# Chemistry 9724y - Special topic

## Micro-spectroscopy and Spectro-microscopy with synchrotron radiation

- X-ray and soft x-ray microbeams
- X-ray and soft x-ray imaging
- IR imaging

# How to achieve a microbeam

Third generation light source can produce X-ray beam of micron size routinely with undulator and KB mirrors. With the use of zone plates, submicron size of the order of $10^2$ nm or even 10 nm can be obtained

**Ingredients for micro beam**

- Undulator
- Optics
    - zone place
    - KB(Kirpatrick-Baze) mirror
    - tapered capillary*

* For confocal X-ray microscopy

# Case studies

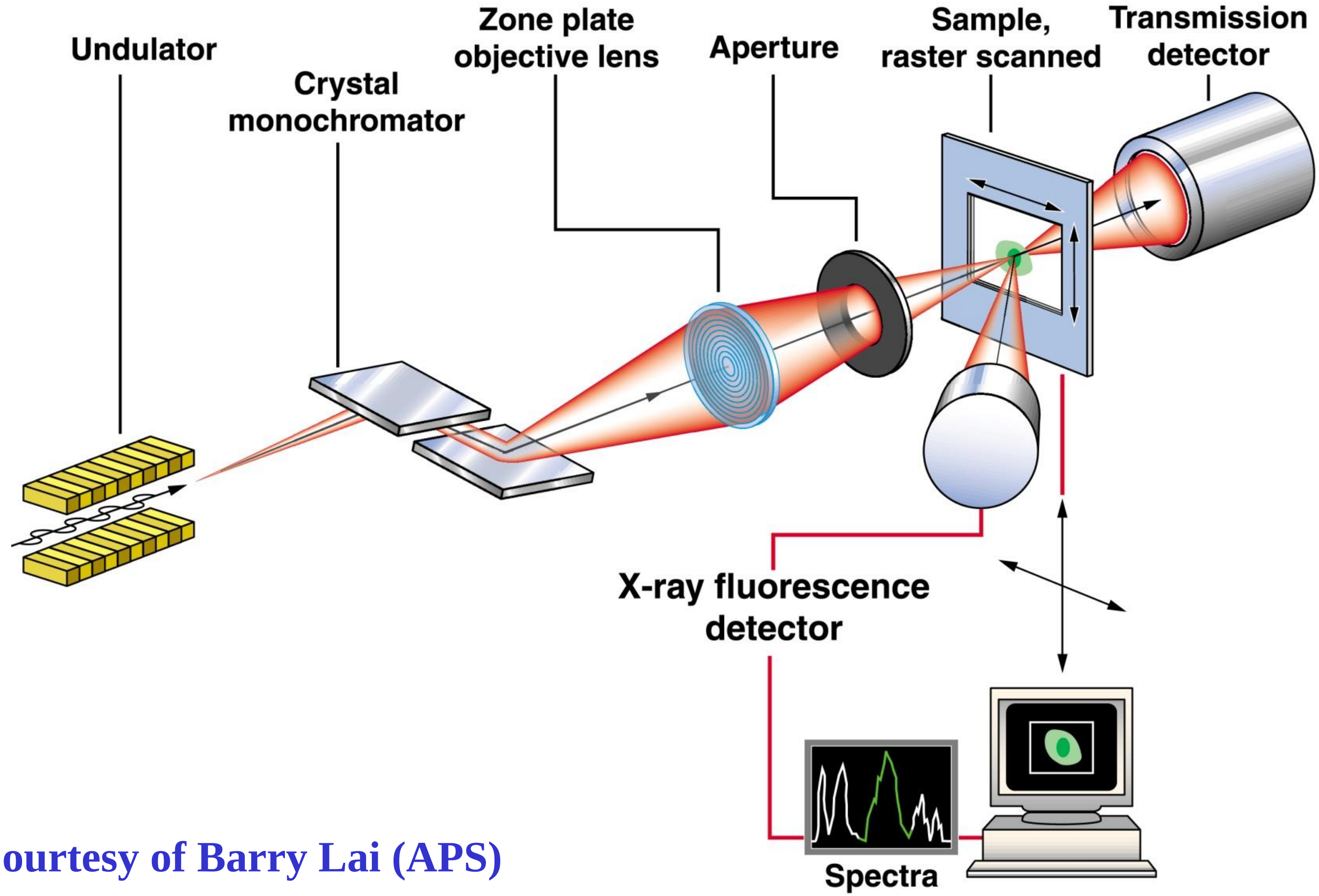
- Zone plate capabilities/submicron spectro-microscopy
- Microprobe analysis of tissues
- IR imaging of tissues
- XEOL imaging

# Microscopy Device: The Zone Plate - The Microscopy line @APS & CLS

+ Sub-micron resolution
+ Capable of micro XANES

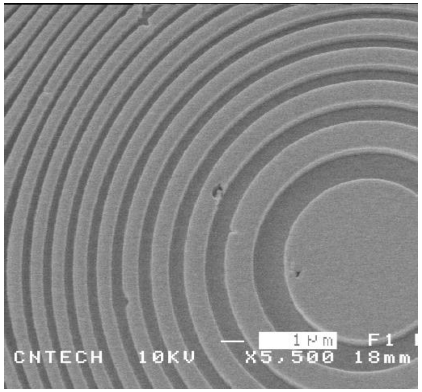
- Not suitable for micro EXAFS

# Schematic of Scanning X-Ray Microprobe @APS




Courtesy of Barry Lai (APS)

# Performance of Fresnel Zone Plates




Parameters: $\Delta r$ = 100 nm
f = 10 cm @ 8keV
N = 385 zones
150 μm diameter

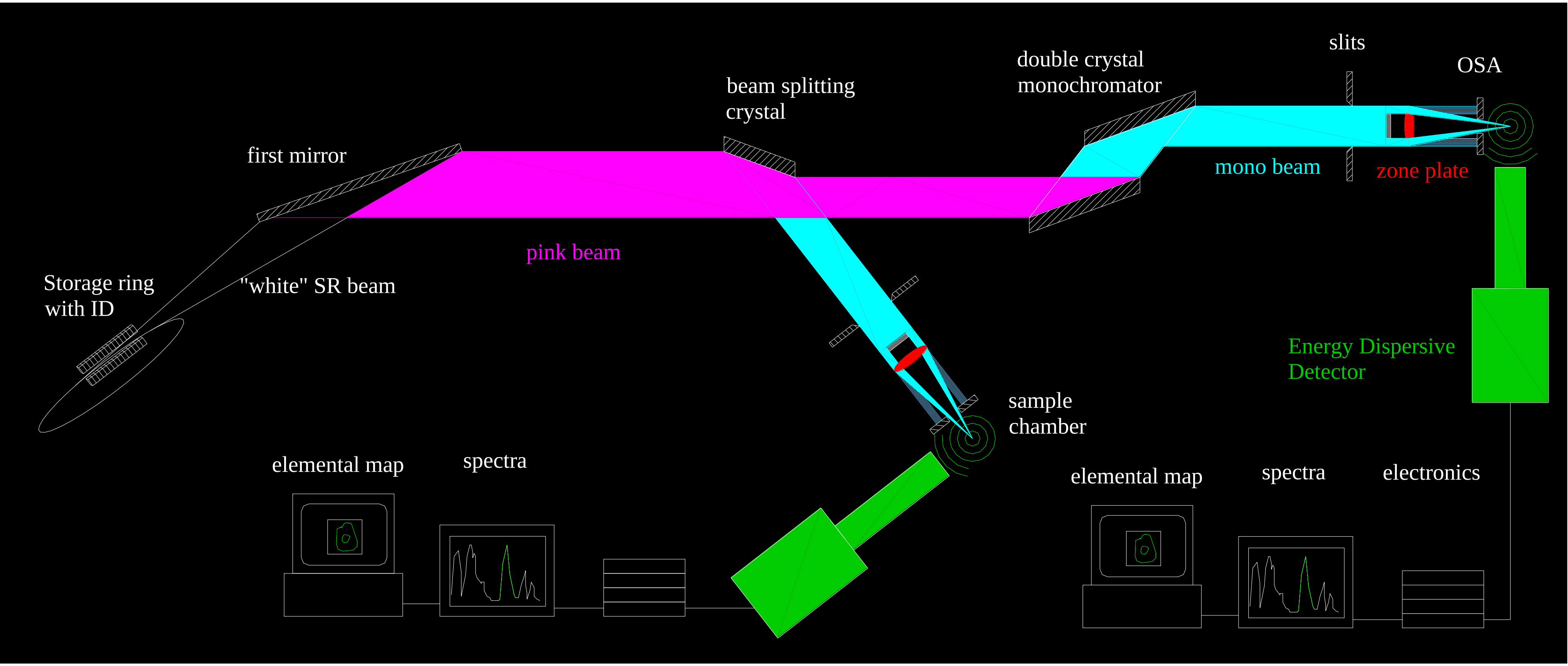
**Spatial Resolution** = **150 nm FWHM**

**Efficiency** = **20-25% (with two Au ZPs)**

**Flux density** = **$2 \times 10^{11}$ photons/sec/$\mu m^2$/0.01%BW**

**Flux density gain** = **$3 \times 10^{4}$**

# Hard X-ray Microprobe Facility, APS sector 2



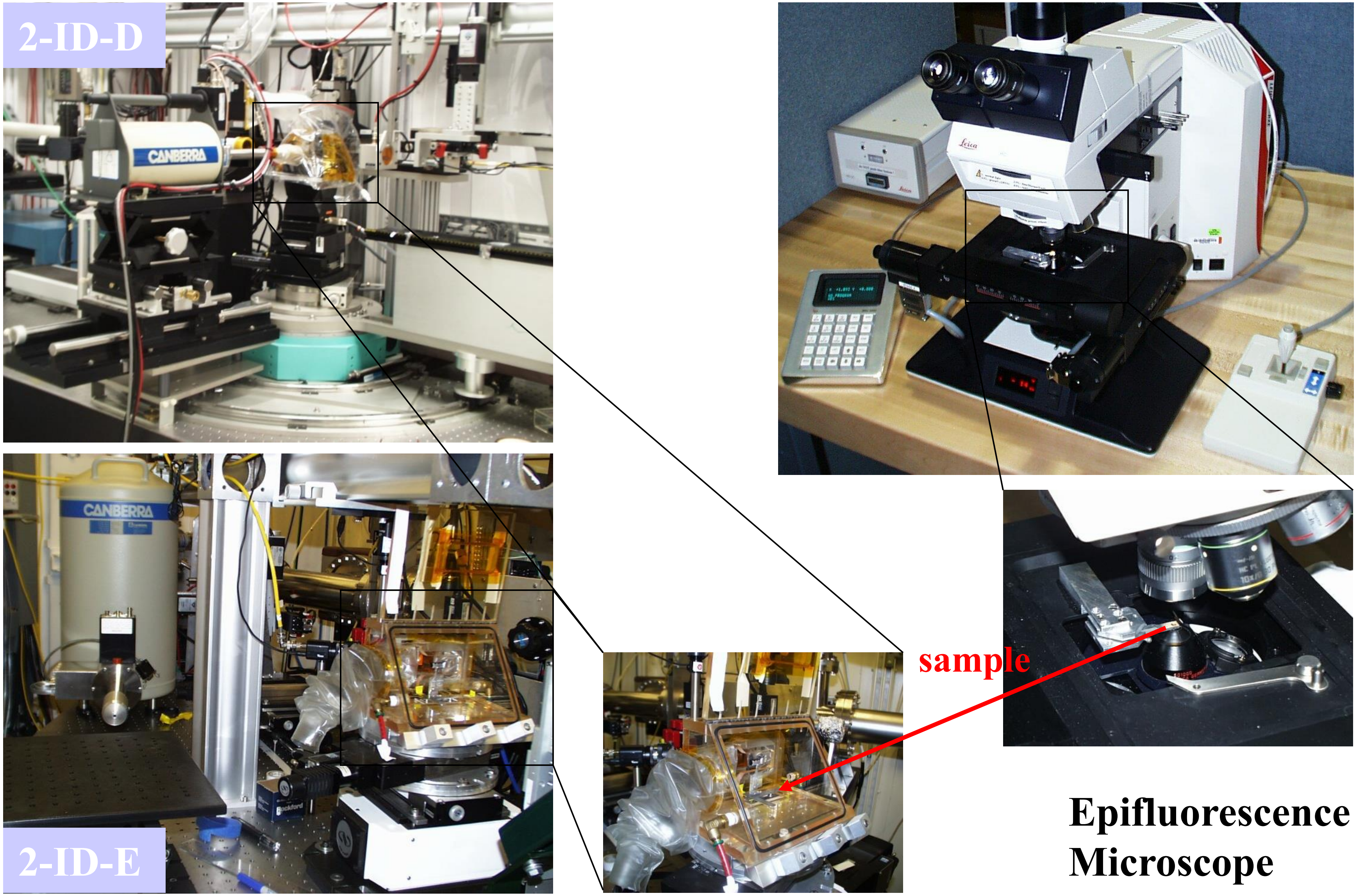

**Main branch 2-ID-D:** **E = 5 – 30 keV,** **$\delta$ = 150 nm ↔** **$2 \cdot 10^9$ phot/s**

**Side branch 2-ID-E:** **E = 7 – 15 keV,** **$\delta$ = 250 nm ↔** **$5 \cdot 10^8$ phot/s**

**Integrated epi-fluorescence microscope**

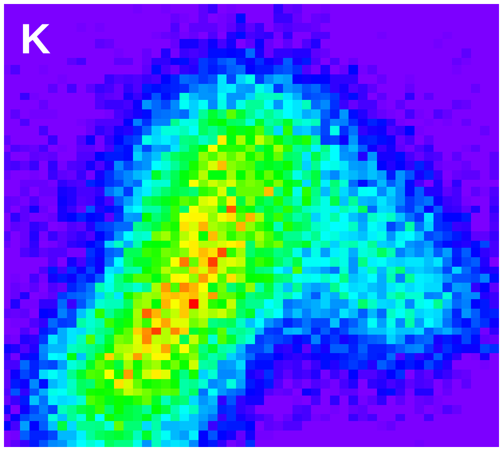
**Courtesy of Barry Lai (APS)**

# 2-ID-D/E Hard X-ray Microprobe Facility

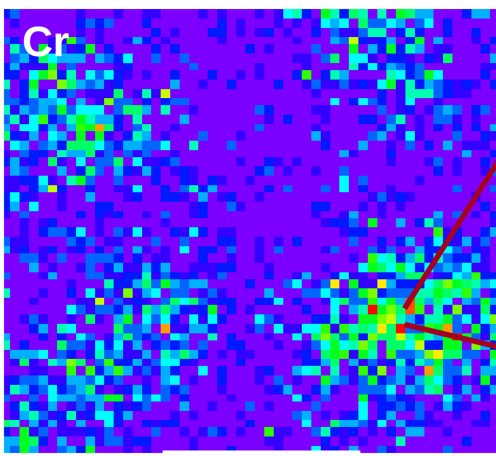


# Hydrated *Pseudomonas fluorescens* treated with Cr(VI)

K

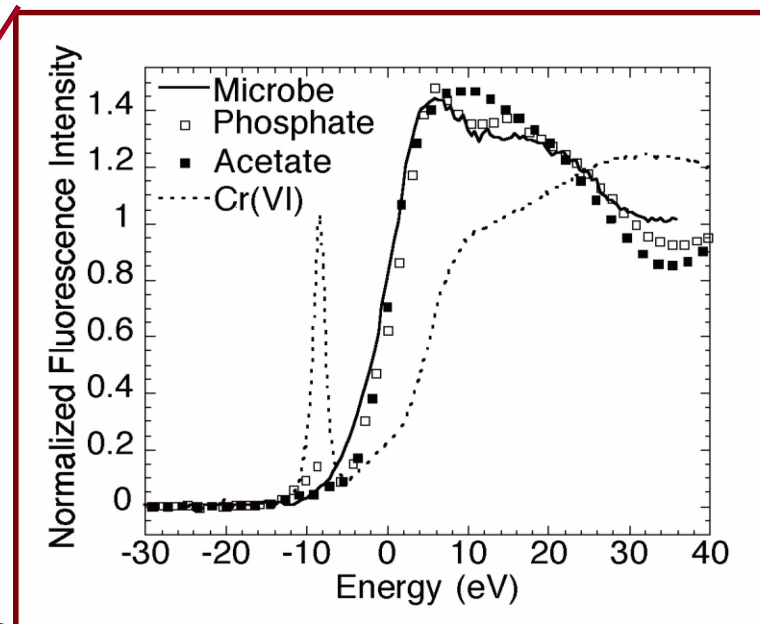



Cr




5 μm

Probe local chemistry *in-vivo* with micro-XANES: Cr(VI) → Cr(III)

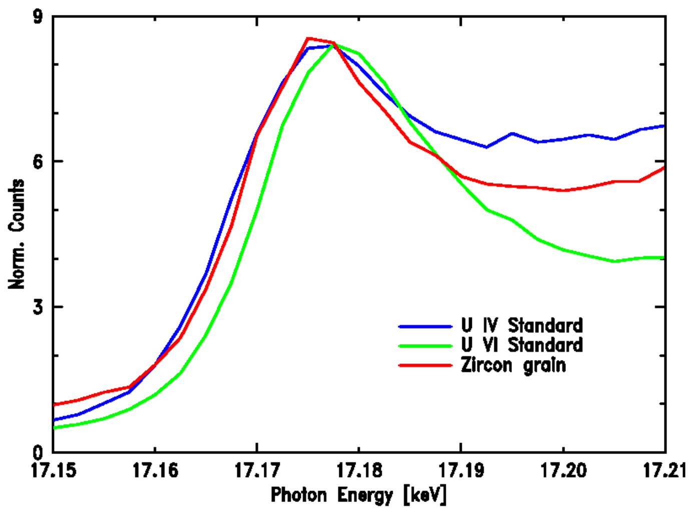


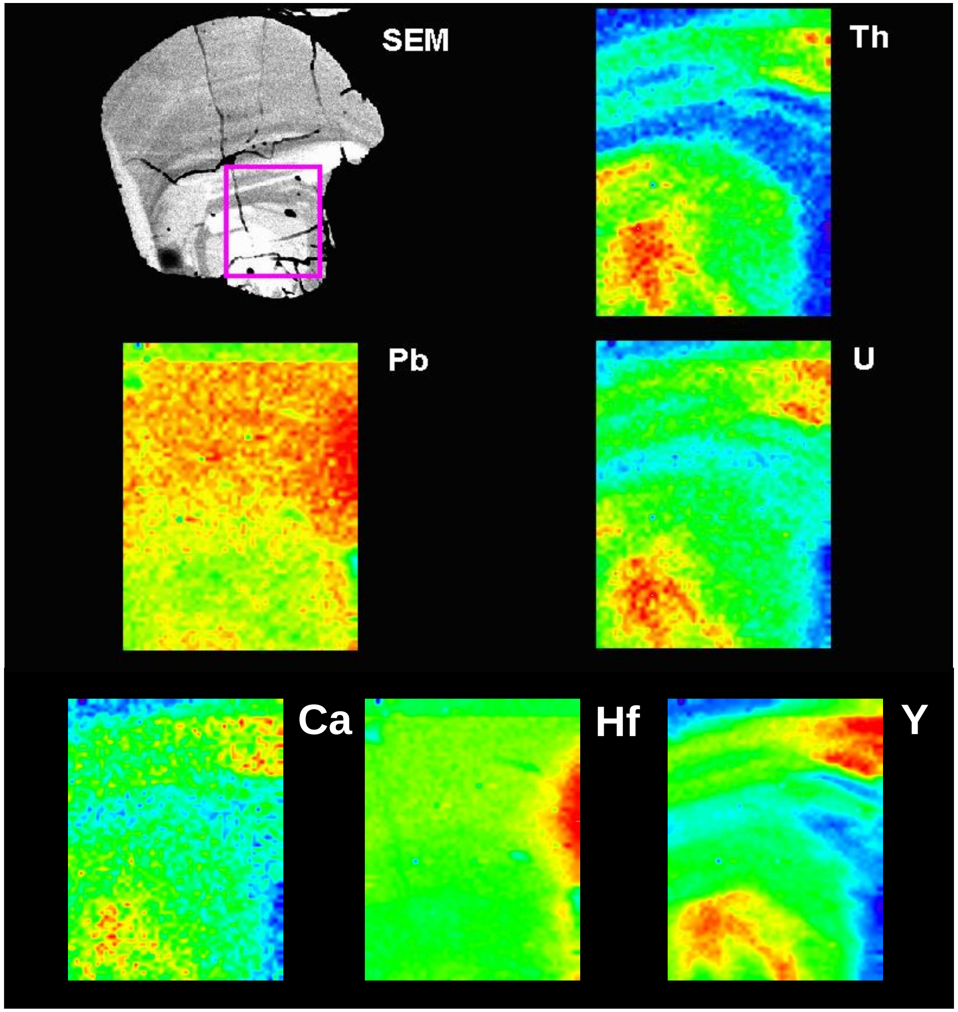

**Pixel size 100 nm**

K. Kemner *et al.*, Science **306**, 686-687 (2004)

# Micro-Imaging and -XANES from Mineral Sands

- Aim: to image and analyse chemical state of radioactive trace elements in minerals: Zircon example
- X-ray Absorption Near Edge Spectroscopy (XANES) determined uranium is present as $U_{IV}$
- Chemical state is needed to design process to remove these elements

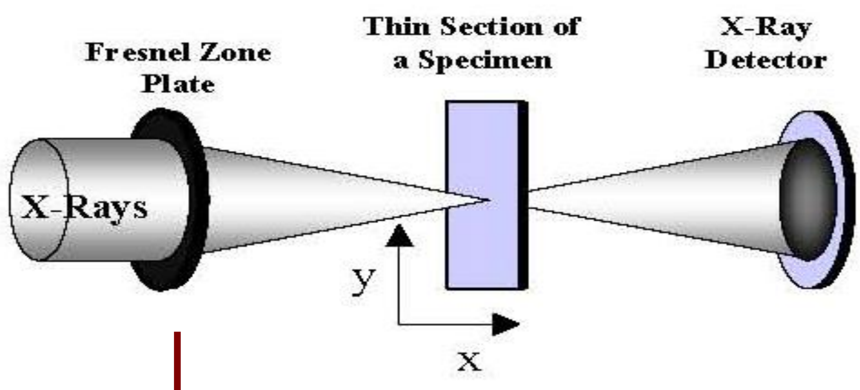


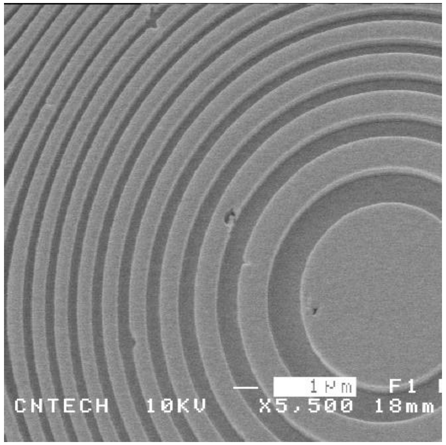




Concentration ranges: U 13 ppm to 33 ppm
Th 3 ppm to 11 ppm
Pb 3 to 4 ppm

AUSTRALIAN
SYNCHROTRON
RESEARCH
PROGRAM

# Microscopy Device: The Zone Plate - The Microscopy line @ CLS

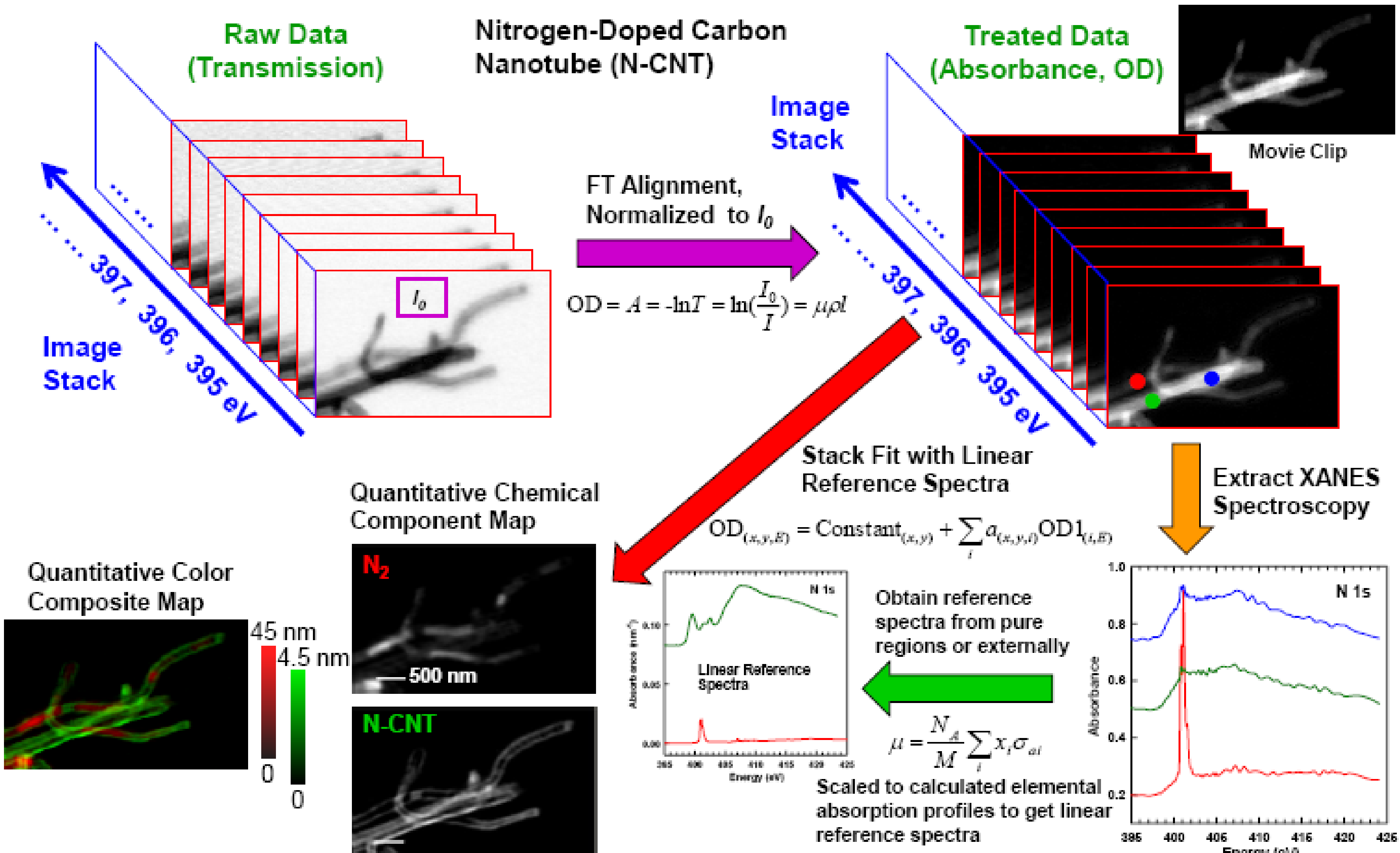



**Fresnel Zone Plates**

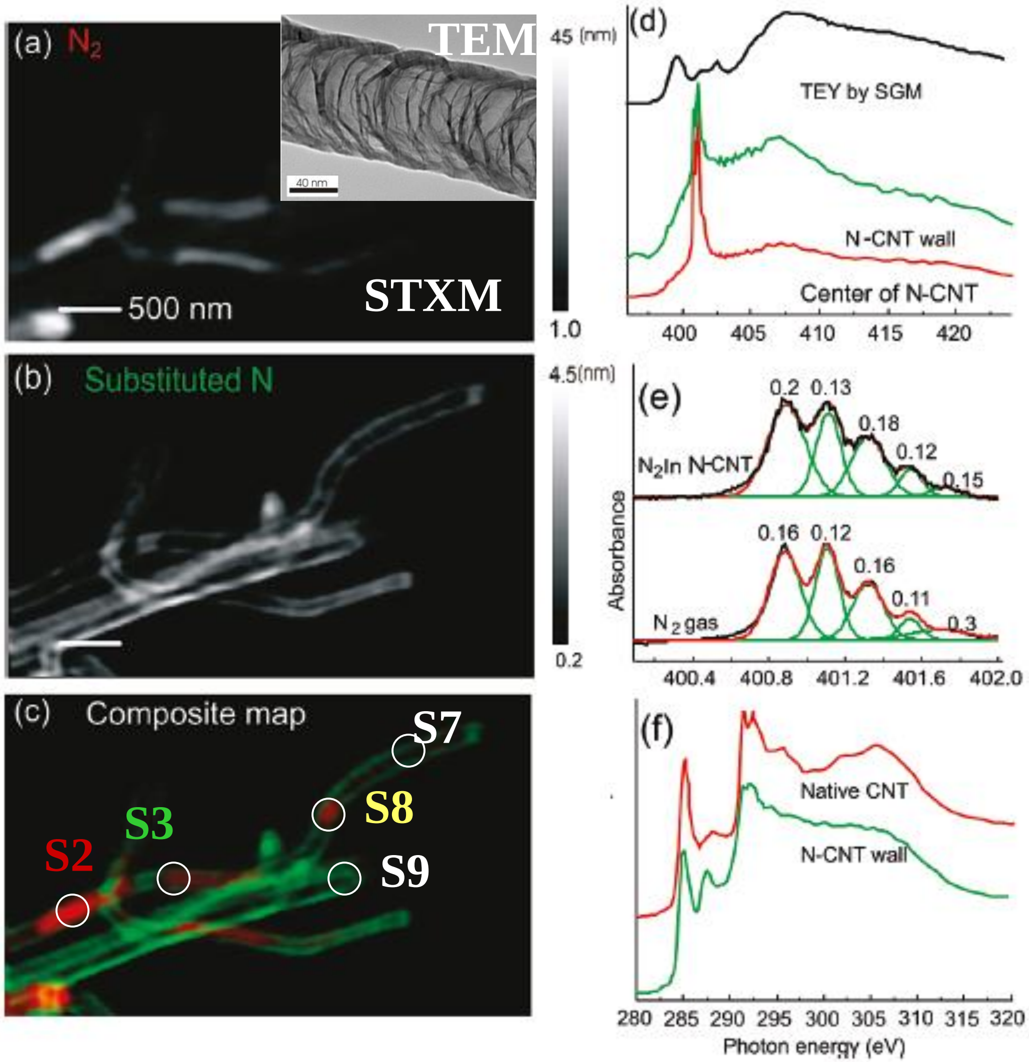
**30nm spatial resolution**

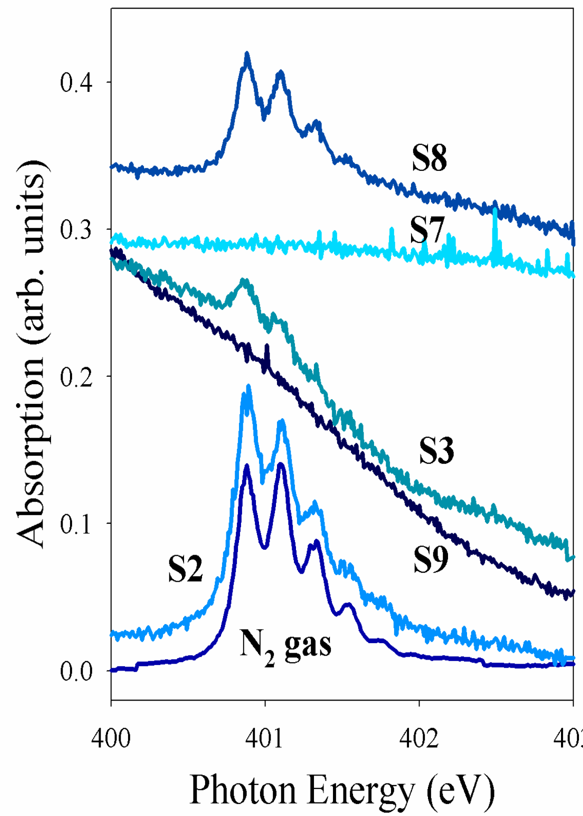
# STXM: Scanning Transmission X-ray Microscopy




J. Zhou et al. *J. Phys. Chem. Lett.* 1 (2010) 1709.

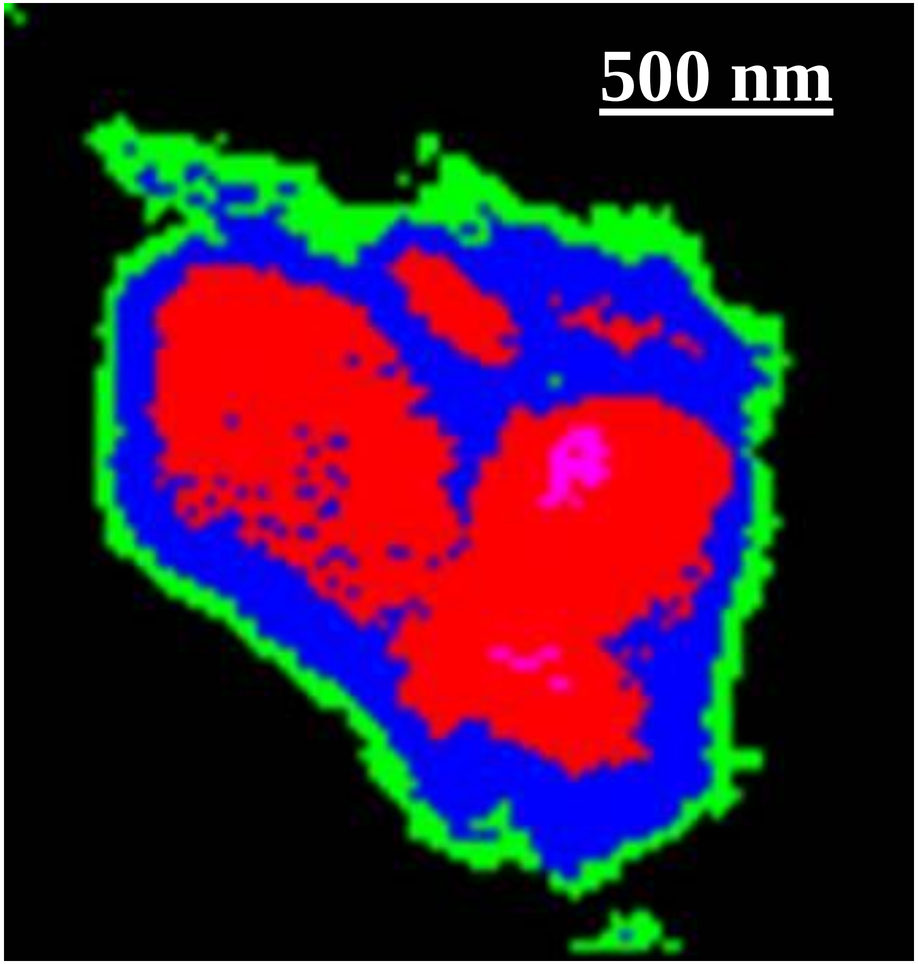
# Mico/nano spectroscopy of N-CNT




J. Zhou et al. **J. Phys. Chem. Lett.** 1, 1709 (2010)

# Chemical imaging a multilayer graphene




**Green**: 1 layer (OD: 1.47-2.2),

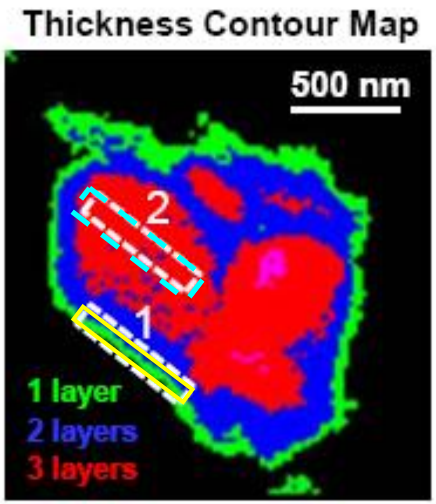
**Blue**: 2 layers (OD: 2.2-3.3),

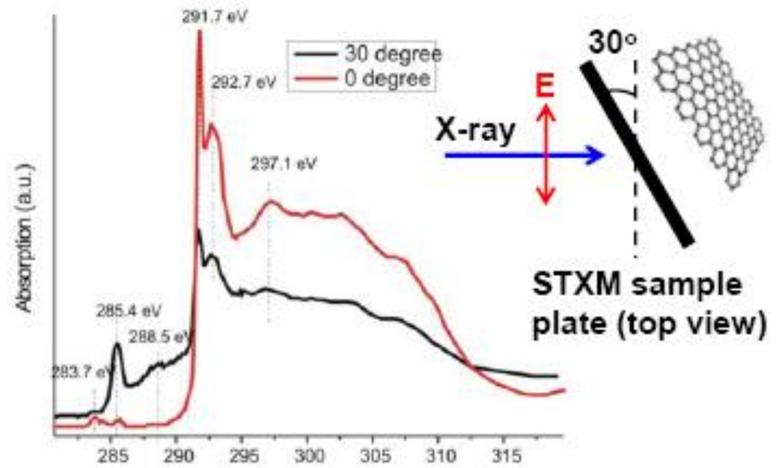
**Red**: 3 layers (OD: 3.3-4.4), and

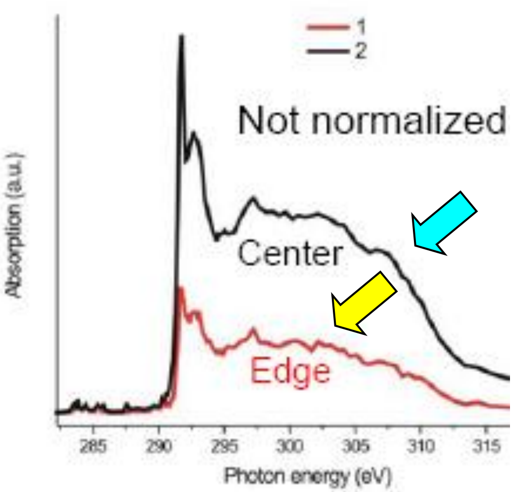
**Purple**: 4 layers (OD: 4.4-4.7).

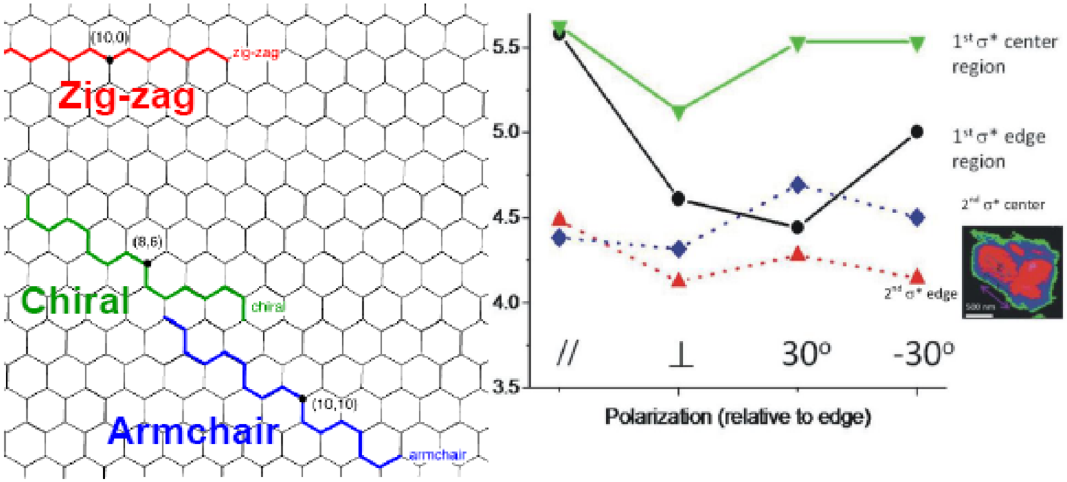
J.G. Zhou, J. Wang, C.L. Sun, J. M. Maley, R. Sammynaiken, T.K. Sham, W.F. Pong, **J. Mater. Chem.** 21, 14622 (2011).

# Nano-spectroscopy of selected regions

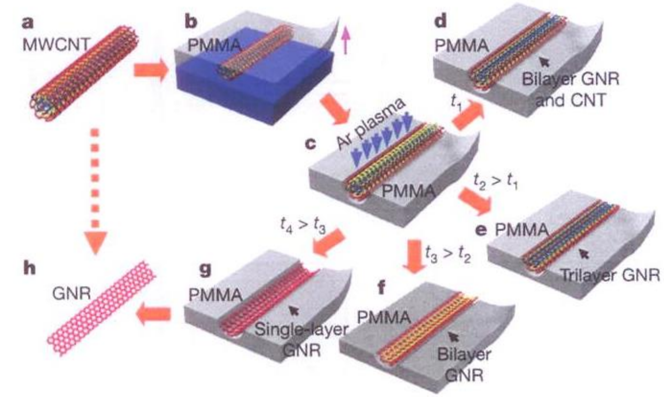


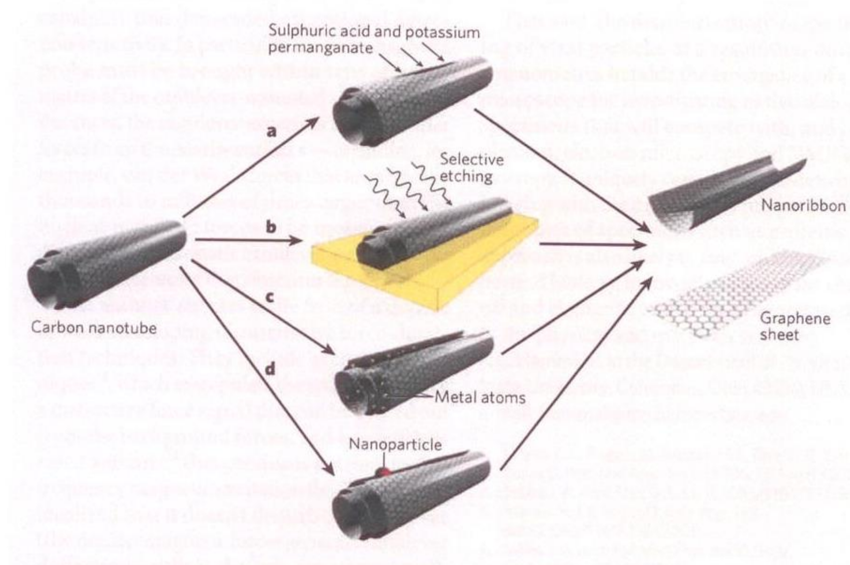
# Unzipping of CNT to form Graphene Nanoribbons (GNR)

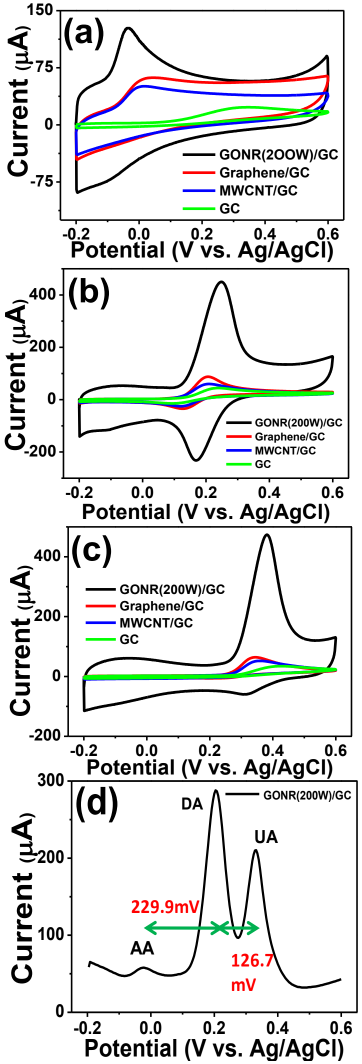

D.V. Kosynkin et al. ***Nature,*** *458*, 872 (2009)

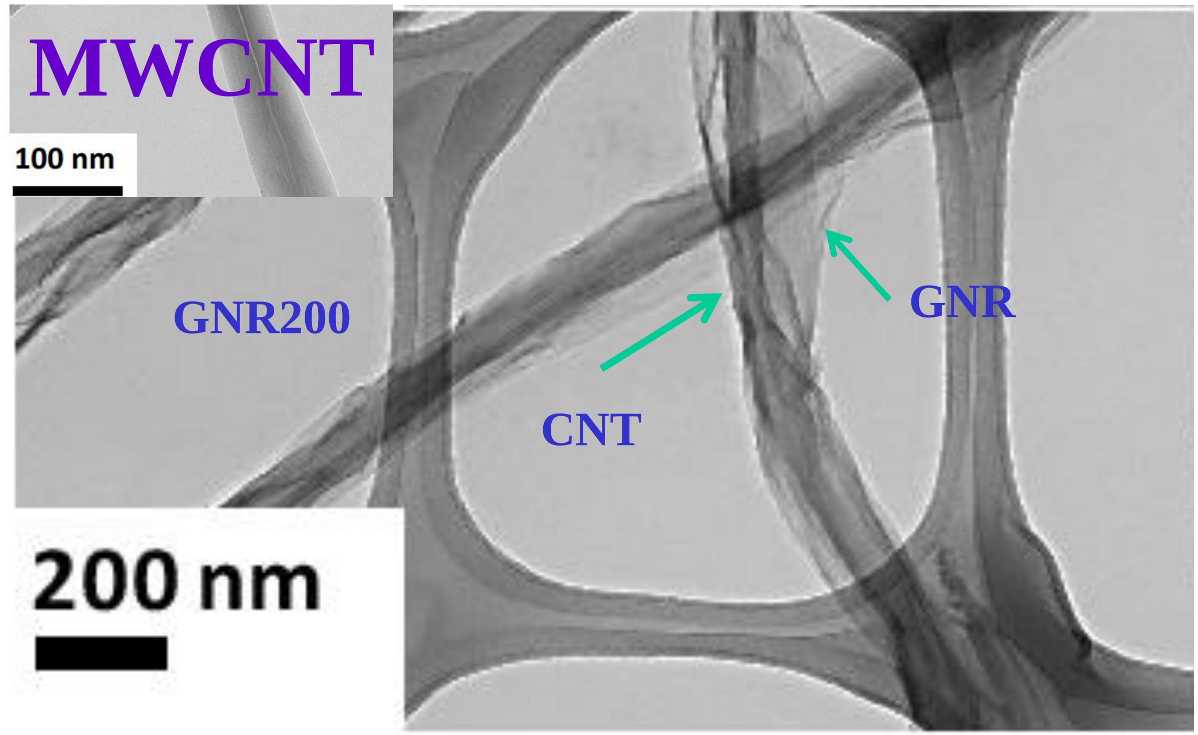
L. Jiao et al. ***Nature***, *458*, 877 (2009)

M. Terrones, ***Nature***, *458*, 845 (2010) News and Views



# Graphene oxide nanoribbons (GONRs) from microwave unzipping of MWCNTs

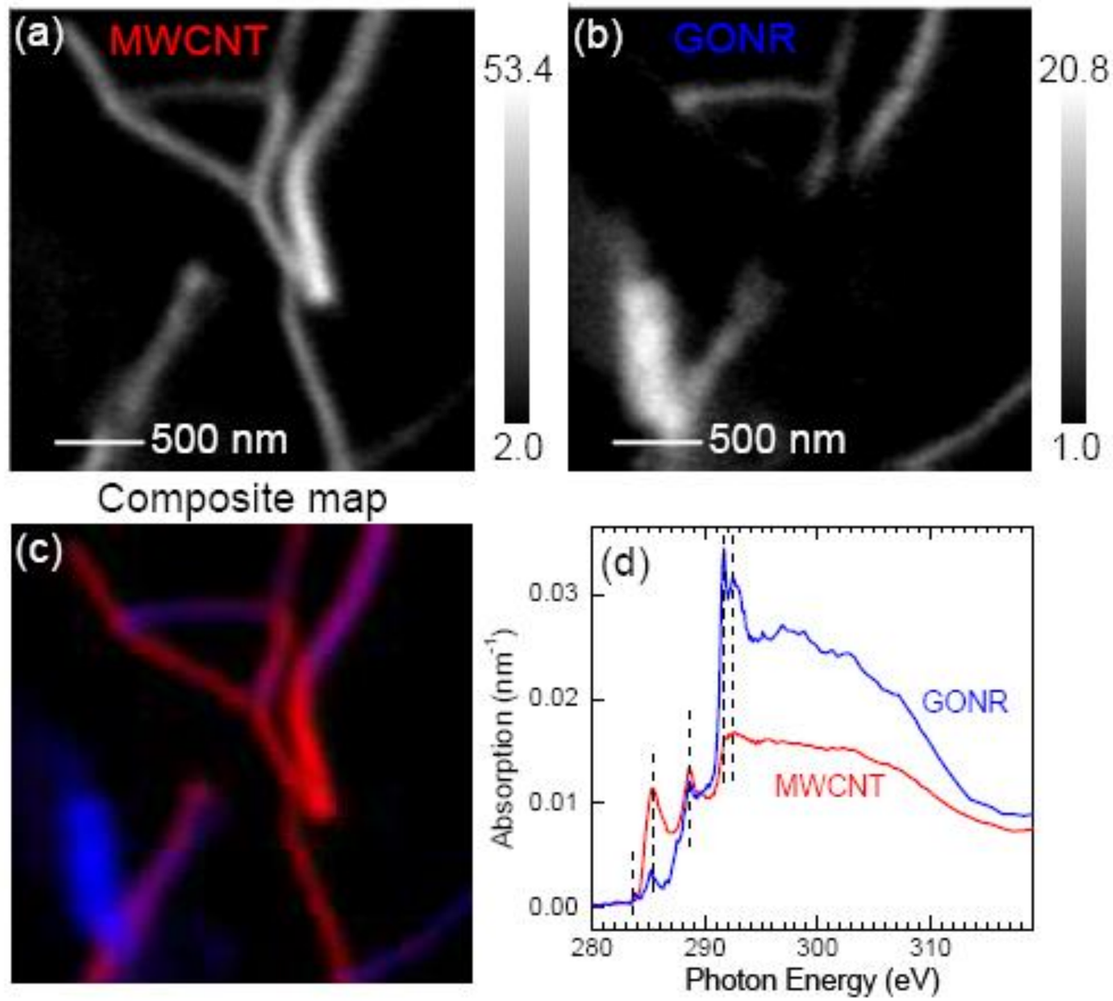



MWCNT/GONR-modified **glassy carbon electrode** was used to electrochemically detect (a) ascorbic acid (AA), (b) dopamine (DA) and (c) uric acid (UA).

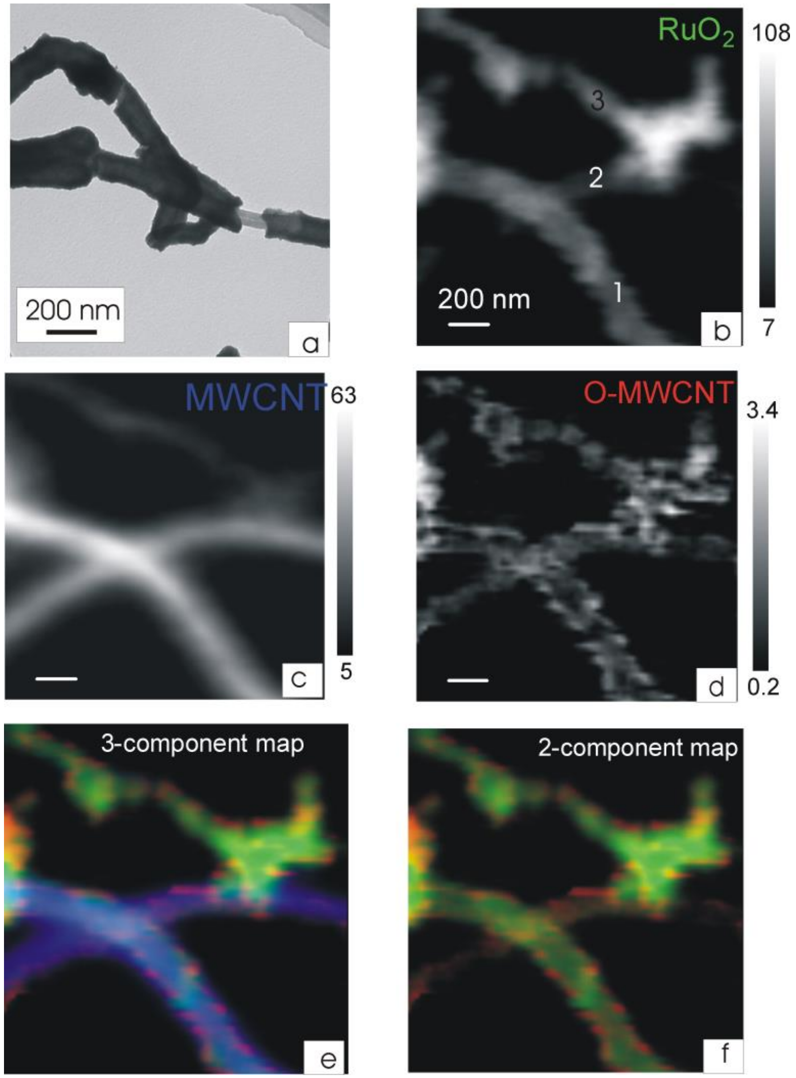
**C.L. Sun et. al. ACS Nano: DOI 10.1021/nn2015908**

# STXM of MWCNT/GONR Nanocomposite




C.L. Sun et al. **ACS Nano** (2011)**DOI:** 10.1021/nn2015908

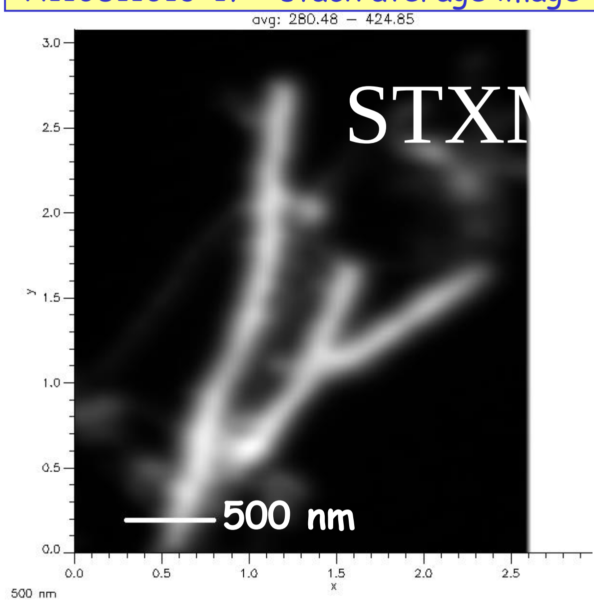
# STXM: $RuO_2$/MWCNT

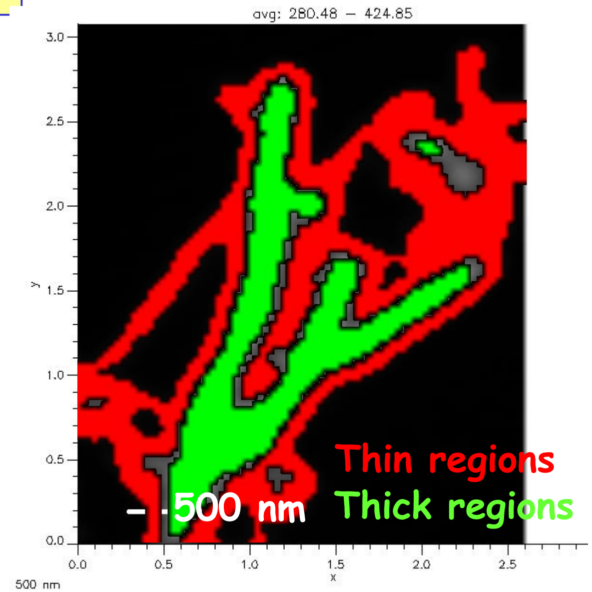


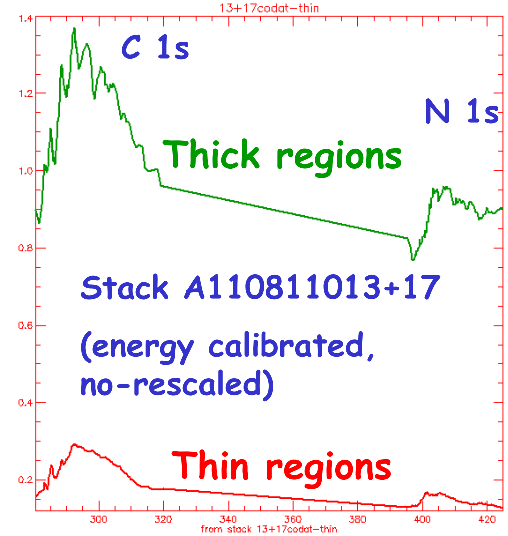

J.G. Zhou, J. Wang, H.T. Fang, C.X. Wu, J.N. Cutler, T.K. Sham, ***Chem. Comm.*** 46, 2778 (2010)

# PtNW/NCNT: C 1s + N 1s stack A110811013 + 17, region B

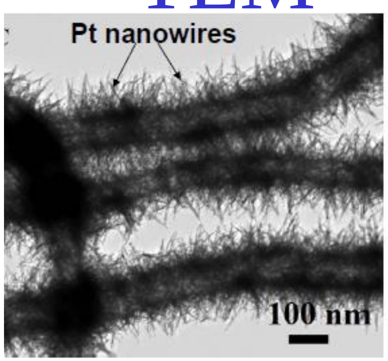
A110811013+17 – stack average image



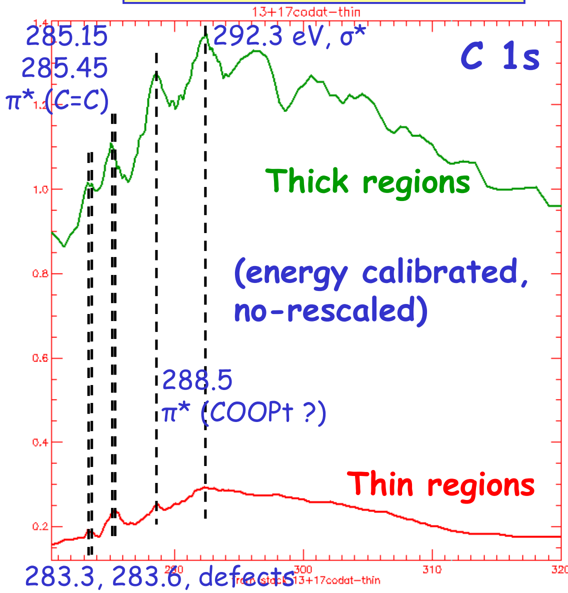



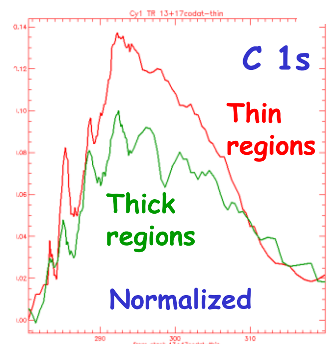

TEM

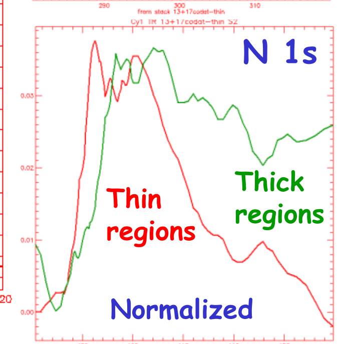



Shuhui Sun et al
to be published

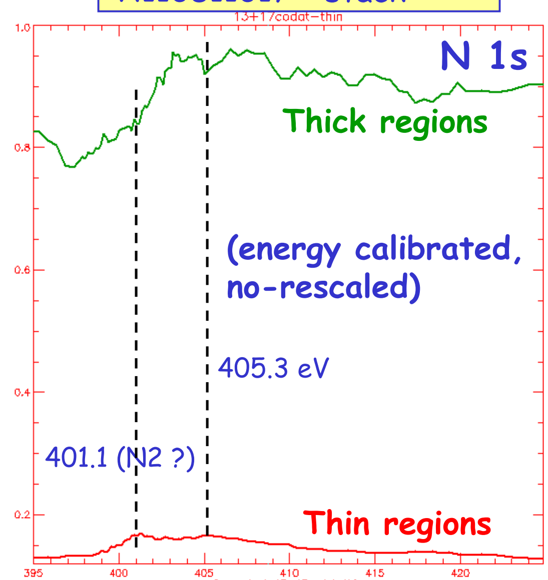
A110811013 – stack



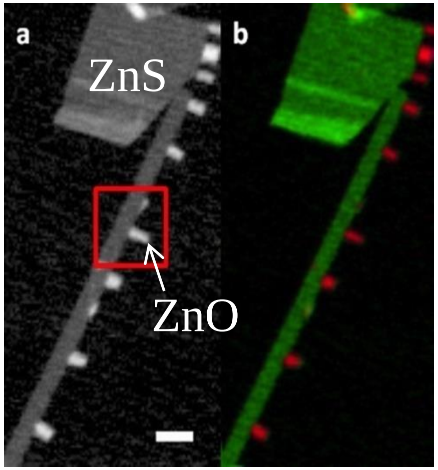



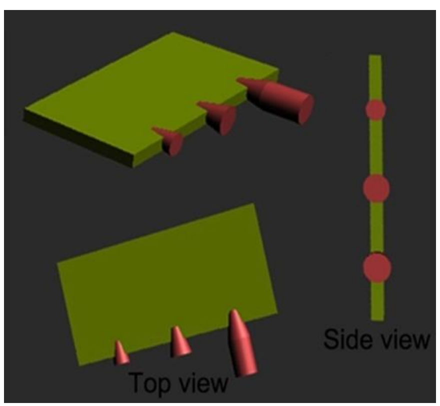



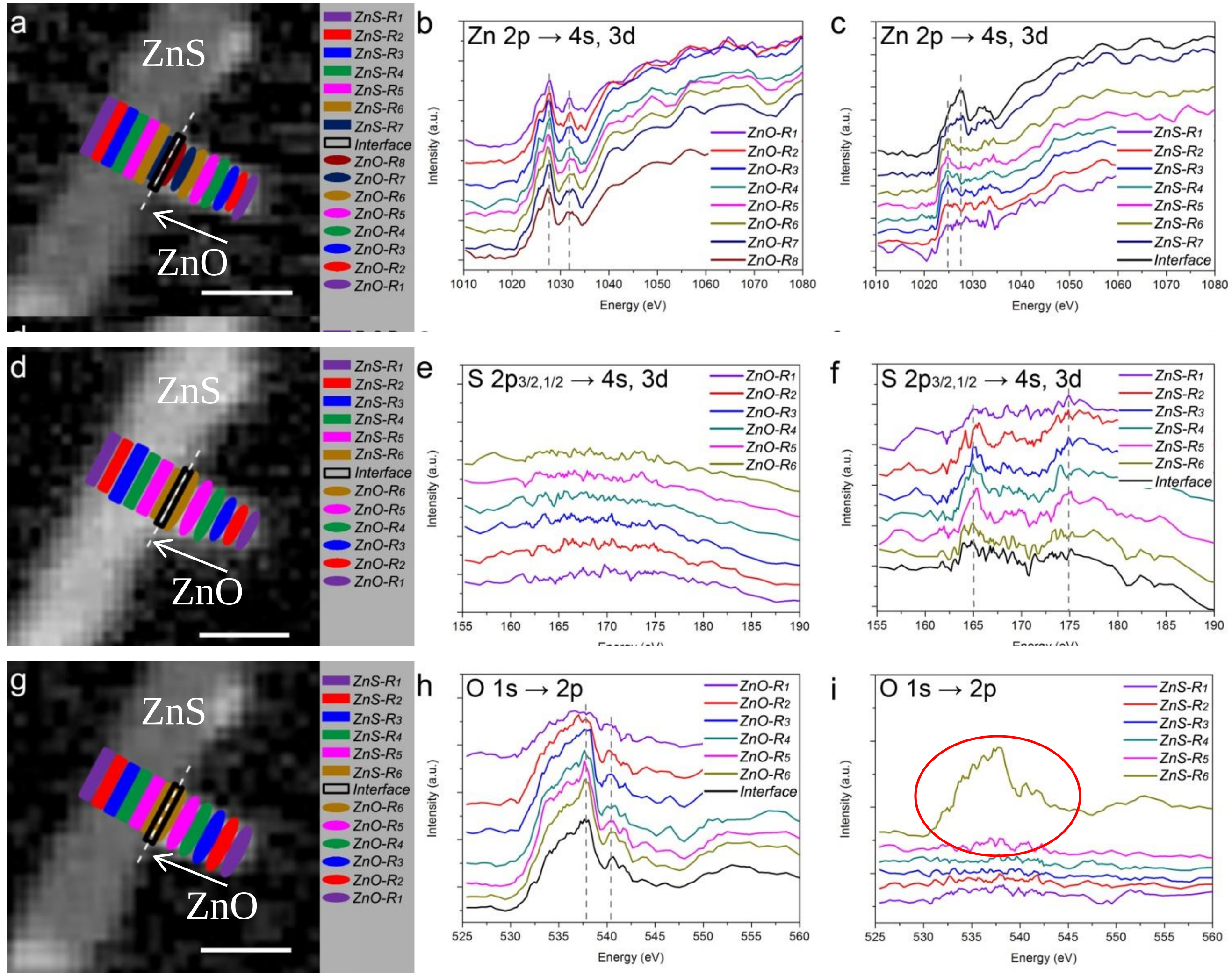

A110811017 – stack



# ZnS/ZnO Nano-Heterostructure Interface




Z. Wang, et al. J. Phys. Chem. C, 116, 10375 (2012)

# Micro Analysis of Tissues
# Imaging and Microspectroscopy

## Synchrotron X-ray and IR Imaging of Hemochromatosis Liver (Human) and Diabetic Mice Tissues: Preliminary Observations

*T.K. Sham[a], P.-S. Grace Kim[a], Huong Ngo[a], Subrata Chakrabarti[b], and Paul C. Adams[b,c]*
*Departments of Chemistry[a], Pathology[b] and Medicine[c], University of Western Ontario*

**T.K. Sham, P.S. G Kim, H. Ngo, S. Chakrabarti and P.C. Adams** Physica Scripta,
**T115, 1047-1049 (2005).**

- **Introduction**
  - Objectives
  - Methodology
- **Human liver tissues -hemochromatosis**
  - Metal images
  - Micro-XAFS at the Fe K-edge
- **Mouse kidney tissues-diabetes**
  - Metal images
  - Micro-XAFS at the Cu K-edge
- **IR spectromicroscopy/microspectroscopy**
- **Summary and outlook**

## Introduction

We aim at the development of a microscopy/ spectroscopy technique to investigate tissue specimens (e.g. iron in liver biopsy tissues) using a ***third generation synchrotron light source – APS/CLS***

To develop the methodology to reveal the ***distribution and local chemical characteristics*** of metals in tissues with a μm x-ray beam.

## Objectives

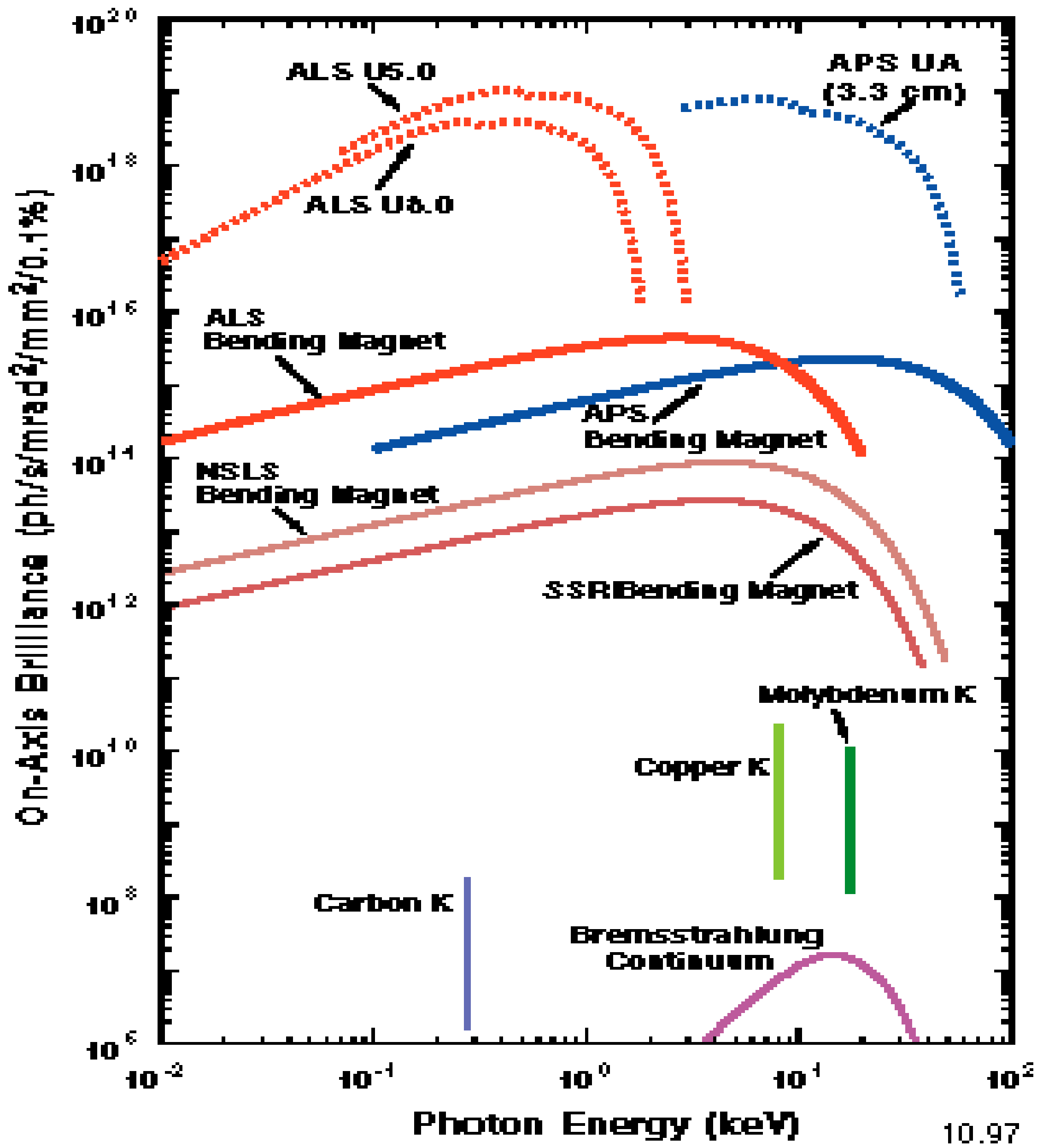
To study the distribution and chemical identity of iron in ***hemochromatosis*** liver and ***related tissue analysis*** using ***X-ray microprobe***

## Methodology

Studies were conducted using a ***micro x-ray beam*** from the ID beamline of the Pacific Northwest Consortium (***PNC***) – ***CAT*** at Advanced Photon Source (***APS***). APS produces bright and tunable X-rays by accelerating electrons to an energy of 7 GeV and wiggling them through a magnetic structure called an insertion device (**ID**).

## The APS Synchrotron Light Source

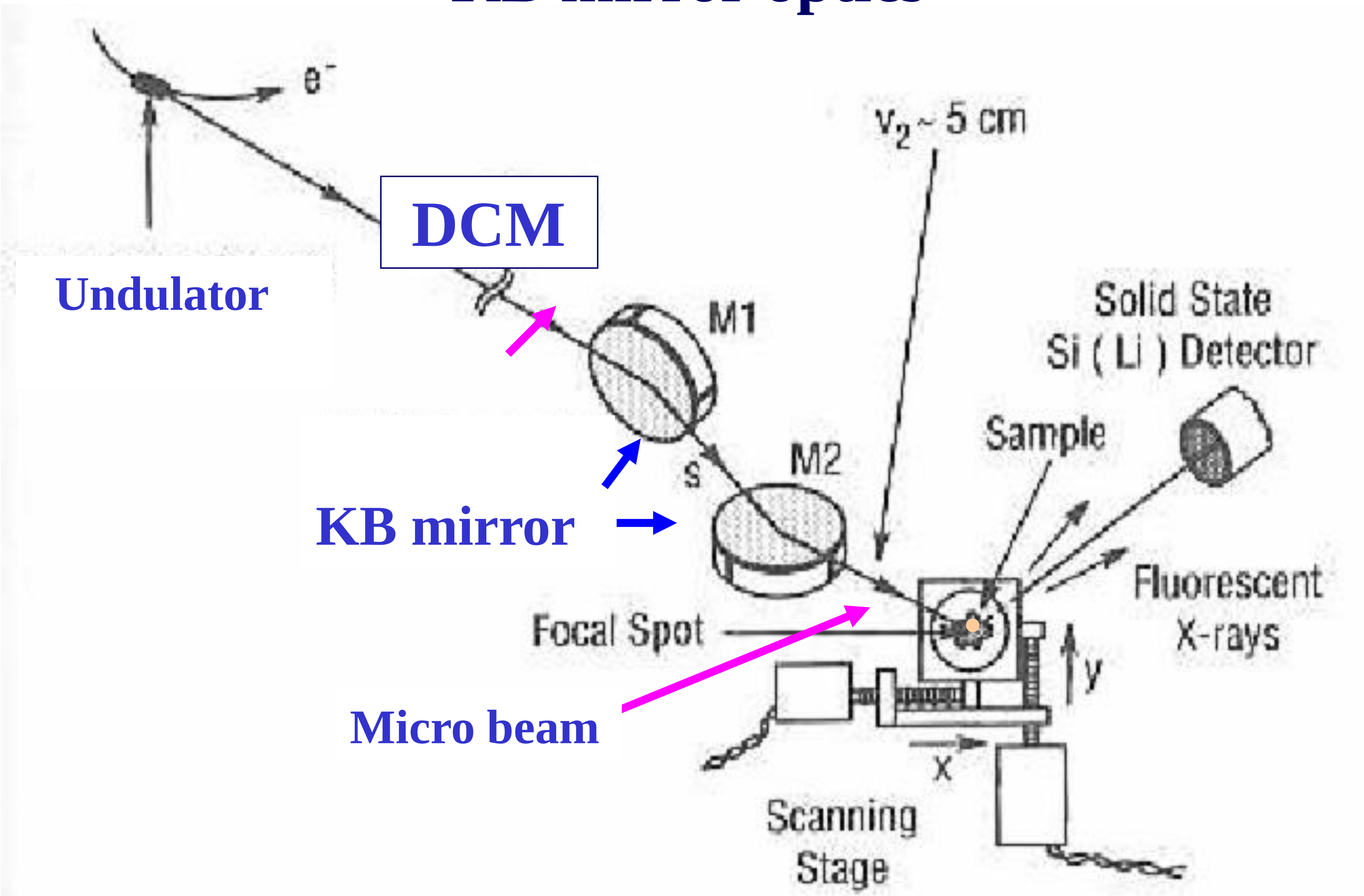
If **brightness** is the concern, as is often the case in microscopy, what can be accomplished in one day by an ID line at the APS will take the best laboratory x-ray (rotating anode) to do in 27 million years (>**$10^9$ gain in brightness**)!

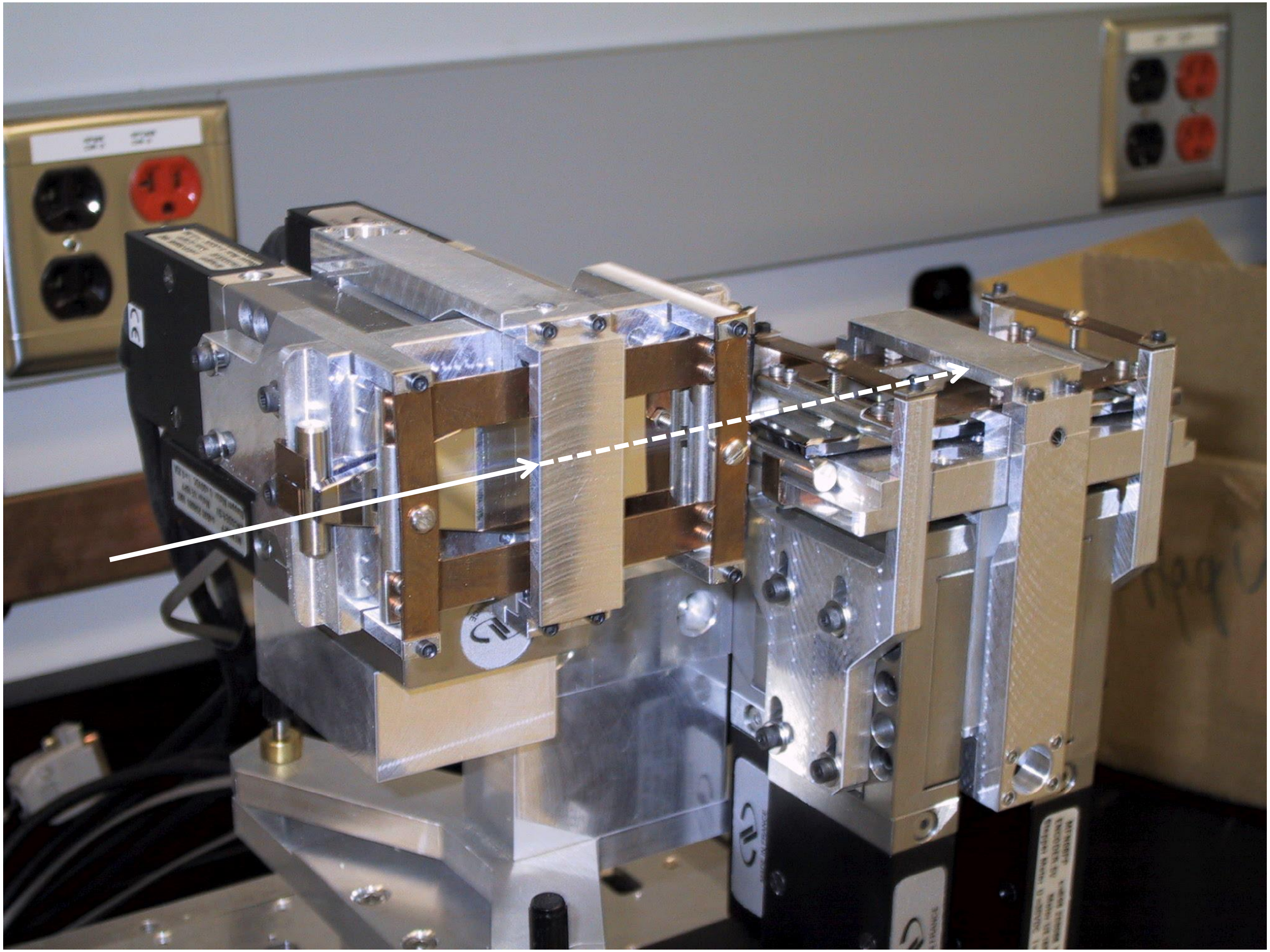



A comparison of **APS** with **ALS** (1.9 GeV) **NSLS** (2.6 GeV) **SSRL** (3 GeV)

Laboratory x-ray source

# KB mirror optics





# What is Hemochromatosis ?

Hemochromatosis is a disease in which the body absorbs excessive iron from the diet. ***Excess iron in the liver eventually leads to cirrhosis of the liver and hepatocellular carcinoma.*** It is the most common genetic disease in Canada (1 in 327 documented in a large population screening in London, Ontario, Canada).

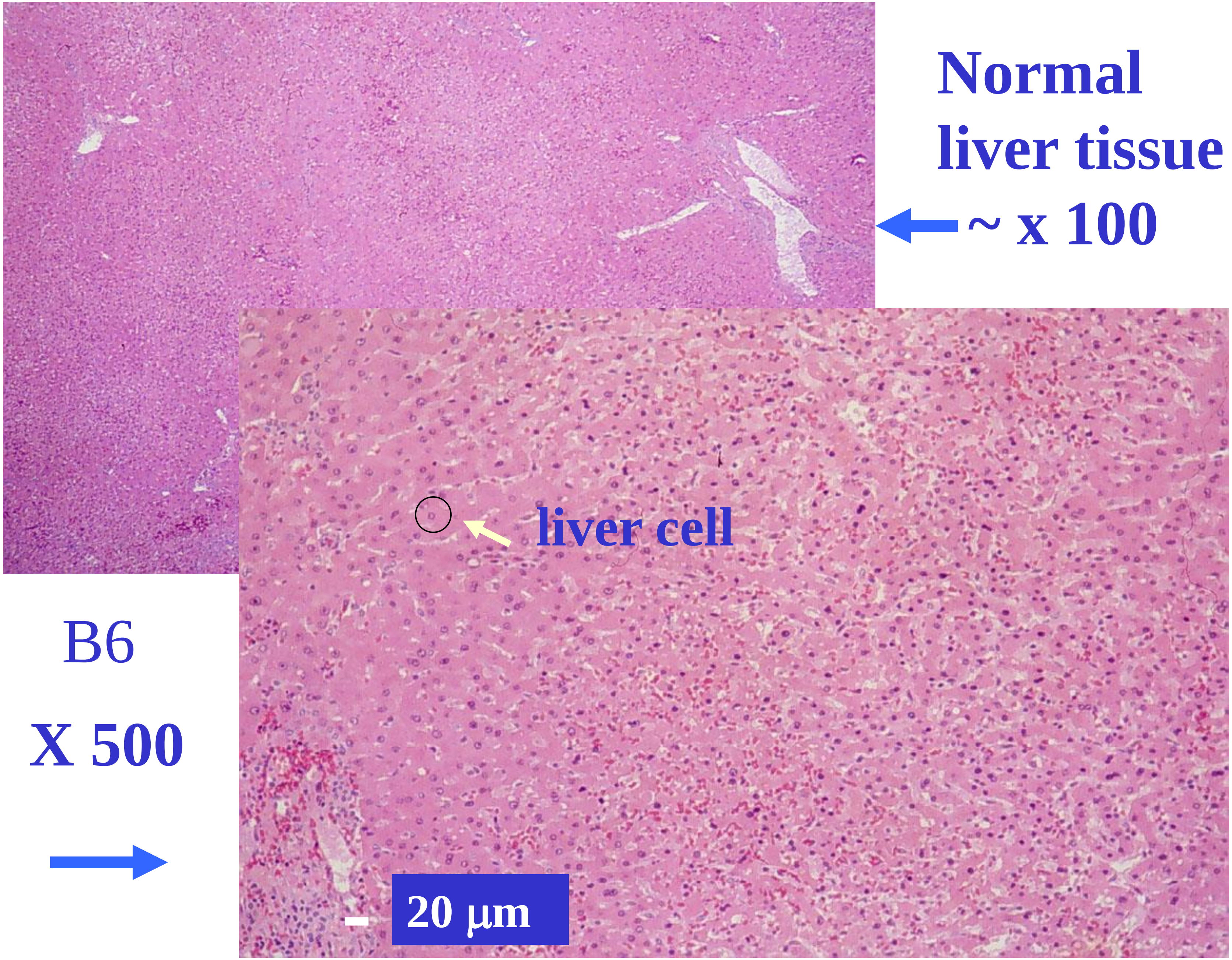
**Hemochromatosis**

An autosomal recessive condition in which a defective gene has been inherited from each parent. Over 90 % of patients have a mutated HFE gene. The exact role of the HFE protein in the pathogenesis of hemochromatosis is not completely understood

W. Griffiths and T. Cox, Human Molecular Genetics, 9, 2377(2000).

# Normal liver tissue

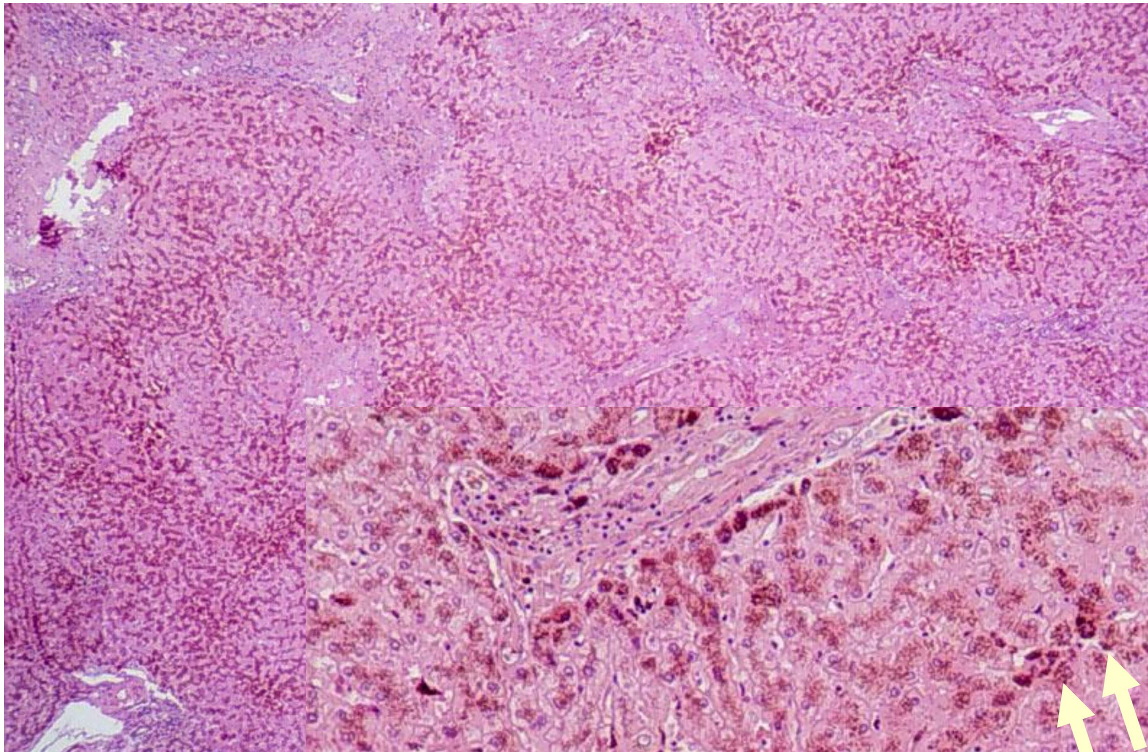
~ x 100

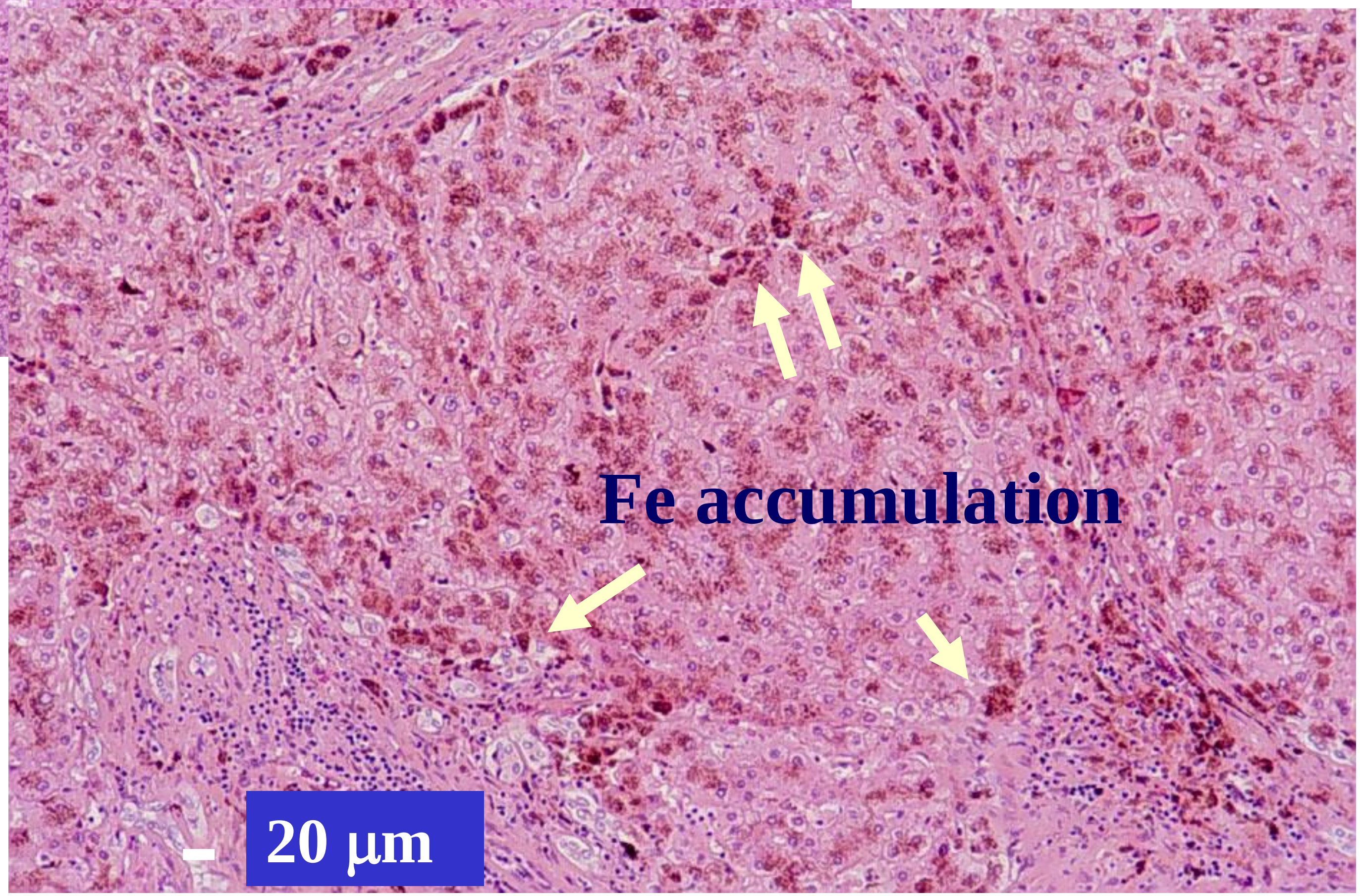



B6

X 500

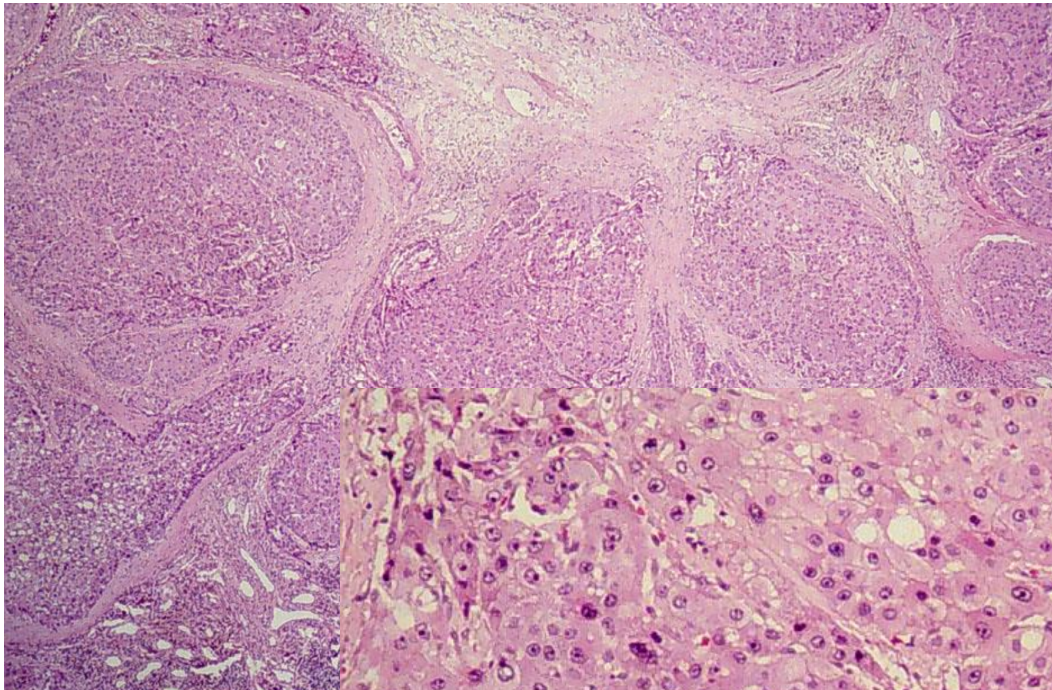
# Hemo-chromatosis liver with cirrhosis



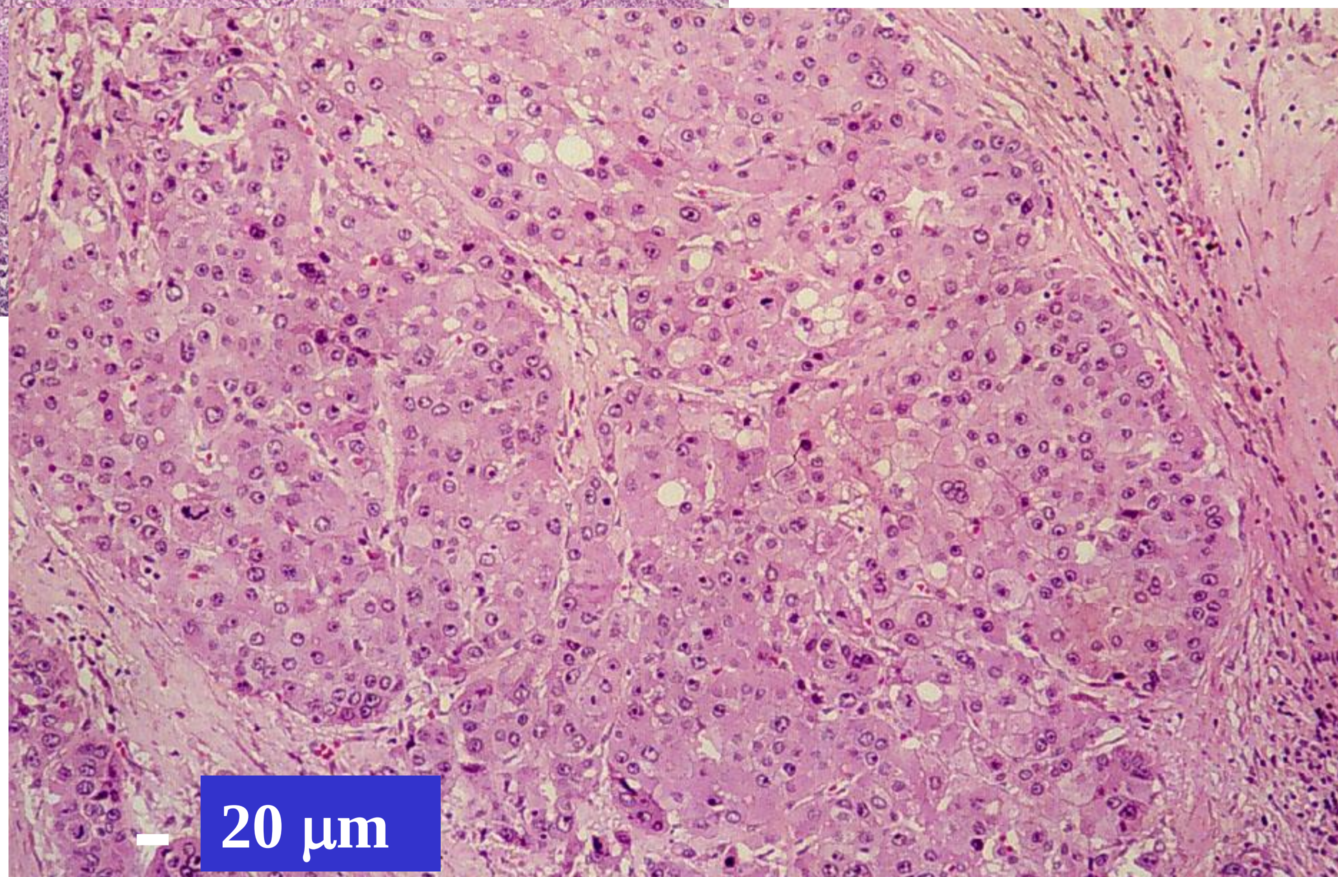



A4

# Hemochromatosis liver with A tumor



A7



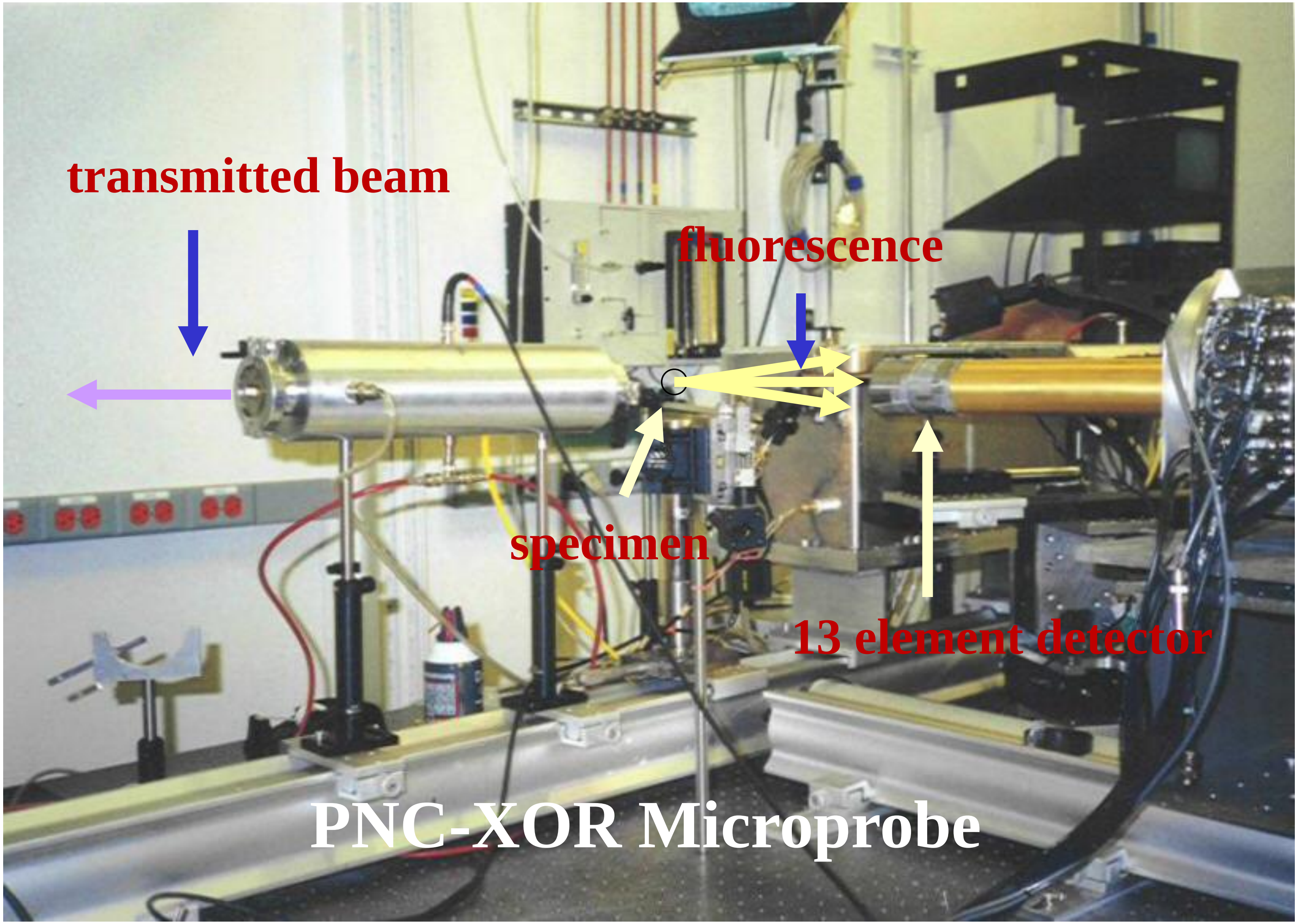
## Instrumentation

The ID line is equipped with

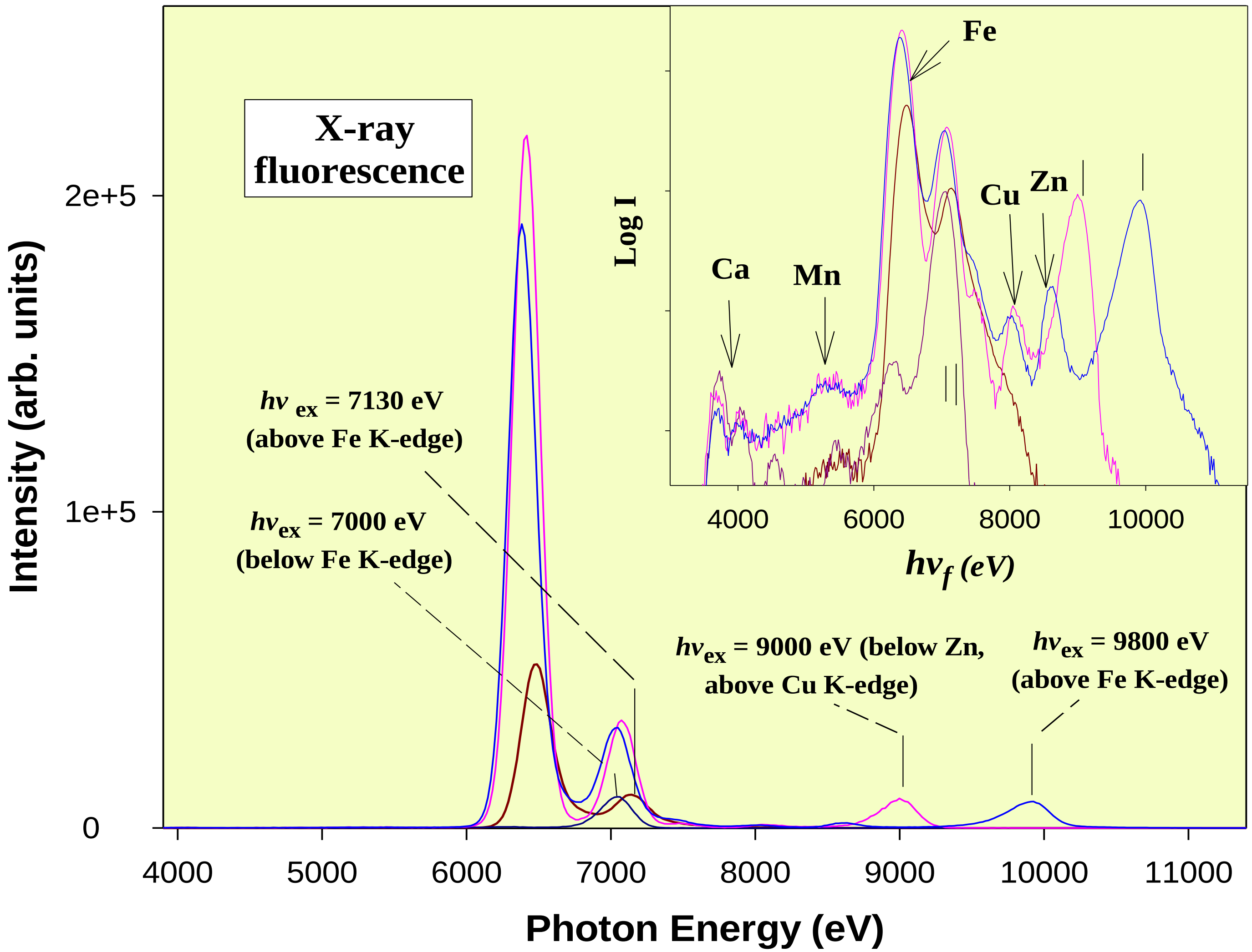
- Si(111) Monochromator
- Kirpatrick-Baze Mirrors
- 13 element solid state fluorescence detector
- High precision specimen scanning stage

**Procedure**

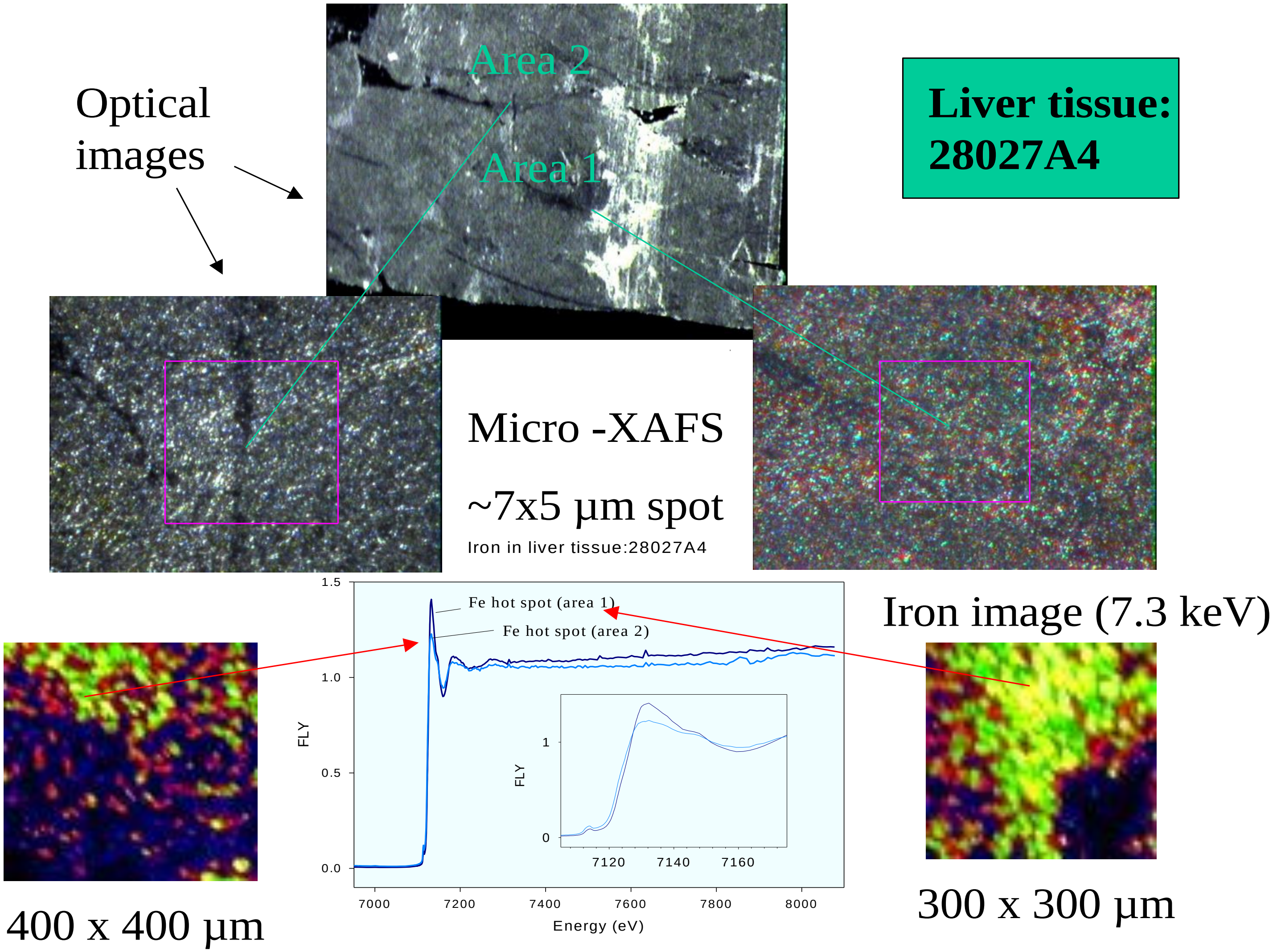
- the selection of a region of interest (optical microscope)
- identification of elements in the specimen using x-ray fluorescence.
- obtain elemental maps with nm step size by simultaneously monitoring the fluorescence signal of the elements of interest.
- Obtain micro-XAFS for an iron hot-spot for example.


transmitted beam
fluorescence
specimen
13 element detector
PNC-XOR Microprobe

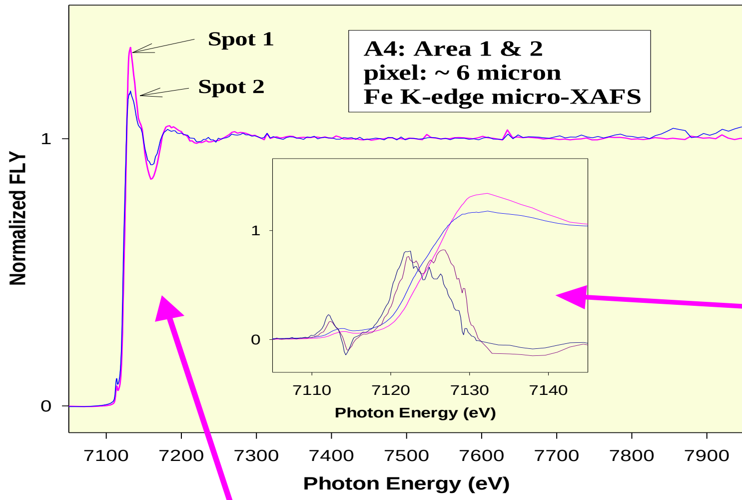
28027A4




**Fluorescence of elements from a liver specimen at selected excitation energies. Inset: (log plot)**




**Optical and Fe maps of a liver tissue (A4). The Fe K-edge XAFS at two hot spots are shown**



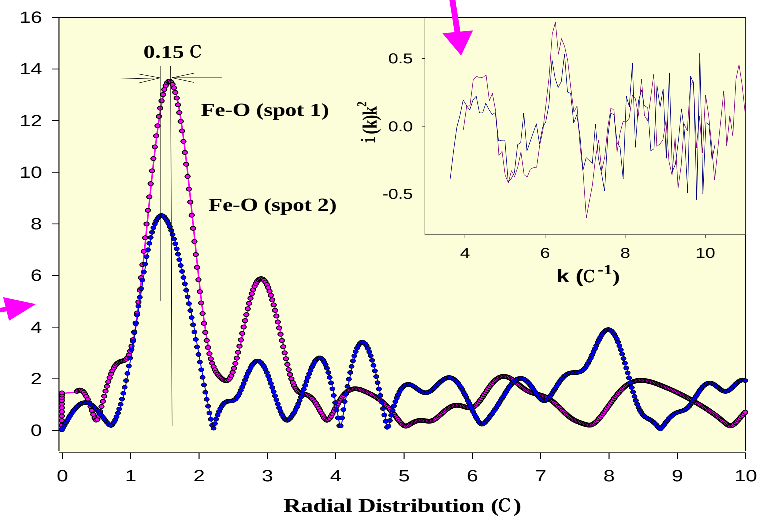

**Specimen A4 micro- XAFS at two spots**

XANES

EXAFS

Normalized Absorption (FLY)

Fourier Transform of EXAFS



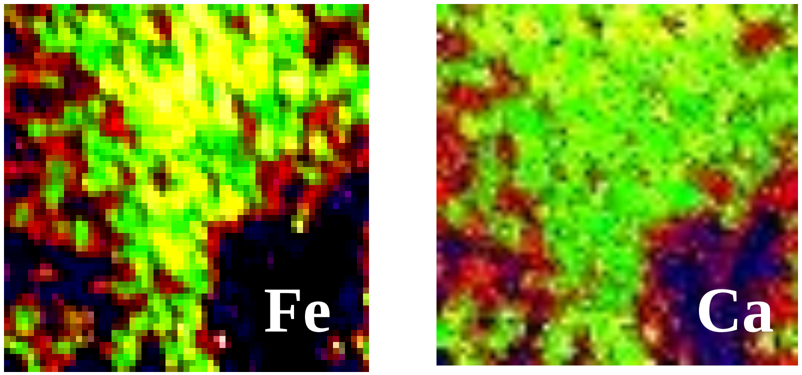

# Micro-XAFS of two Fe spots in a liver specimen

**Results (A4)(hemochromatosis)**

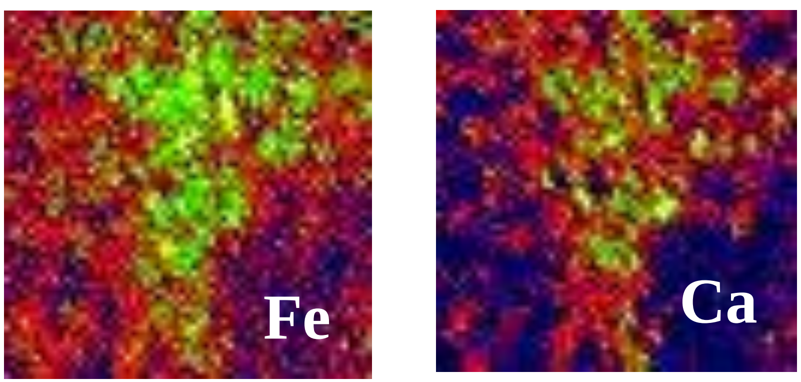
The X-ray Absorption Near Edge Structure (XANES) and EXAFS (Extended X-ray Absorption Fine Structures) exhibit different features indicating that the chemical identity of Fe in the two spots is not identical. A preliminary analysis of the EXAFS by Fourier Transform shows that the Fe-ligand bond at the two sites is noticeably different (by more than 0.1 C).

## Element map A4 : 300 x 300 μm

$h\nu_{ex}$ = 7.2 keV, above the Fe K-edge

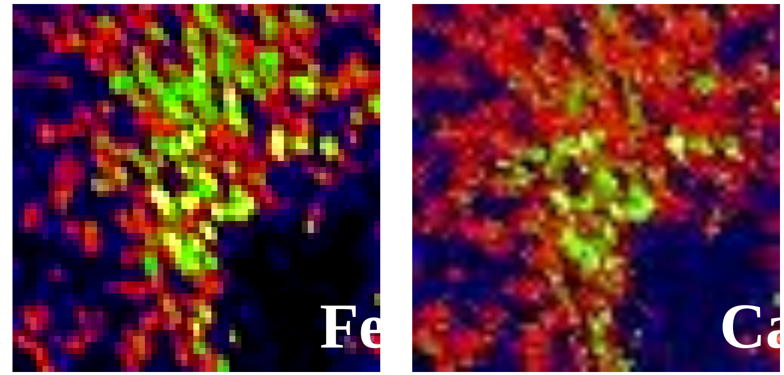


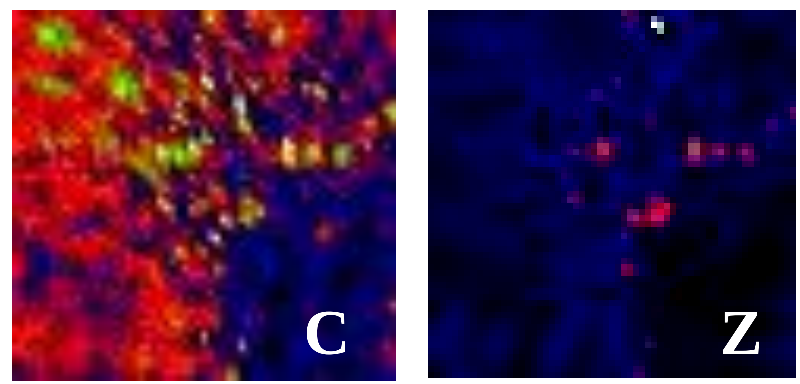

$h\nu_{ex}$ = 7.0 keV, below the Fe K-edge




## Element map A4 : 300 x 300 μm

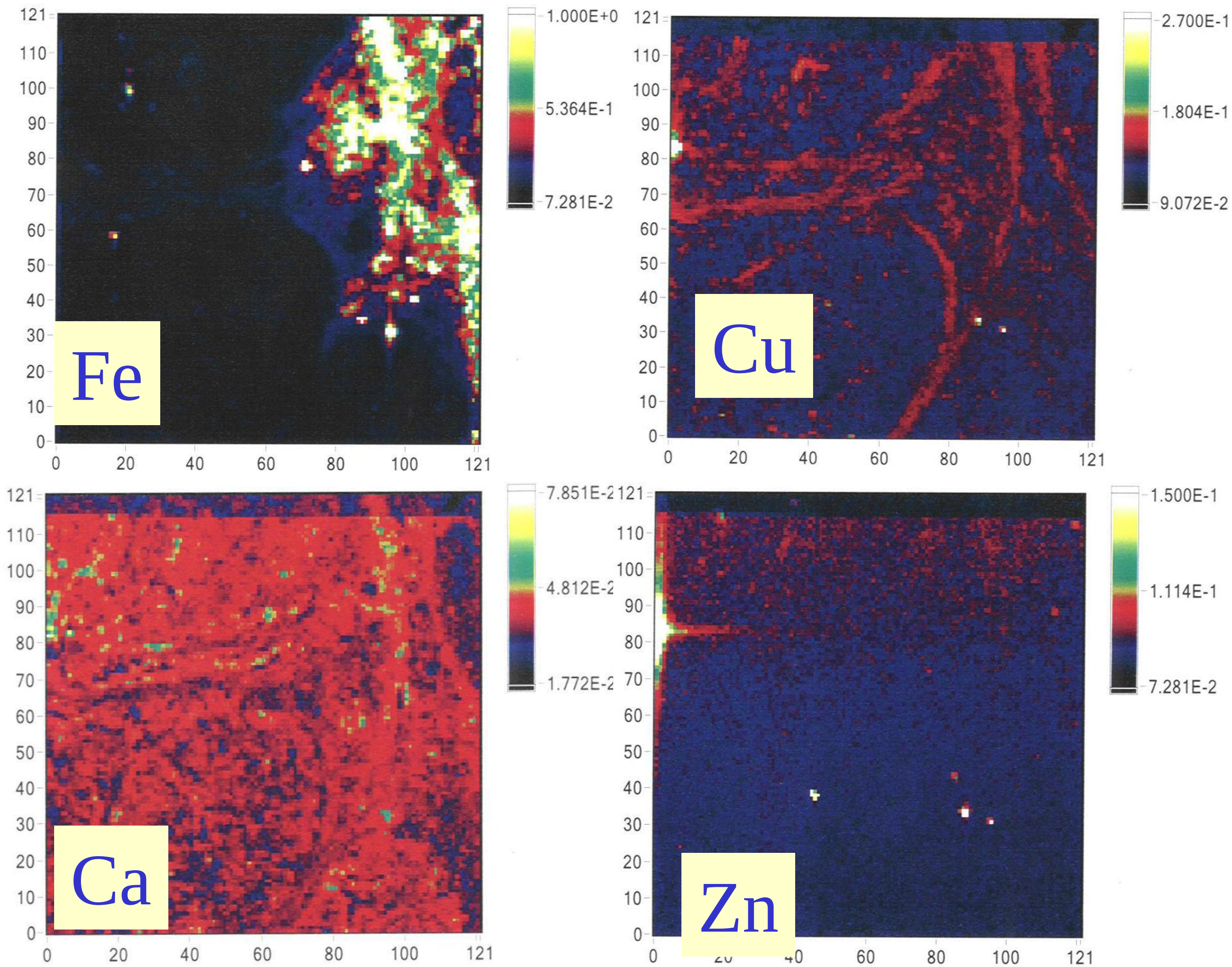
9800 eV excitation



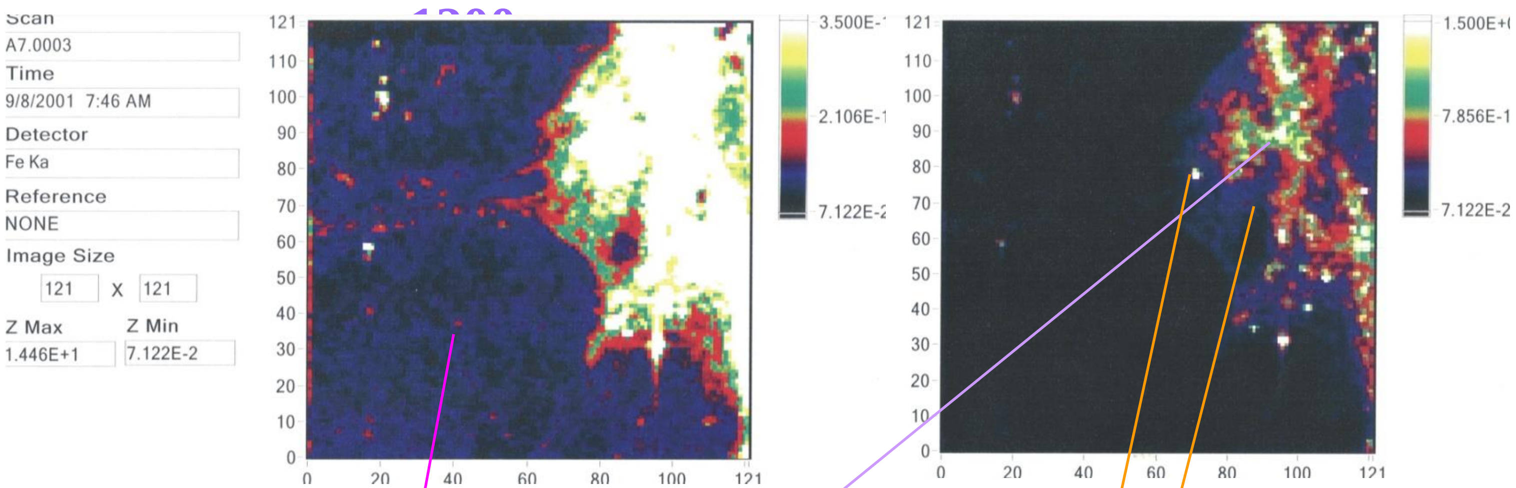

# Element maps of liver (A4) tissues

# Element map of liver tissue A7 (cancer)

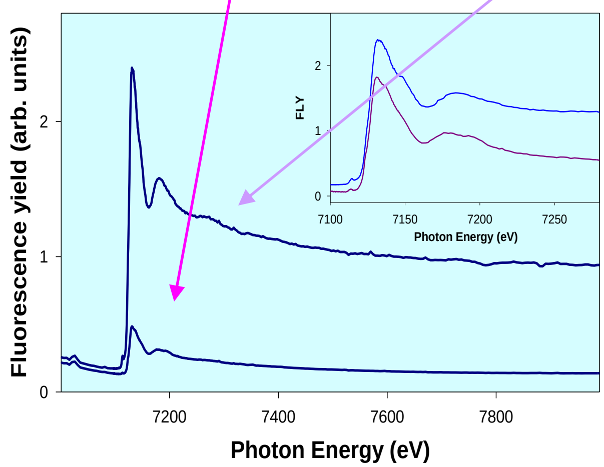


# X-ray microprobe analysis of liver tissue

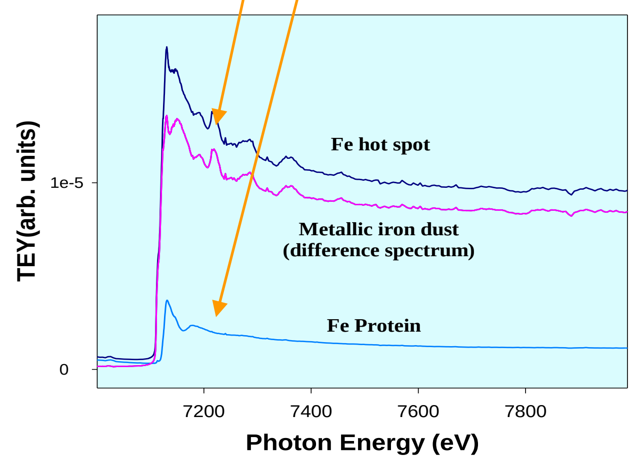
## Fe map of liver tissue (A7) 1200 µm x

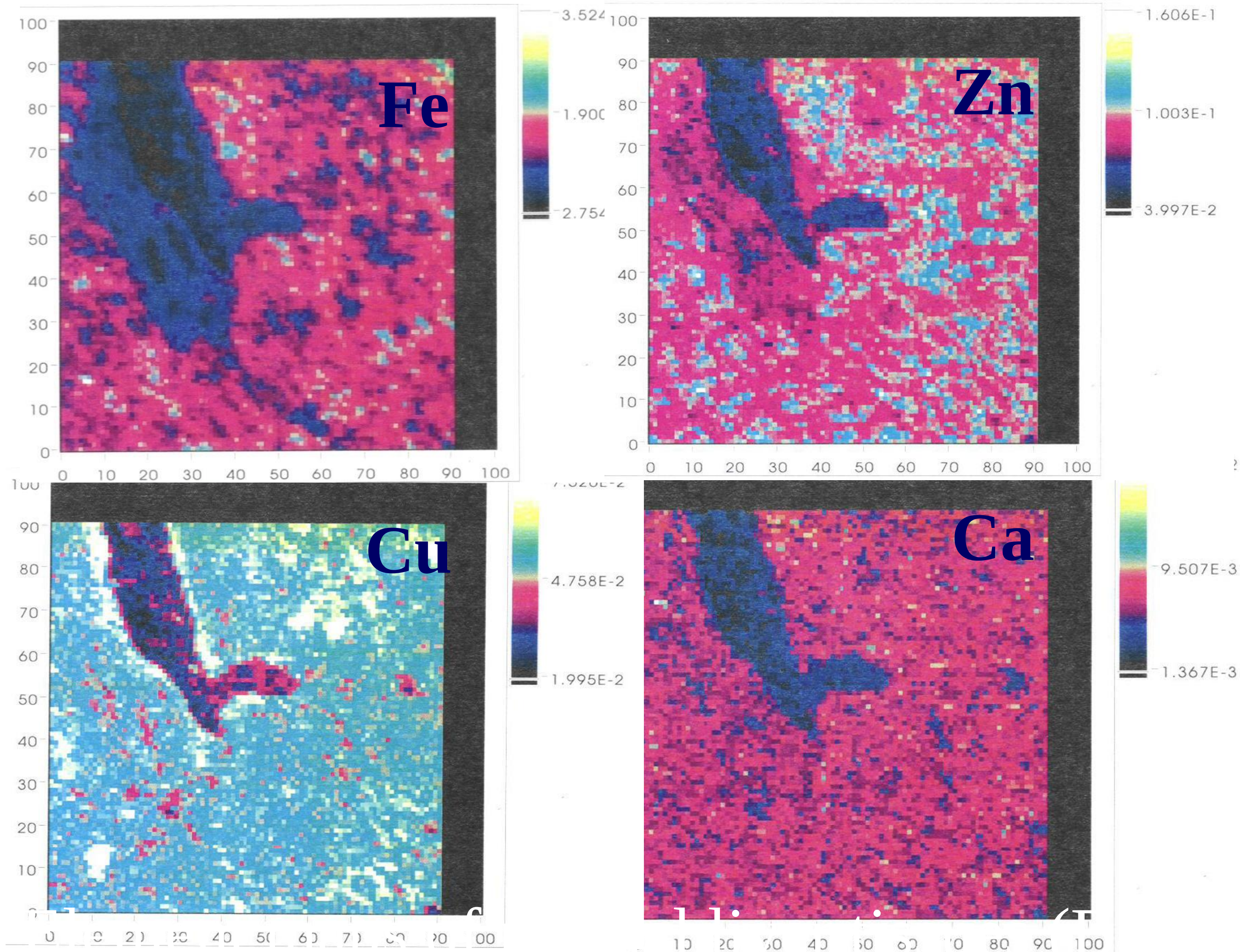



## Fe K-edge micro-XAFS, beam spot size: 5 µm x 6 µm

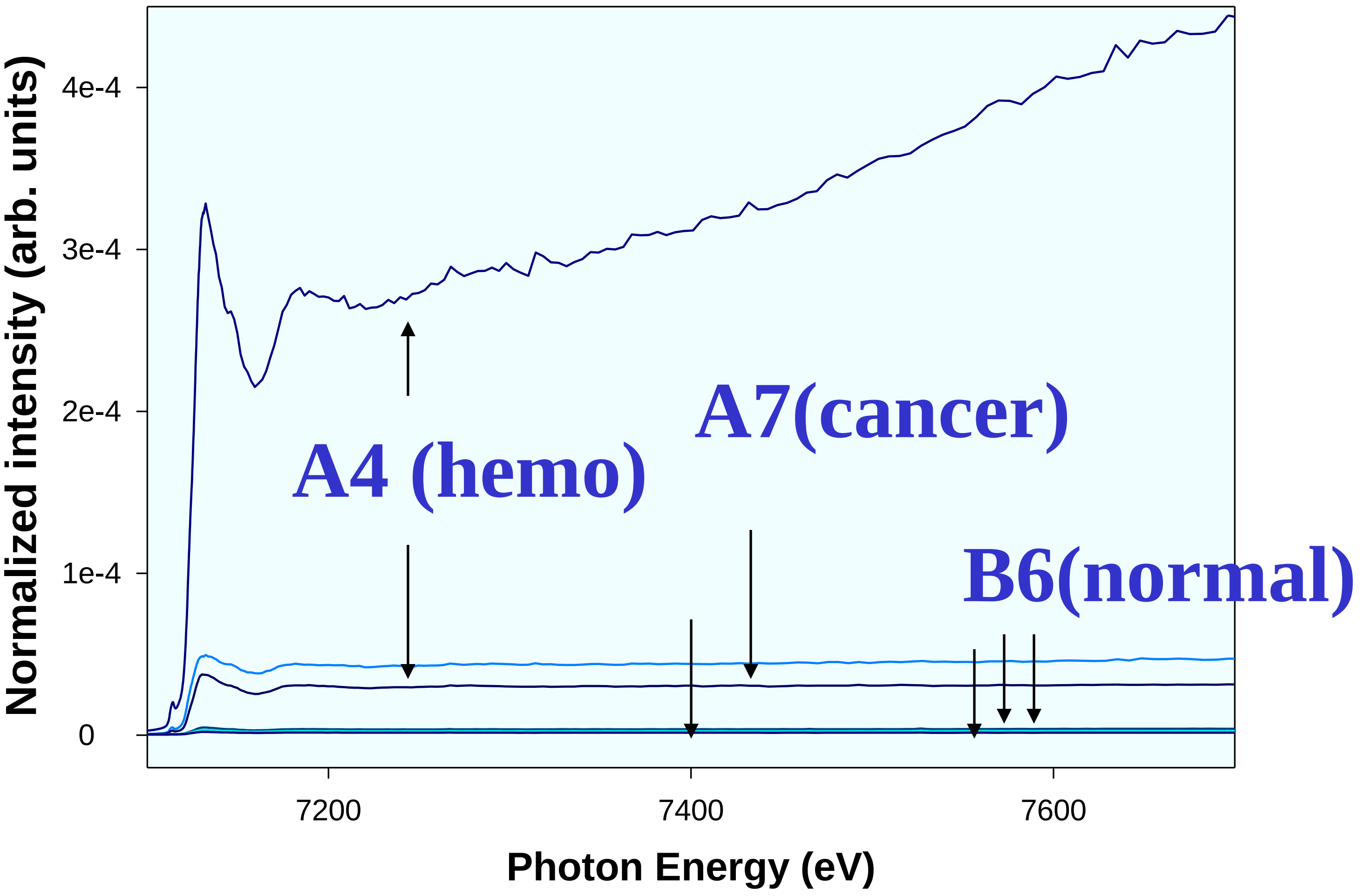



## Fe K-edge micro-XAFS, of an iron dust

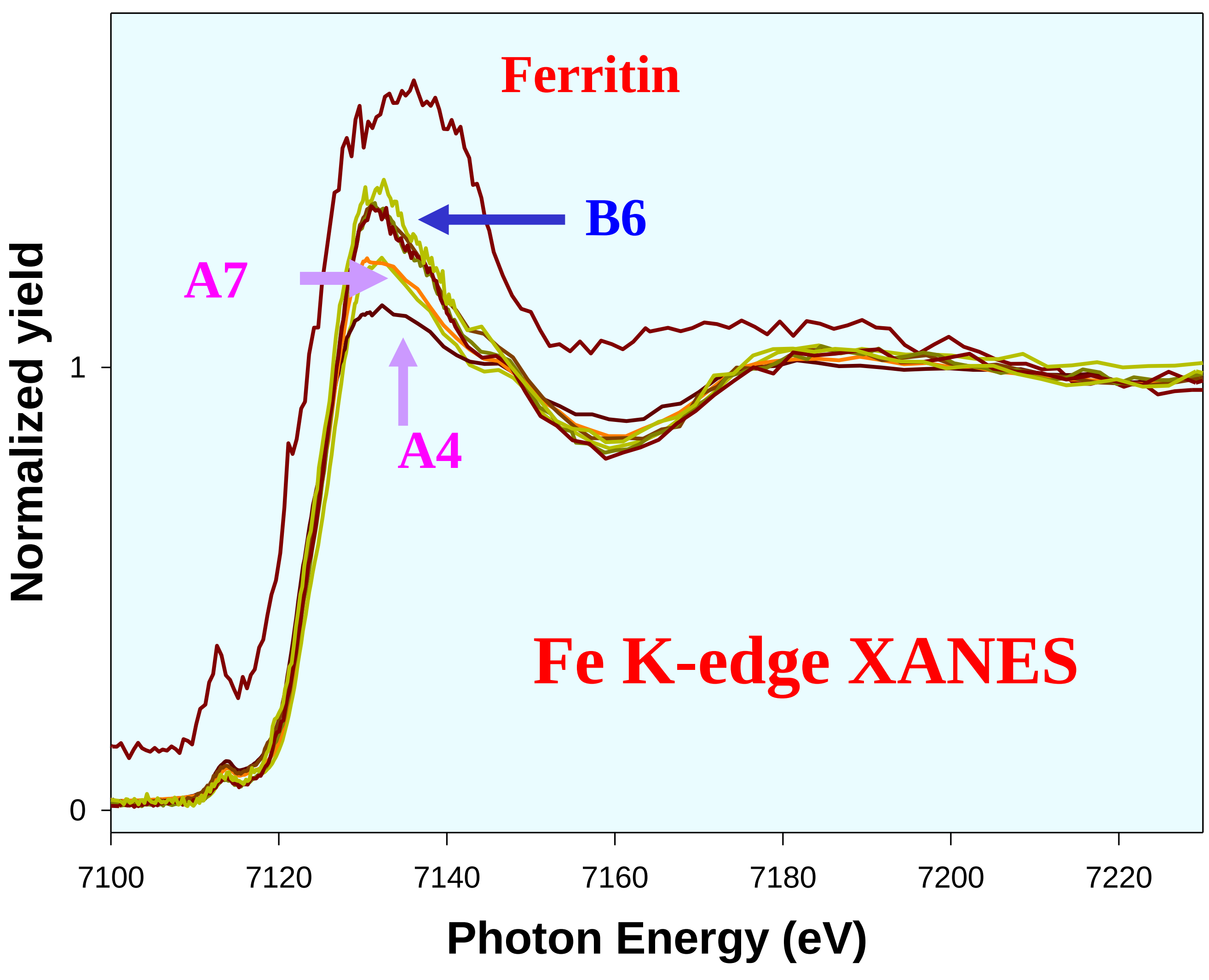



Fe
3.524
1.900
2.754
Zn
1.606E-1
1.003E-1
3.997E-2
Cu
4.758E-2
1.995E-2
Ca
9.507E-3
1.367E-3
0
10
20
30
40
50
60
70
80
90
100

# Comparison of Fe K-edge XANES

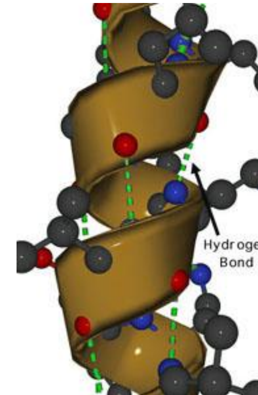


# Comparison of Fe K-edge XANES

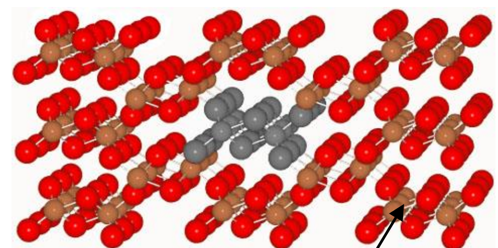


# Ferritan

Peptide



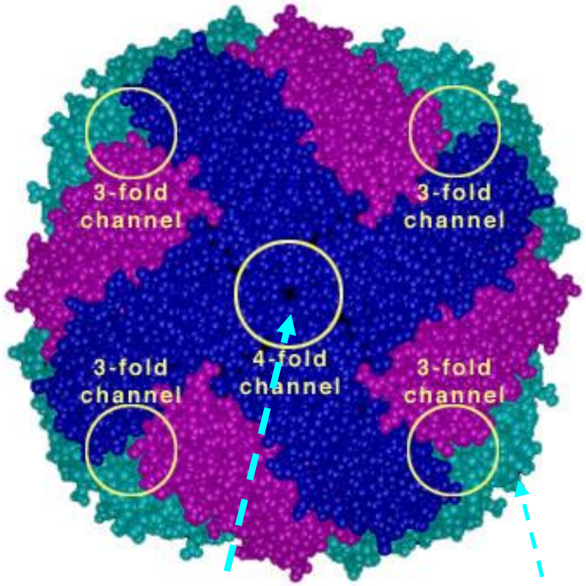

$[FeO(OH)]_8[FeO(H_2PO_4)]$.

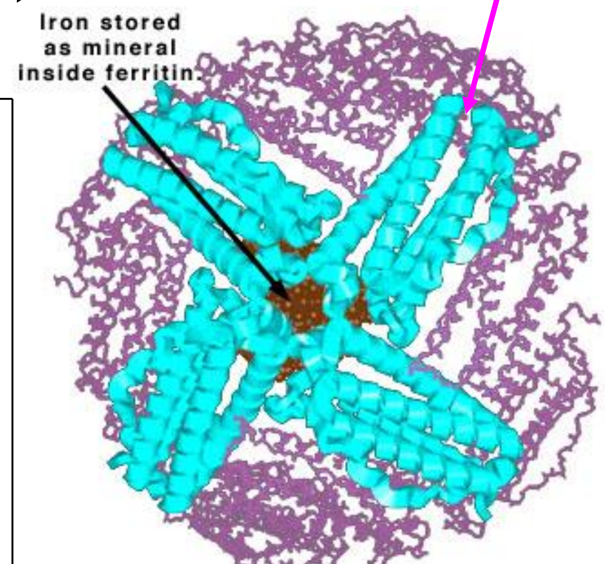


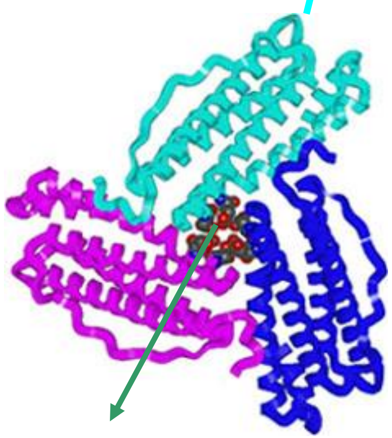
Fe(III)

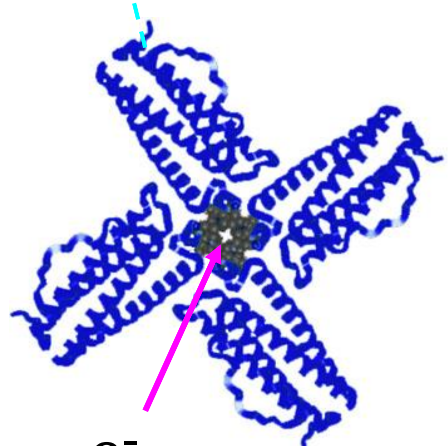
Fe

O



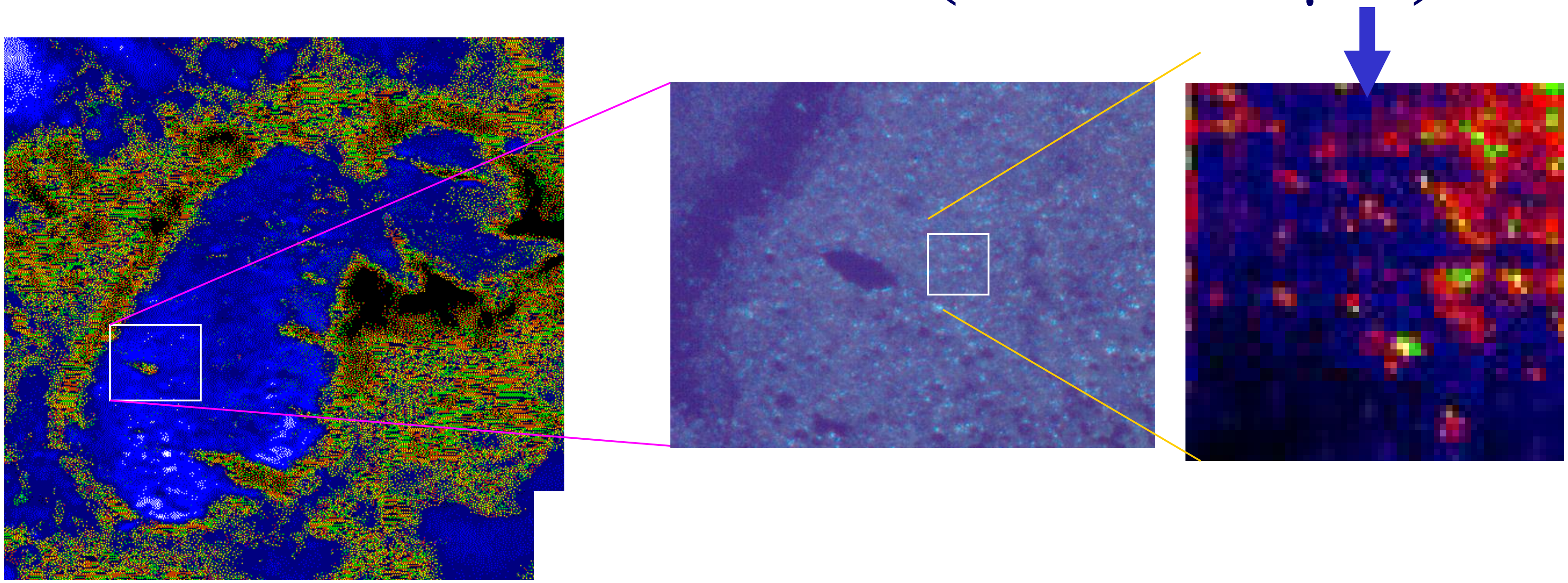





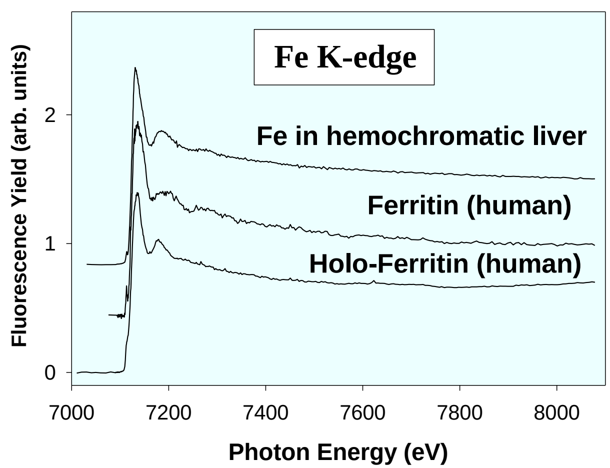
Fe(II)

$e^-$

# **Hemochromatic Liver** Fe (300 x 300 μm)



**Fe K-edge XAFS at a hot spot and standards (ferritin and holo-ferritin)**




**Information**: **Fe is largely in the form of Ferritin**

This specimen has also be investigated with IR microscopy

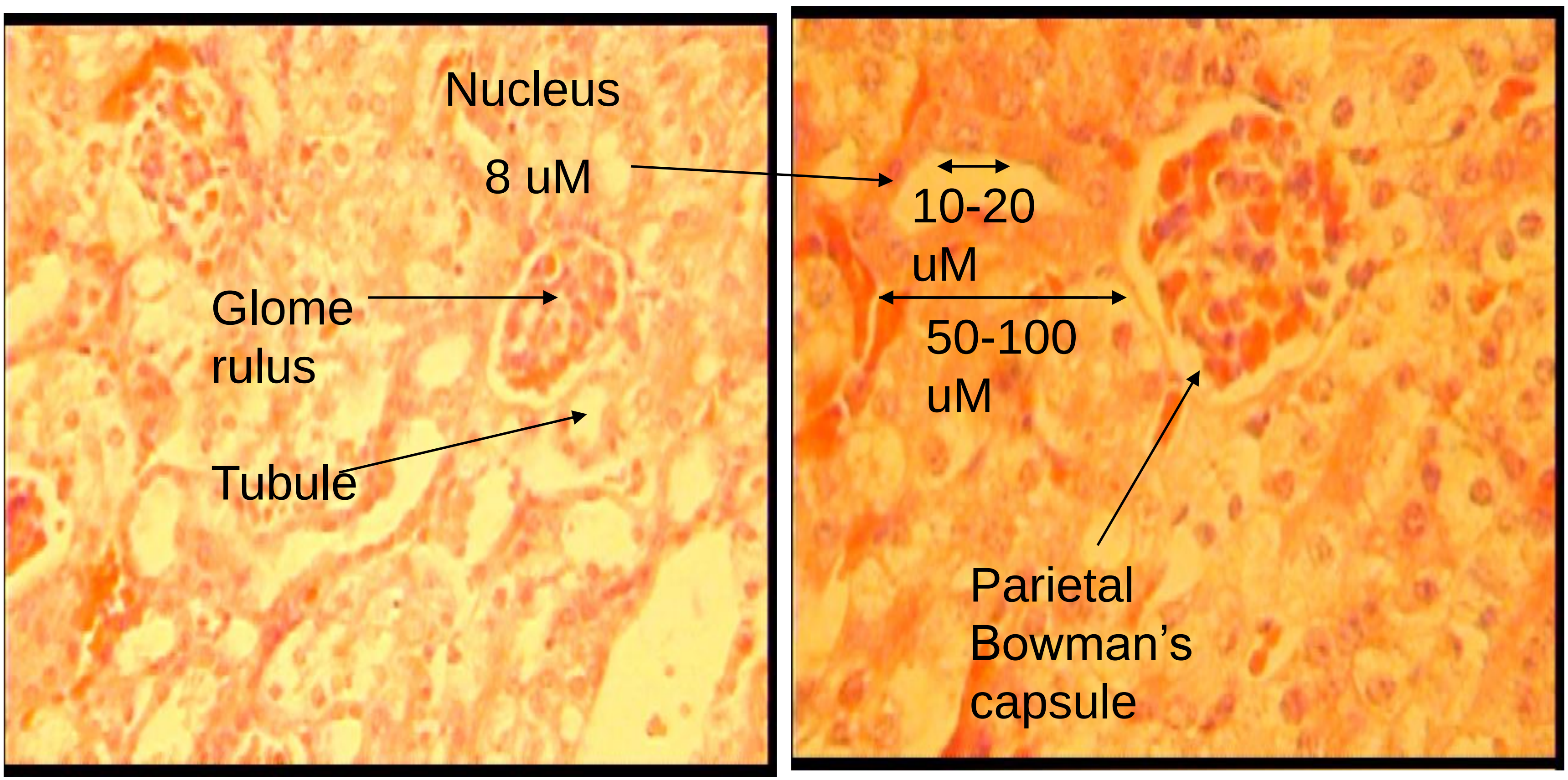
# Cu Metallothionein and Diabetes

**The Hypothesis**: Diabetes induced renal damage is mediated by alternation of Metallo-thionein (MT) and transition metal in the kidney

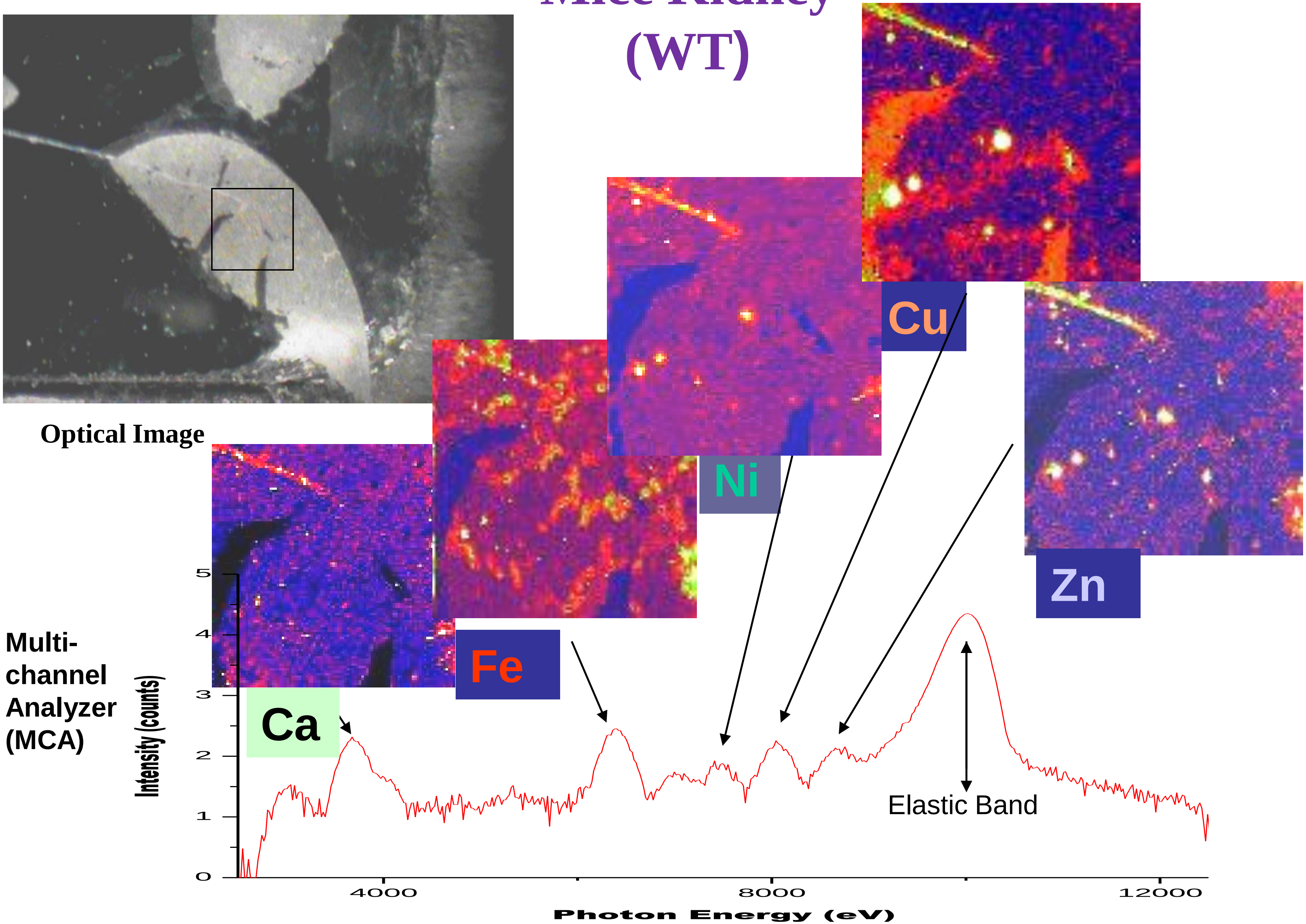
**The Strategy**: Investigate kidney tissues of transgenic mice with MT over-expression, MT knockout (KO) and wild type (WT) mice

**X-ray Microscopy**: Distribution and chemical identity of Cu in mice kidney tissues

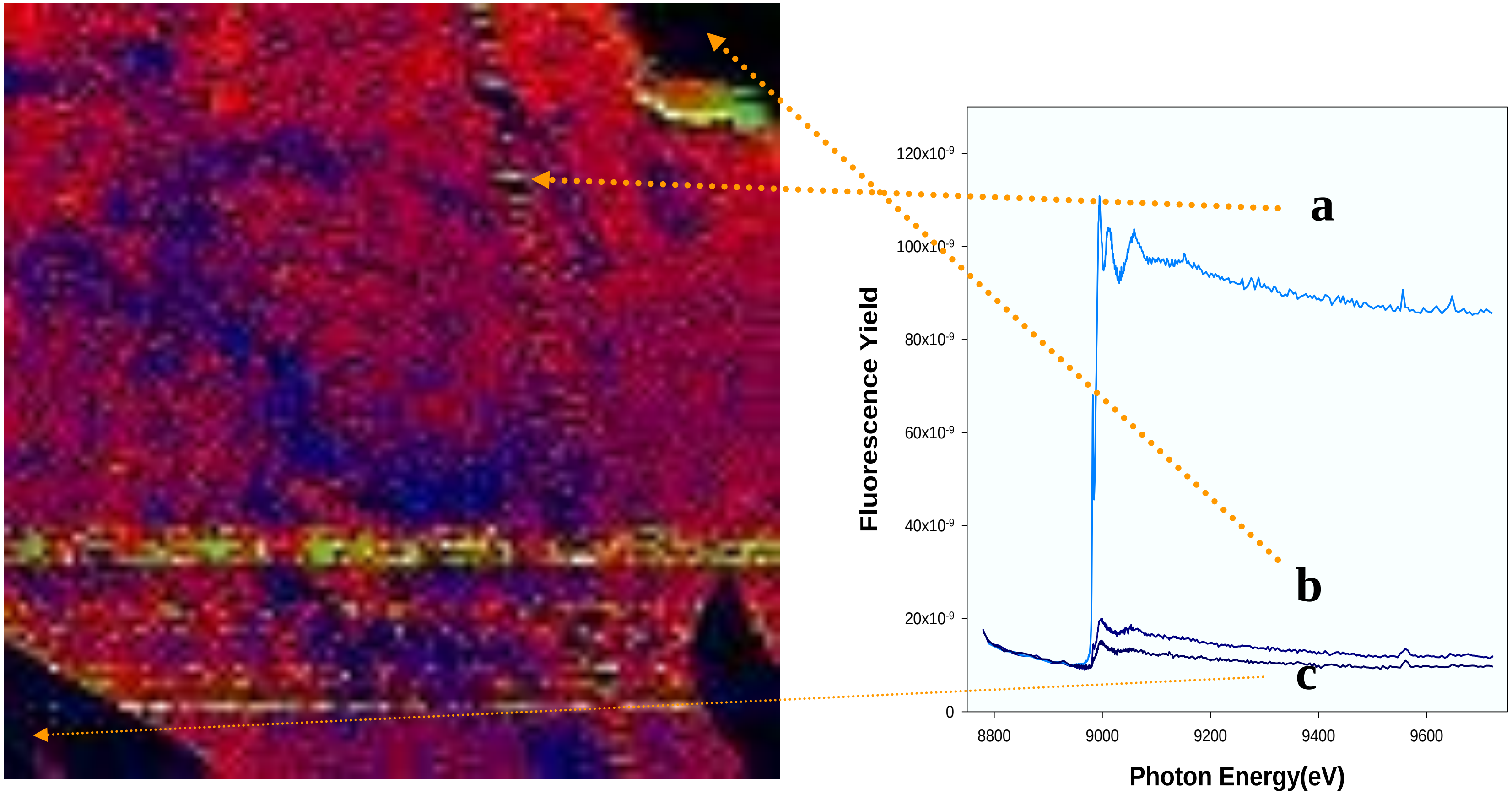
# The Optical Images of the Mouse Kidney



# Mice Kidney (WT)



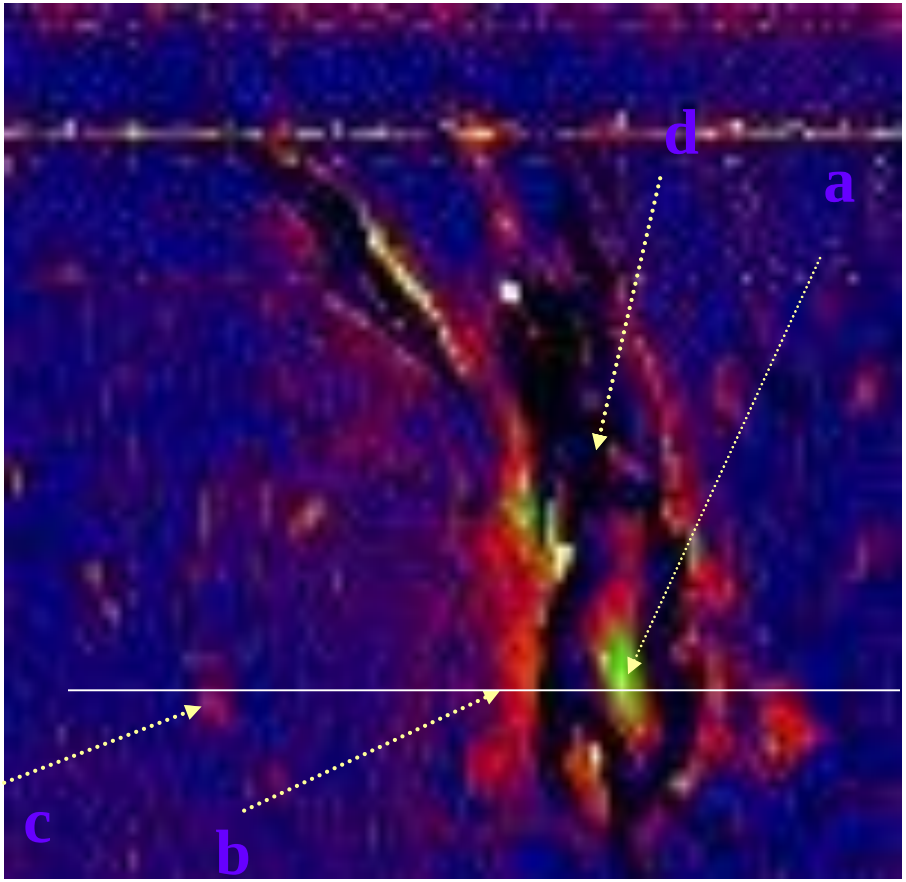
# Cu distribution in WT kidney tissue (normal)



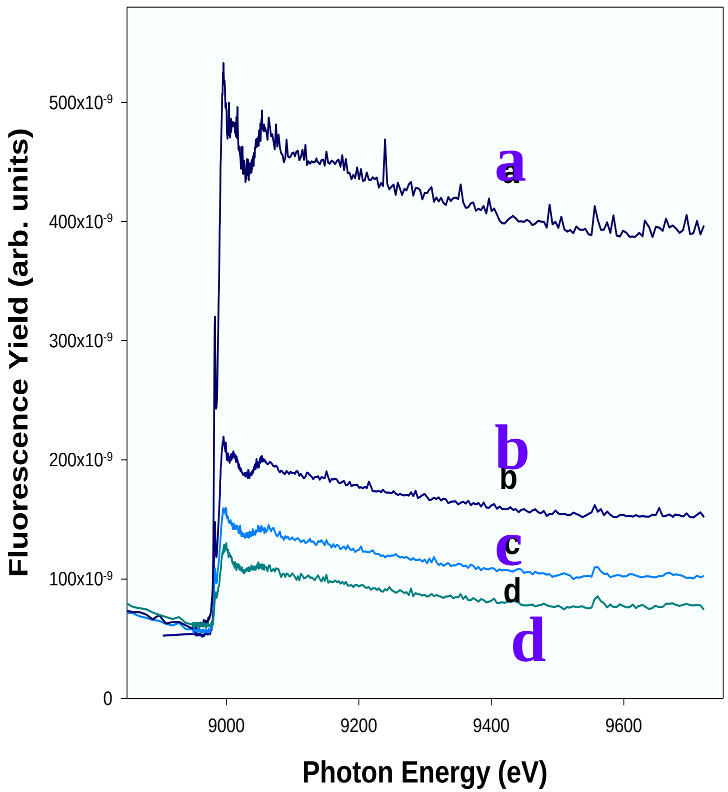

Cu element map (beam size ~ 7 µm ) 1000 x 1000 µm, 10 µm step; lighted color associates with more intense fluorescence

Cu K-edge XAFS

# Cu distribution in KO mouse kidney tissue

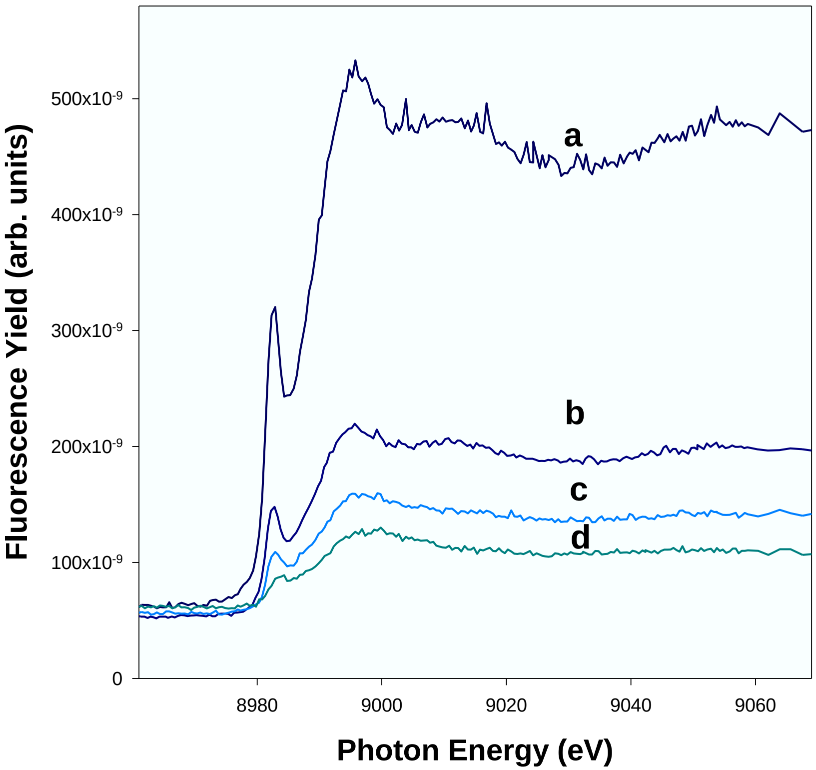


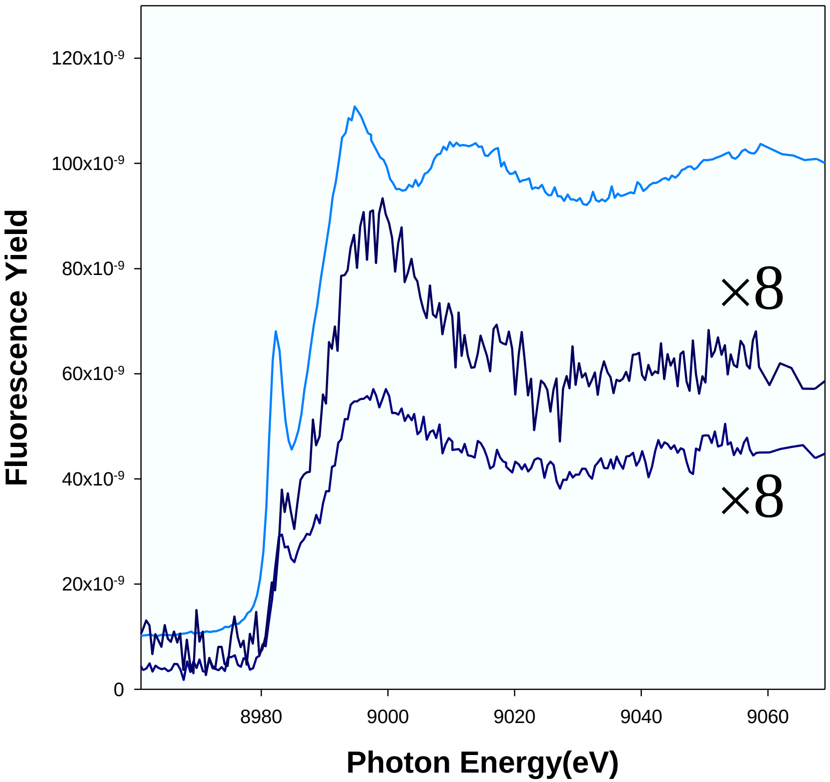

Cu element map (beam size ~ 7 µm )
1000 x 1000 µm$^2$, 10 µm step; lighted color associates with more intense fluorescence




Cu K-edge XAFS

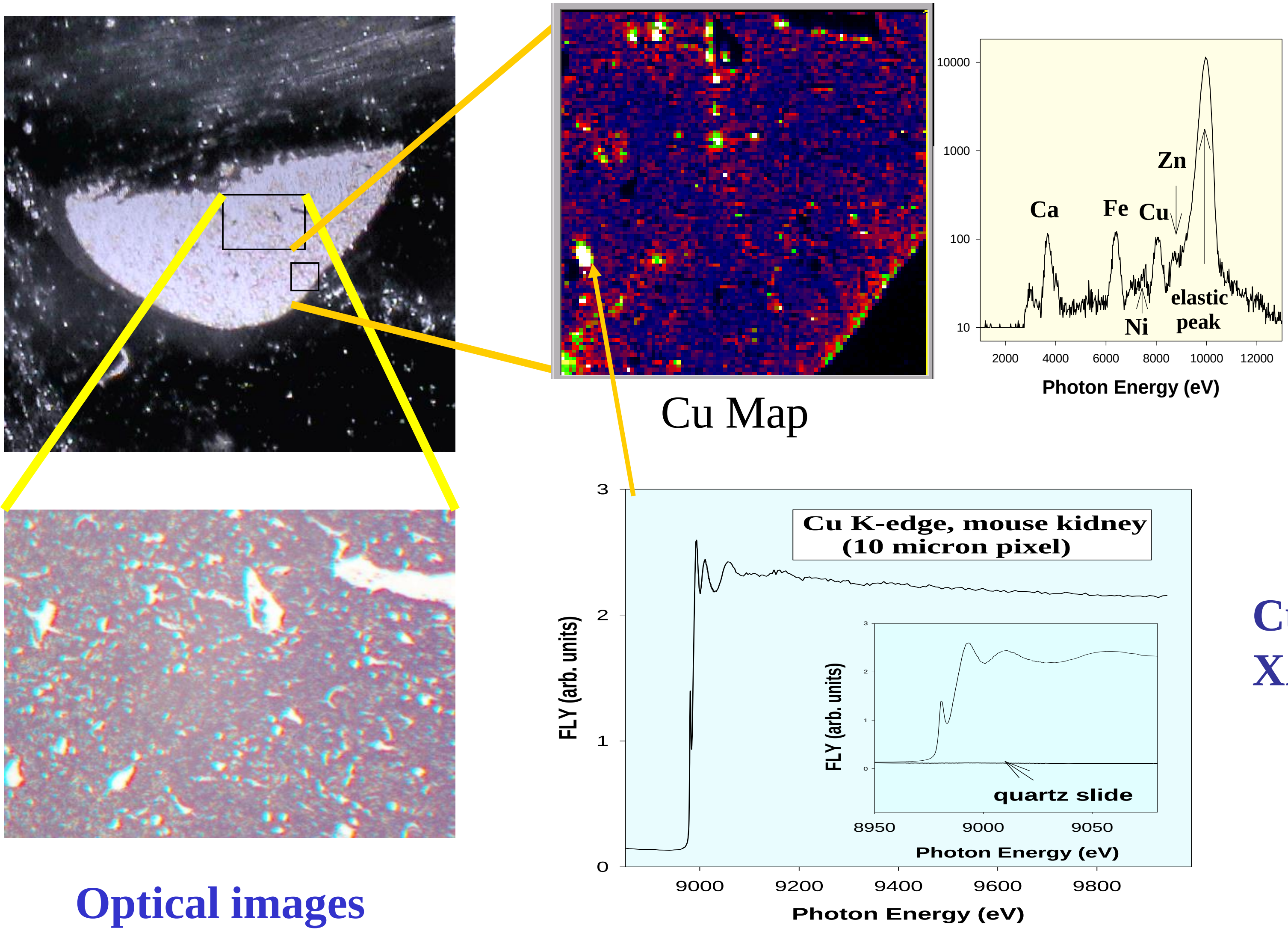
# Cu K-edge XANES




**Knockout tissue**

**Normal (WT) tissue**




Cu XAFS

Optical images

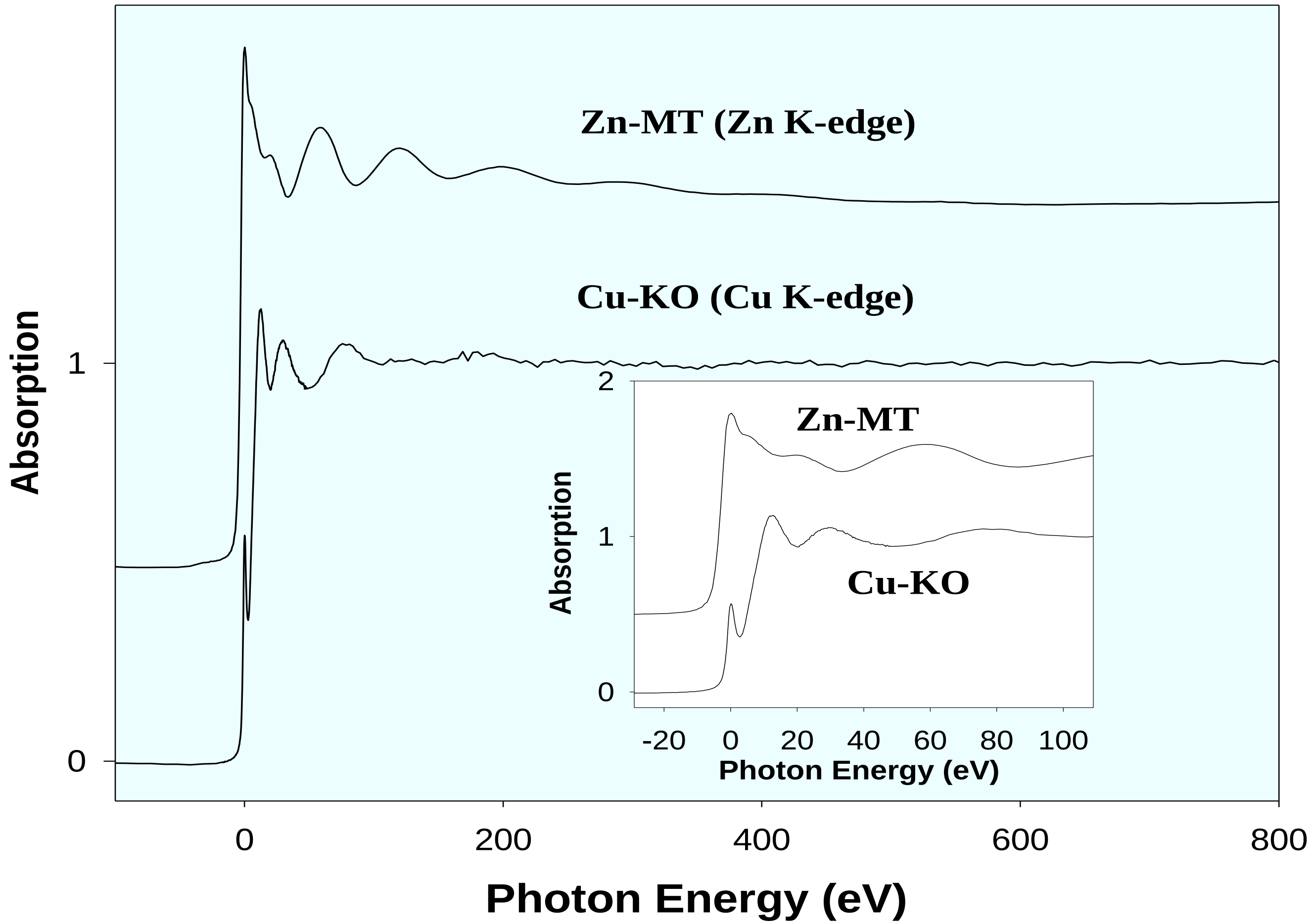
# .X-ray analysis of mouse kidney tissue (KO)

**Observations:**

- In contrast to expectation, the KO mouse kidney has a higher Cu concentration Cu than the WT

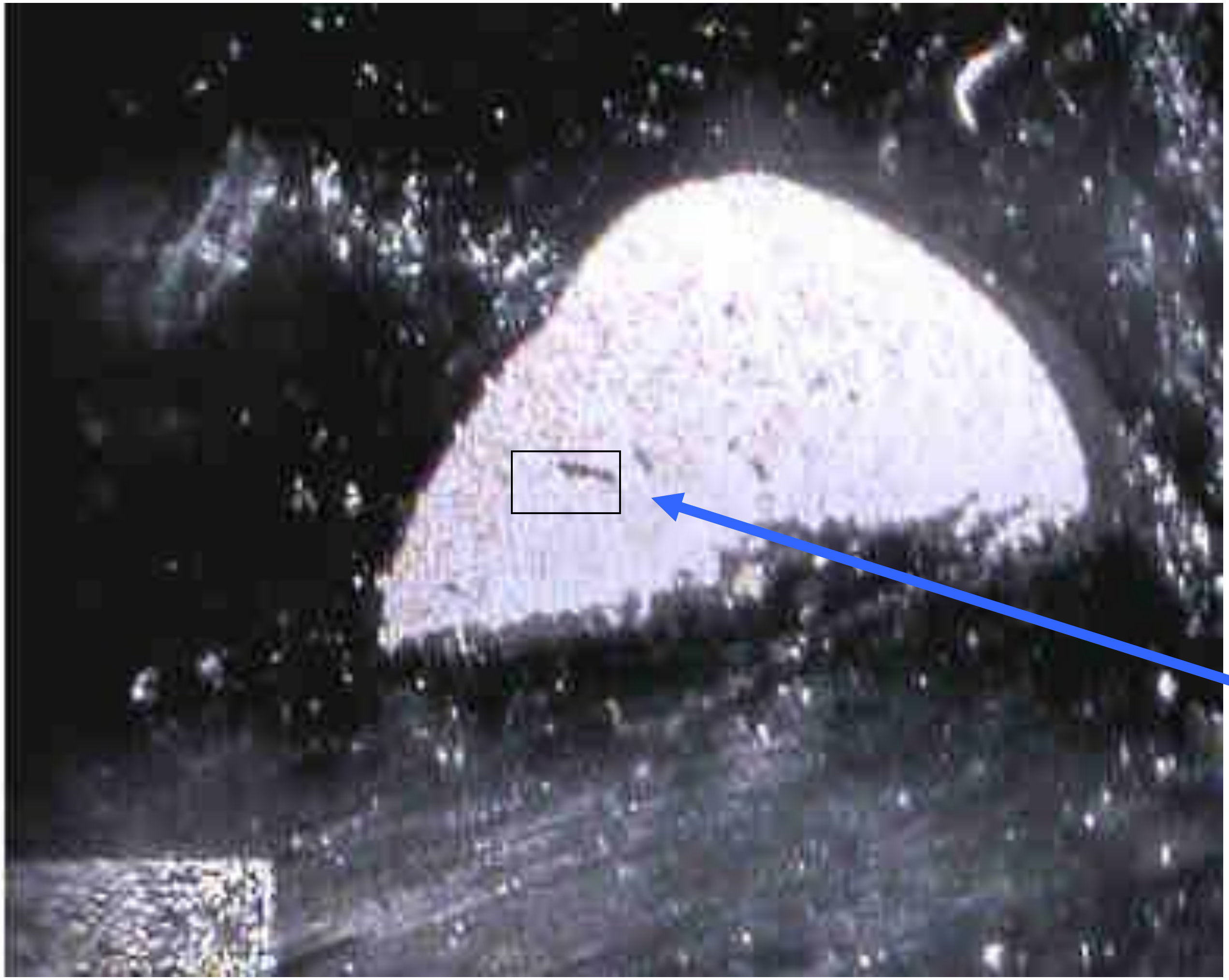
- The Cu local environment is more complex than Cu metallothiolein

# Comparison with Zn metallothionein

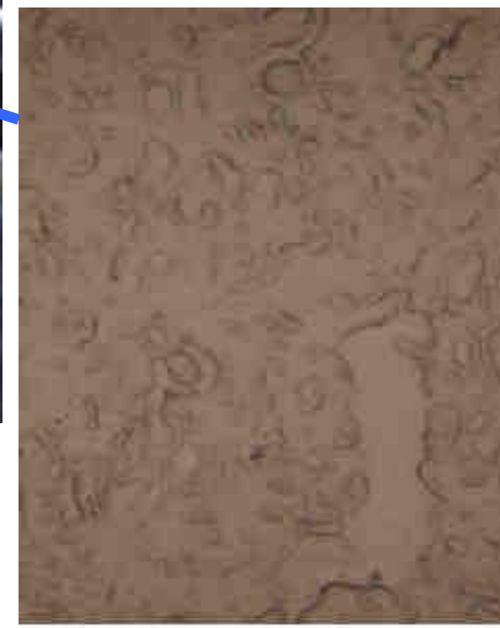


# Summary

- X-ray micro analyses of Fe in human liver tissues and mouse kidney tissues Show that the technique is feasible
- Preliminary results show that Fe in liver is largely ferritin and there is some degradation in hemochromatosis liver
-Cu in KO mice is more abundant than in WT mouse in contrast to expectation
-The tracking of x-ray image with IR and visible is entirely feasible



**Kidney KO Optical Image after x-ray Image was obtained**



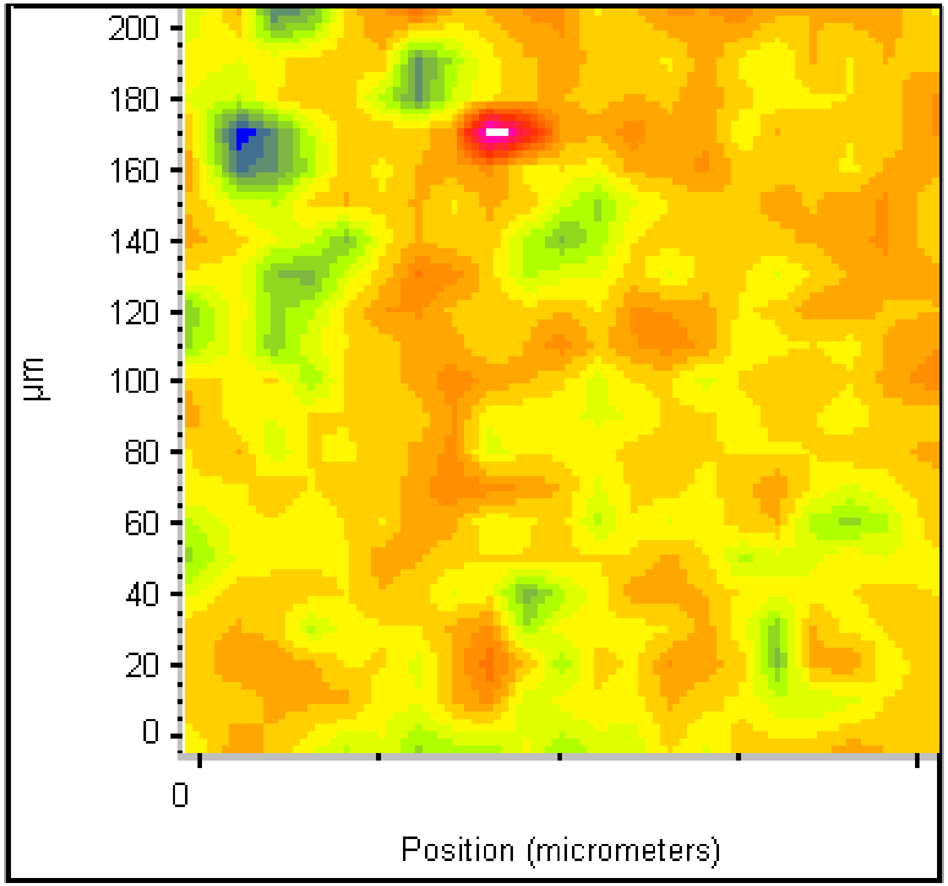
**Tracking x-ray image with visible**

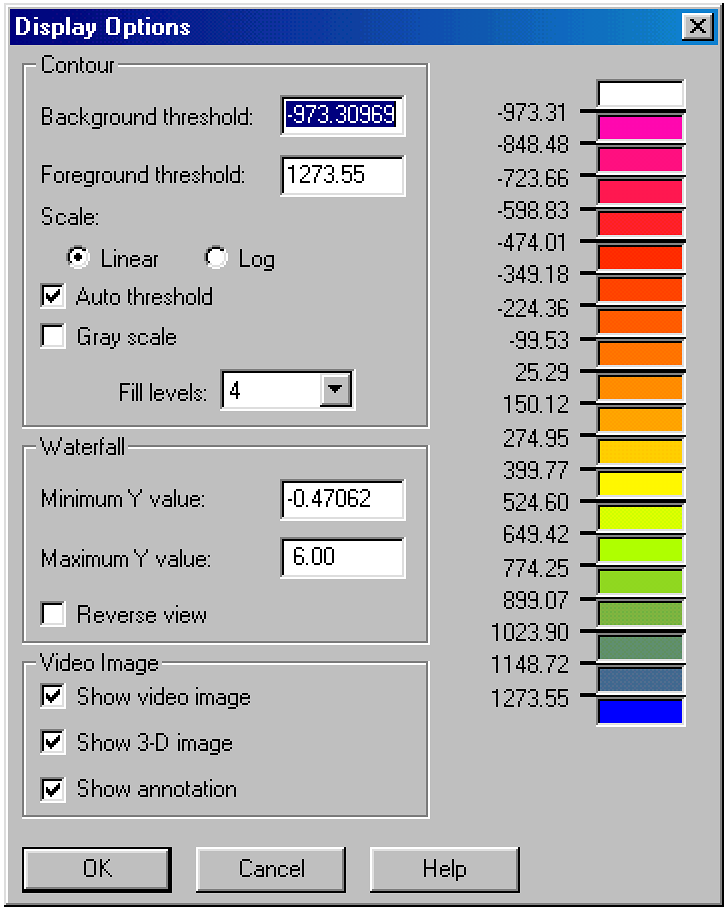
# IR Imaging

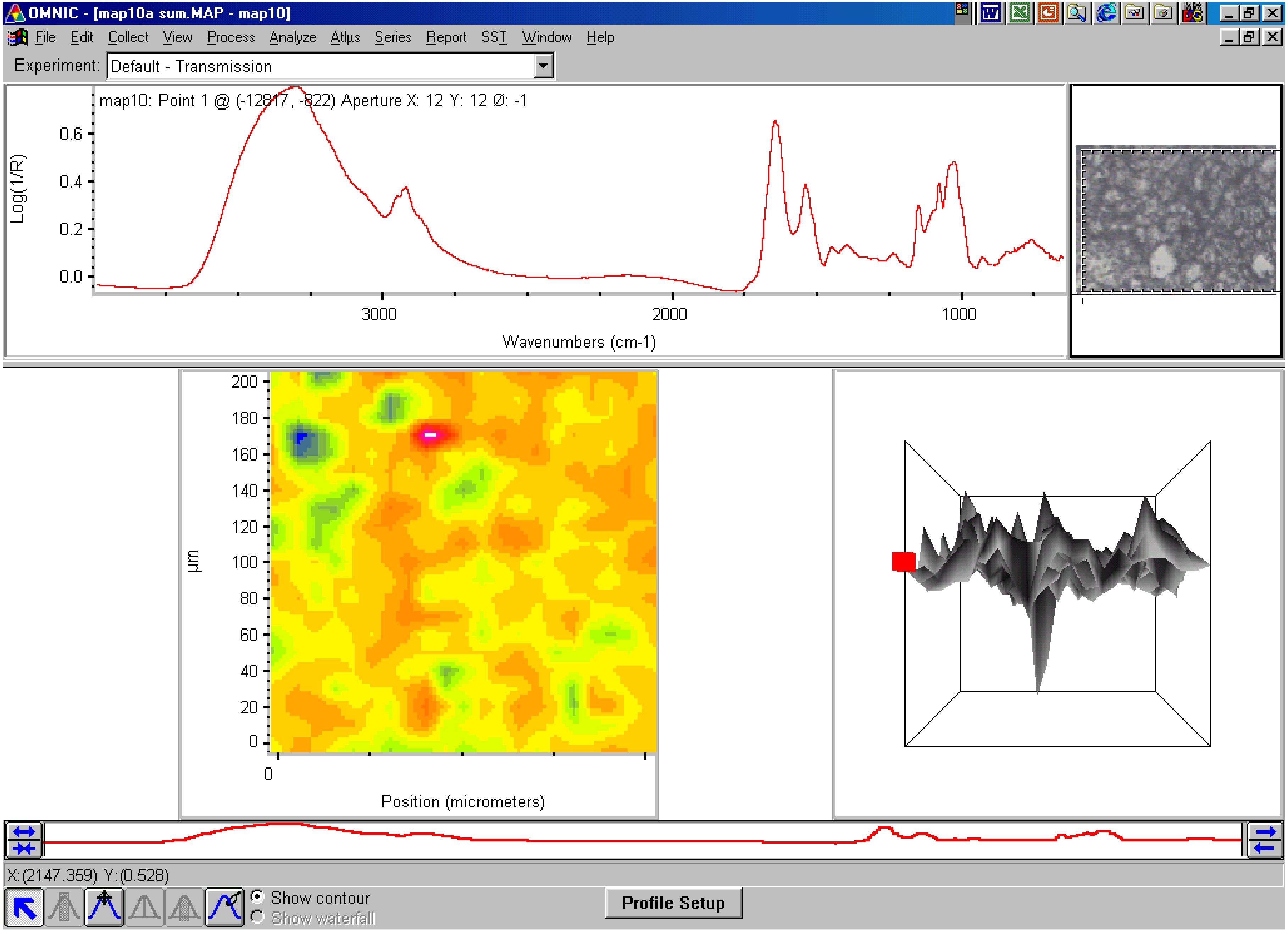
Comparing to commercial IR, SR gains in brightness, an ideal scenario for imaging. The spatial resolution is ~ 10 microns. IR imaging is functional group specific, complementary to x-ray which is element specific. IR has been widely used to study tissues.

# Typical IR map

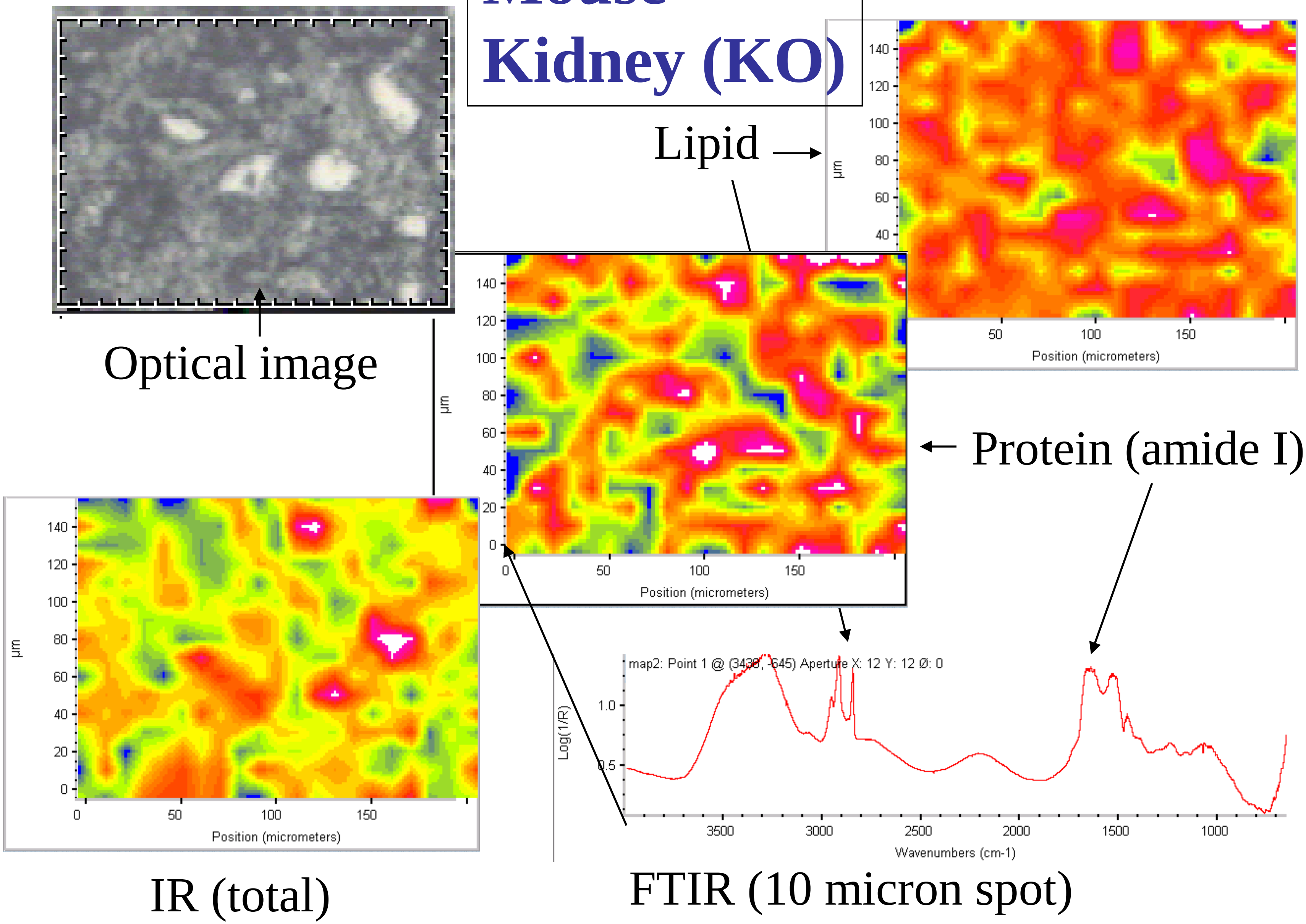
Map10a_sum.map



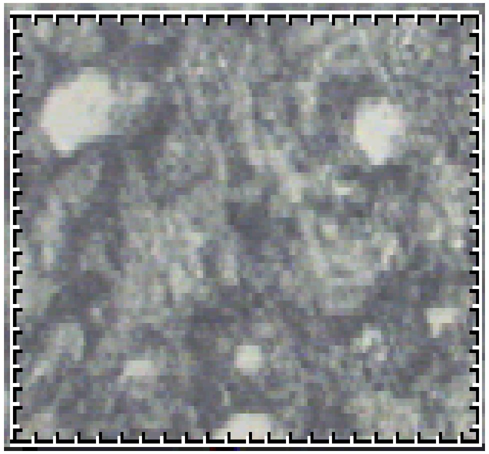




OMNIC - [map10a sum.MAP - map10]
File Edit Collect View Process Analyze Atlμs Series Report SST Window Help
Experiment: Default - Transmission
map10: Point 1 @ (-12817, -822) Aperture X: 12 Y: 12 Ø: -1
Log(1/R)
0.6
0.4
0.2
0.0
3000
2000
1000
Wavenumbers (cm-1)
200
180
160
140
120
100
80
60
40
20
0
μm
Position (micrometers)
X:(2147.359) Y:(0.528)
Show contour
Show waterfall
Profile Setup

# Mouse Kidney (KO)

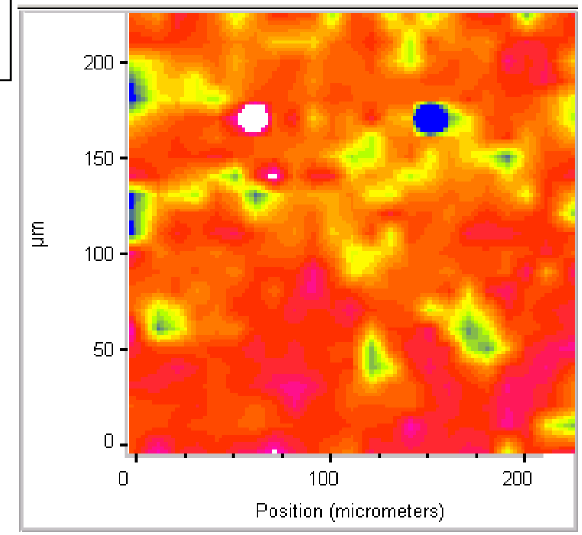


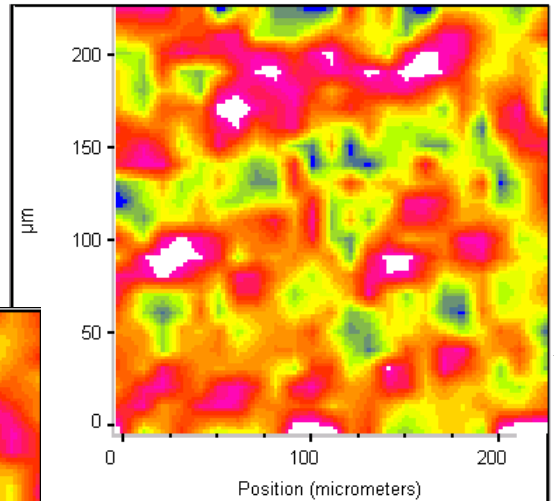
# Mouse Kidney (WT)



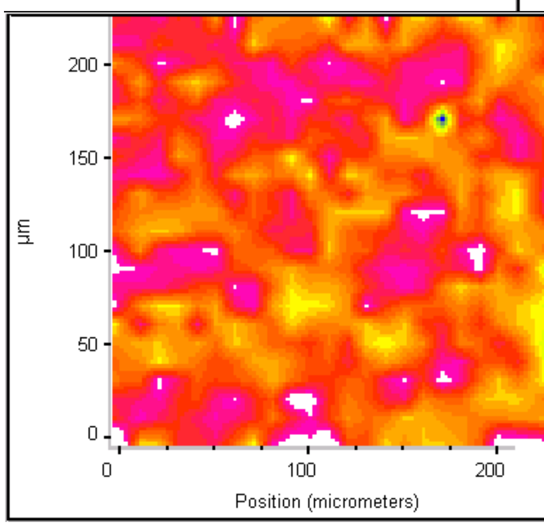
Optical Image

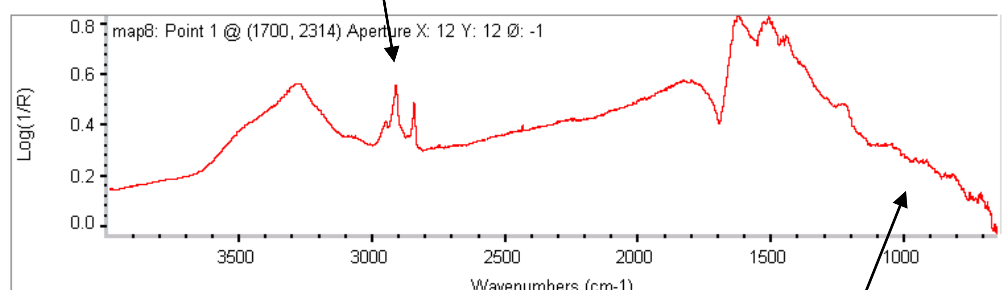
Lipid




Protein (amide I)



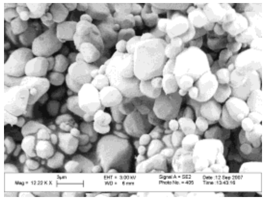



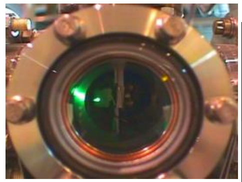

Other (900-1199 $cm^{-1}$)

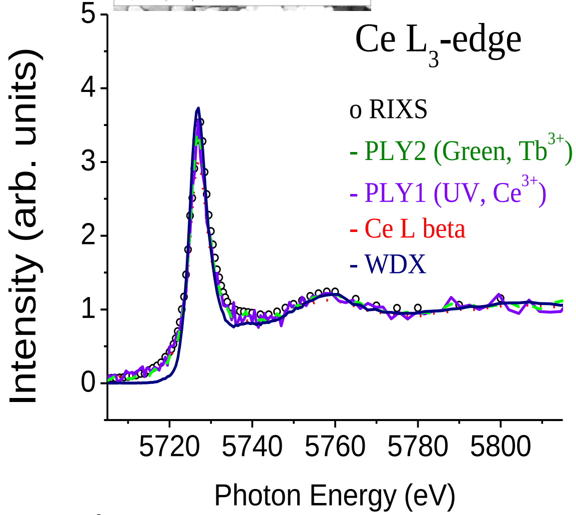
# XEOL Imaging

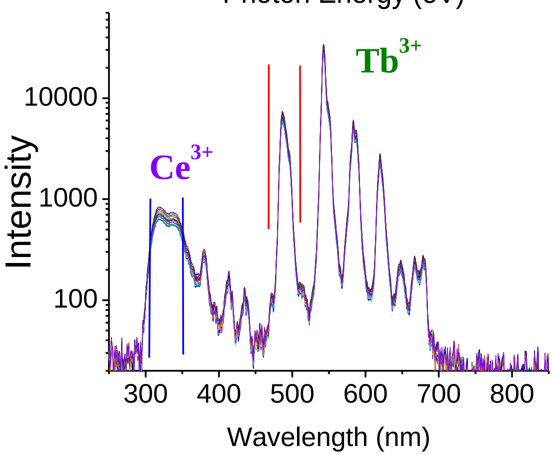
Light emitting materials can also be used for imaging. Rare earth scintillators for example can be excited by X-ray and emit bright light in the visible which in turn can be used to produce image

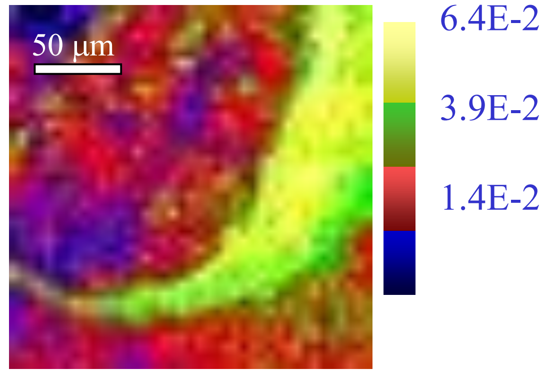
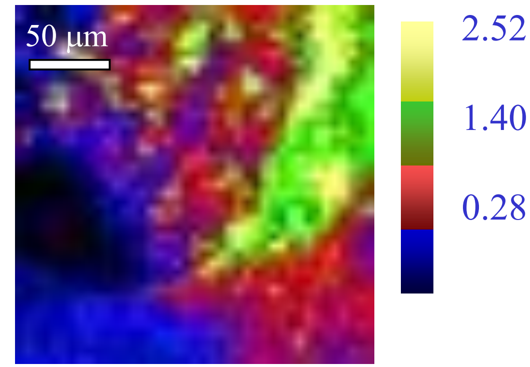
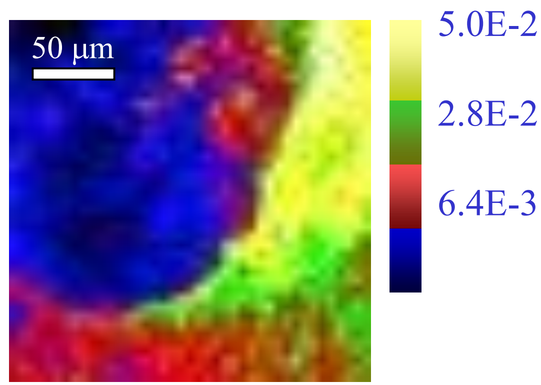
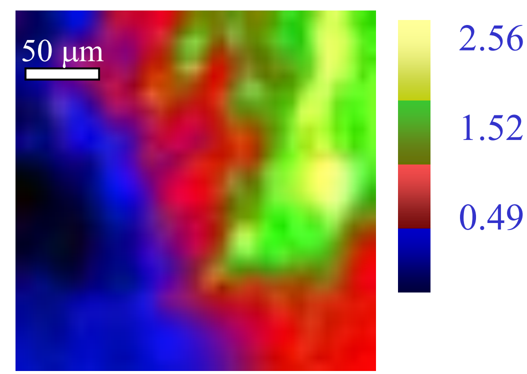
# XRF and XEOL map → TRXEOL map








| X-ray Map | Optical Map |
|---|---|
|  |  |
| WDX: *Ce Lα* | *$Ce^{3+}$:300 – 363 (nm)* |
|  |  |
| Si: *Tb Lα* | *$Tb^{3+}$:450 – 475 (nm)* |









T.K. Sham and R.A. Gordon, *SRI2009 proceedings*, 1234, 133(2010).