BIOMEDICAL APPLICATIONS OF MICROPROBE ANALYSIS

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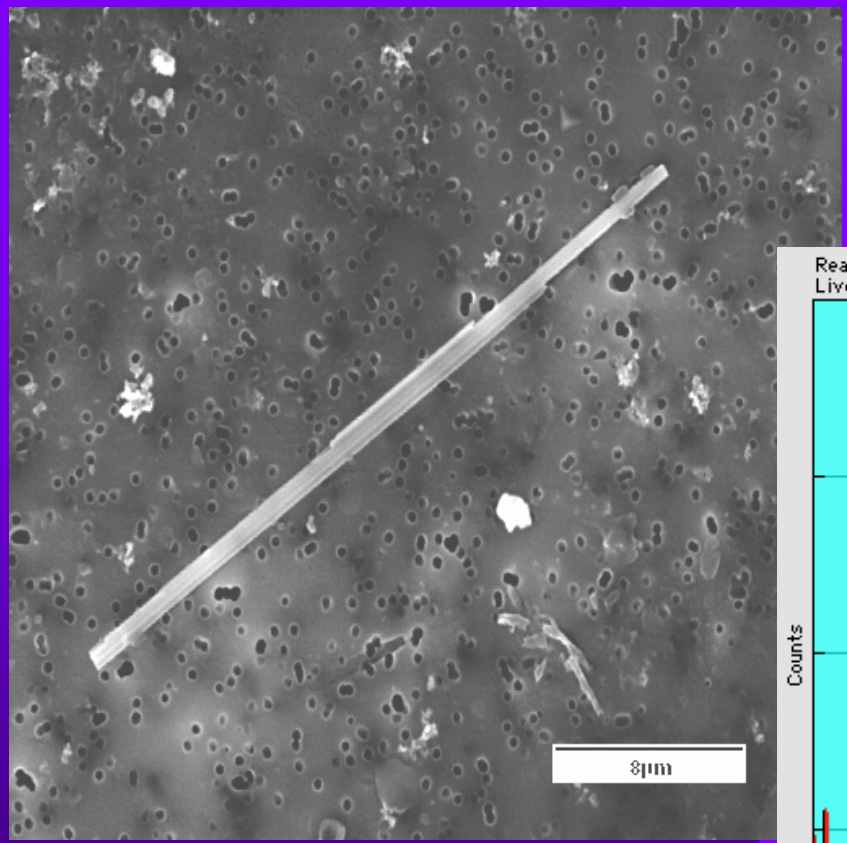
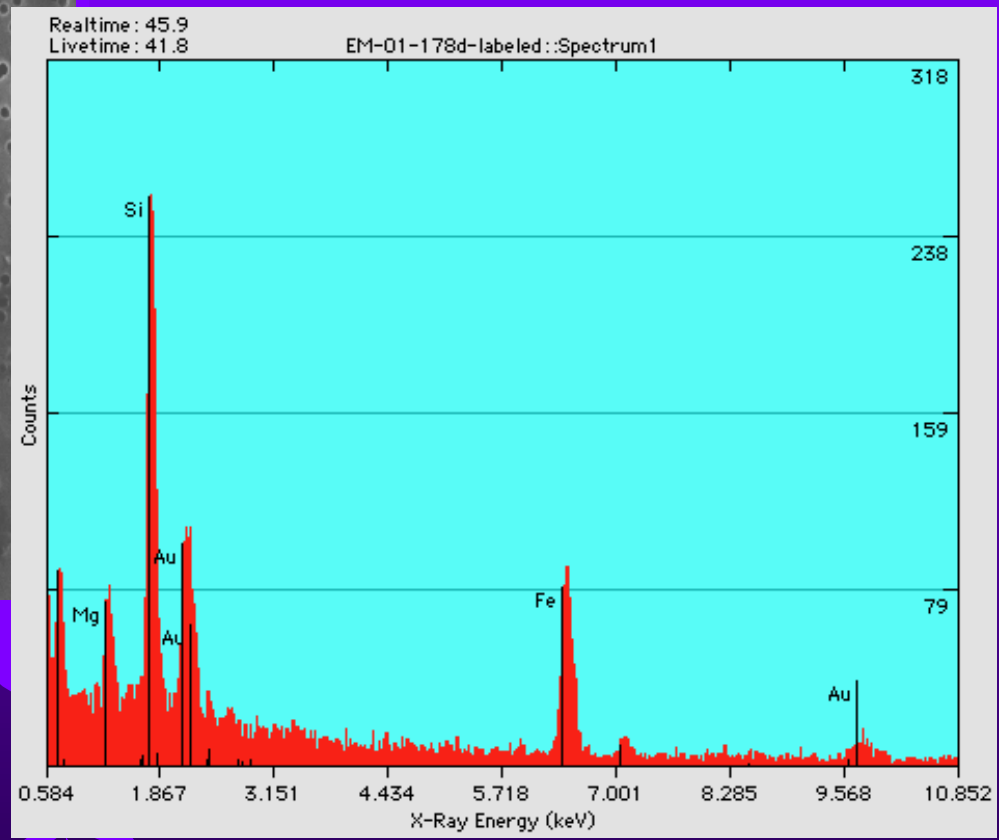
Microanalysis as a Diagnostic Standard of Care

- Unexplained Particulates, Inclusions, Granulomas
- **Mineral Pneumoconioses (Asbestosis)**
- **Renal Stone Disease**
- Reactions to Element-Based Anti-arrythmics, Chemotherapeutics, Contrast Agents, Poisons

Asbestos Fiber
Amosite





NYC Firefighter Exposed to WTC Dust

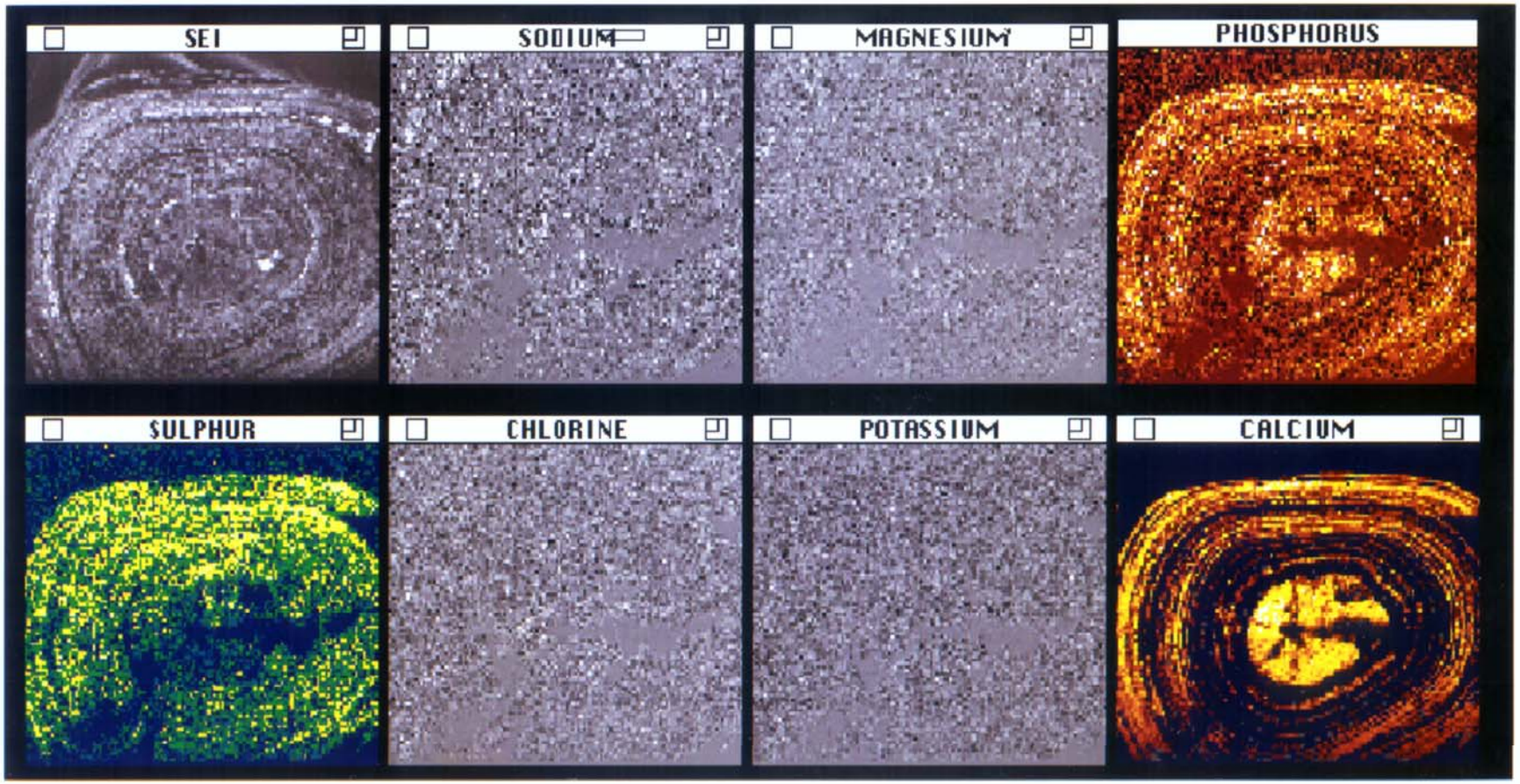
Roggli et al., Am J Resp Crit Care Med 166:797-800, 2002

Microanalysis as a Diagnostic Standard of Care

- Renal Stone Disease
  - Calcium Oxalate
  - Calcium Phosphate
  - Magnesium Ammonium Phosphate
  - Uric Acid
  - Cysteine
  - Bilirubin

Renal Stone

RenalStone

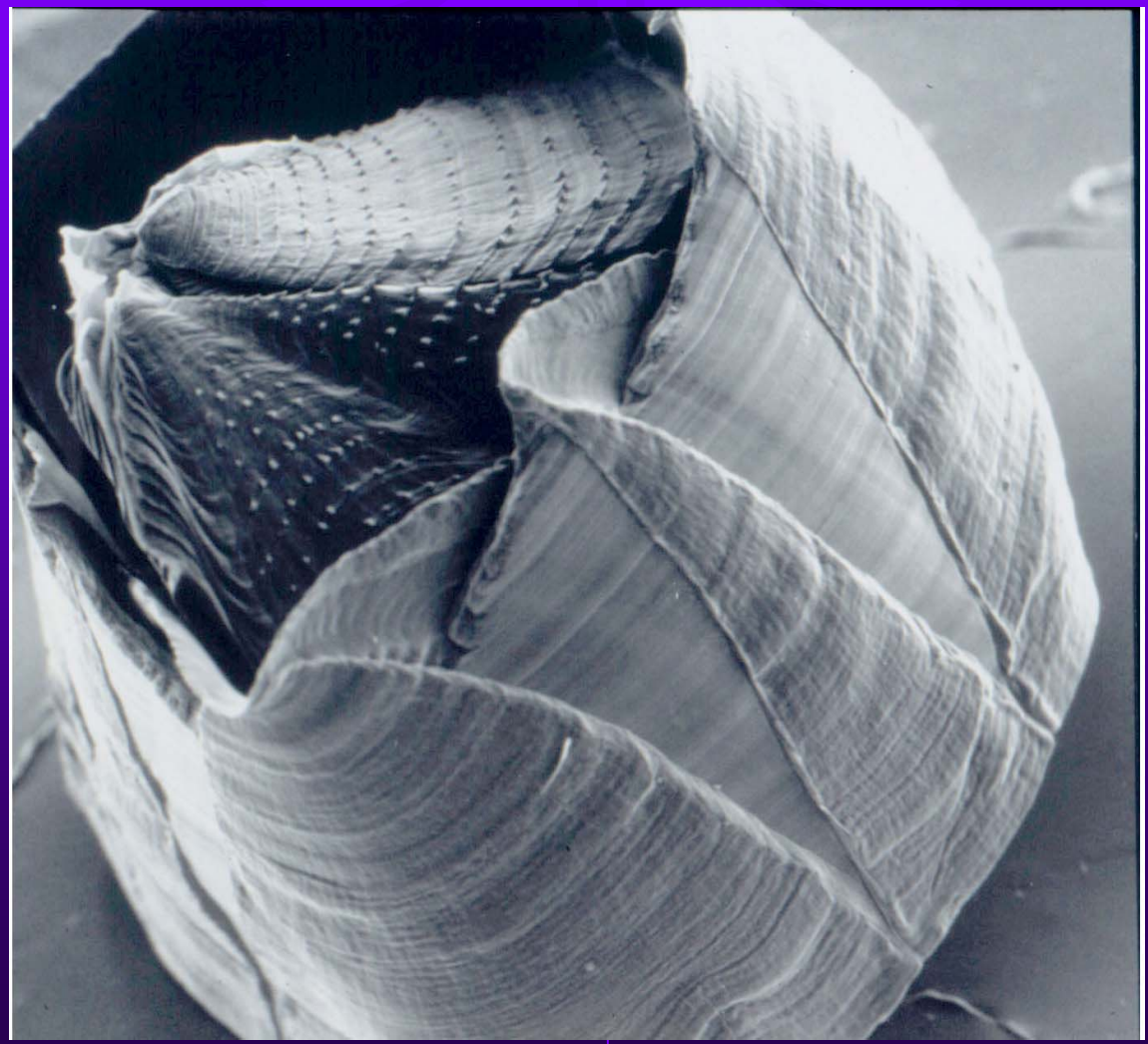

SEI
SODIUM
MAGNESIUM
PHOSPHORUS
SULPHUR
CHLORINE
POTASSIUM
CALCIUM

Barnacle Biomineralization as Bioindicator

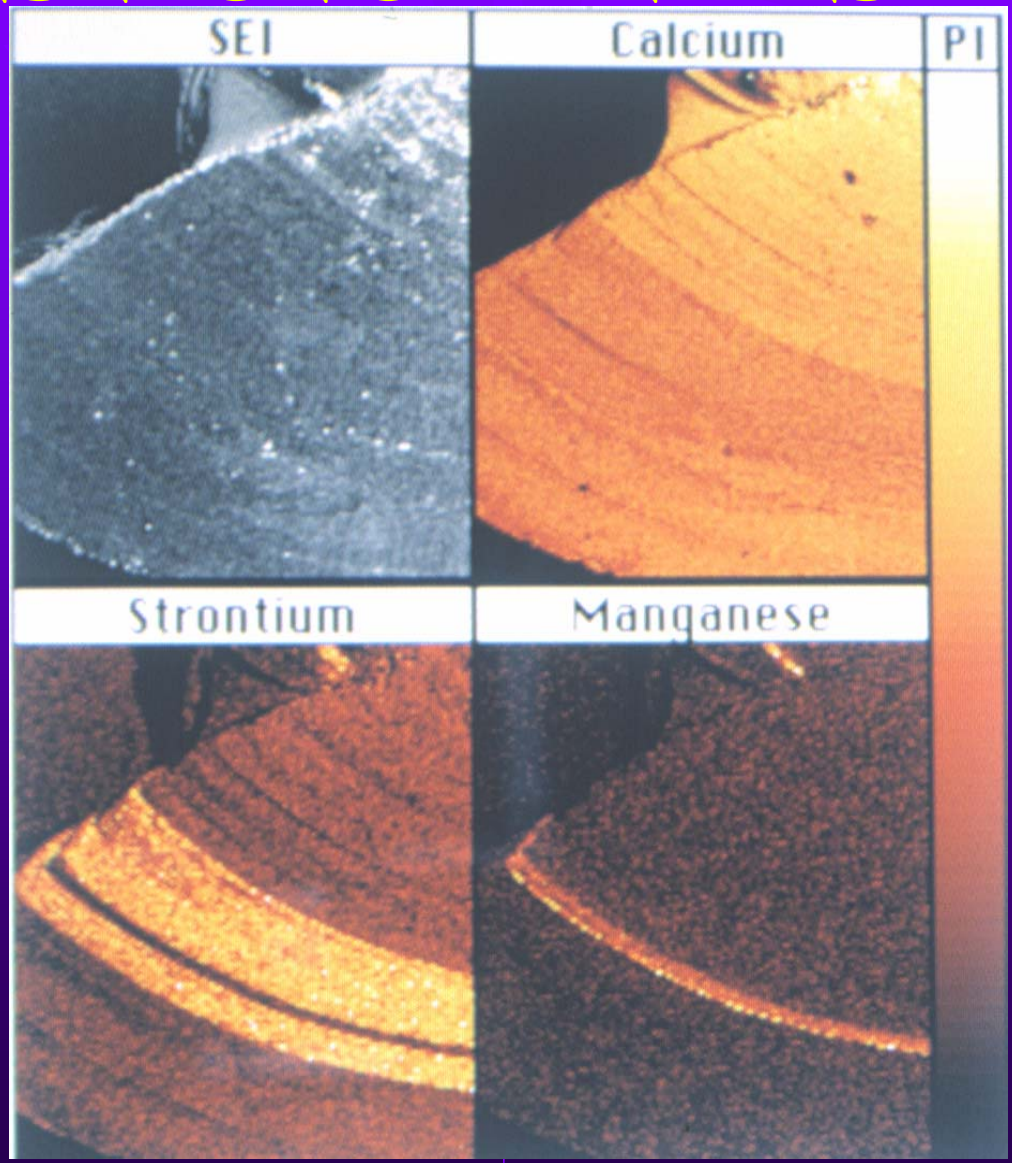
- Integrates water content (quality) over time
- Indicative of exposures **to other organisms**

- **Reveals time course and location of biomineralization front**

Adult Barnacle Biomineralized Shell



Biomineralization in the Presence of Metals

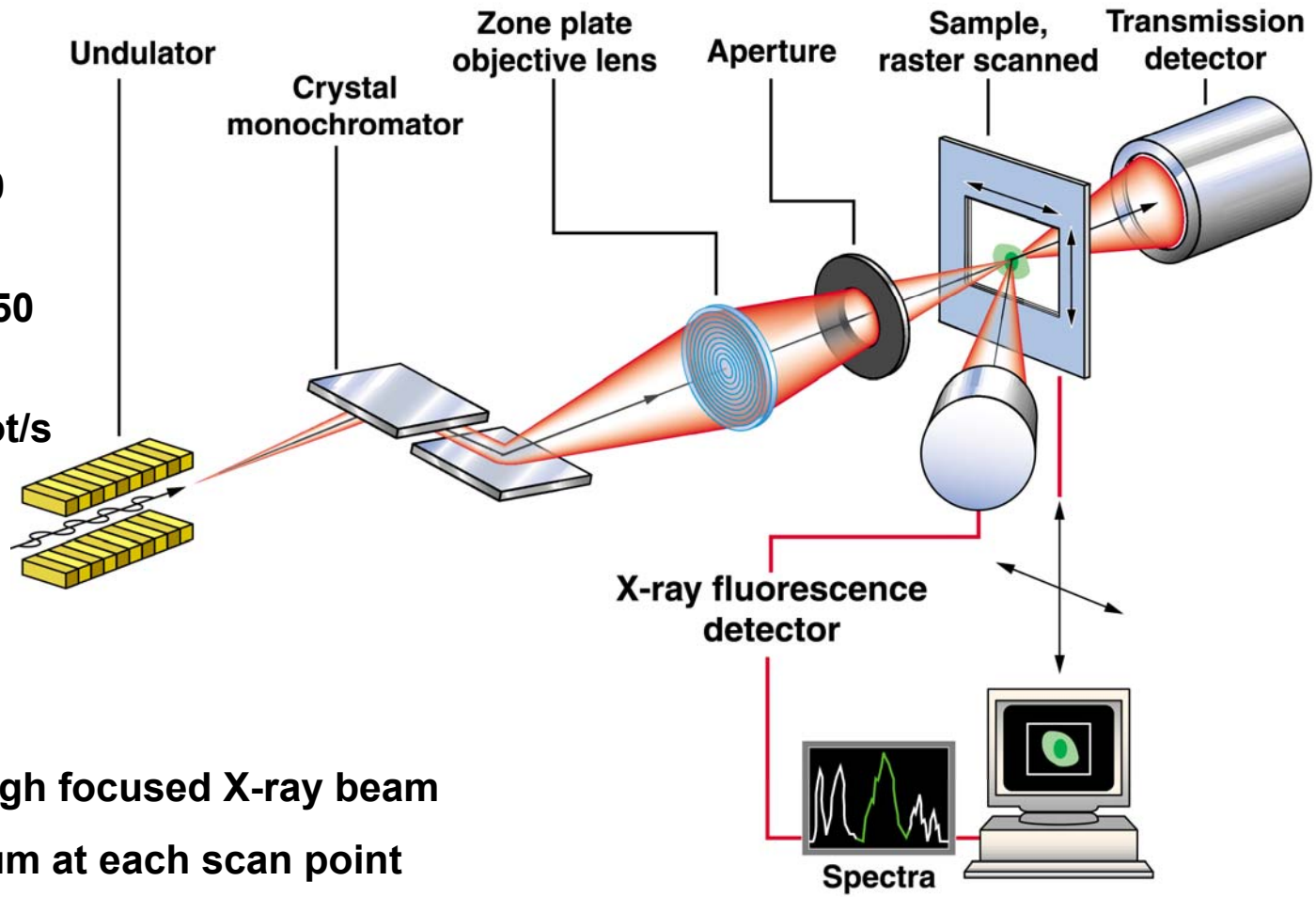


HIGH SPATIAL RESOLUTION GENERALLY REQUIRES THIN SECTIONS IN EPXMA OF BIOLOGICAL MATERIALS

XPXMA schematic

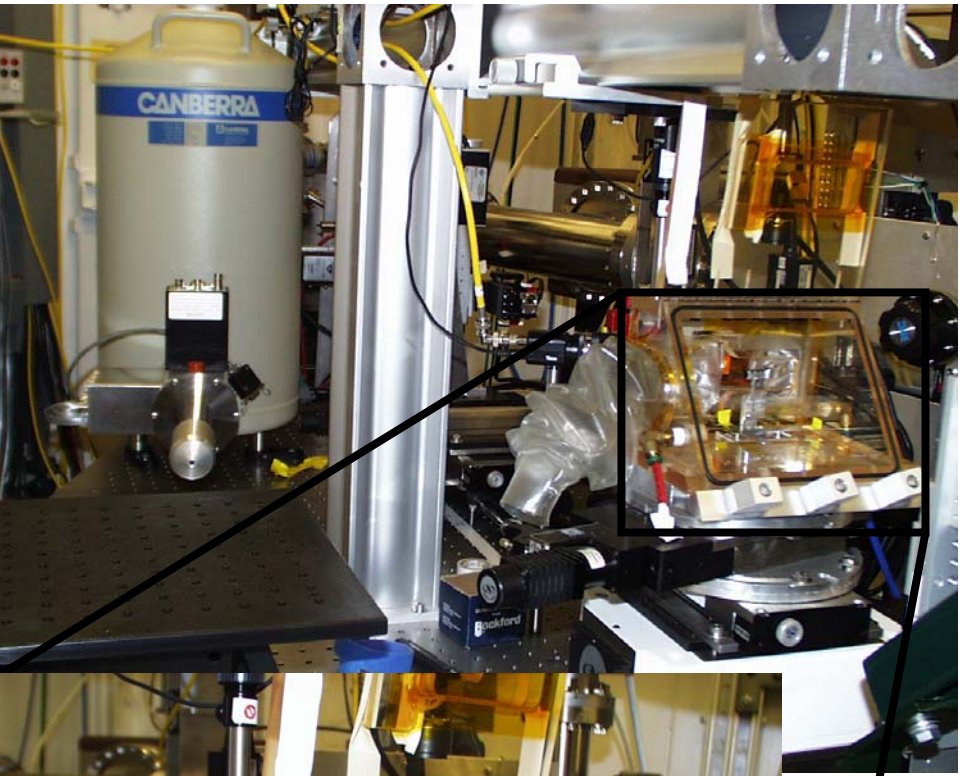
Example APS 2-ID-D:

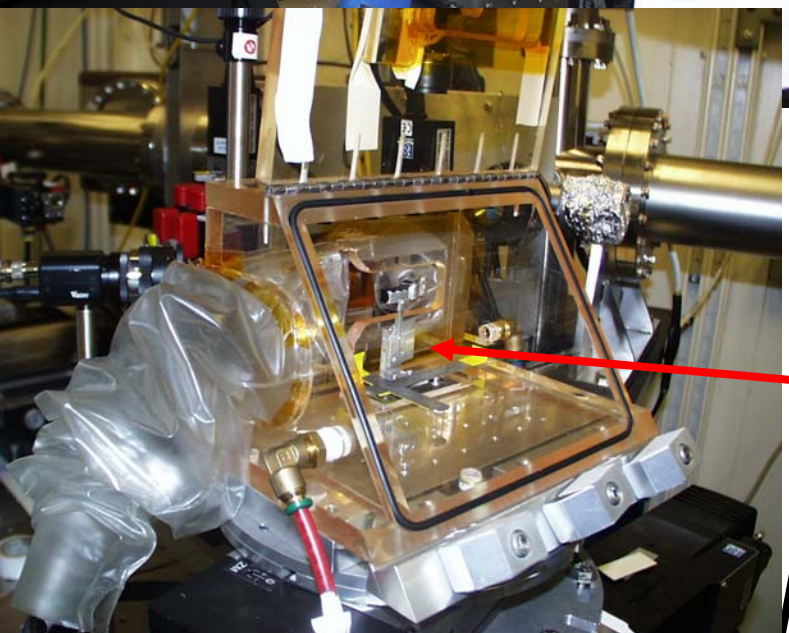
- **energy range E = 5 – 30 keV**
- **spatial resolution δ = 150 nm**
- **focussed flux $2 \cdot 10^9$ phot/s (can trade flux for resolution)**



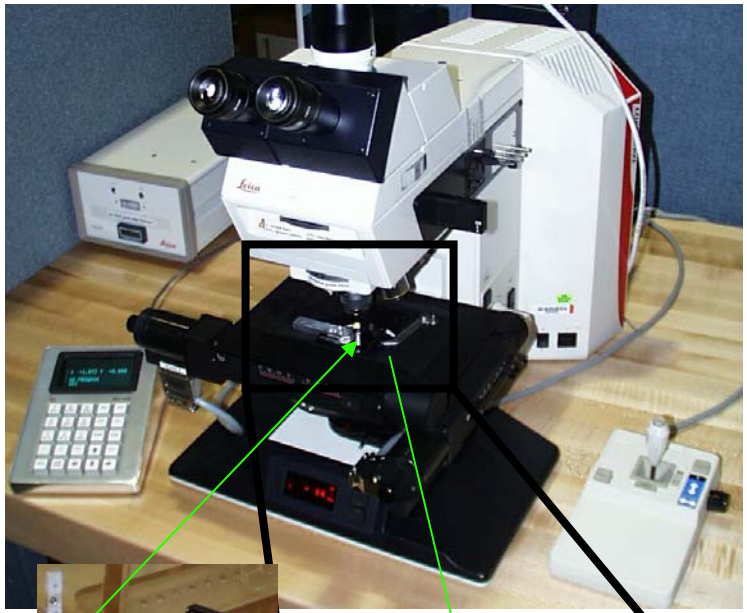
- **stepscan sample through focused X-ray beam**
- **record full XRF spectrum at each scan point**
- **compare specimen counts/spectra to calibration curve, to quantify to area density**

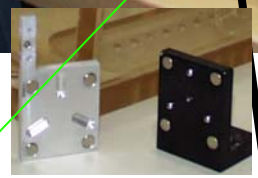
2-ID-E Hard X-ray Microprobe Facility





Epi-Fluorescence Microscope

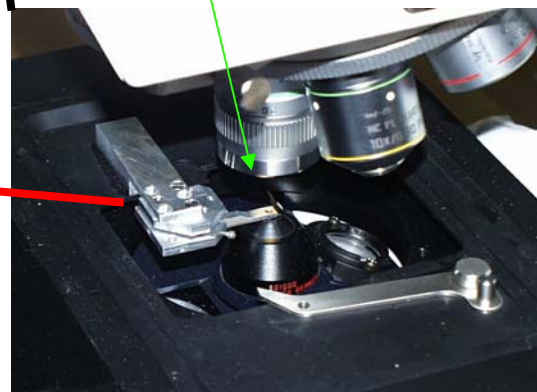




Sample Holder

Kinematic Mount

sample

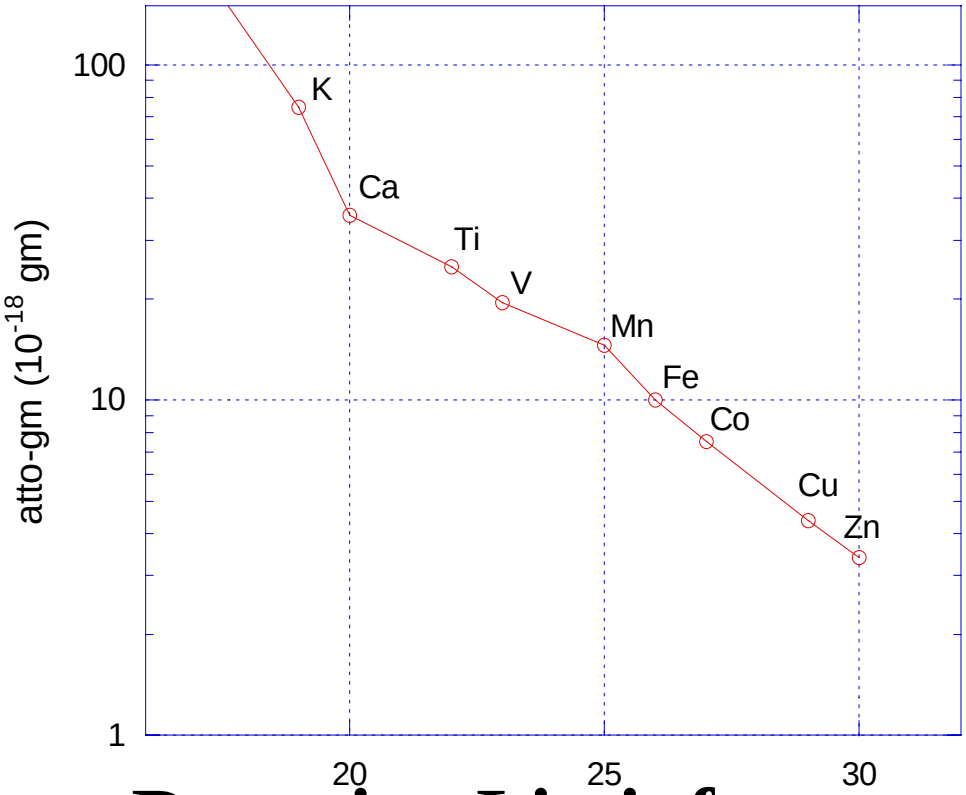


Trace elements / metals in biology & life sciences

- essential cofactors in proteins
- linked to diseases
- in therapeutic drugs
- as intracellular labels
- contaminants in the environment with adverse impact on human health

Why use x-ray-induced fluorescence to study trace metals?

- **Simultaneously map 15+ elements**
- **No dyes necessary**
- **very high sensitivity (sub part-per-million)**
- **quantitative**
- **large penetration depth (> 100 µm)**
- **chemical state mapping & micro-XANES**



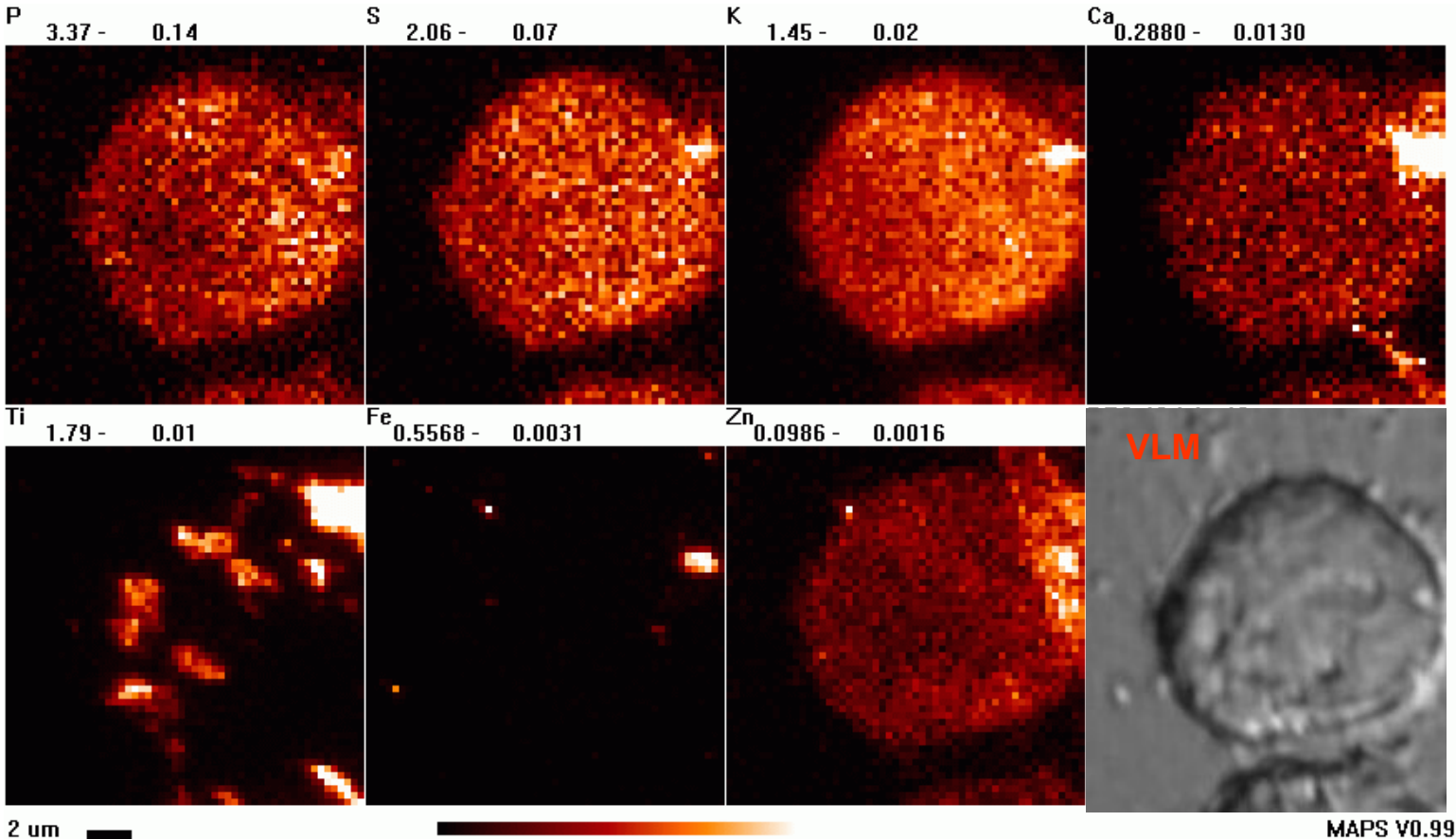
Detection Limit for Transition Elements: for 1 sec. acquisition time, 0.2 x 0.2 µm^2 spot, E=10 keV

Example: Ti nanocomposites as intracellular probes

T. Paunesku, G. Woloschak et al

- attach inorganic TiO_2 nanoparticle (4.5 nm diameter) to DNA strand to combine DNA specific biochemistry with semiconductor properties of TiO_2
- ➔ carrier-particle that can bind to a specific chromosomal region w/ ability to release bound DNA & cause targeted strand breaks upon illumination

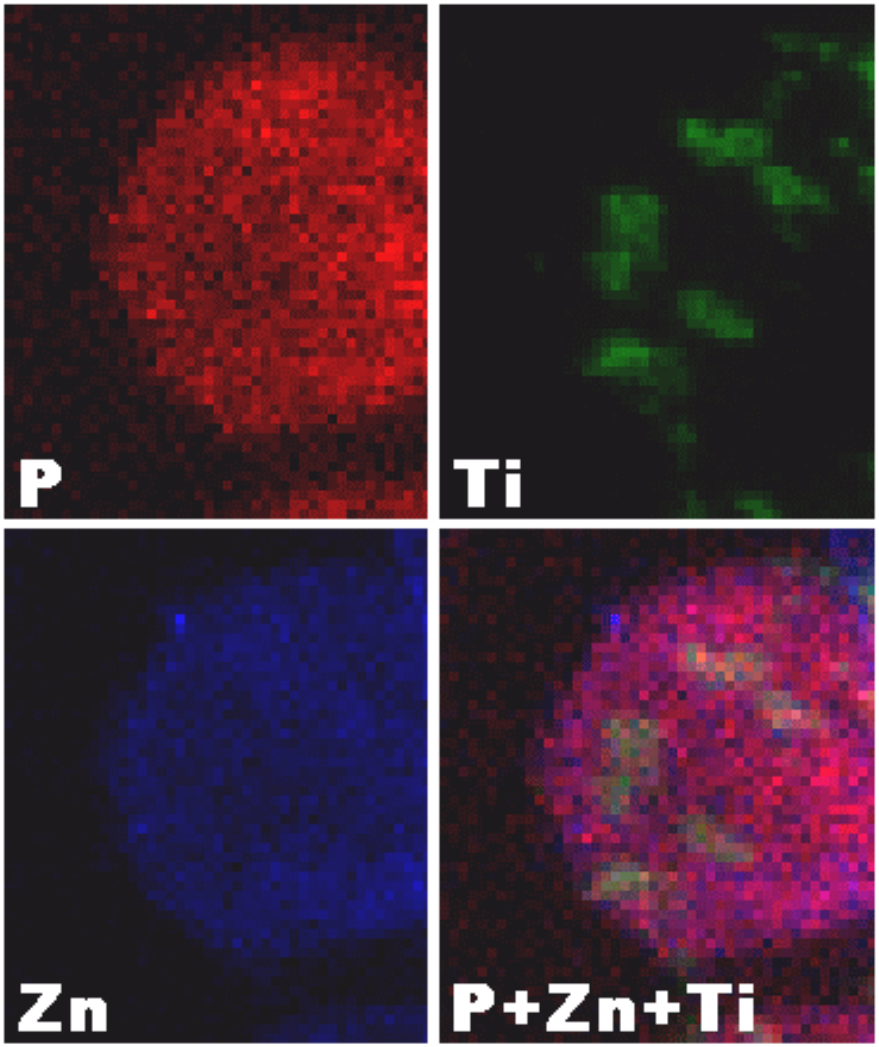
PC-12 cell transfected with nanocomposite combining mitochondrial DNA w. TiO_2.

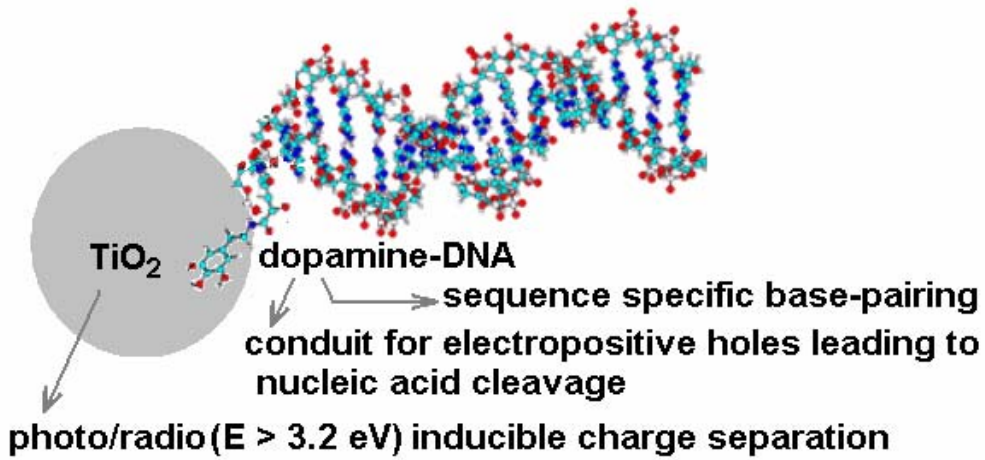


See also,* T. Paunesku et al, *Nature Materials 2, 343-346 (01. May 2003)

Why study trace metals in life sciences:

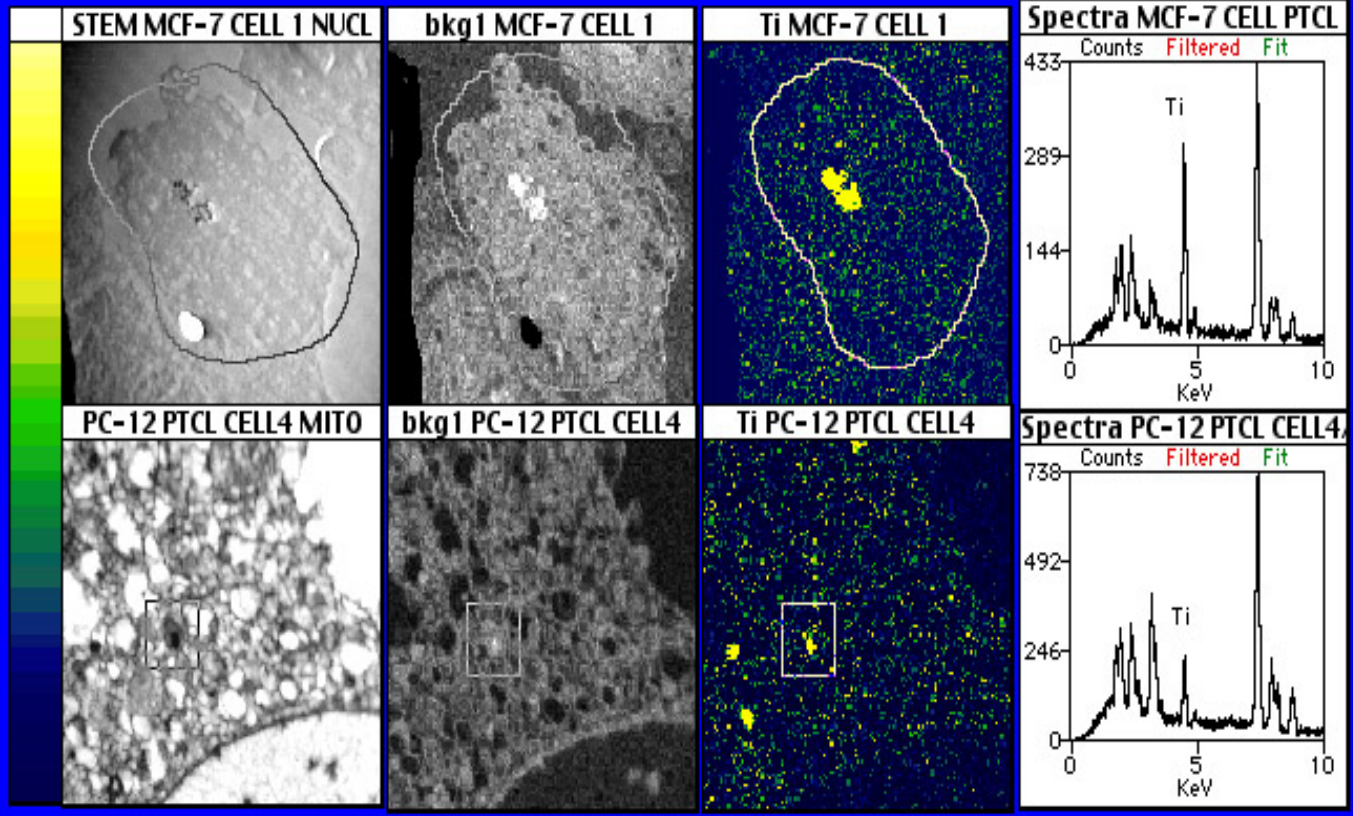
Nanocomposites as intracellular tools

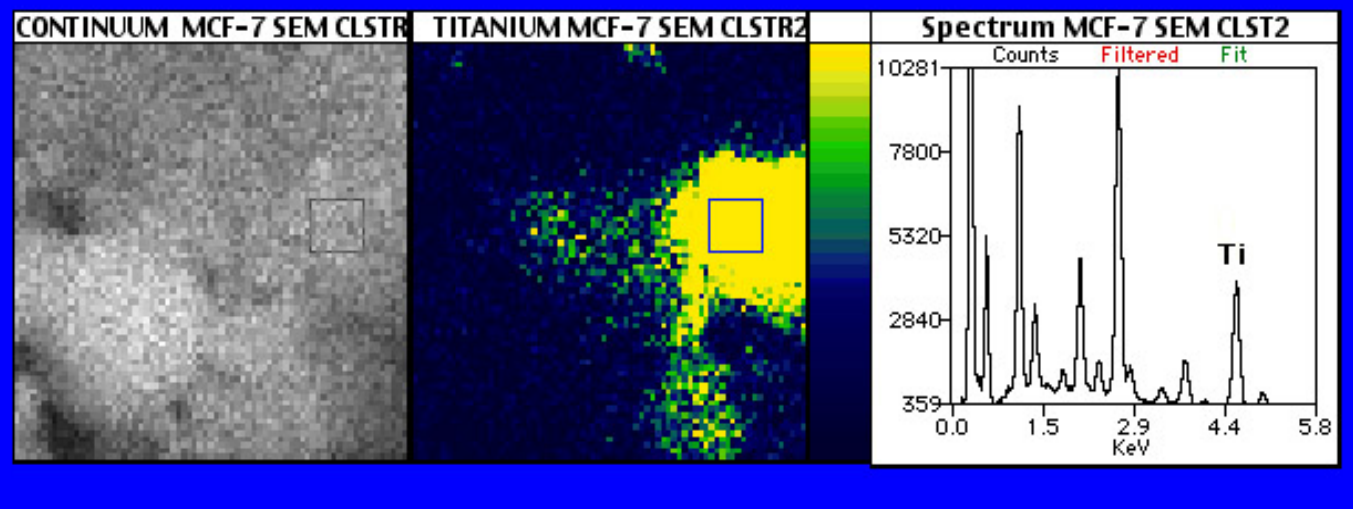




- attach TiO_2 nanoparticle (4.5 nm diameter) to DNA
- combine DNA biochemistry with semiconductor properties of TiO_2
- ➔ carrier-particle that can bind to a specific chromosomal region w/ ability to cleave it upon illumination

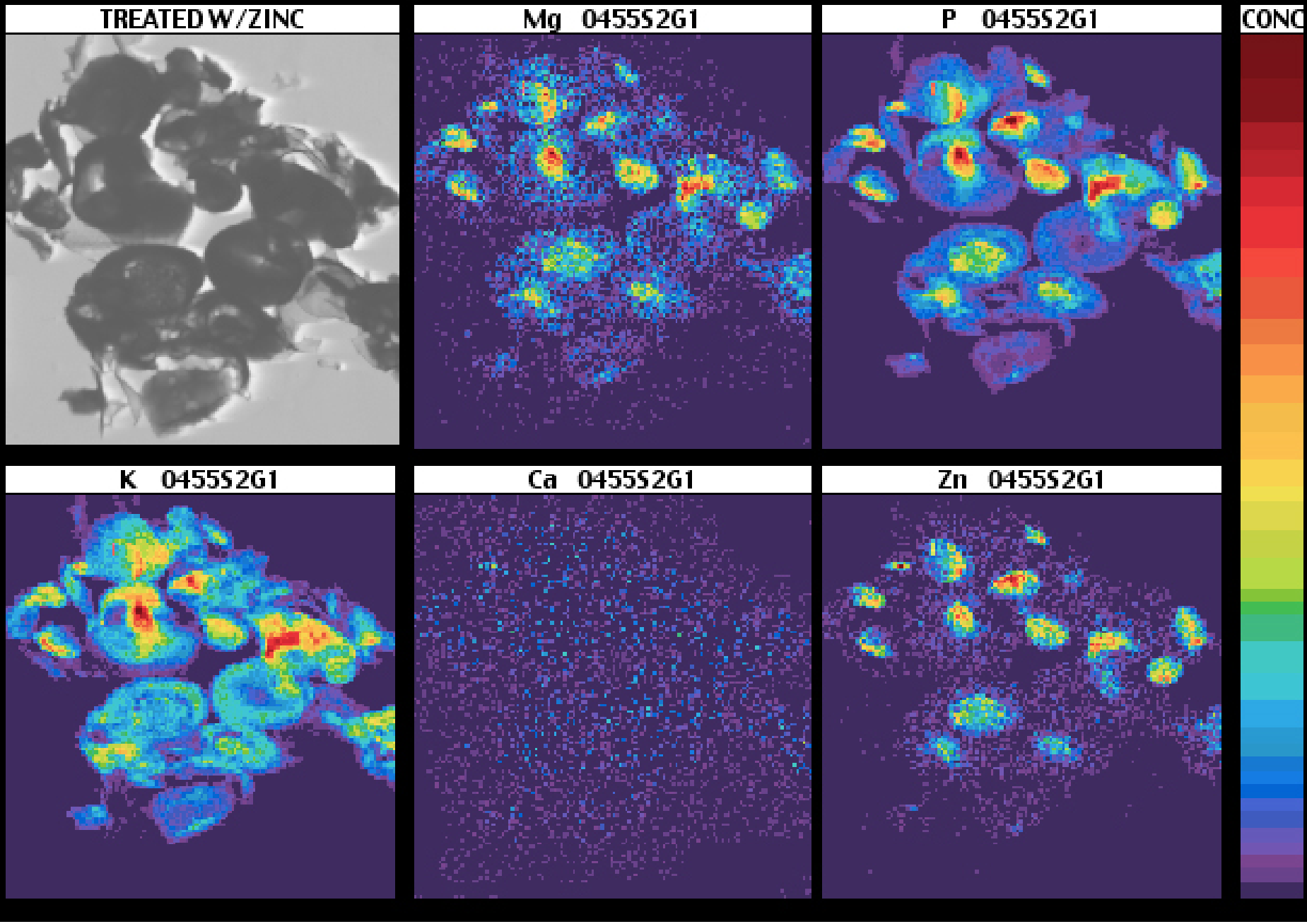
T. Paunesku et al, *Nature Materials 2, 343-346 (01. May 2003)*



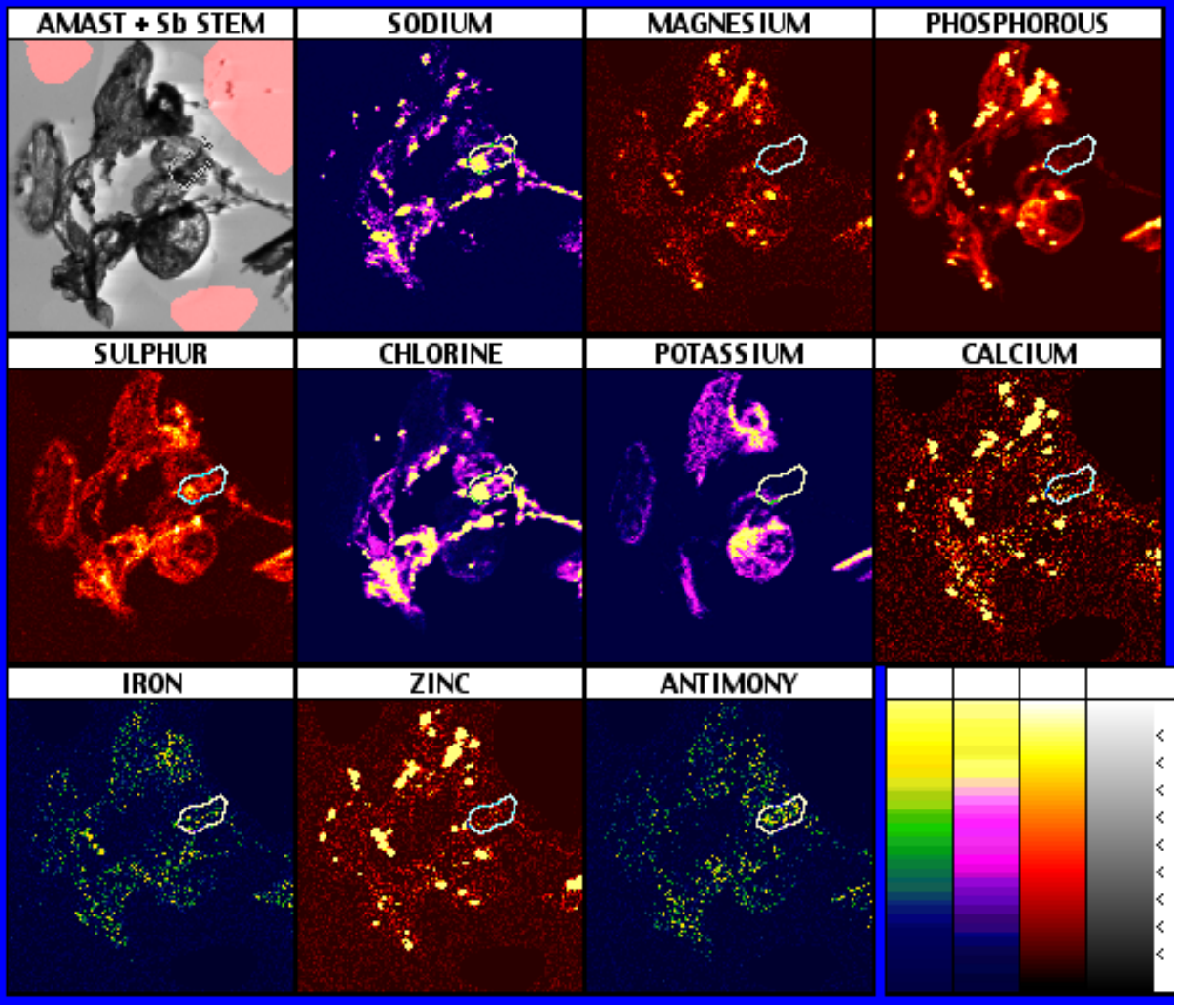


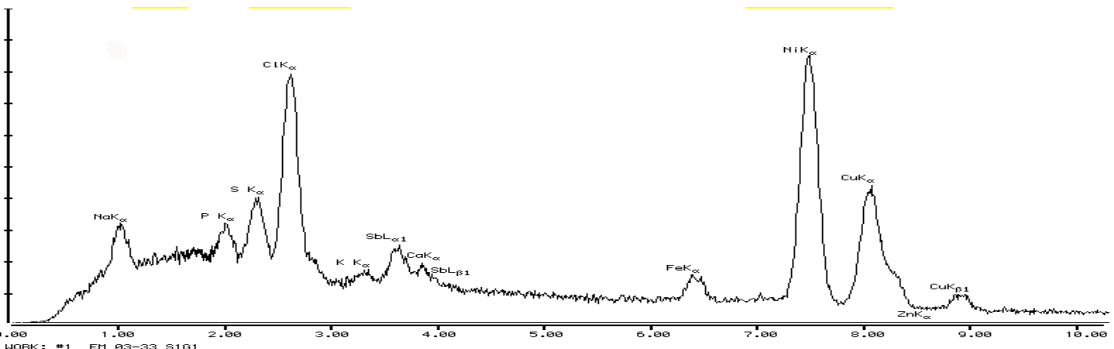
Elemental images and EPXMA spectra of MCF-7 breast cancer cells transfected with TiO_2 nanoparticles conjugated to **nuclear** DNA (top 4 panels) and to **mitochondrial** DNA (lower 4 panels). STEM at 80 Kev.

MCF-7 cells, similarly treated to those above, but air dried on to a carbon support and imaged with EPXMA in a scanning electron microscope at 20 Kev..



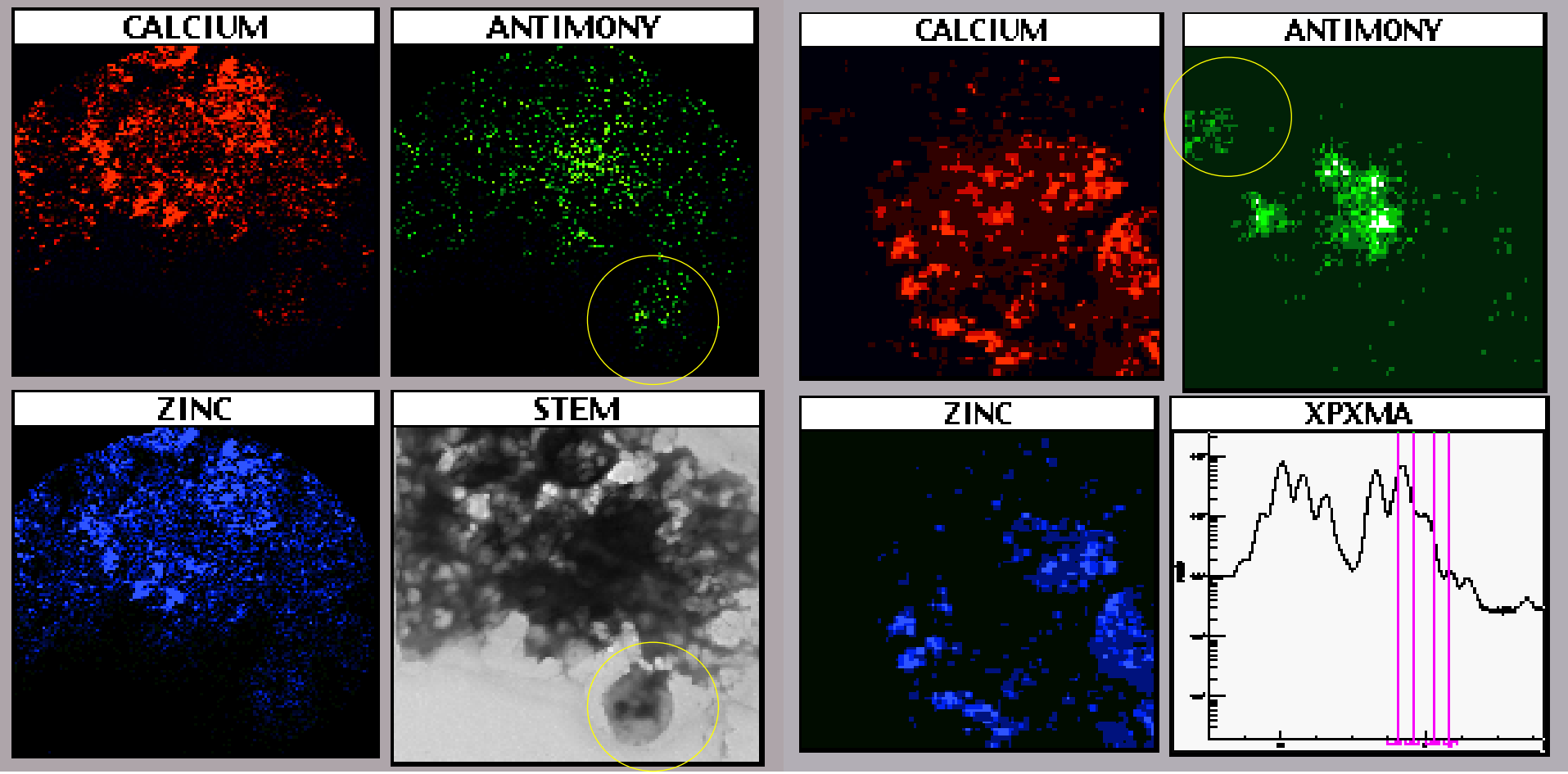
EPXMA OF YEAST CELLS INCUBATED IN **ZINC**-RICH MEDIA





Elemental EPXMA images from several cryoprocessed Sb-treated protozoa (*L. donovani*, amastigote stage).

The color scales are fully quantitative but have been adjusted to accentuate related elements. The spectrum was taken from the region outlined.



EPXMA **XPXMA**

Comparative images of the same cells from several Sb-treated protozoa (*L. donovani*, amastigote stage 1000µg/ml SbV, for 1 hr).. The intensity of the colors qualitatively reflect the content of the elements. The yellow circled region is from the same area of the specimen. Note that the EPXMA set on the left is a mirror image of the XPXMA set on the right.

MICROANALYSIS METHOD	ELECTRON MICRO-PROBE	PROTON MICRO-PROBE	SIMS MICRO-PROBE	LAMMA MICRO-PROBE	X-RAY MICRO-PROBES
MDL (ppm)*	100	0.5	0.005	0.005	0.01
MDL (μM)	75	3	0.003	0.003	0.05
QUANTITATION	GOOD	GOOD	V. DIFF-ICULT	POSS-IBLE	GOOD
RESOLUTION (μm)	0.01	1.0	0.04	0.5	0.03** 0.05#
HYDRATED SAMPLES	NO##	DIFFI-CULT	NO	DIFFI-CULT	POSS-IBLE
BEAM DAMAGE	HIGH	MED	HIGH	HIGH	LOW

*In part, after Spanne and Rivers (1987)
**Predicted for the APS Bio-Nanoprobe
##Except for water content from frozen specimens

Transmission Imaging has demonstrated ~ 0.05 μm between 6 to 8 keV. Bilderback et al. (1994)

Comparison of XPXMA with other sub-cellular biological microprobe analysis methods