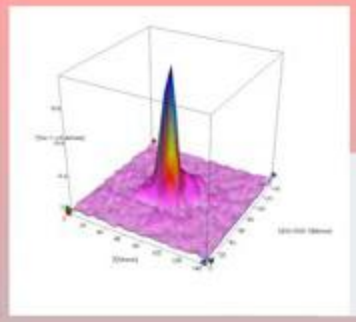

PAUL SCHERRER INSTITUT

PSI

BIOZENTRUM
Universität Basel

SOLEIL
SYNCHROTRON

INFRA
E
D

WORKSHOP ON IR SPECTRO-MICROSCOPY

Neuronal deficiencies studied by Synchrotron-assisted Infrared Micro-spectroscopy

László Forró

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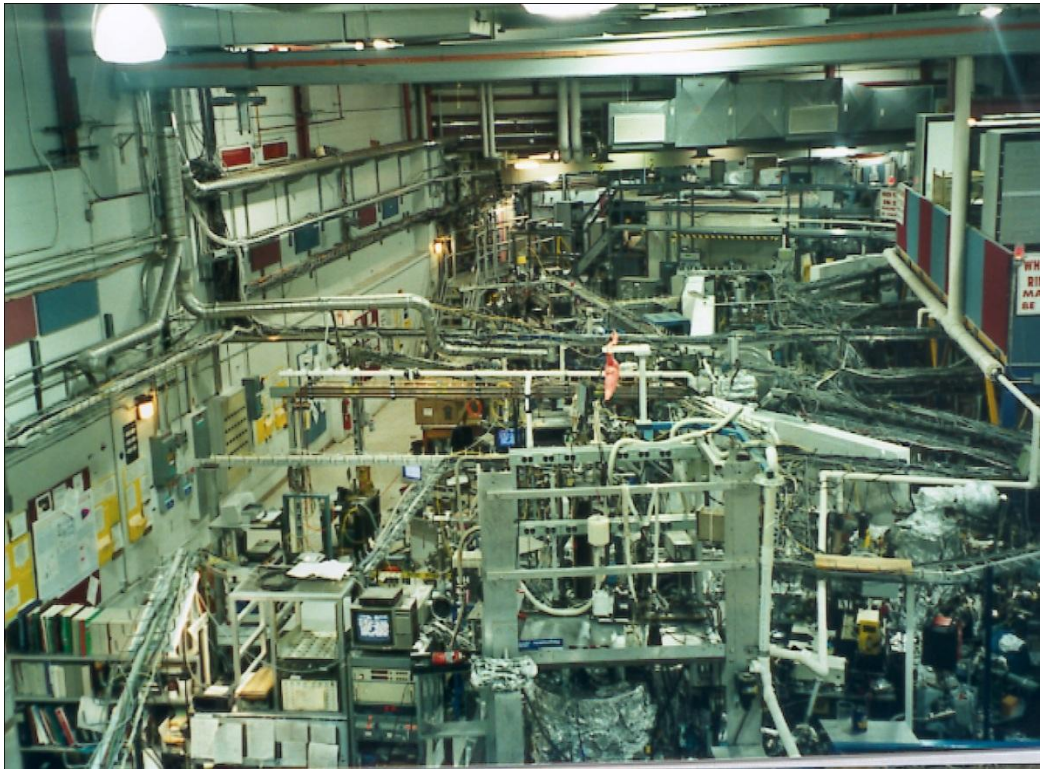
École Polytechnique Fédérale de Lausanne
Lausanne Switzerland

NN research group

EPFL ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Where did we do it?

- Brookhaven NL, NSLS



Short overview of systems studied at IR beamlines of NSLS

Complex systems which we studied with synchrotron light source?

Quasi-onedimensional organic conductors: TTF-TCNQ, $(TMTSF)_2ClO_4$

Quasi-onedimensional inorganic conductors: $K_{.3}MoO_3$

Two-dimensional superconductors: 2H-$NbSe_2$

Fullerenes

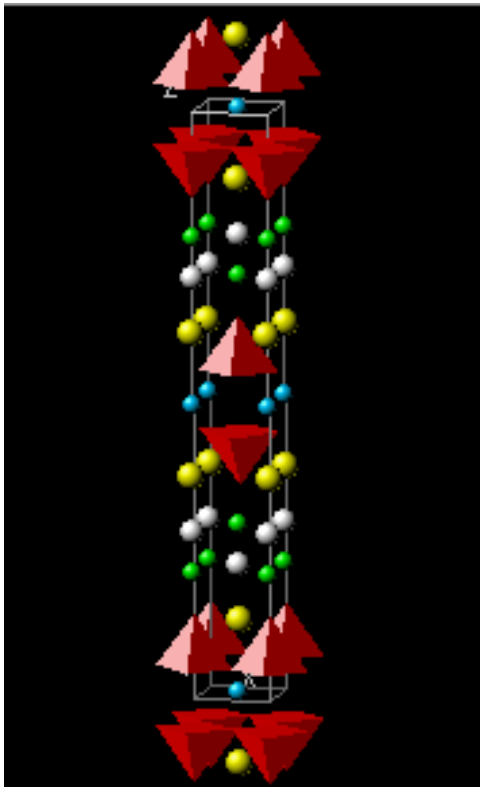
Carbon nanotubes

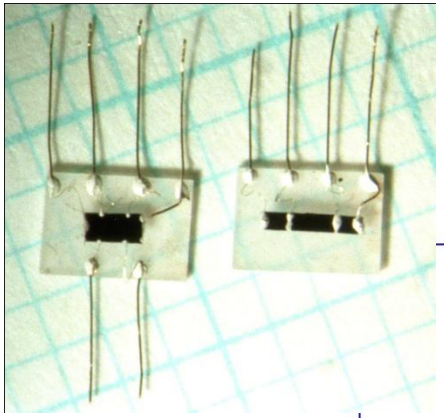
λ-DNA

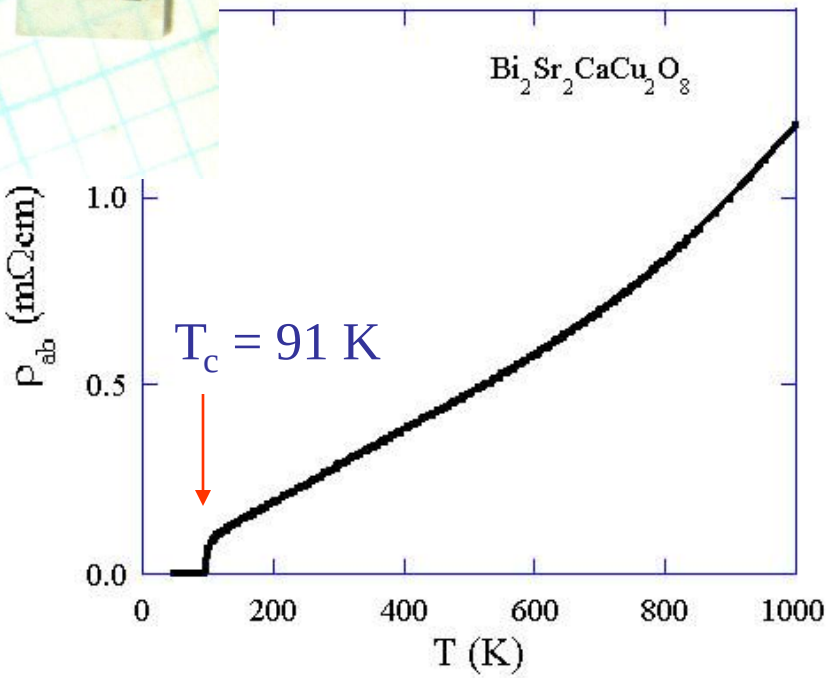
Cuprate superconductors

Strongly correlated d-element metals: BaVS3

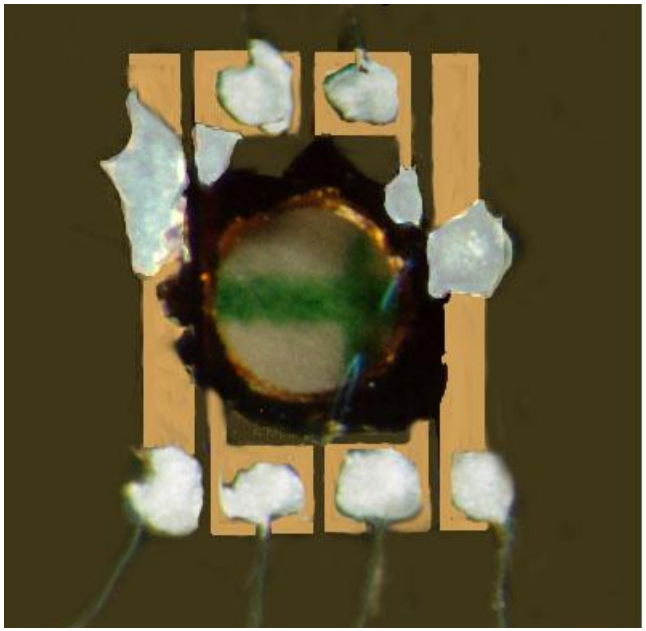
High T_c sample: $Bi_2Sr_2CaCu_2O_8$



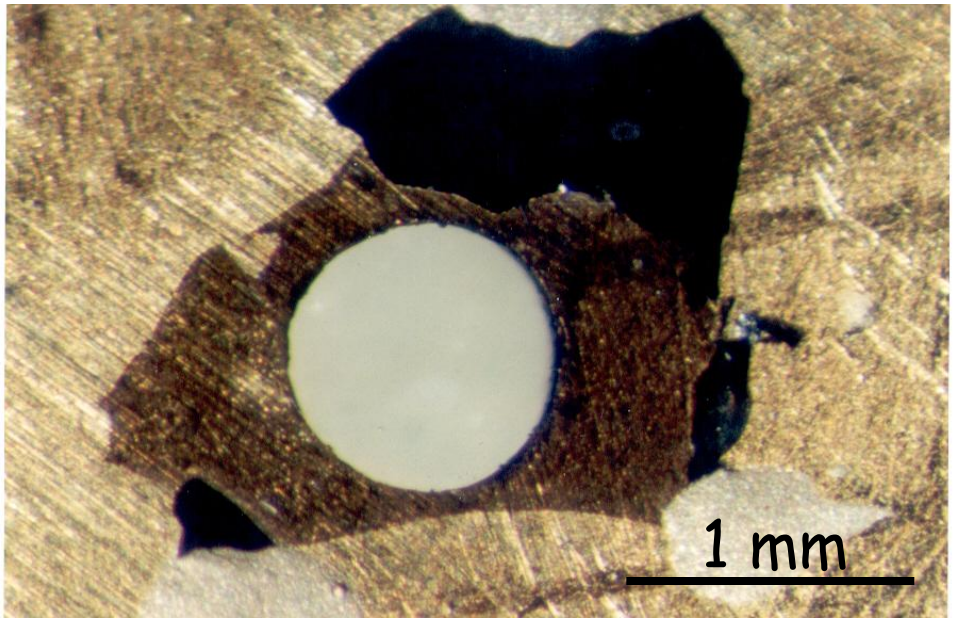




Transparent single crystals of $Bi_2Sr_2CaCu_2O_8$

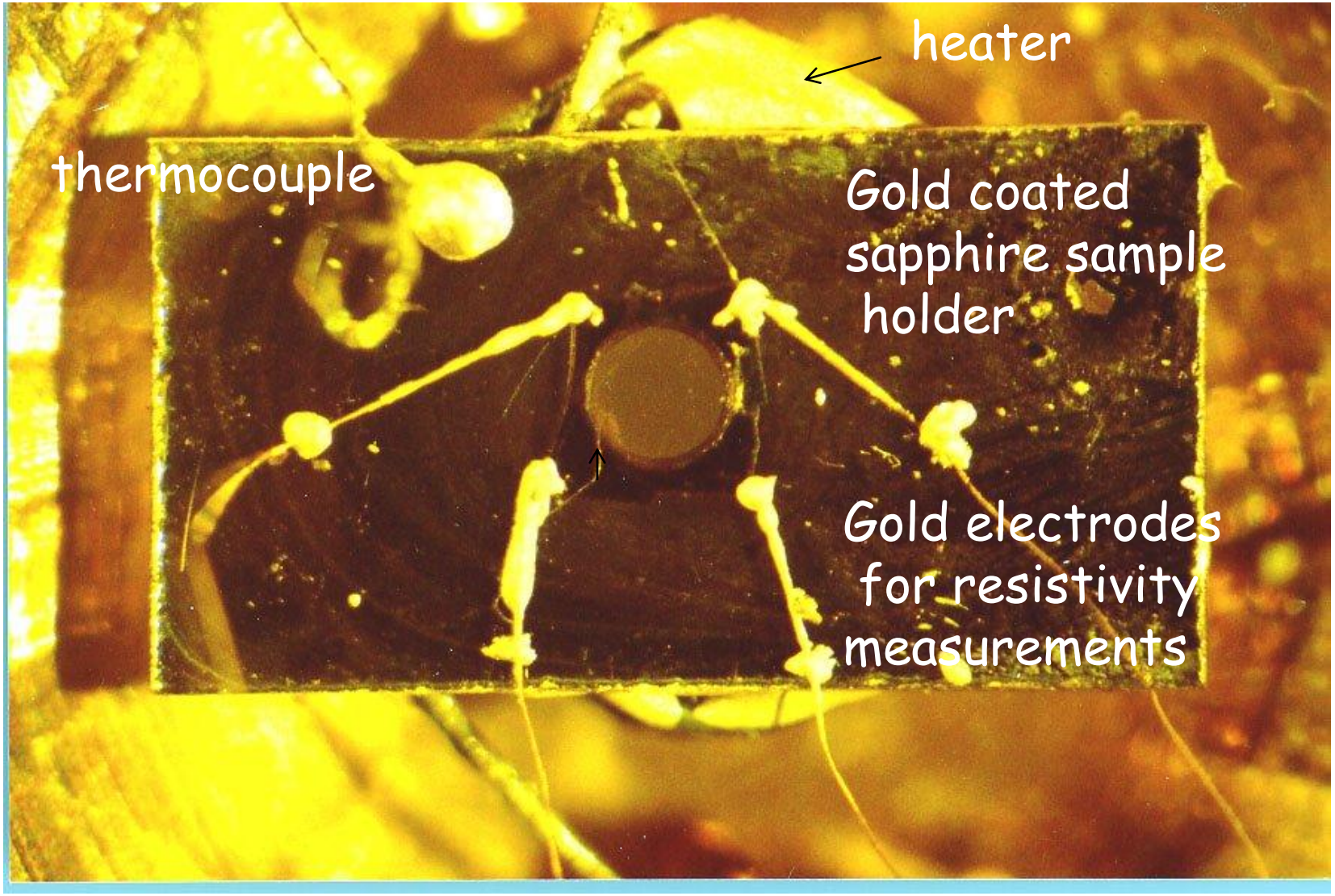


2212 150 nm thick single cystal, selfsupported over 0.6 mm hole, contacted for resistivity, sapphire substrate

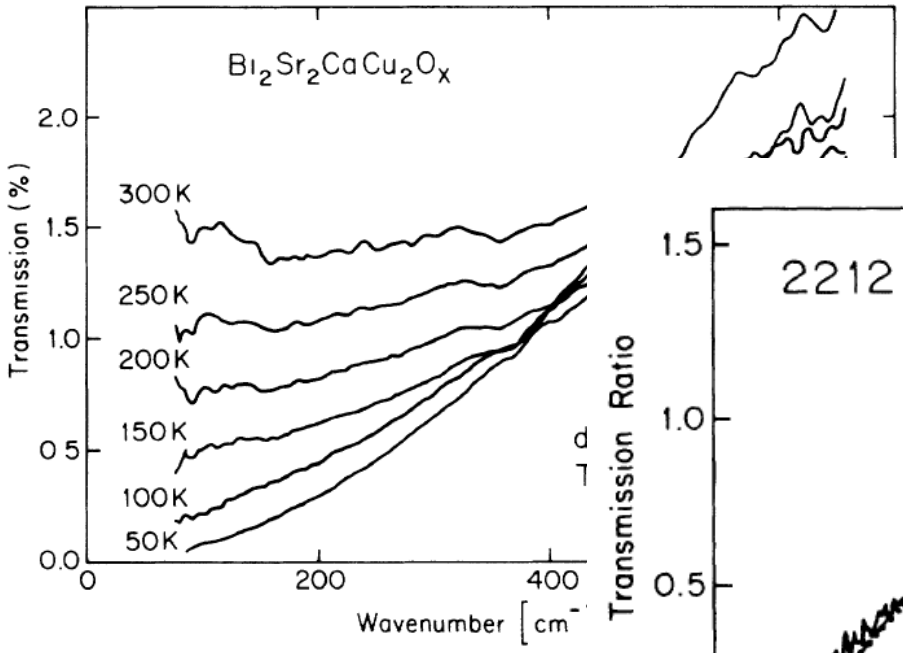


2212 100 nm thick single cystal, selfsupported over 0.9 mm hole, platinum substrate

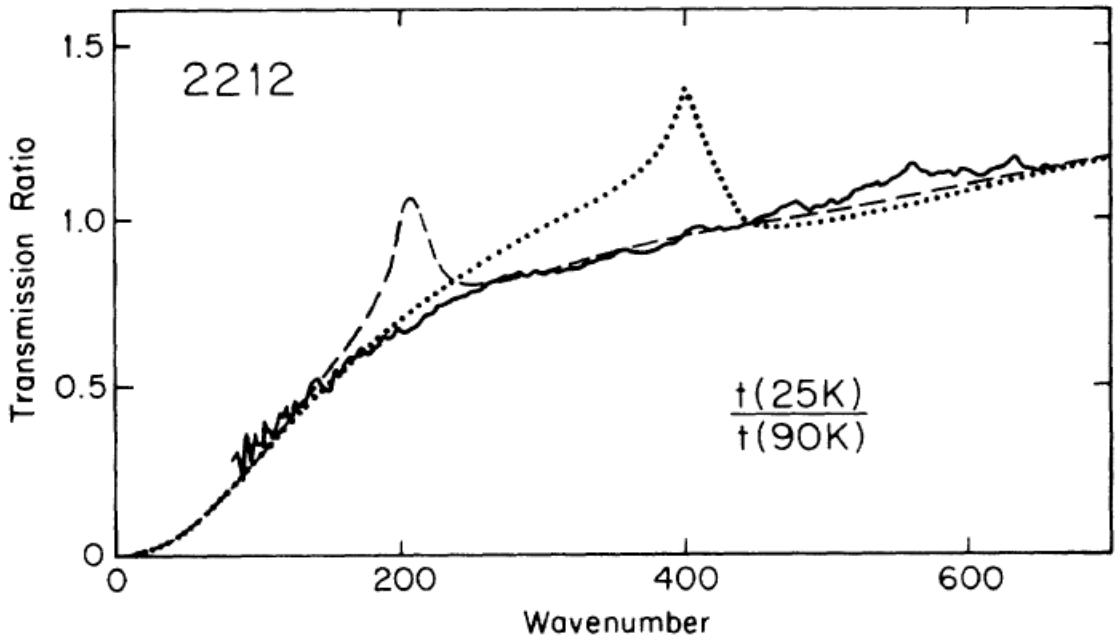
Infrared transmission of 2212 up to high temperatures (500 °C)



The first « good » results on High T_c



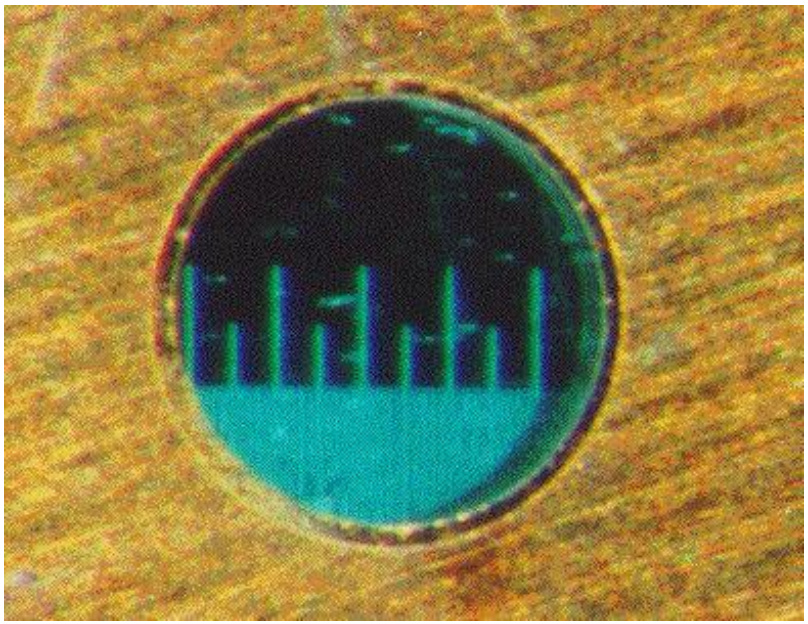
No peak in transmission ratios -> gapless superconductivity!



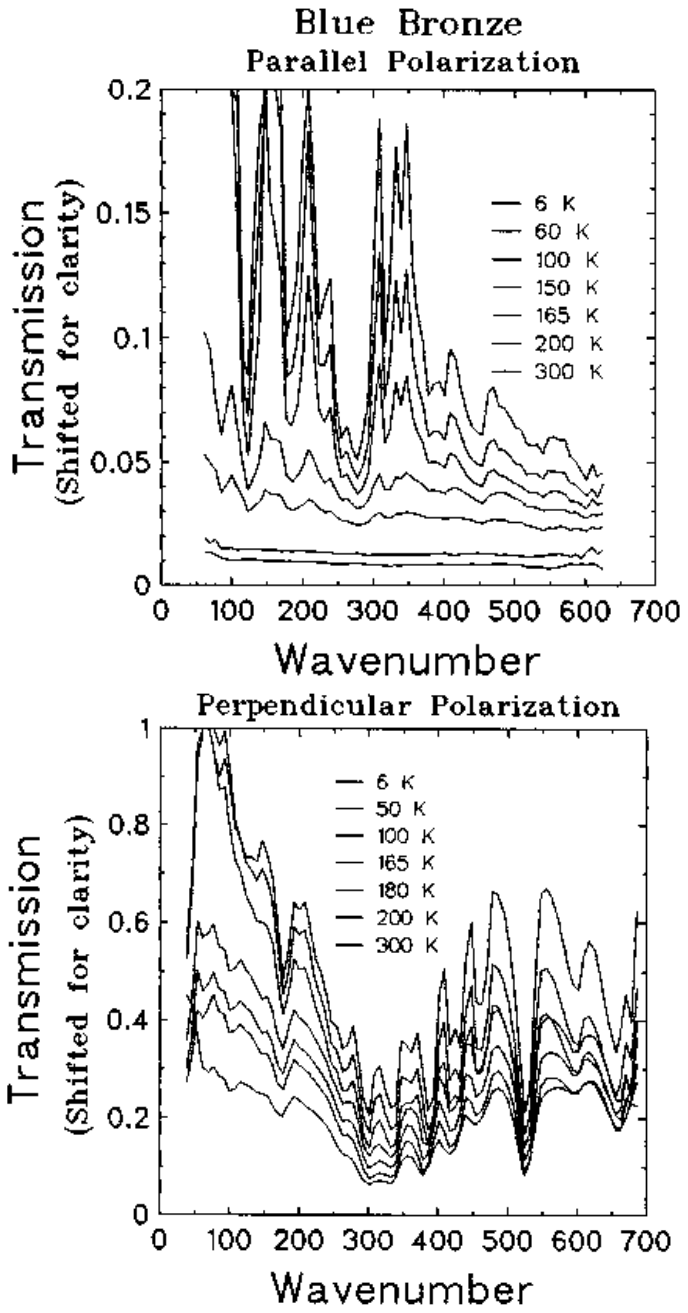
Forró et al., PRL, 65, 1941 (1990)

Blue bronze

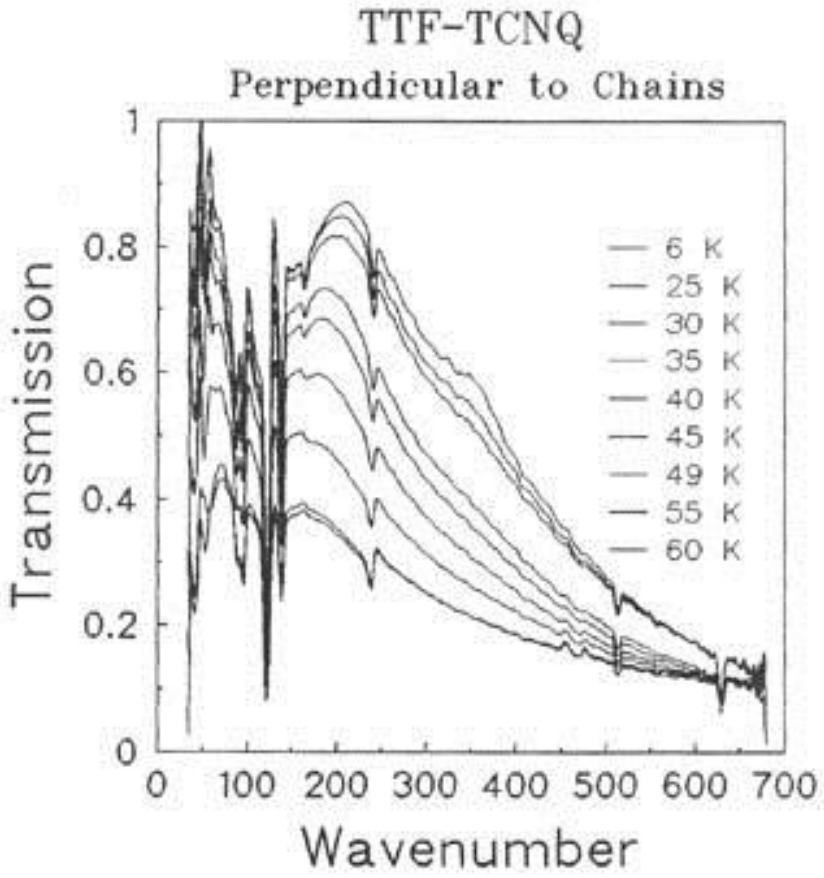
Peierls transition at 180 K

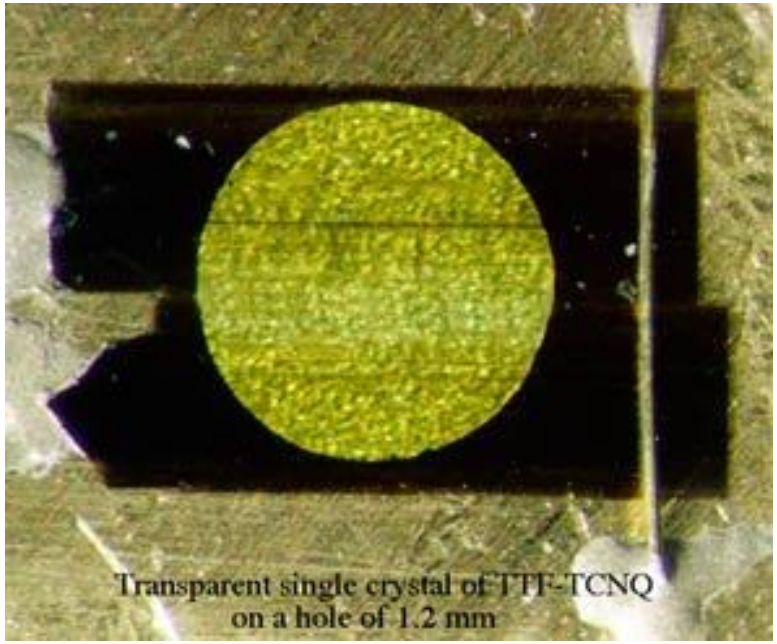


K_3MoO_3, blue bronze, 200 nm thick single cystal, selfsupported over 0.9 mm hole,.

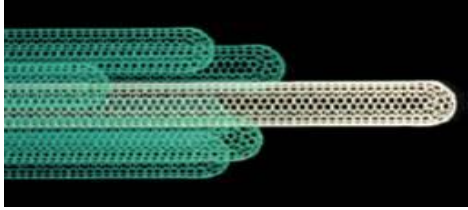


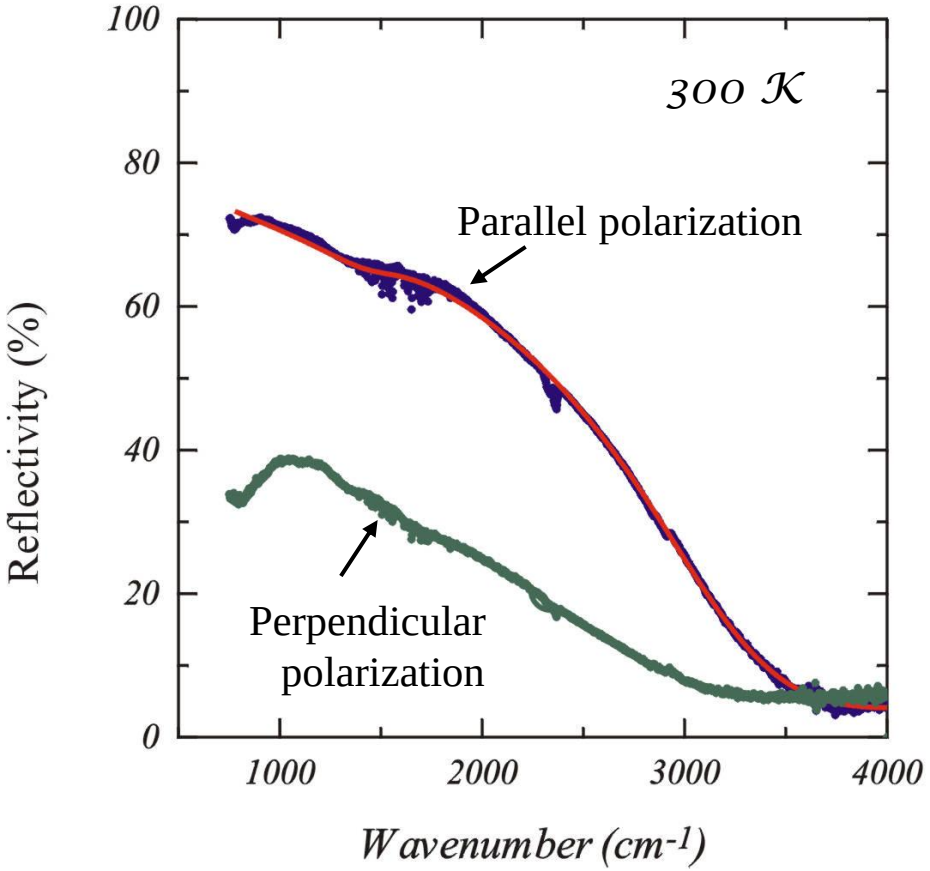
Quasi-1D organic CDW conductors

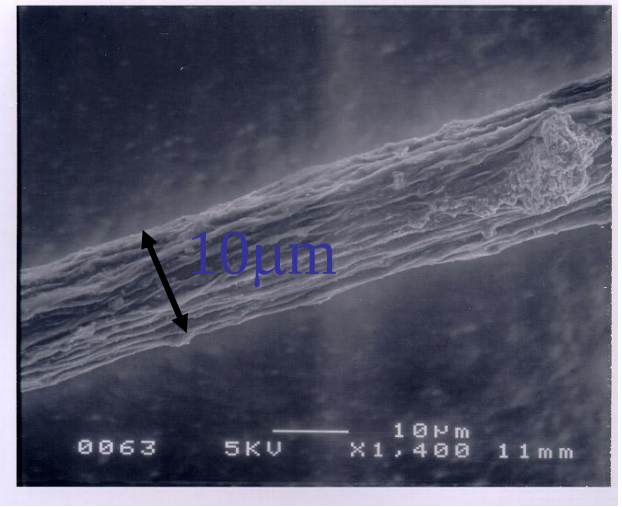


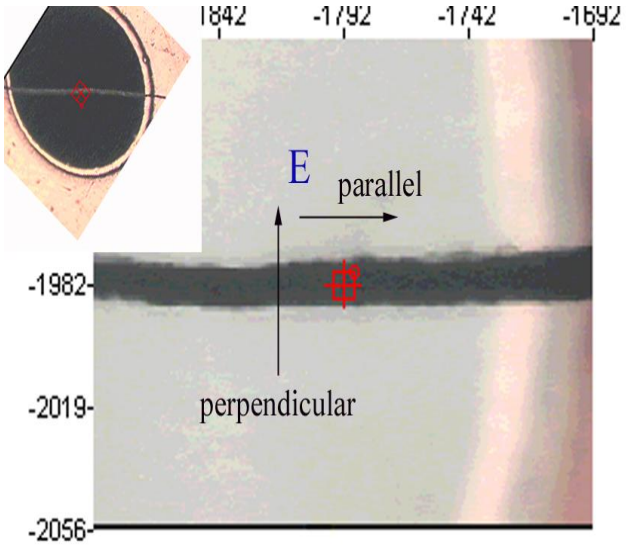


Aligned bundle of SWNT

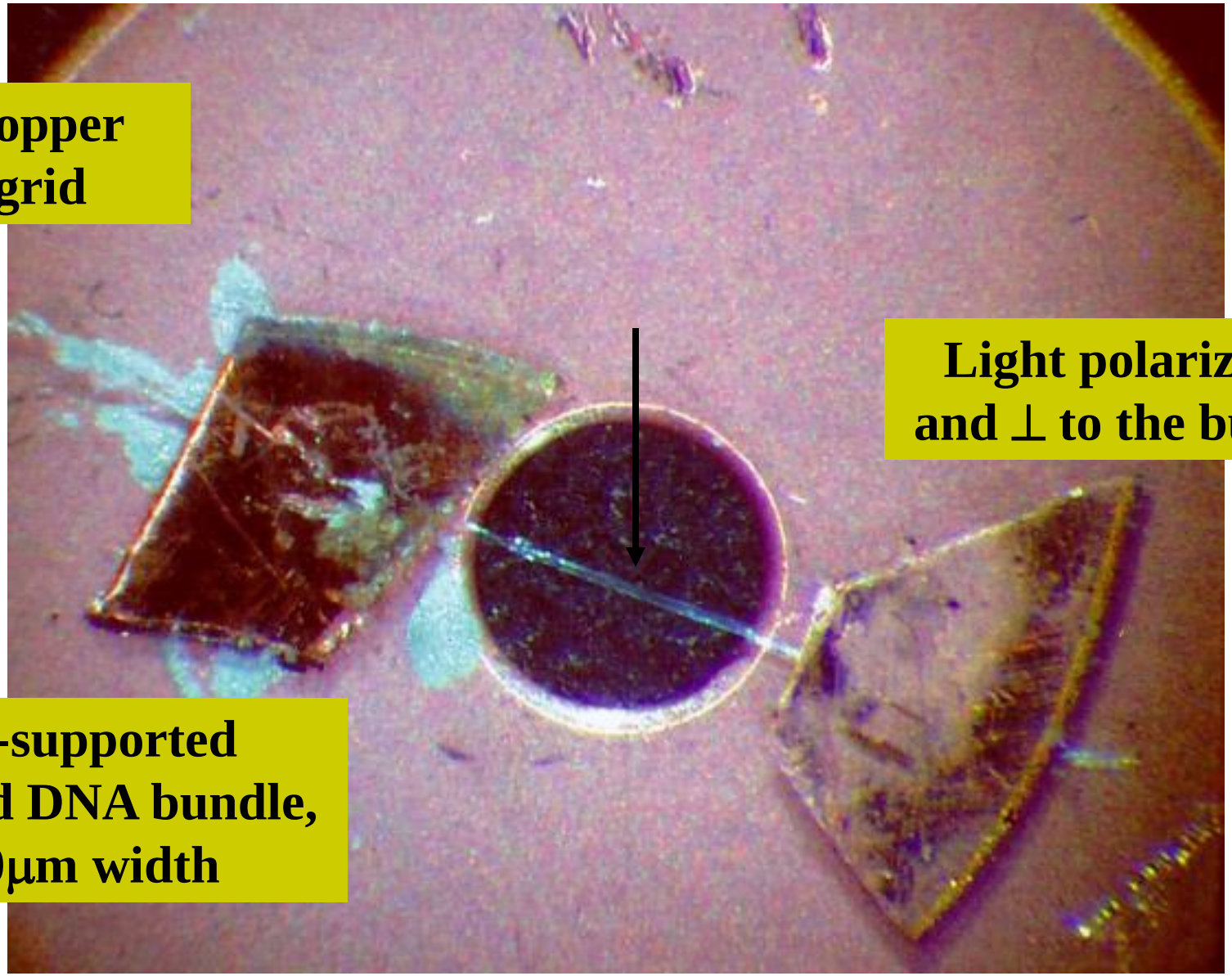








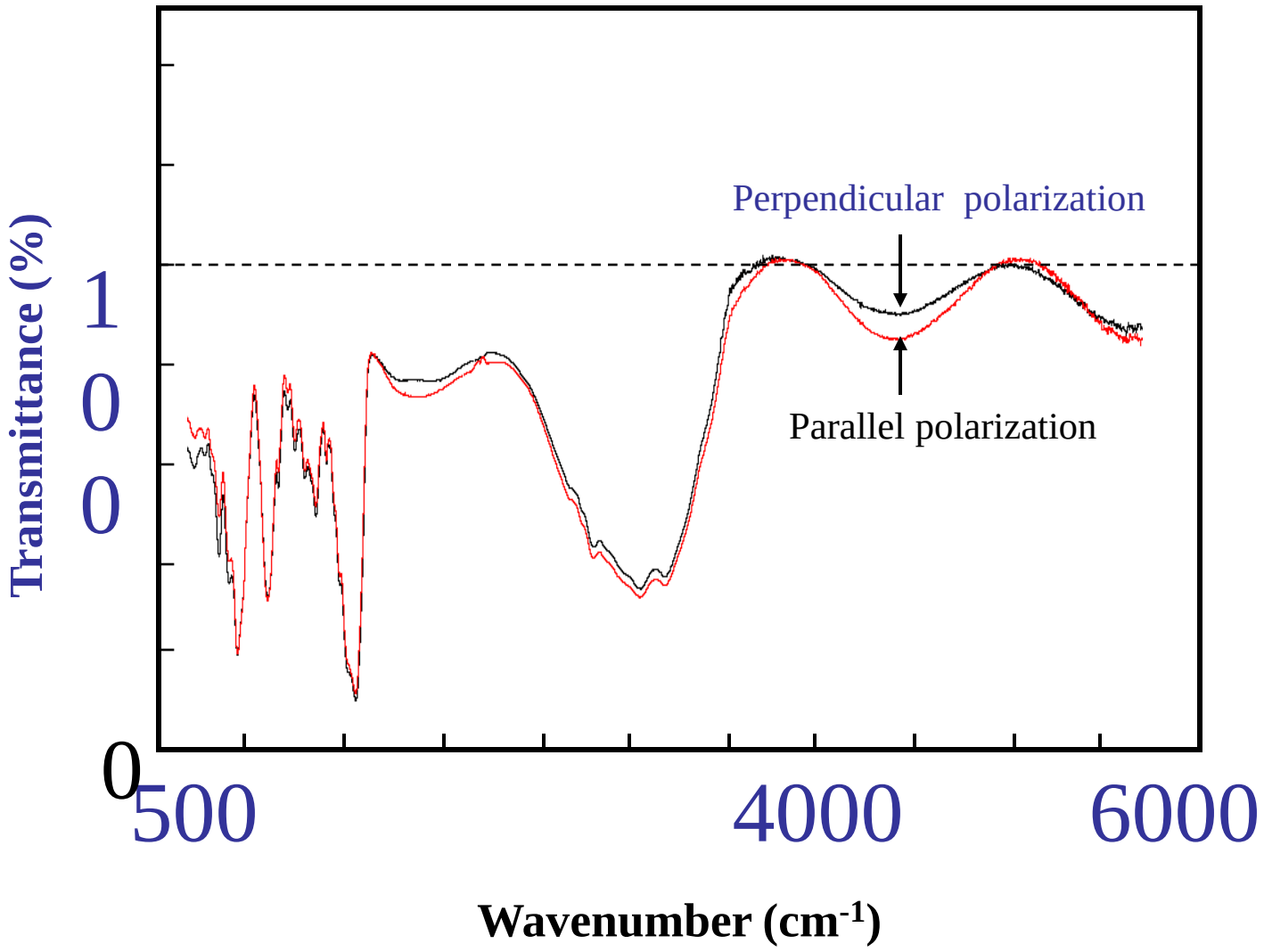
Aligned bundle of λ-DNA

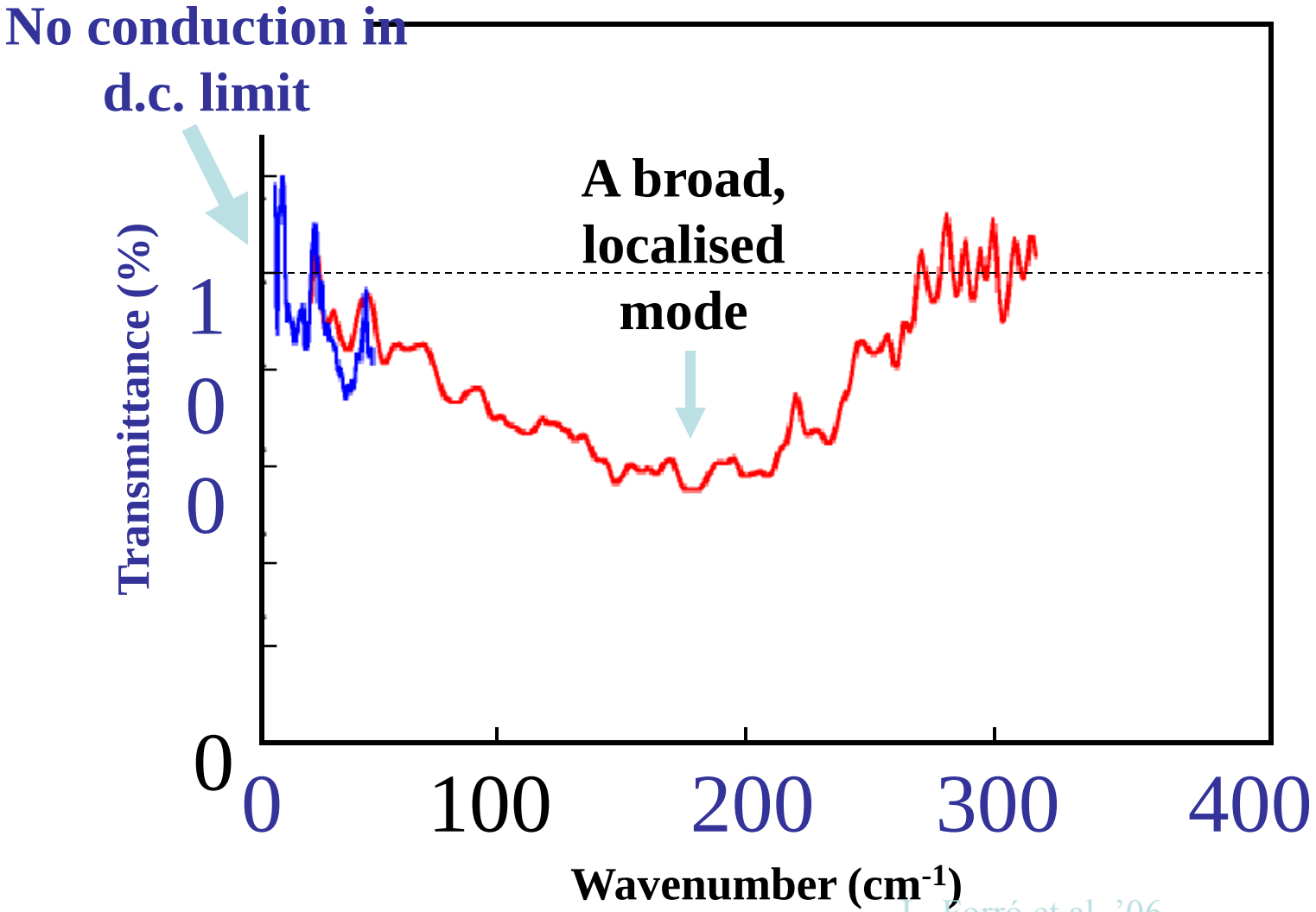


Copper grid

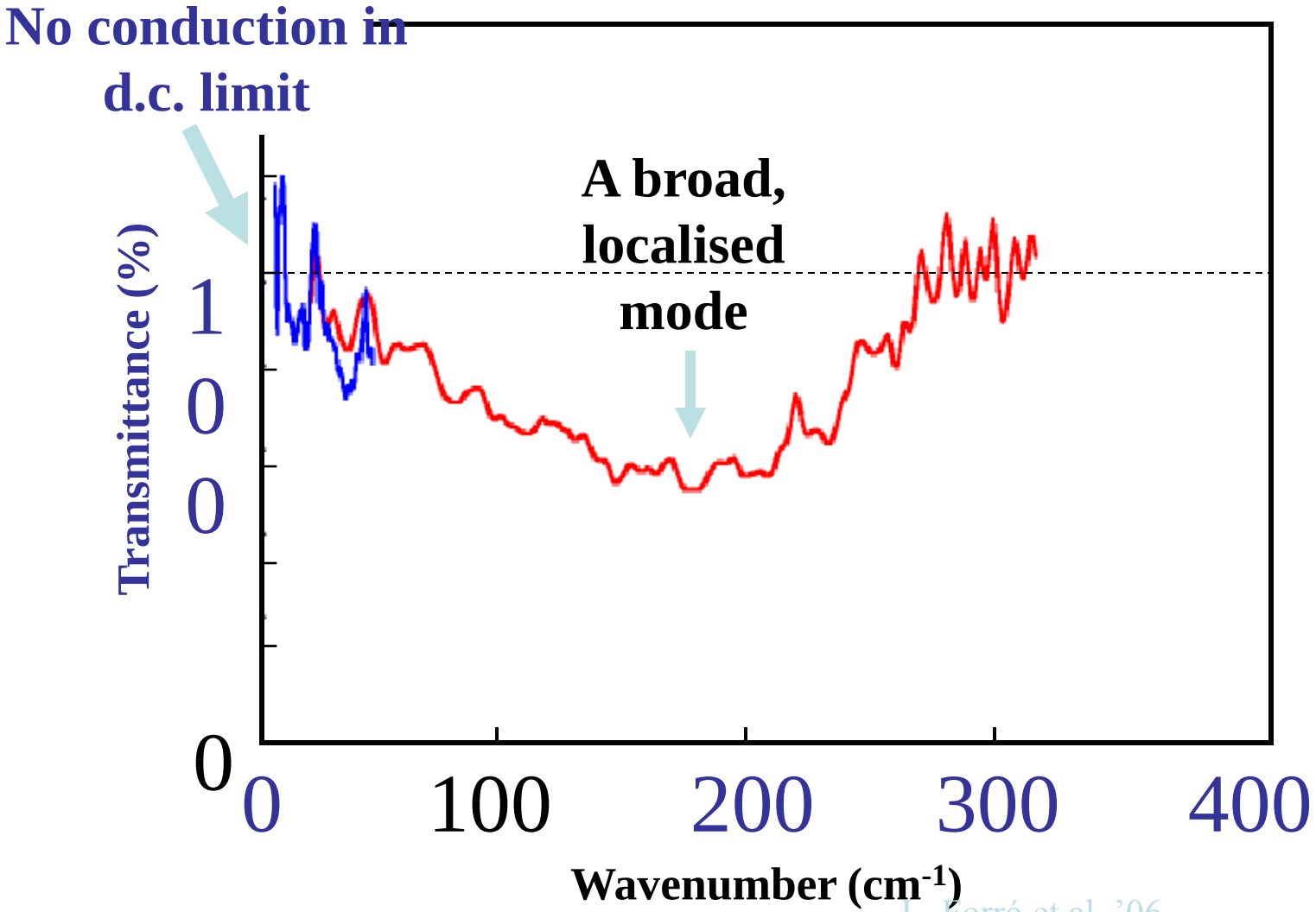
Light polarized ∥ and ⊥ to the bundle

Self-supported oriented DNA bundle, ~10μm width


Perpendicular polarization
Parallel polarization
Transmittance (%)
100
0
500
4000
6000
Wavenumber (cm-1)



L. Forró et al.,'06



L. Forró et al.,'06

Synchrotron-assisted Infrared Micro-spectroscopy is an ideal tool for studying biological tissues with deficiencies

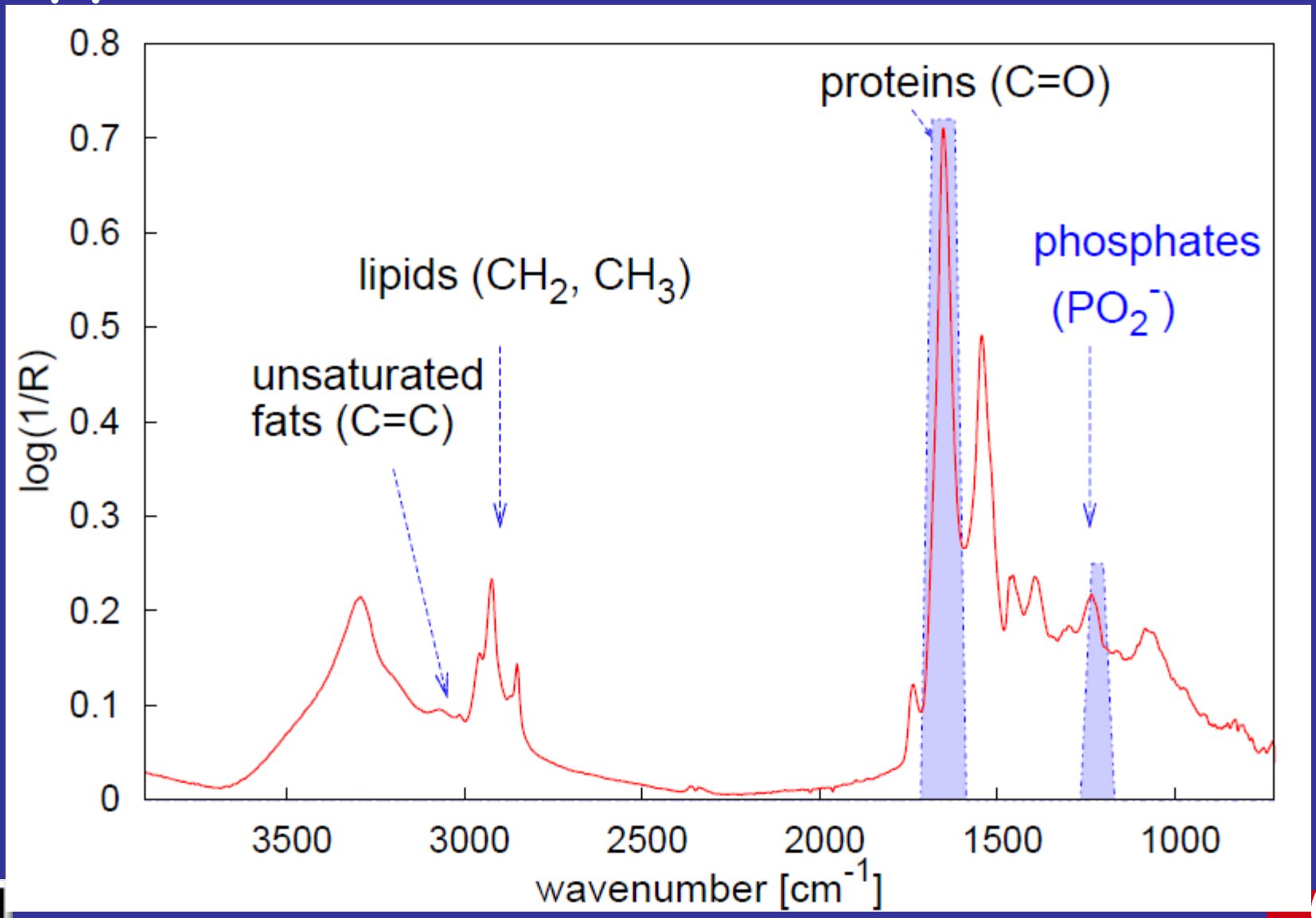
Illustration: Protein misfolding in AD tissue

Miller LM, REVIEW OF SCIENTIFIC INSTRUMENTS 73, 1357-1360 (2002)

Miklossy et al. NEUROBIOLOGY OF AGING 27, 228-236 (2006)

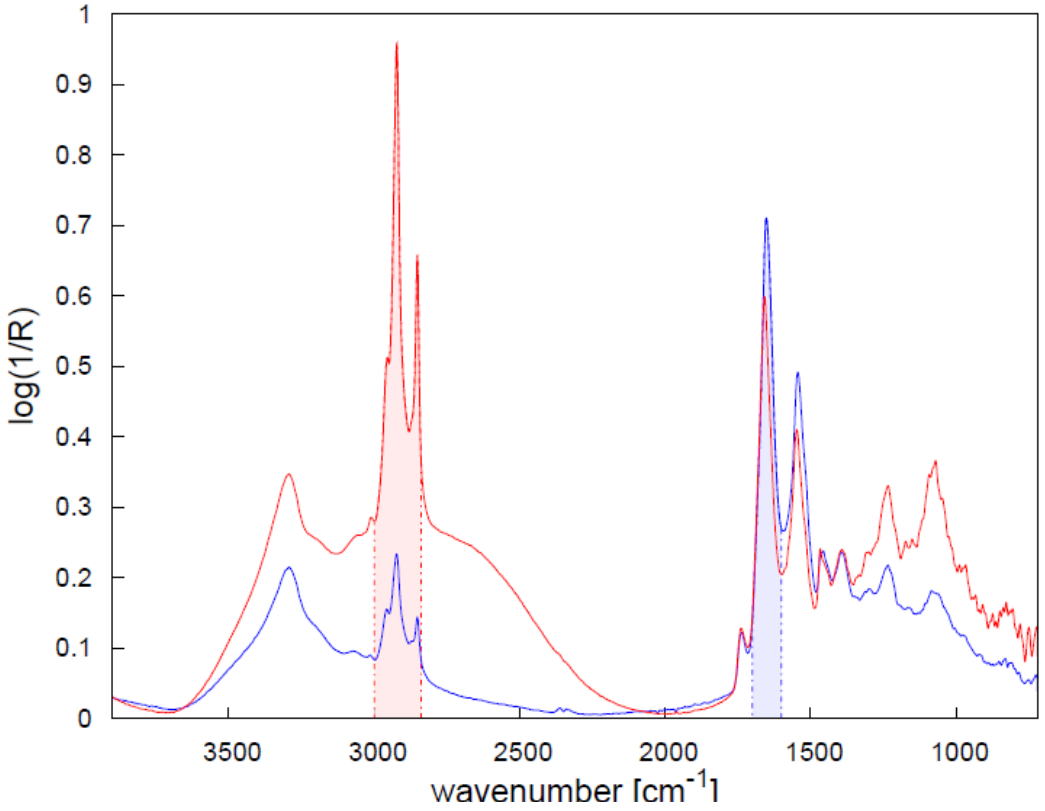
Miklossy et al.,NEUROBIOLOGY OF AGING 31, 1503-1515 (2010)

Approach


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
log(1/R)
proteins (C=O)
lipids (CH_2, CH_3)
unsaturated
fats (C=C)
phosphates
(PO_2^-)
3500
3000
2500
2000
1500
1000
wavenumber [cm^{-1}]

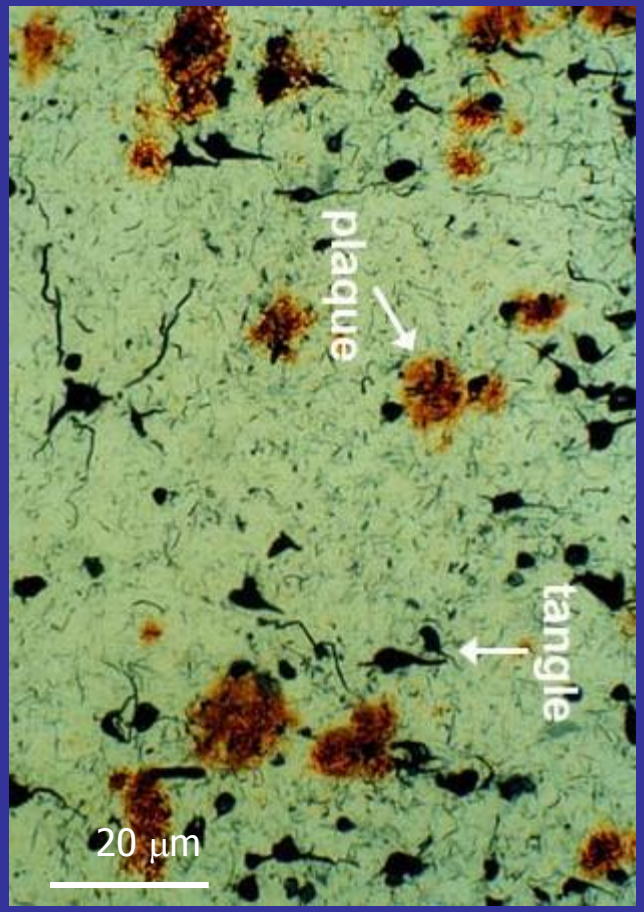
Multivariante Analysis: Principal Component Analysis (PCA)

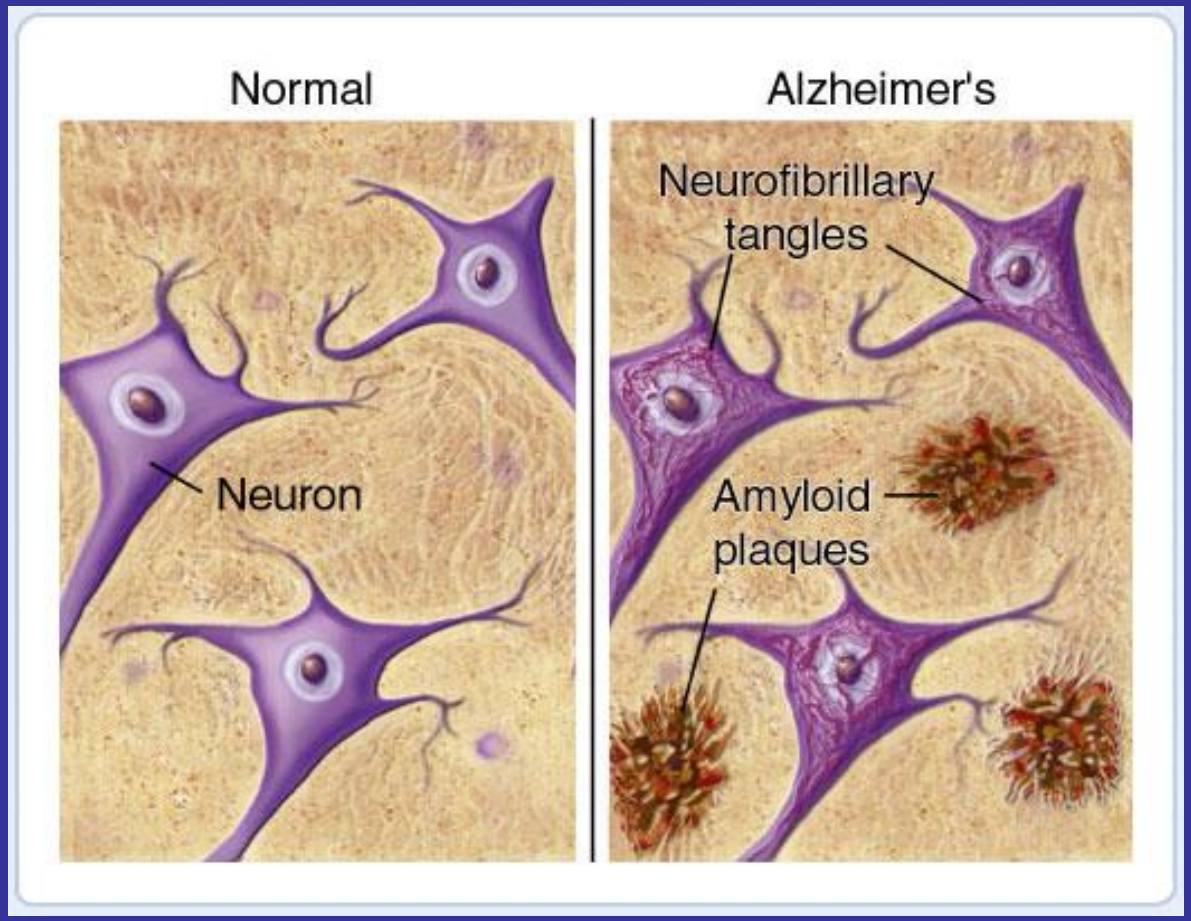
PCA reduces complexity to only a few wavenumbers:



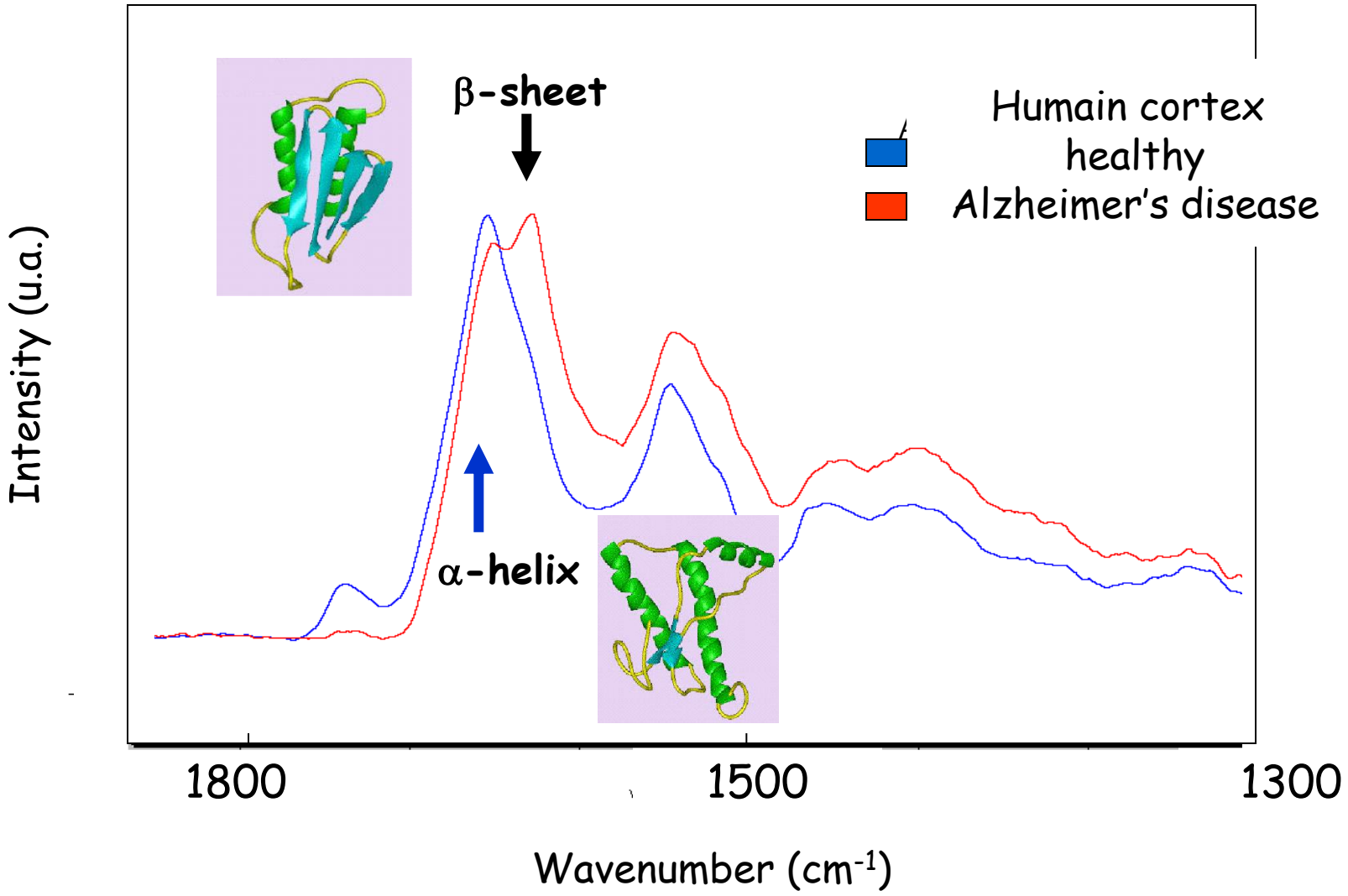
. . . the [wavenumber] x [sample] space is reduced to a subspace according to largest variance.

Alzheimer's Disease



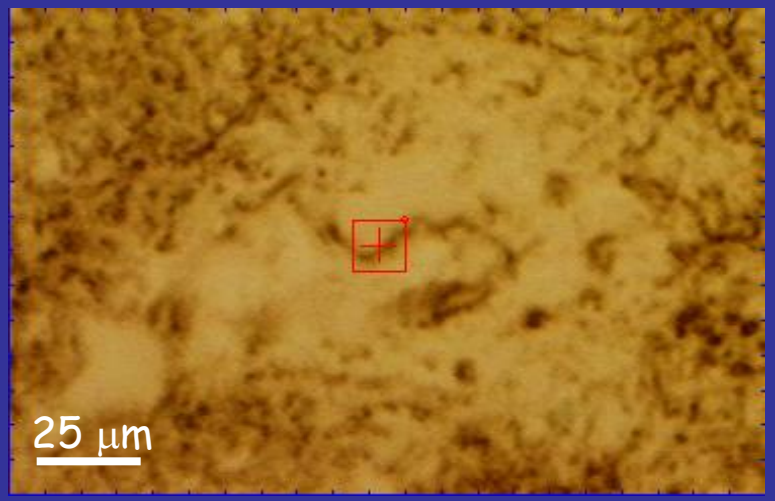


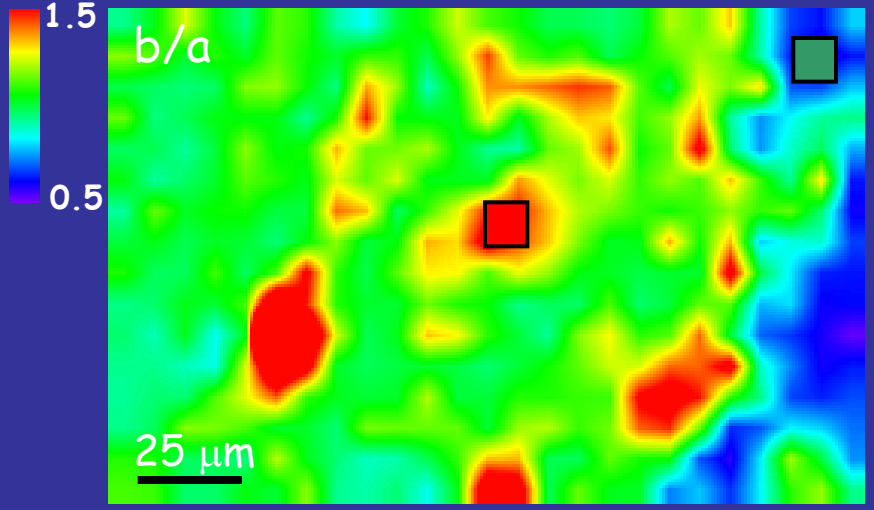
Conformation change of a protein

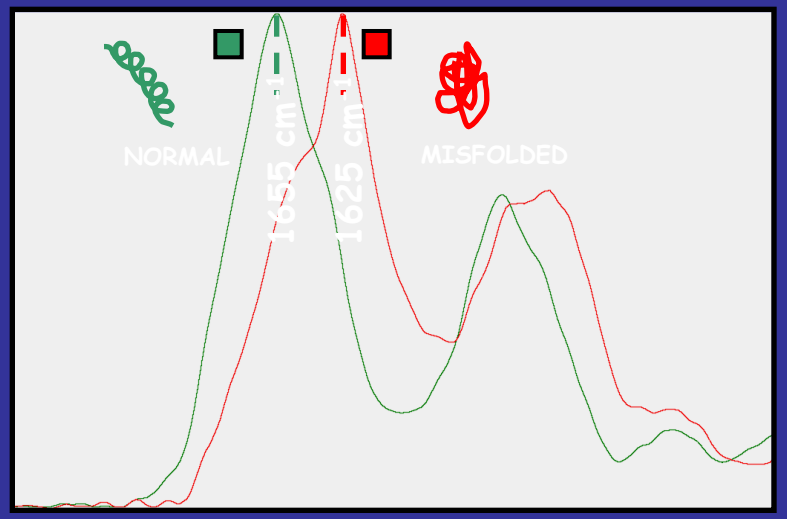


Protein misfolding in AD diseased brain tissue

Diseased frontal lob (5μm thick)

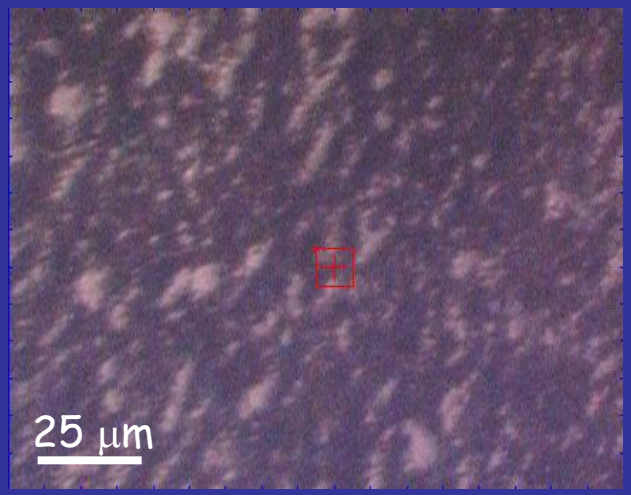


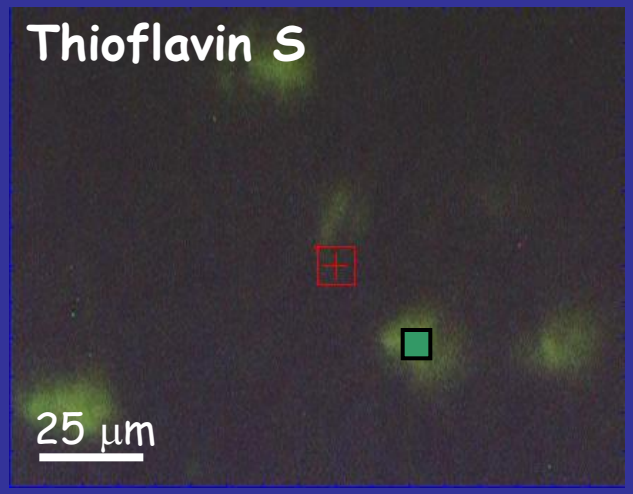


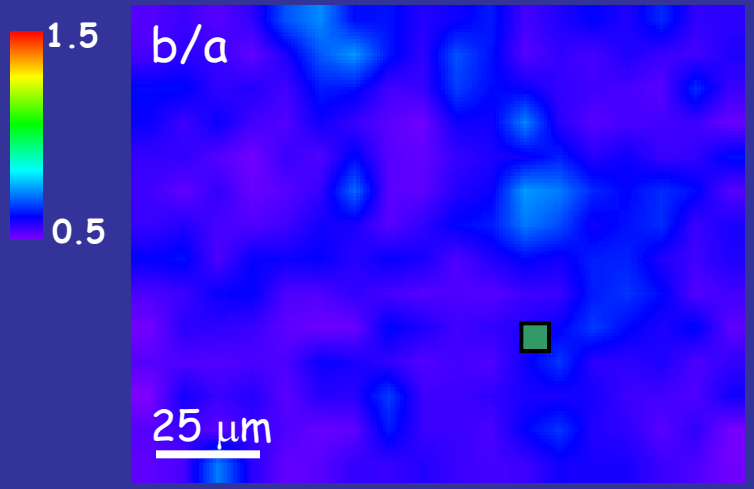


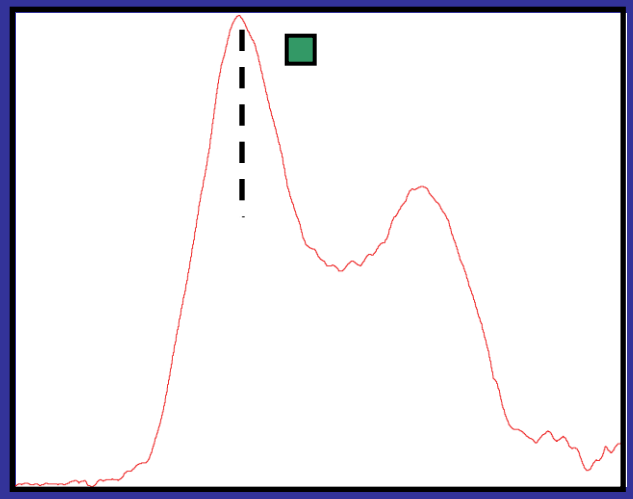
Negative control

Frontal lob: healthy tissue (5μm thick)



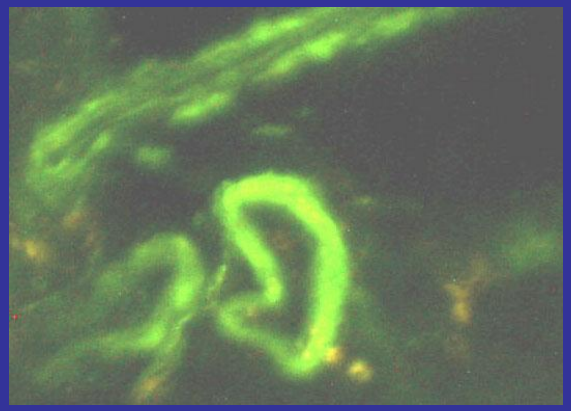






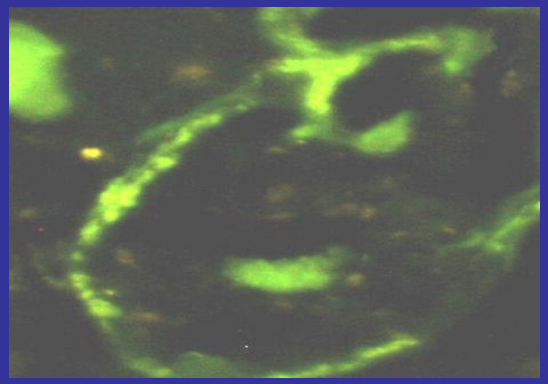
β - amyloid deposits at other places

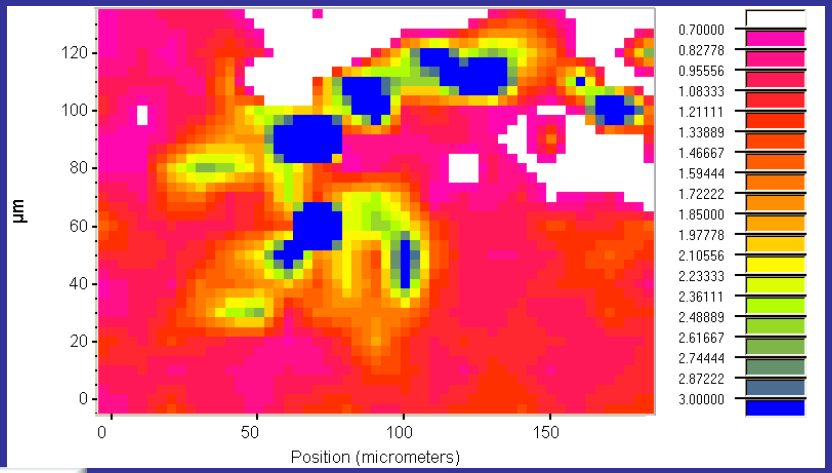
β - amyloid deposits in blood vessels

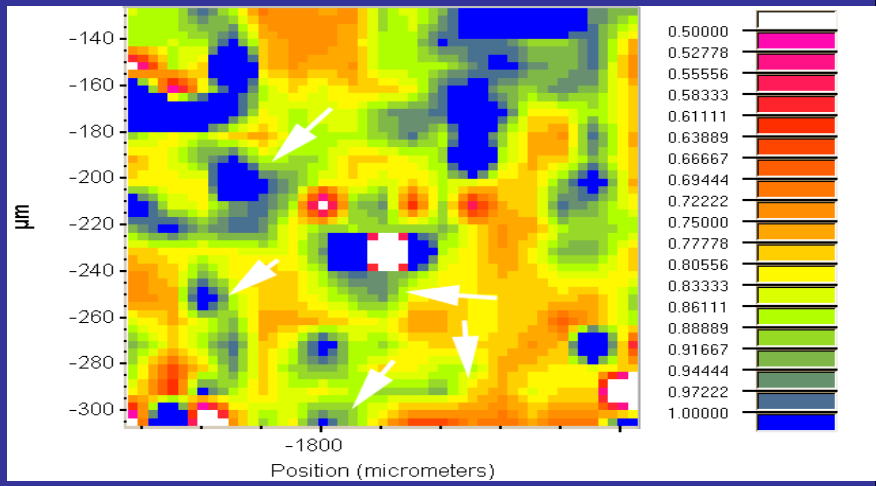


Fluorescence images

β - amyloid deposits in plexus

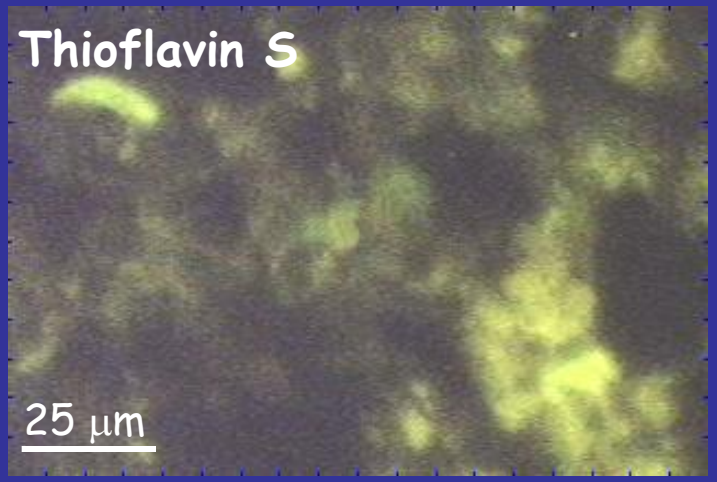


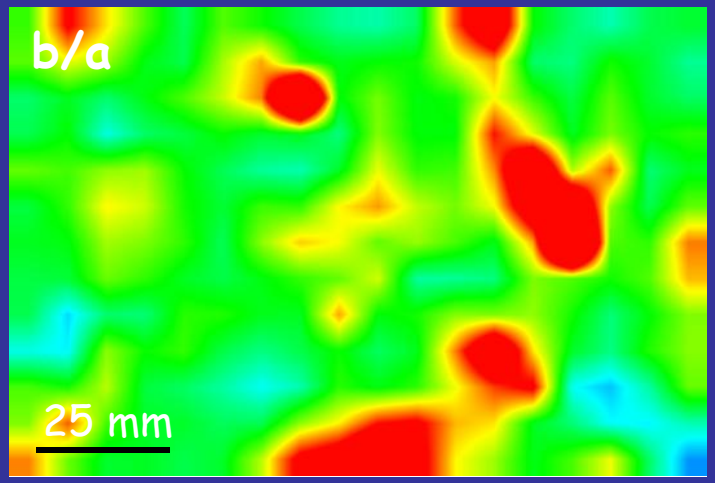




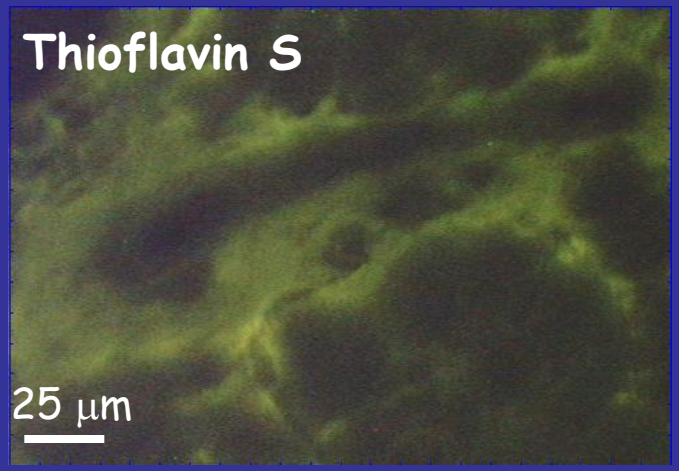
Protein misfolding in AD diseased pancreatic tissue

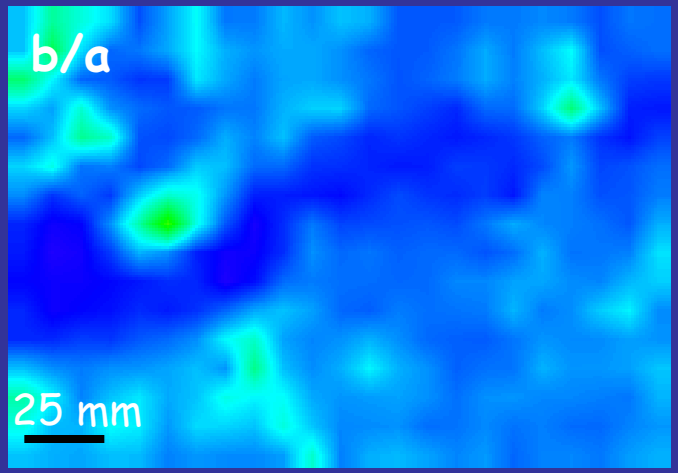
AD pancreatic tissue (5μm thick)

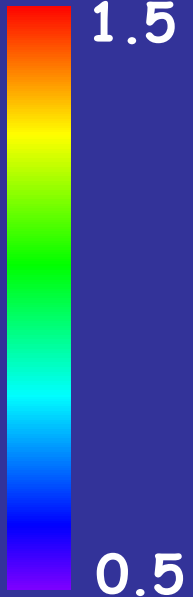




Negative Control

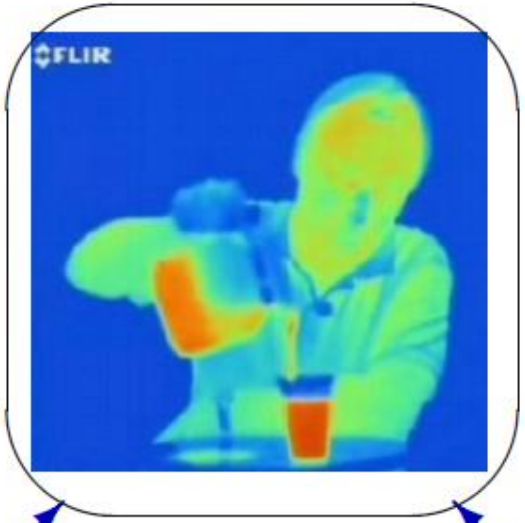




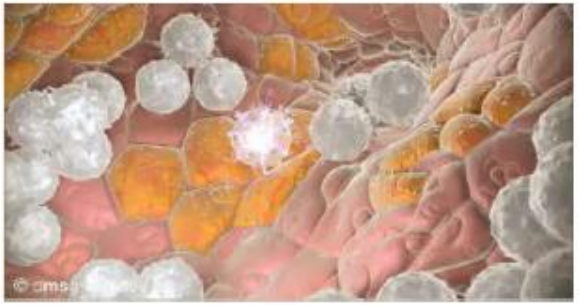


This study…

Huntington's Disease



Multiple Sclerosis

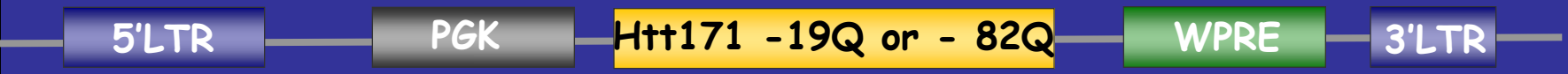


1. Huntington Disease (HD)

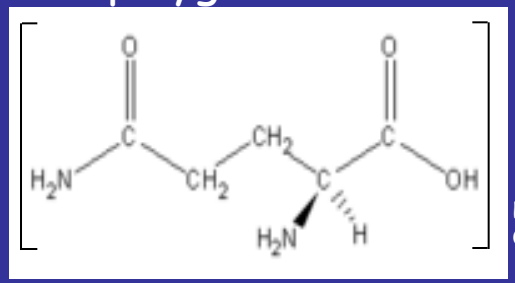
Facts & Figures about HD

- HD is completely of hereditary origin.
- HD is caused by a trinucleotide repeat expansion in the in the Huntingtin gene located on the short arm of chromosome 4
- The gene product is a 348 kDa cytoplasmic protein called Huntingtin (htt), which function is not fully known.
- Htt has a characteristic sequence of fewer than 40 glutamine (Q) residues (CAG codon) in the normal form.
- The mutated form of htt that causes the disease has more than 40-155 Q residues. The severity of the disease is proportional to the number of extra residues.
- The aggregation of htt molecules inside neurons causes them to die off in selected regions of the brain.

A rat model for HD



polyglutamine

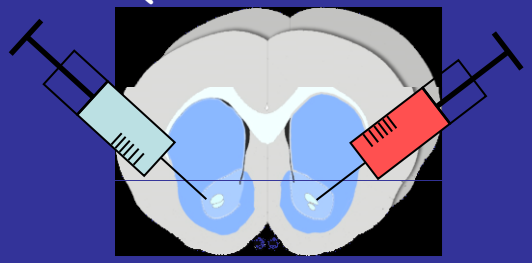


n=19
or 82

Lentiviral-based gene delivery in rodents

Htt171-19Q

Htt171-82Q

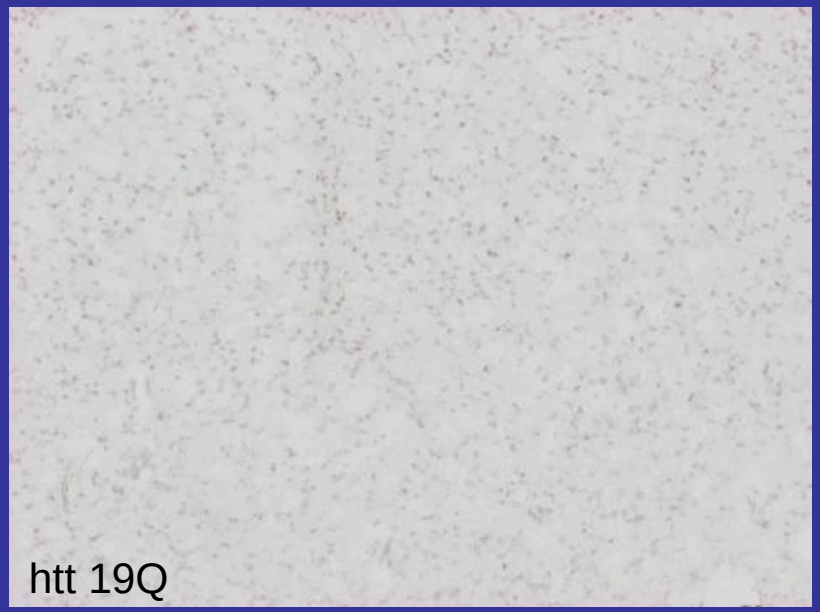


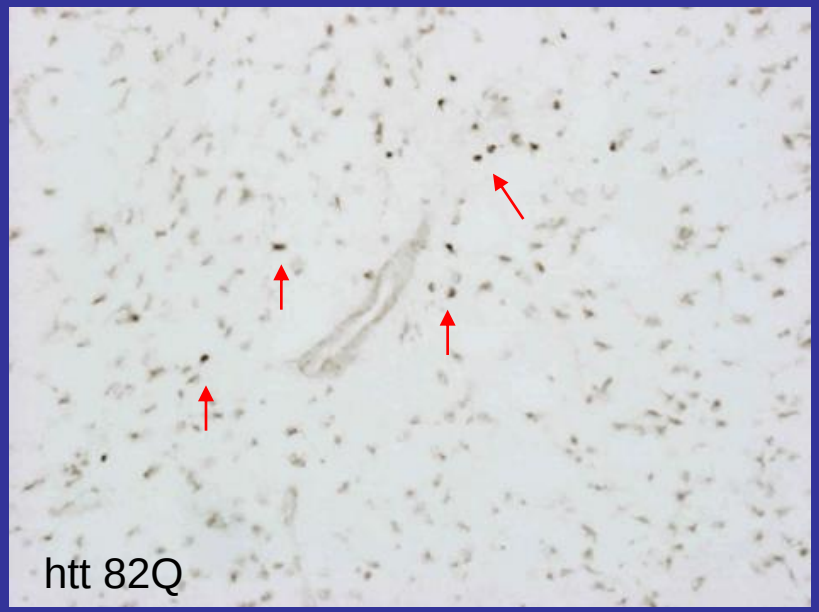
ctrl HD

Staining infected brain slices

Provided by Valerie Perrin / Vérène Pignat

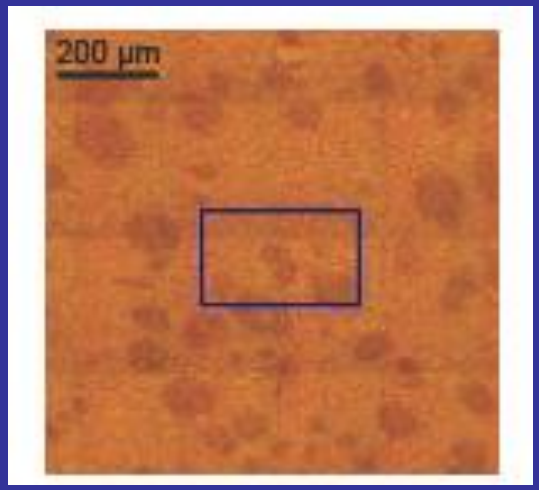
Aggregates of misfolded protein form intracellular inclusions (size up to 7μm)

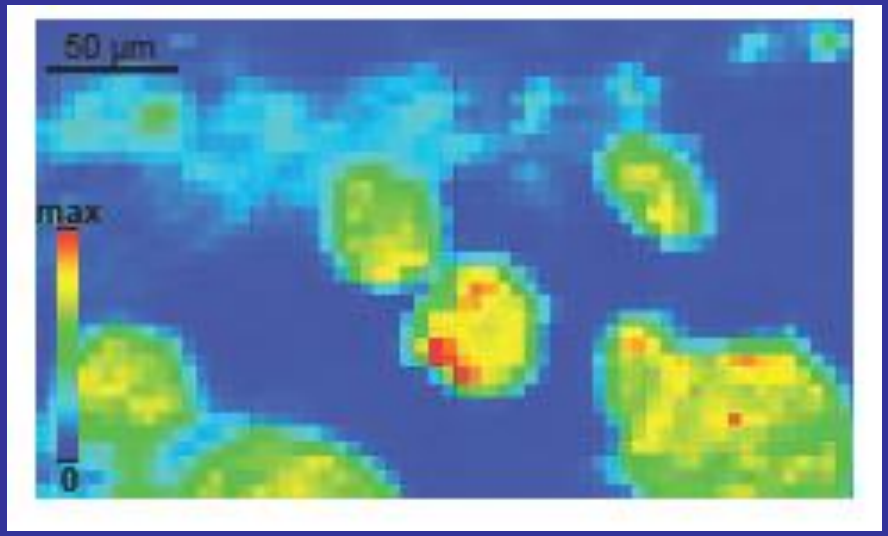




Evidence for grey and white matter in IR

- grey matter (protein rich) and white matter (lipid rich)
- needs to be separated in analysis.





IR image with lipid-to-protein ratio showing grey and white matter in tissuea

Grey Matter

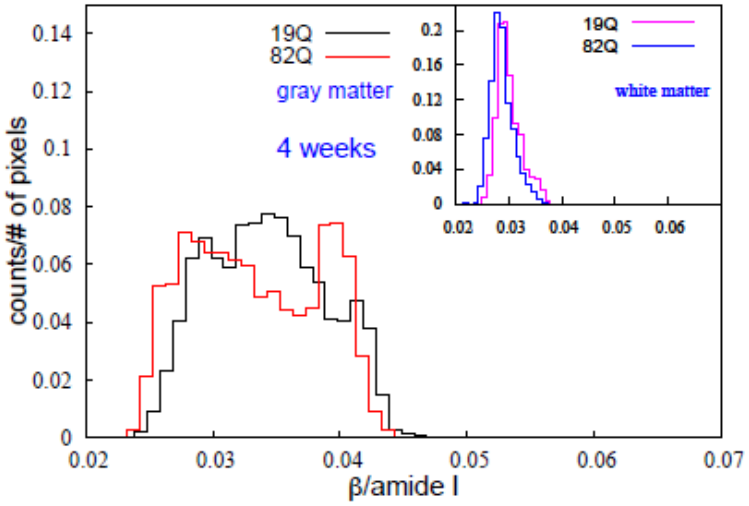
Change in β-sheet and α-helix structure
in grey matter of HD-infected rat brain.

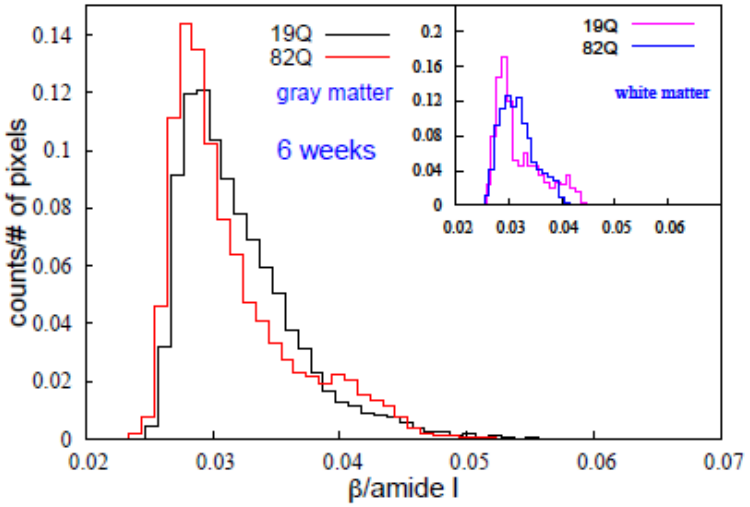
β-sheet content in gray matter increases

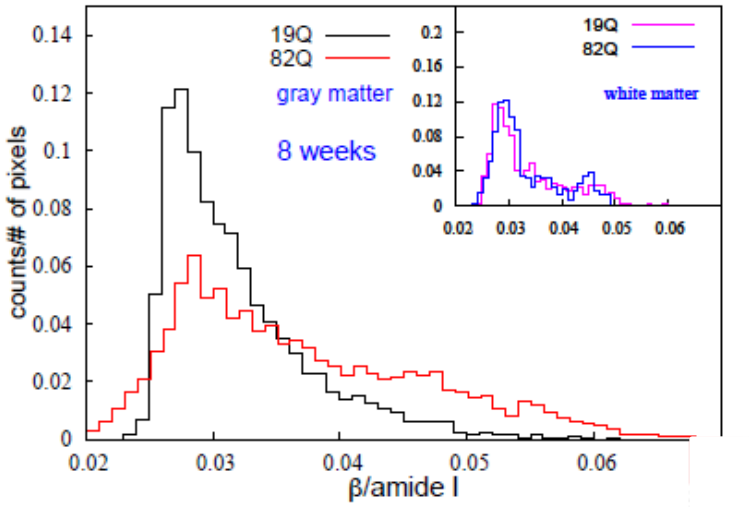
evolution of β-sheet content

at 4, 6 and 8 weeks

normalized by protein content
(from top right to bottom right)







White matter

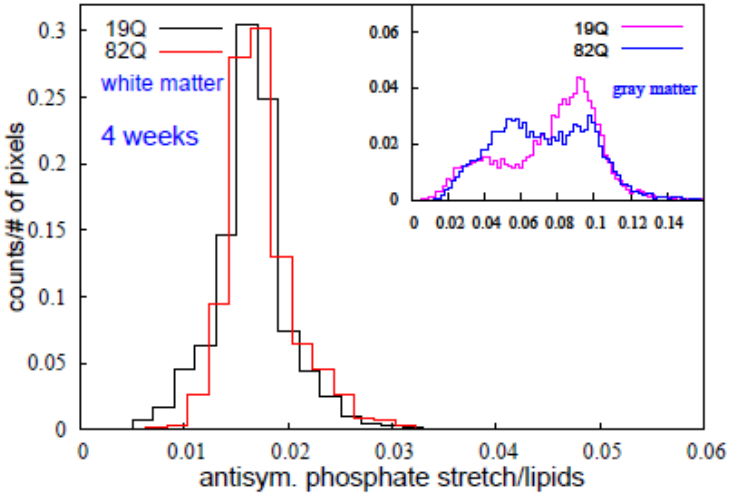
Change in phosphorylation in white matter of HD-infected rat brain.

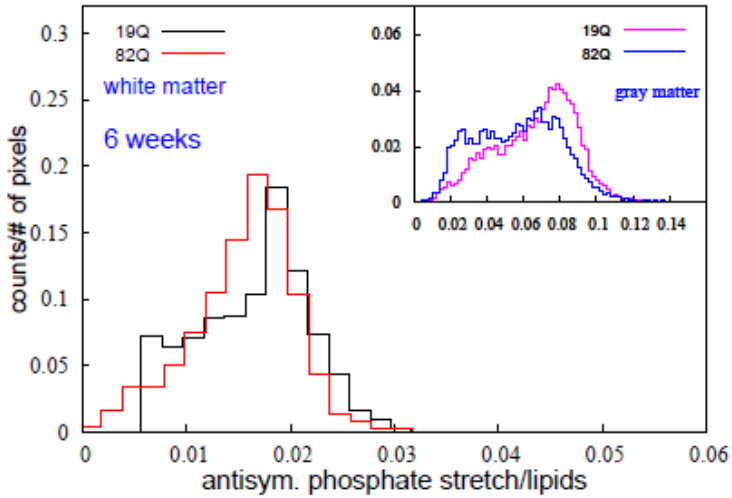
antisymmetric phosphate stretching

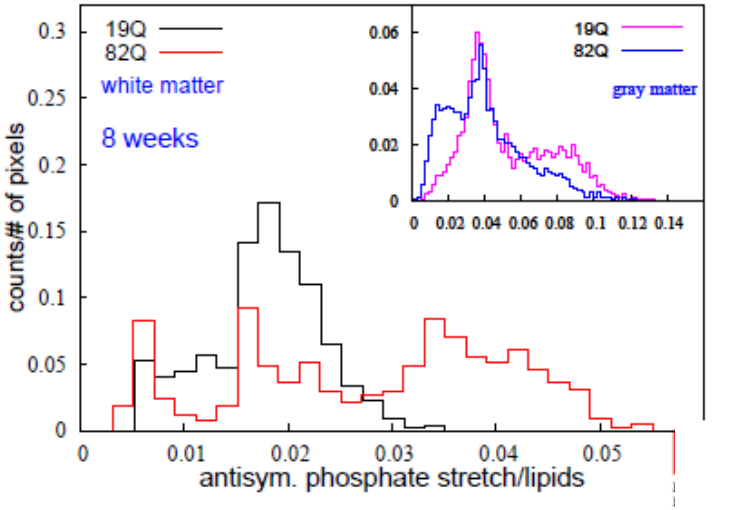
evolution of phosphates

at 4, 6 and 8 weeks

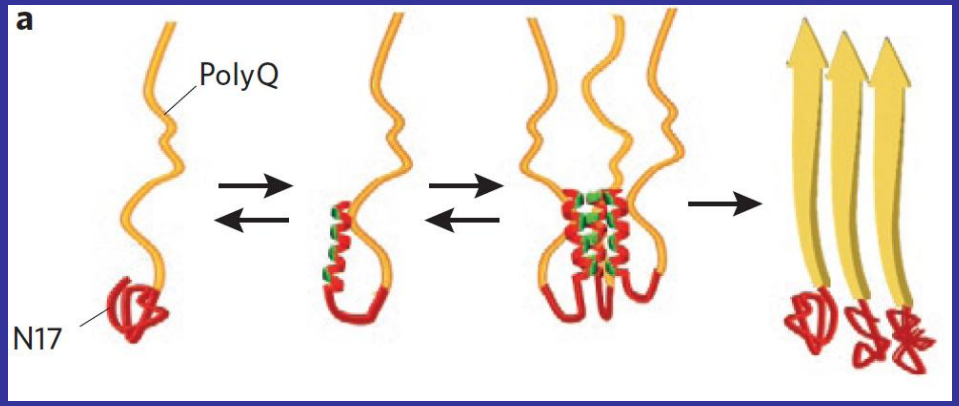
normalized by lipid content
(from top right to bottom right)





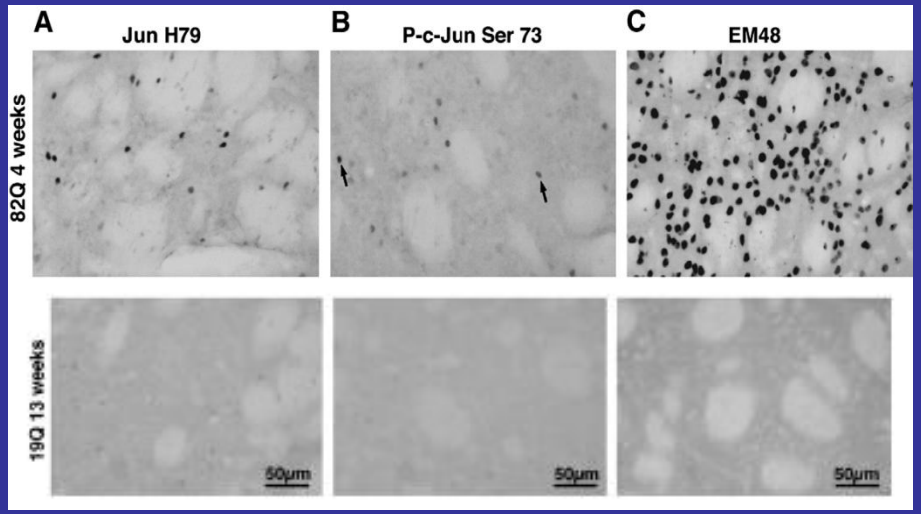


Biochemical explanation



Aggregation of htt in gray matter is caused by conformational change from α-helix to β-sheet in neurons.

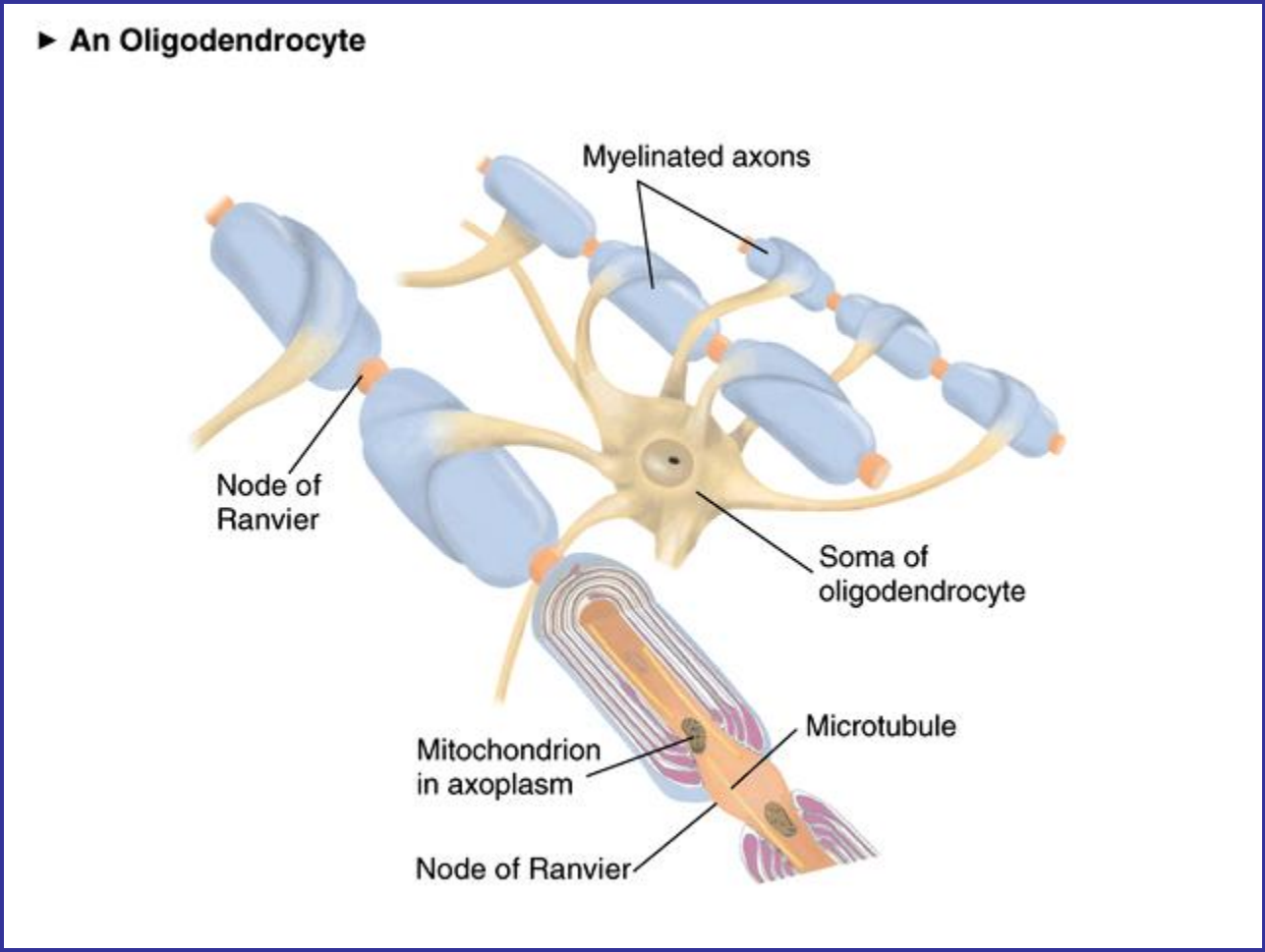
[Nature Chem.Bio., 6(2010)]



Apoptosis in white matter is induced by a phosphorylation pathway.

[Exp.Neur., 215(2009)]

Dying white matter cells?



our results suggest oligodendrocytes are affected

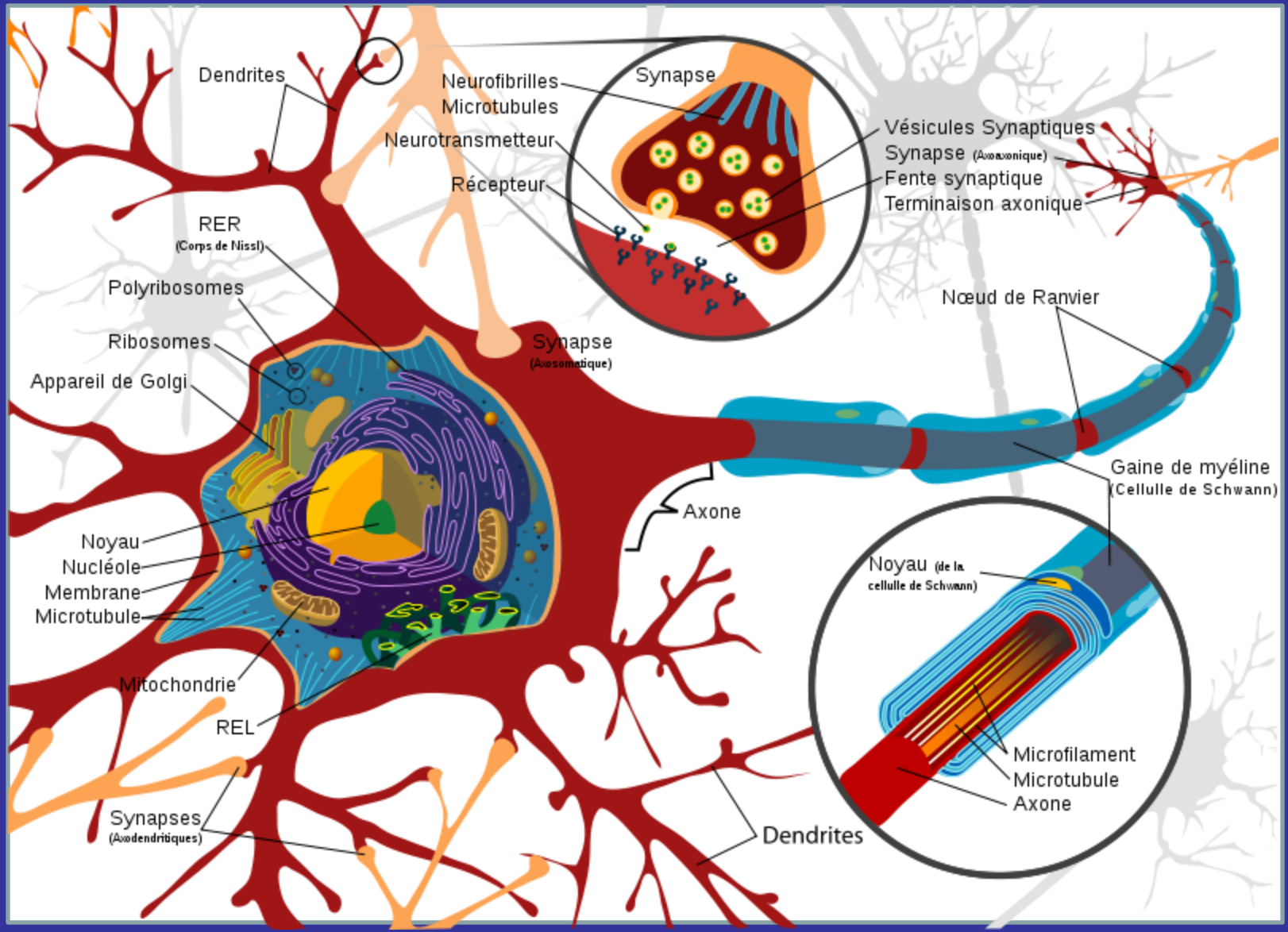
Summary of HD study

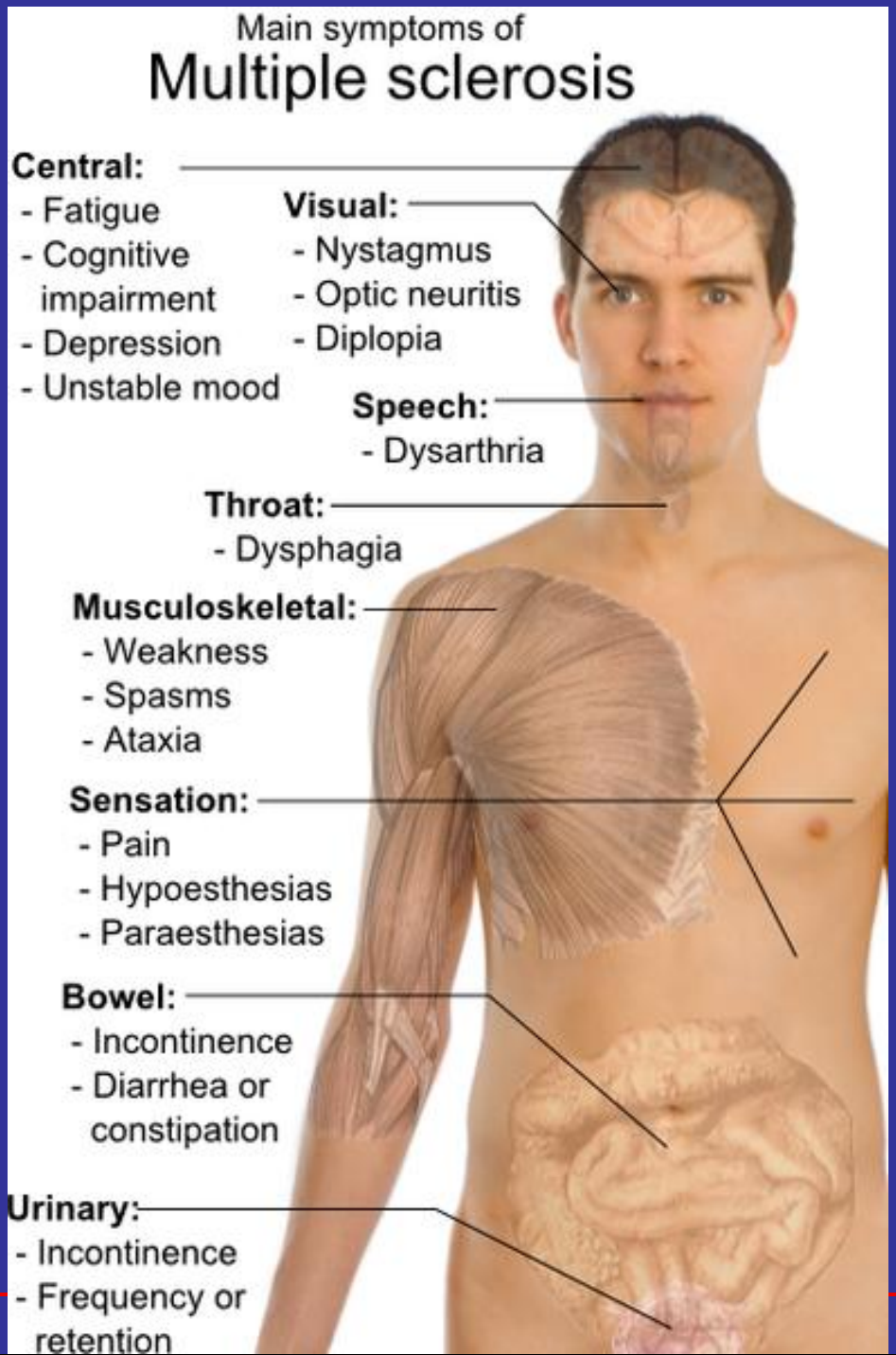
In grey matter tissue: Drop in α-helix content along with the increase in β-sheet content. No significant changes in other chemical species.

In white matter tissue: no significant change in β-sheet nor in α-helix content. But: dramatic increase in phosphorylation .

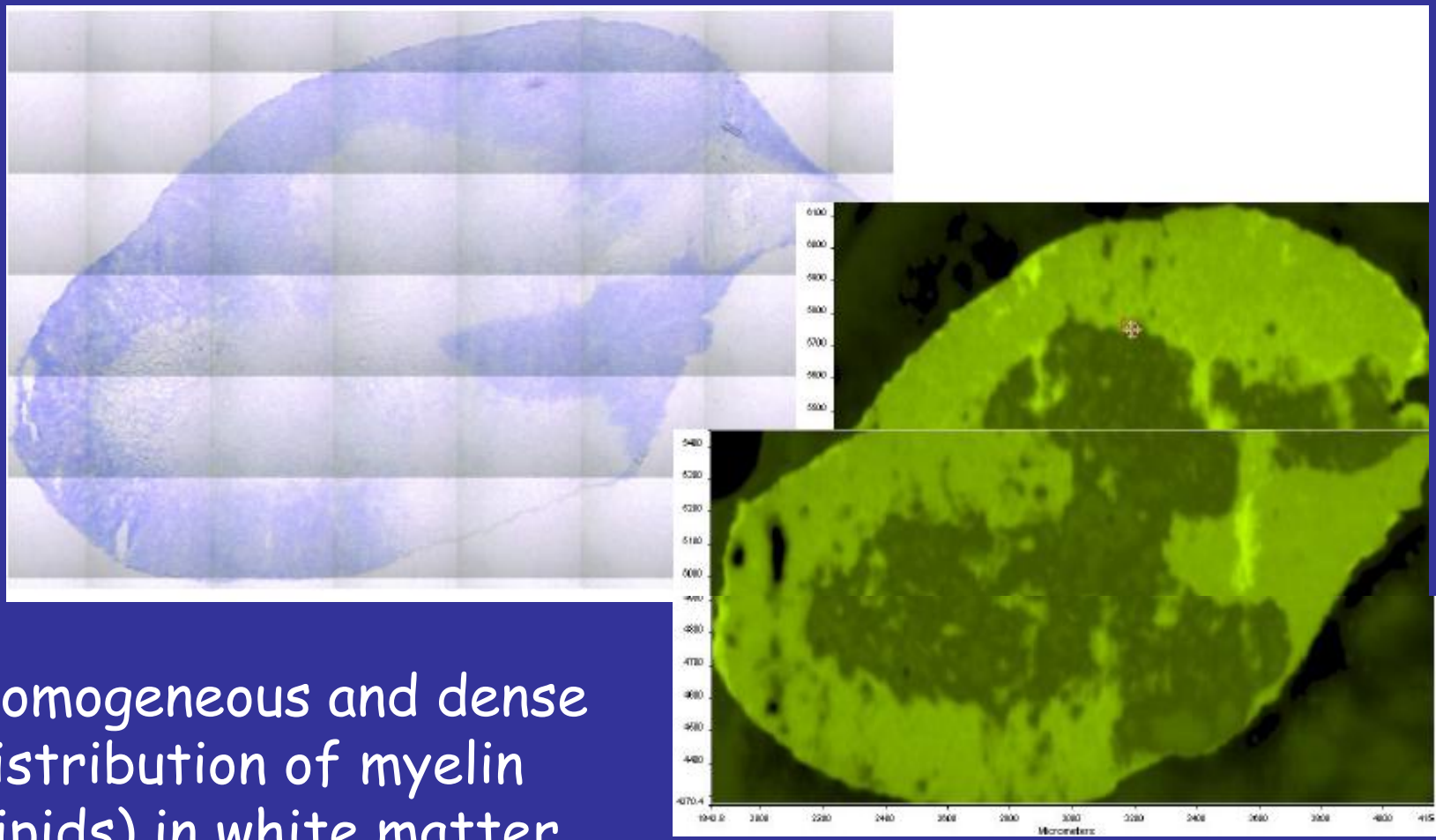
Cultured neurons: Same findings as for grey matter.

2. Multiple Sclerosis (animal model: Experimental Autoimmune Encephalomyelitis)


Dendrites
Neurofibrilles
Microtubules
Neurotransmetteur
Récepteur
Synapse
Vésicules Synaptiques
Synapse (Axoaxonique)
Fente synaptique
Terminaison axonique
RER
(Corps de Nissl)
Polyribosomes
Ribosomes
Appareil de Golgi
Synapse
(Axosomatique)
Nœud de Ranvier
Gaine de myéline
(Cellulle de Schwann)
Axone
Noyau
Nucléole
Membrane
Microtubule
Mitochondrie
REL
Noyau (de la cellulle de Schwann)
Microfilament
Microtubule
Axone
Synapses
(Axodendritiques)
Dendrites

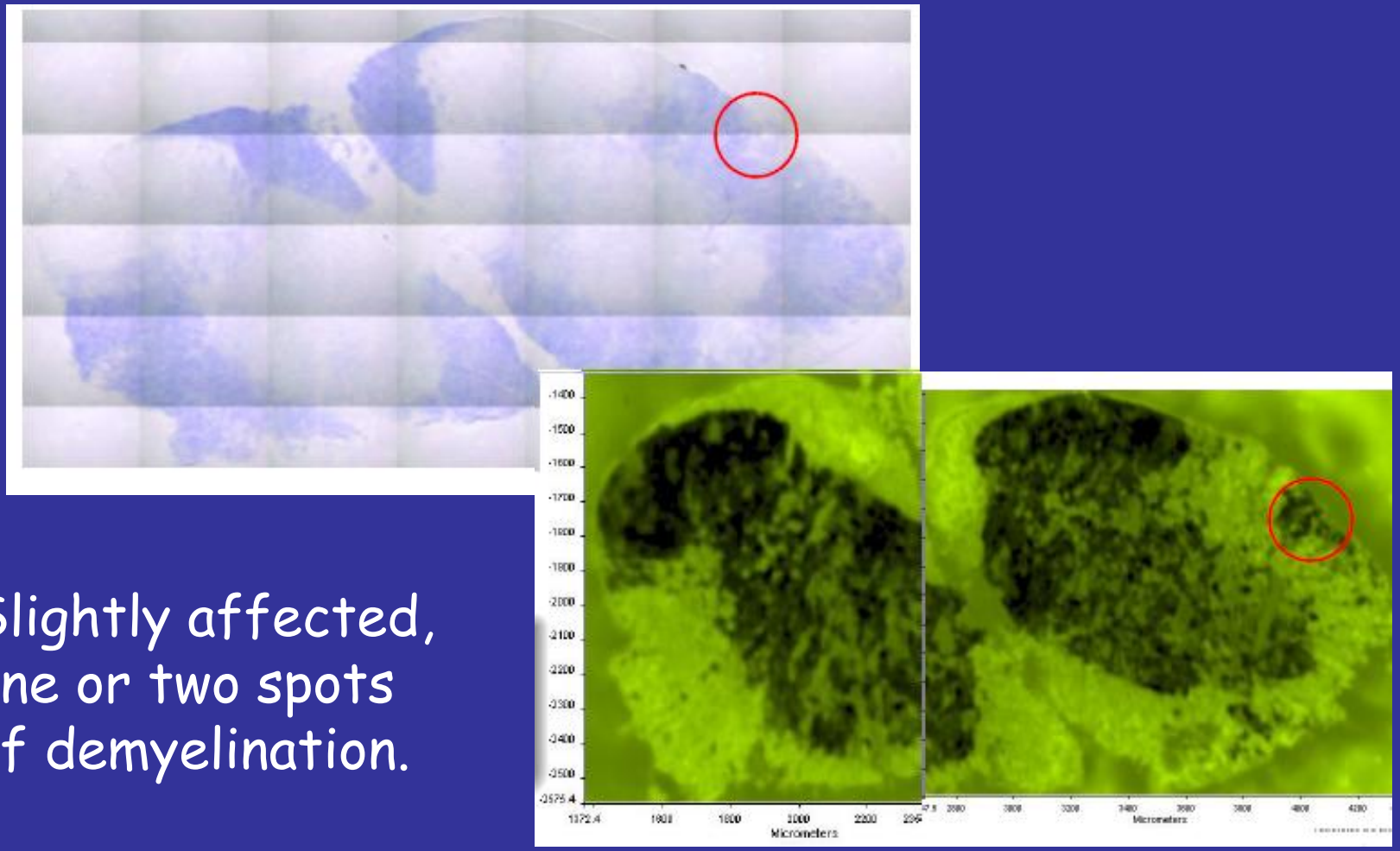

Main symptoms of
Multiple sclerosis
Central:
- Fatigue
- Cognitive impairment
- Depression
- Unstable mood
Visual:
- Nystagmus
- Optic neuritis
- Diplopia
Speech:
- Dysarthria
Throat:
- Dysphagia
Musculoskeletal:
- Weakness
- Spasms
- Ataxia
Sensation:
- Pain
- Hypoesthesias
- Paraesthesias
Bowel:
- Incontinence
- Diarrhea or constipation
Urinary:
- Incontinence
- Frequency or retention

Luxol Blue®™ and PCA in sample 5: healthy



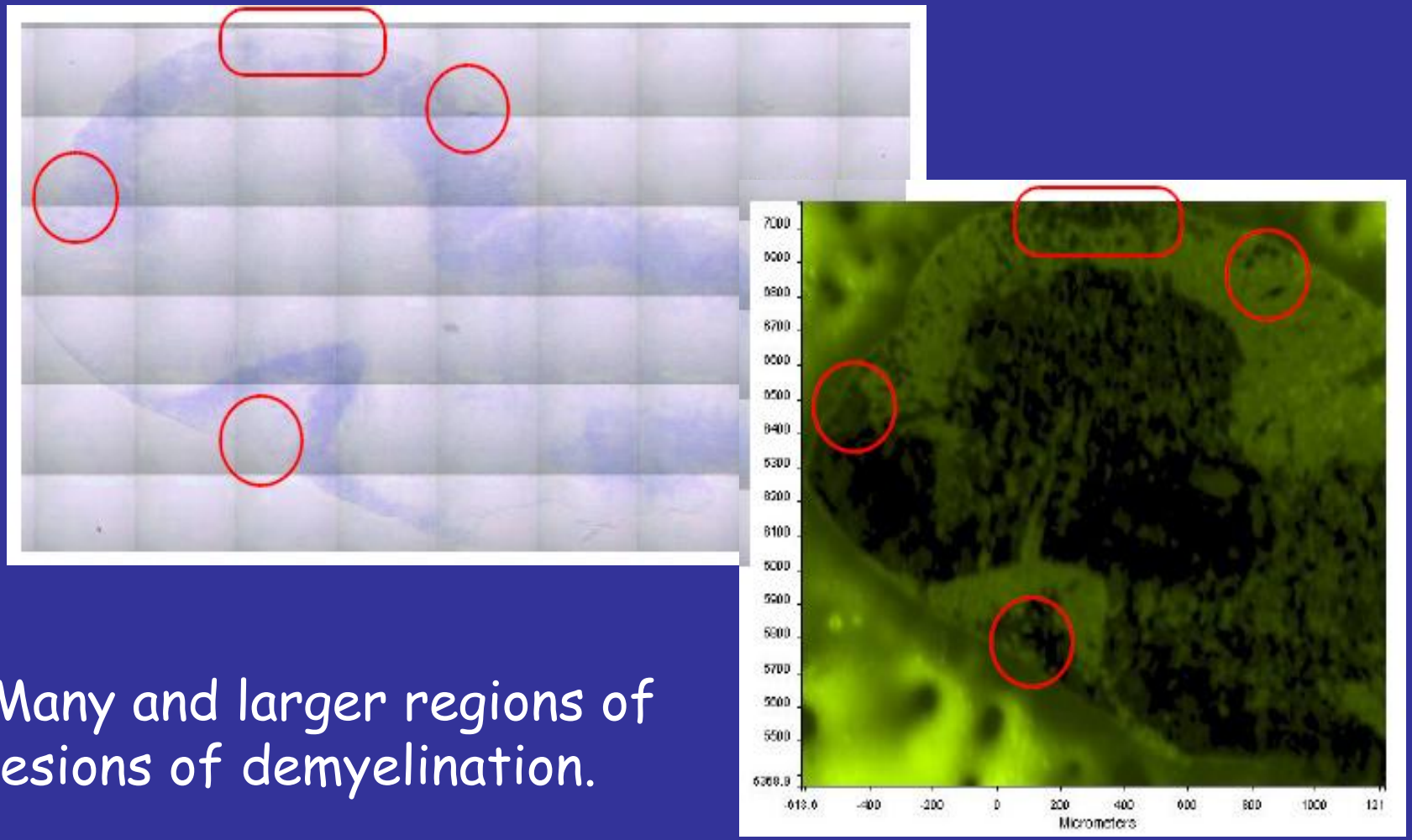
Homogeneous and dense distribution of myelin (lipids) in white matter.

Luxol Blue®™ and PCA in sample 1: medium



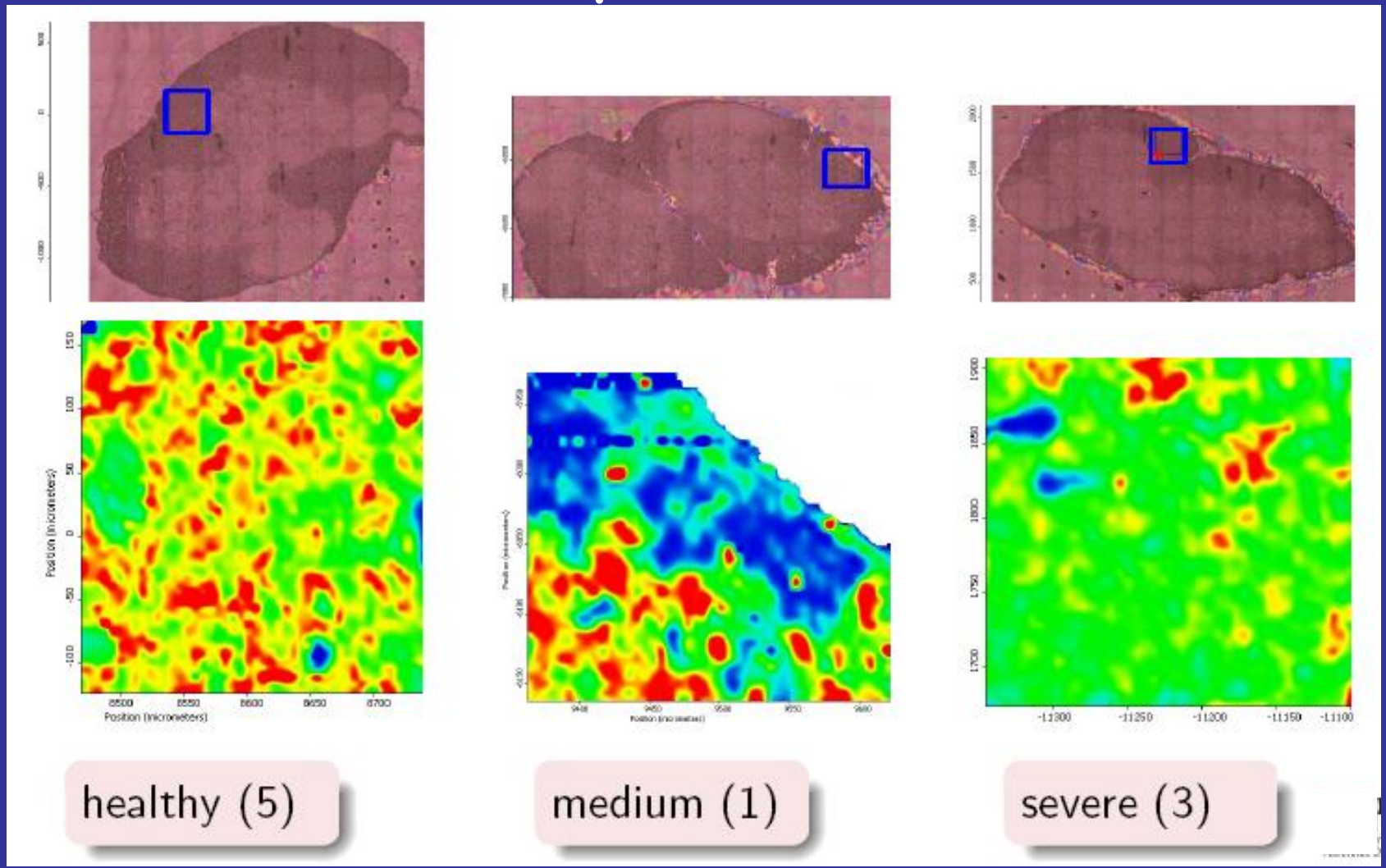
Slightly affected, one or two spots of demyelination.

Luxol Blue®™ and PCA in sample 3: severe



Many and larger regions of lesions of demyelination.

Lipids/proteins in SIRMS for samples 1, 3, 5



Summary of MS study

MS: Perfect compliance of biological marker and IR-measurements. Sharp decrease (↘) of lipids, phosphates and alkenes due to demyelination and peroxidation in white and in gray matter.

Acknowledgements

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BNL, NSLS

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CHUV

Thank you for your attention