



**The Abdus Salam  
International Centre for Theoretical Physics**



**2332-29**

**School on Synchrotron and FEL Based Methods and their Multi-Disciplinary  
Applications**

*19 - 30 March 2012*

**Transmission X-ray Microscopy contrast approaches and applications in life  
science**

Alessandra Gianoncelli  
*ELETTRA - Sincrotrone Trieste*

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# Transmission X-ray Microscopy contrast approaches and applications in life science

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## Different X-ray microscopy techniques:

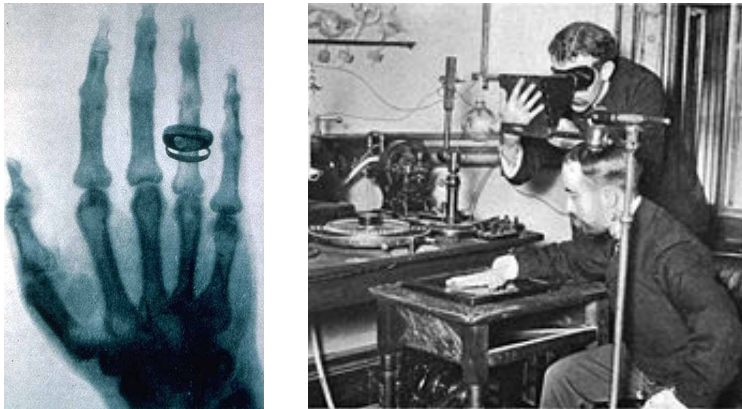
- **Most of XRM**s have an optical analogon to other wavelengths techniques (visible light microscopy, electron microscopy). This means that optical rules can in many cases be applied and transferred from one technique to another.
- Same is valid for applying absorption and phase-related imaging contrasts.

**We will try to explore now different transmission XRM techniques...**

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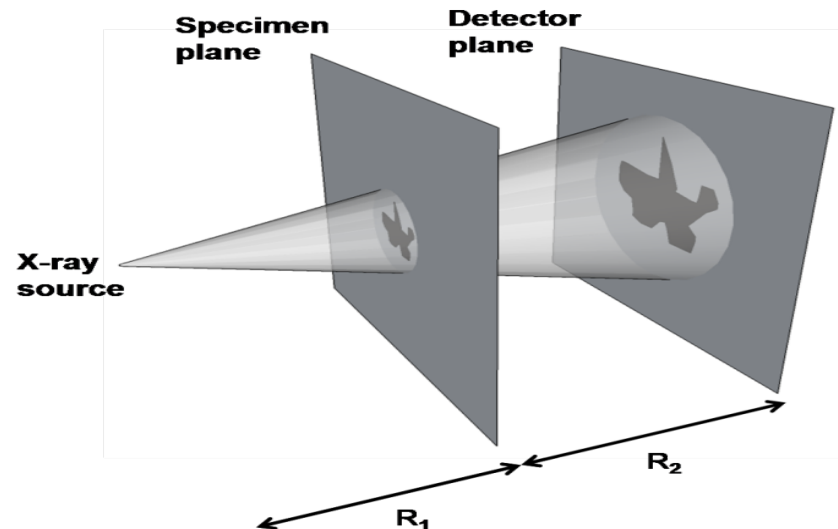
## The classic and most common: X-ray projection imaging



**Old Crooke's apparatus**



**Modern digital radiograph**



The magnification  $M$  is given by  $M=1+R_2/R_1$ , the optical resolution is determined by source size

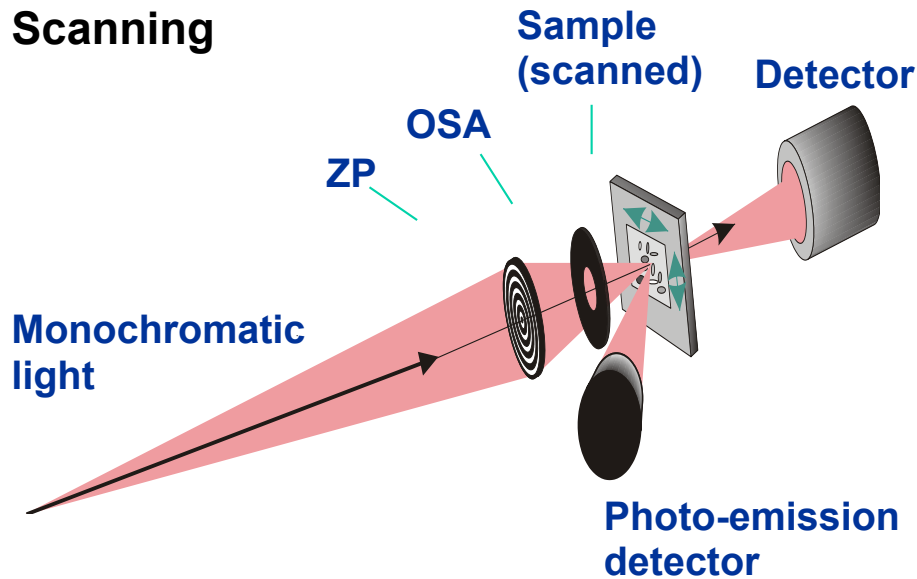
**Problem: Fresnel diffraction**  
(unsharpening of the image)

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# Background info: X-ray microscopy types

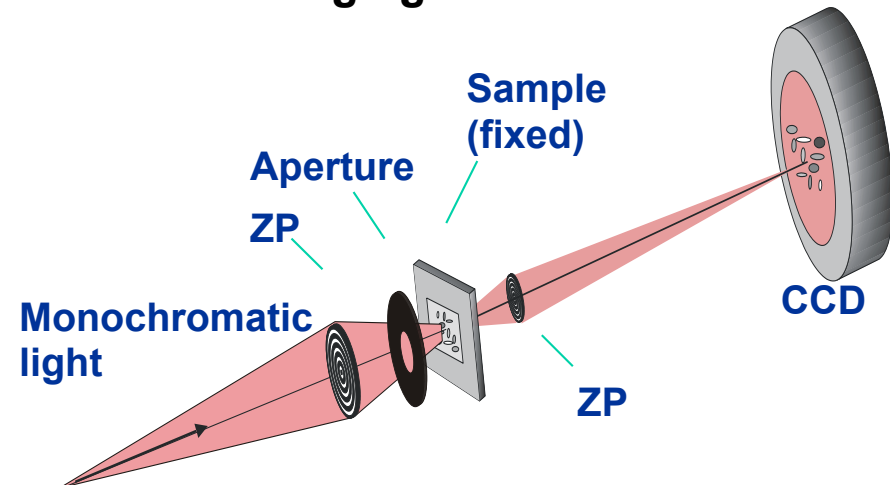
## Scanning



- + versatile detectors can run simultaneously;
- + easier optics set-up;
- long exposure time;
- complex electronics.

**Ideal for spectromicroscopy**

## Full-field imaging



- + short exposure time;
- + higher resolution
- static system;
- complex optical alignment.

**Ideal for dynamic studies  
and tomography**

## Which lenses to use to focus X-rays ?

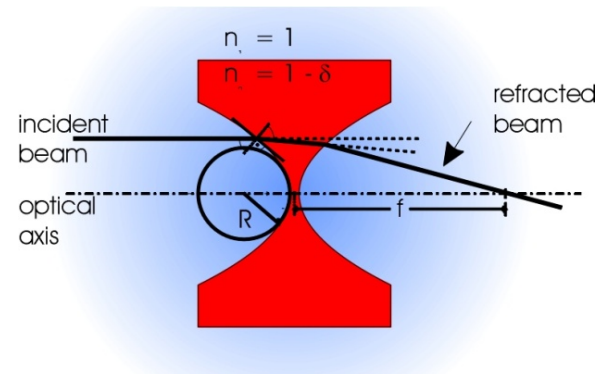
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Conrad Wilhelm Roentgen

**When Roentgen discovered X-rays, he immediately tried to focus them with refractive lenses but did not succeed!**

**Why? ...**

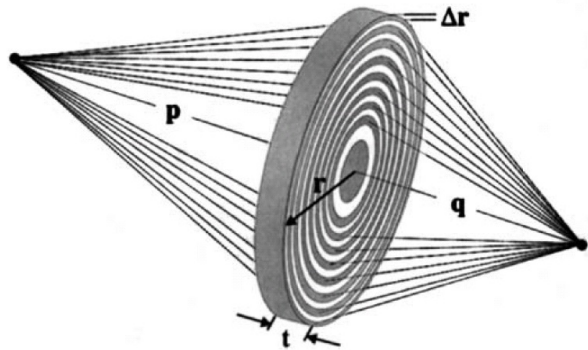


$$f = \frac{R}{2\delta}$$

**For example: Al lens with  $R=500\mu\text{m}$ ,  $E=10\text{keV}$ ,  $\delta=5.46 \cdot 10^{-6}$ :**

$$f = 92\text{m}$$

## A reminder towards X-ray microscopy: The optics?



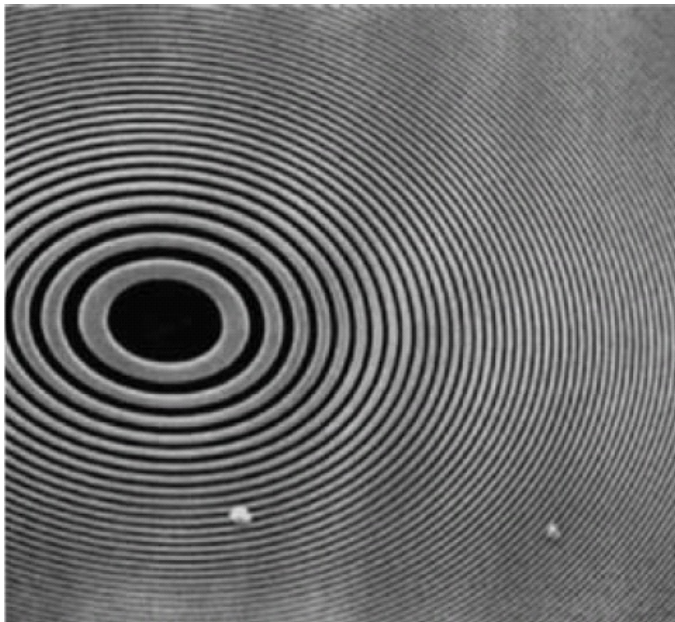
A zone plate (ZP) is a circular diffraction grating with radially increasing line density

$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q} \quad \text{if } n > 100; \quad f = \frac{2r\Delta r}{\lambda}$$

Lateral resolution of a ZP (Rayleigh):

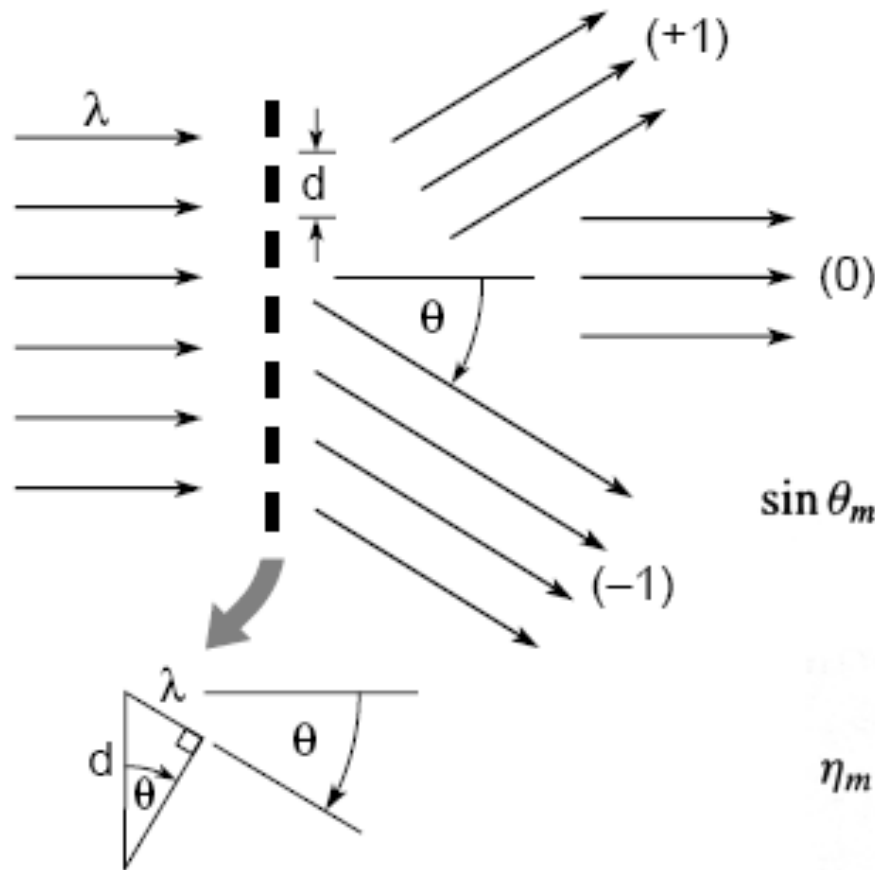
$$NA \equiv \frac{r}{f} = \frac{\lambda}{2\Delta r}$$

$$\partial_{\text{Rayleigh}} = \frac{0.61\lambda}{NA} = 1.22\Delta r$$



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## Background info: Diffraction by a grating



$$\sin \theta_m = \frac{m\lambda}{d} ; \quad m = 0, \pm 1, \pm 2, \pm 3, \dots \quad (9.2)$$

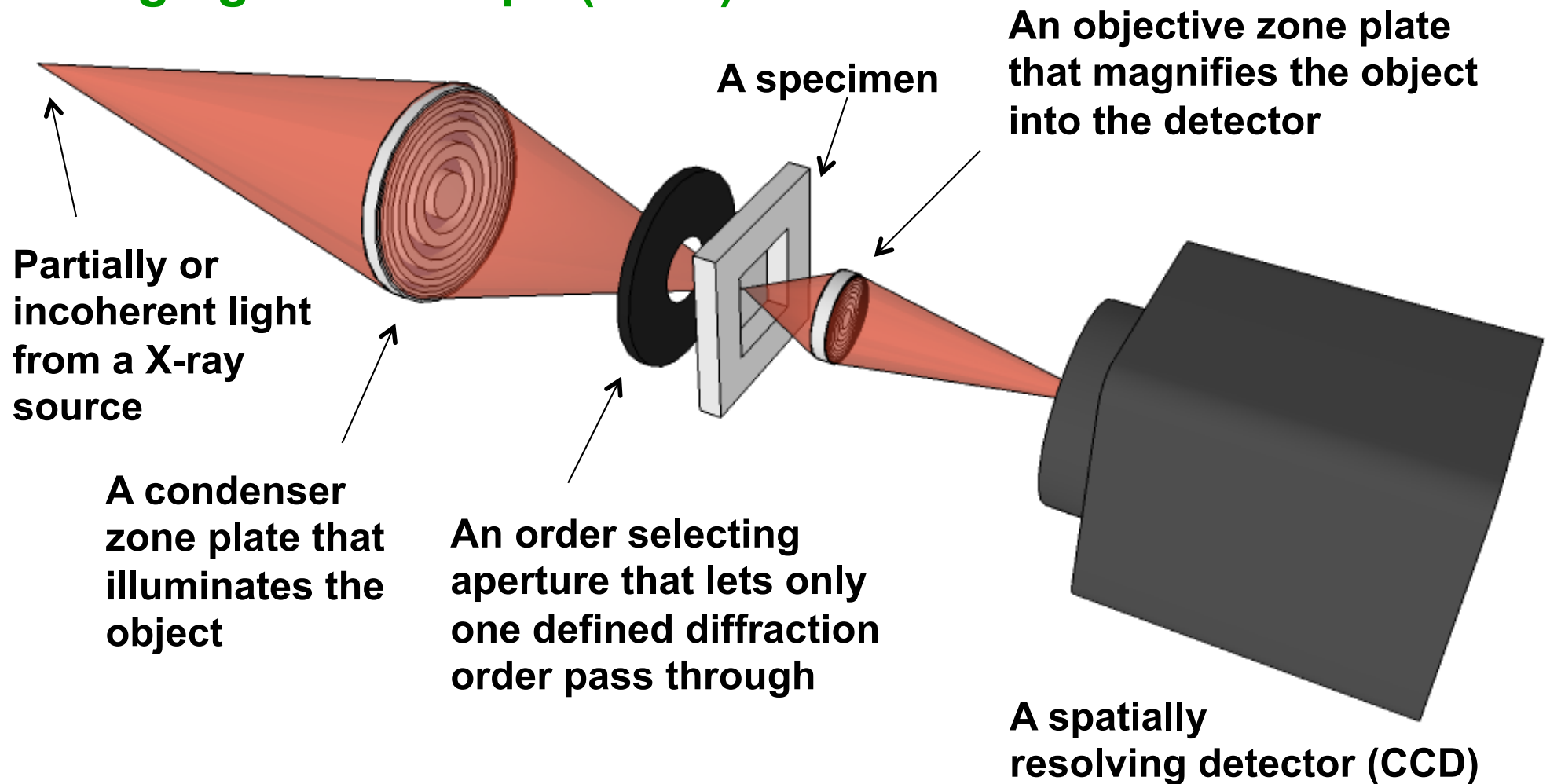
$$\eta_m = \begin{cases} \frac{1}{4} & m = 0 \\ 1/m^2\pi^2 & m \text{ odd} \\ 0 & m \text{ even} \end{cases} \quad (9.24)$$

(50% absorbed)

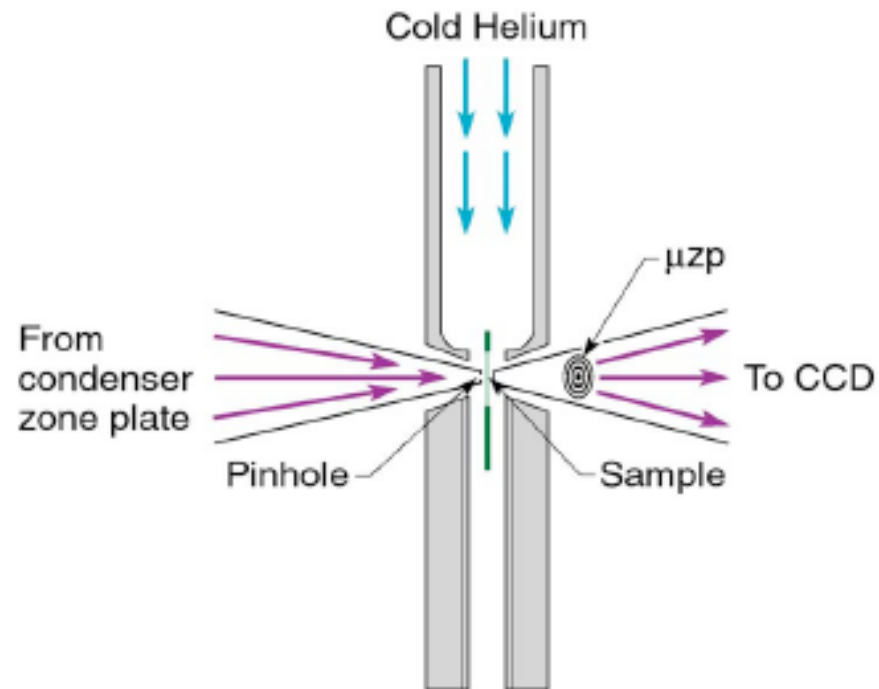
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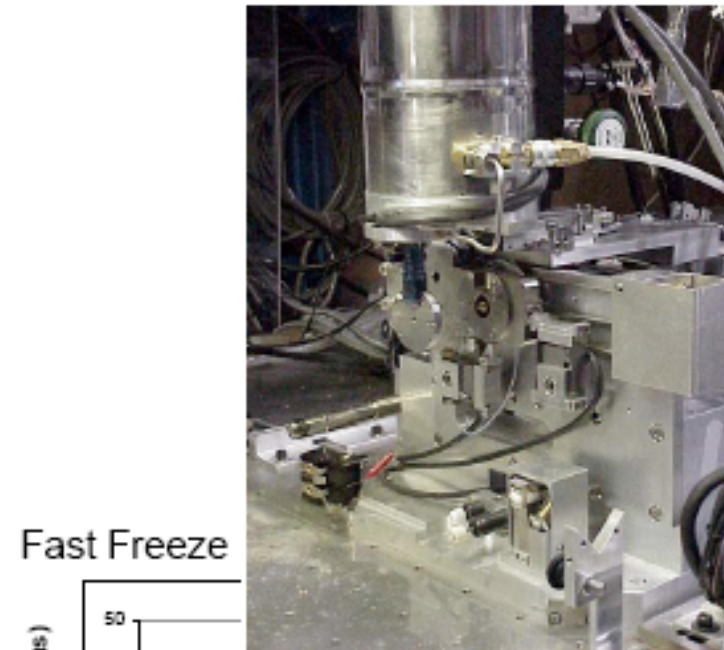
## The transmission X-ray microscope (TXM) or full-field imaging microscope (FFIM)



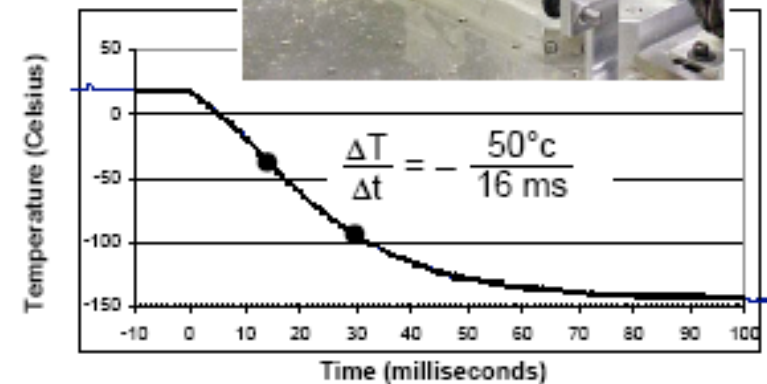
## The XM-1 microscope at the ALS, LBL, CA, US:



Helium passes through LN, is cooled,  
and directed onto sample windows

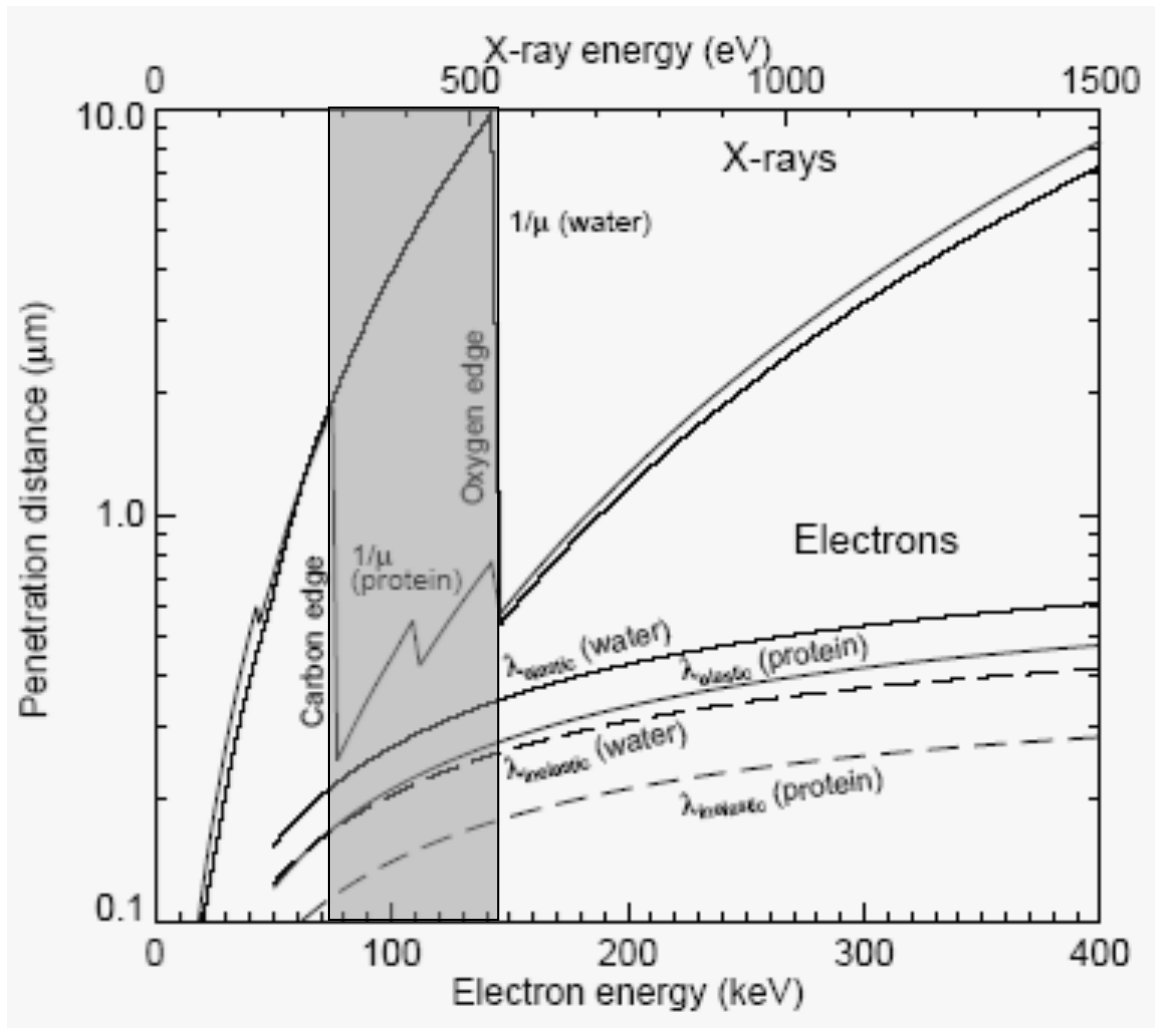


Fast Freeze





## Natural amplitude contrast between water and organic matter



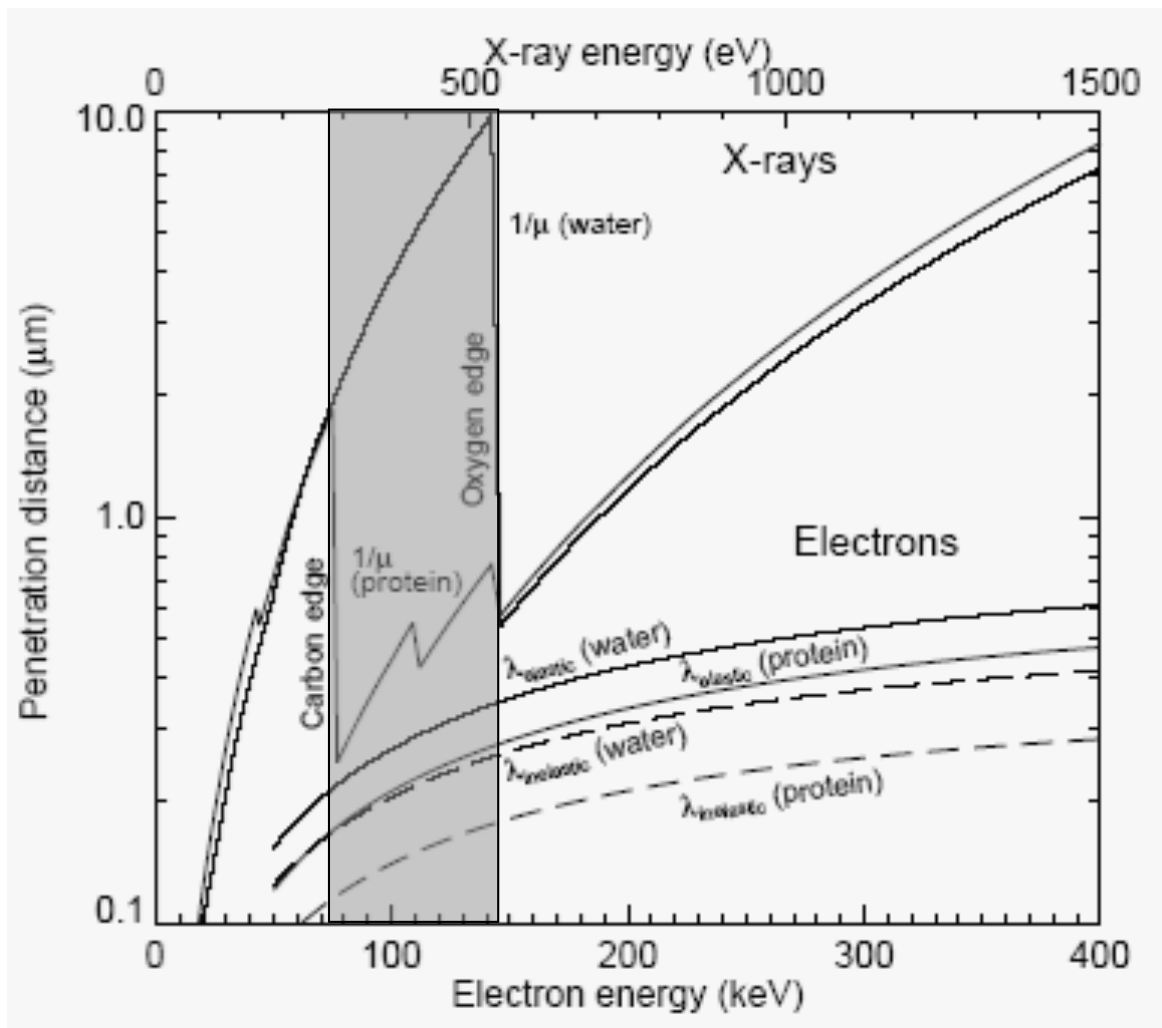
### The “Water Window”:

Due to dramatic difference in the  $f_2$  values of two materials, especially water and organic matter between the C and O K-absorption edges.

Note the penetration distance compared to electrons !!!

**H. Wolter: *Spiegelsysteme streifenden Einfalls als abbildende Optiken fuer Roentgenstrahlen*, Ann. Phys. 10, 94-114, 286 (1952)**

## Natural amplitude contrast between water and organic matter



### The “Water Window”:

Organic matter absorbs approximately one order of magnitude more strongly than water

Chemical contrast agents are not required

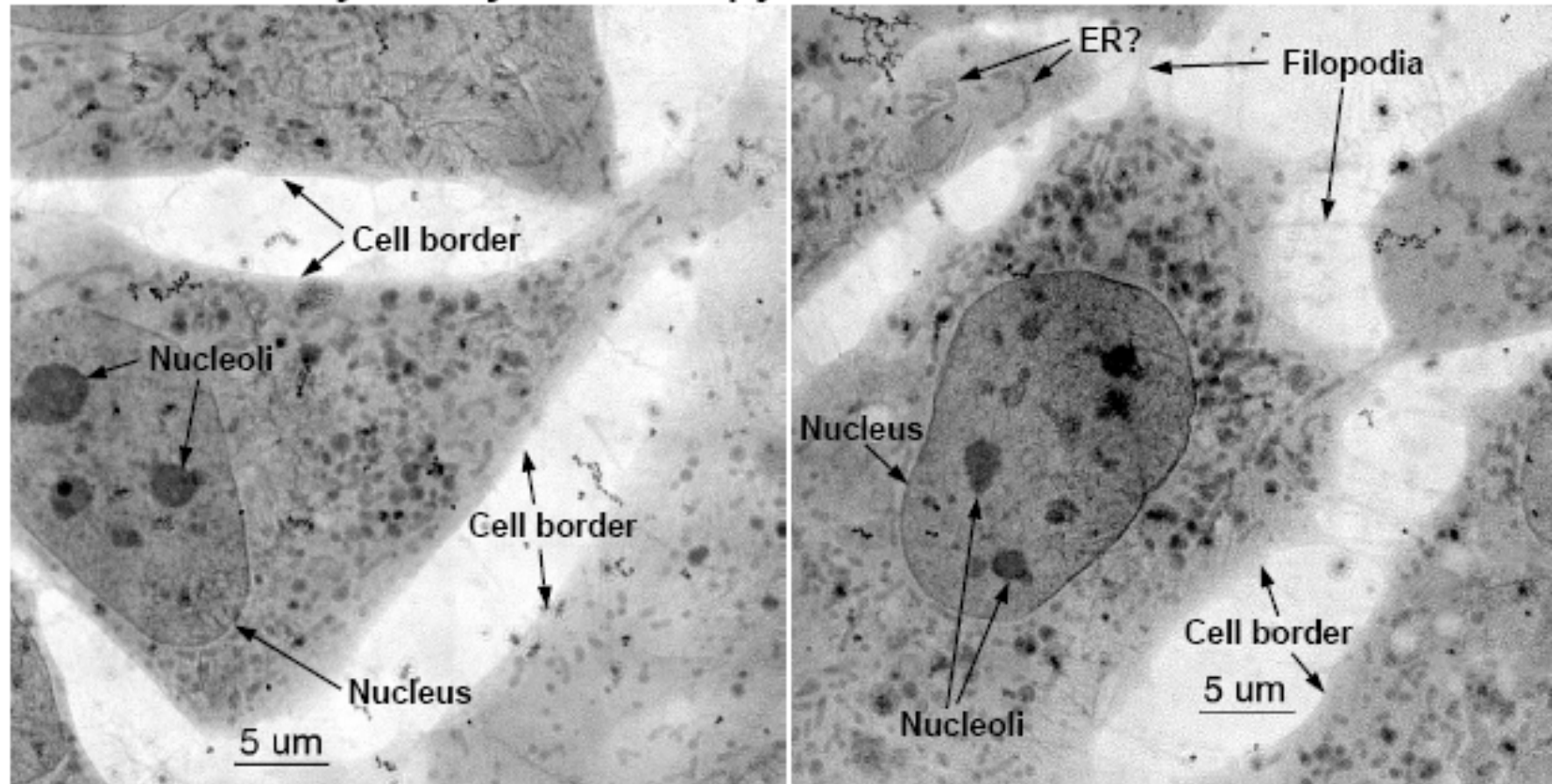
**H. Wolter: *Spiegelsysteme streifenden Einfalls als abbildende Optiken fuer Roentgenstrahlen*, Ann. Phys. 10, 94-114, 286 (1952)**



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# The XM-1 microscope at the ALS, LBL, CA, US:

## Cryo x-ray microscopy of 3T3 fibroblast cells

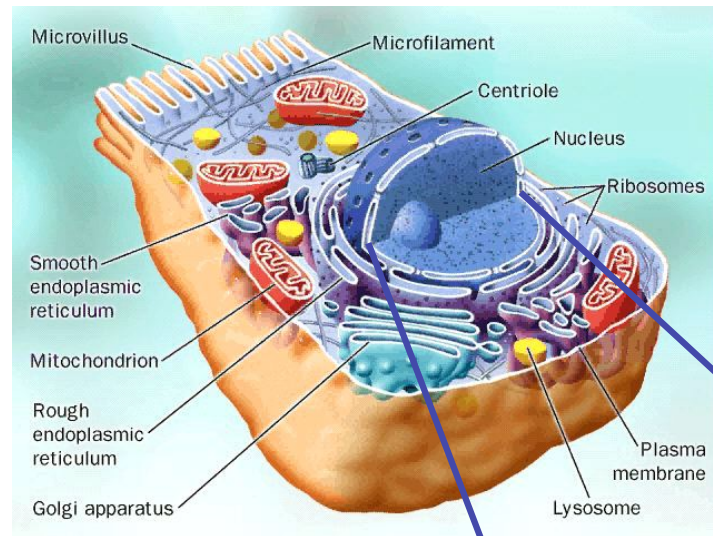


C. Larabell, D. Yager, D. Hamamoto, M. Bissell, T. Shin (LBNL Life Sciences Division)  
W. Meyer-Ilse, G. Denbeaux, L. Johnson, A. Pearson (CXRO-LBNL)

**E=517 eV**

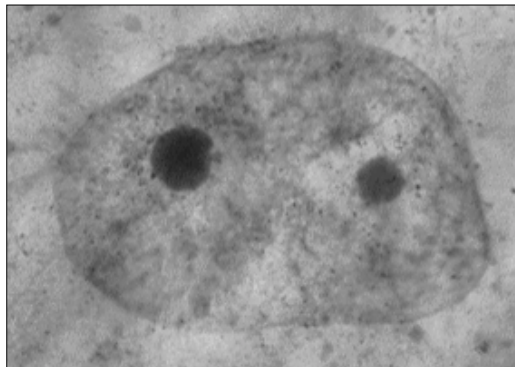
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# Major full-field imaging instruments for biological applications

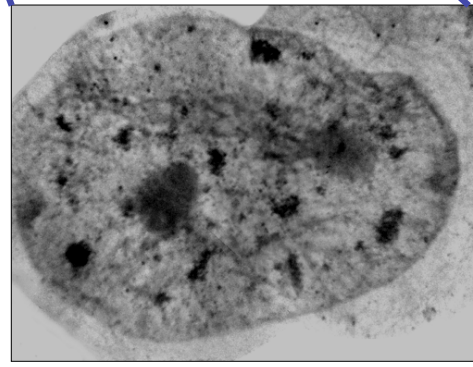


## Location of Splicing Factors in whole, hydrated human mammary epithelial Cells (ALS, XM1)

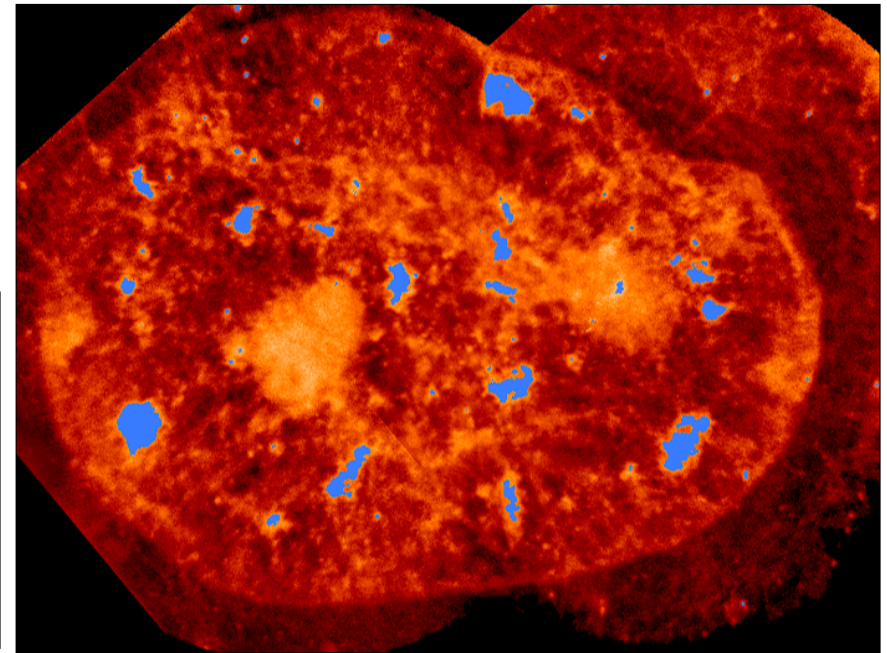
Rapidly frozen samples



Control nucleus, no primary antibody



Single nucleus labeled using antibodies specific for splicing factors



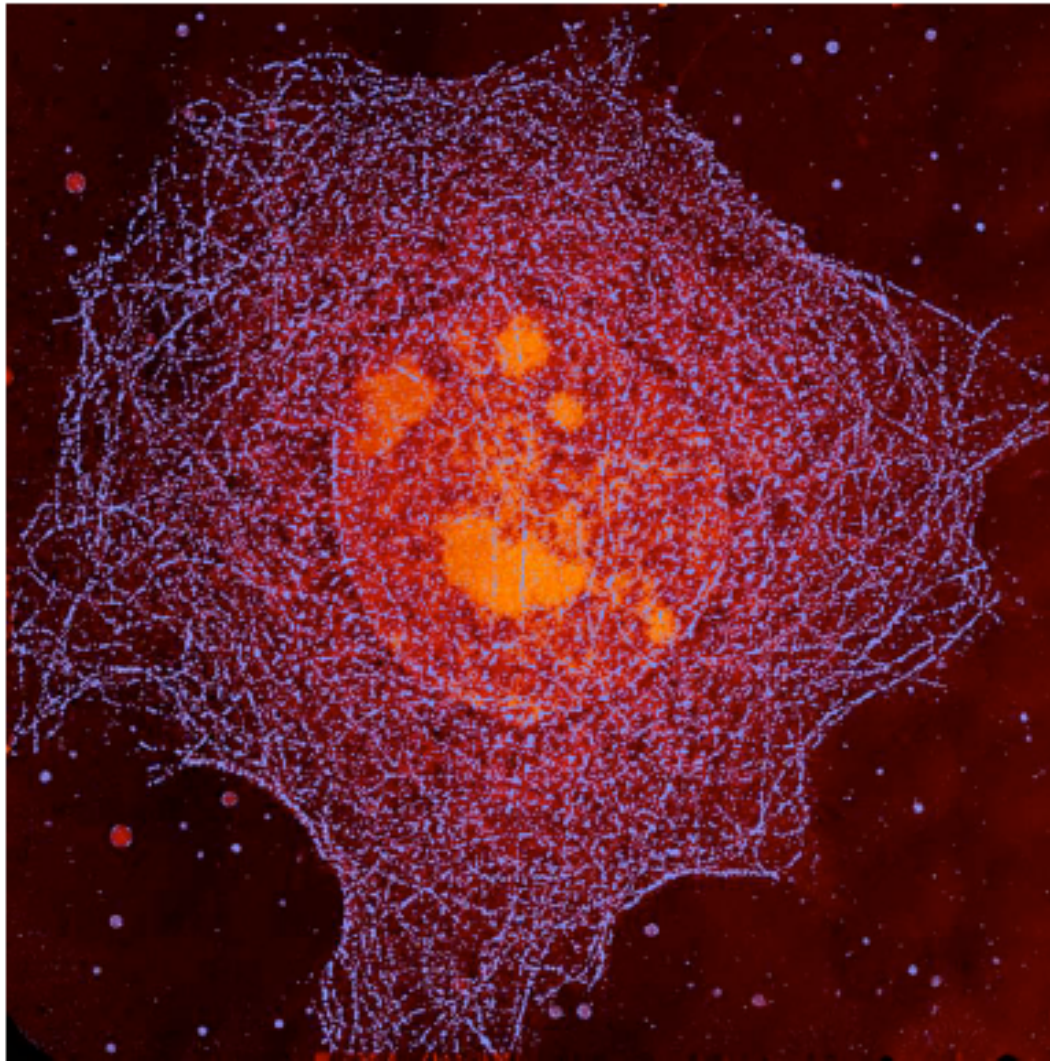
Same nucleus, splicing factors colored blue

**Resolution better than 50nm**

**Image courtesy: C. Larabell, LBL, US**



## The XM-1 microscope at the ALS, LBL, CA, US:



### Localization of proteins by utilizing gold-labelled antibodies

$\hbar\omega = 520 \text{ eV}$

$32 \mu\text{m} \times 32 \mu\text{m}$

Ag enhanced Au labeling  
of the microtubule network,  
color coded blue.

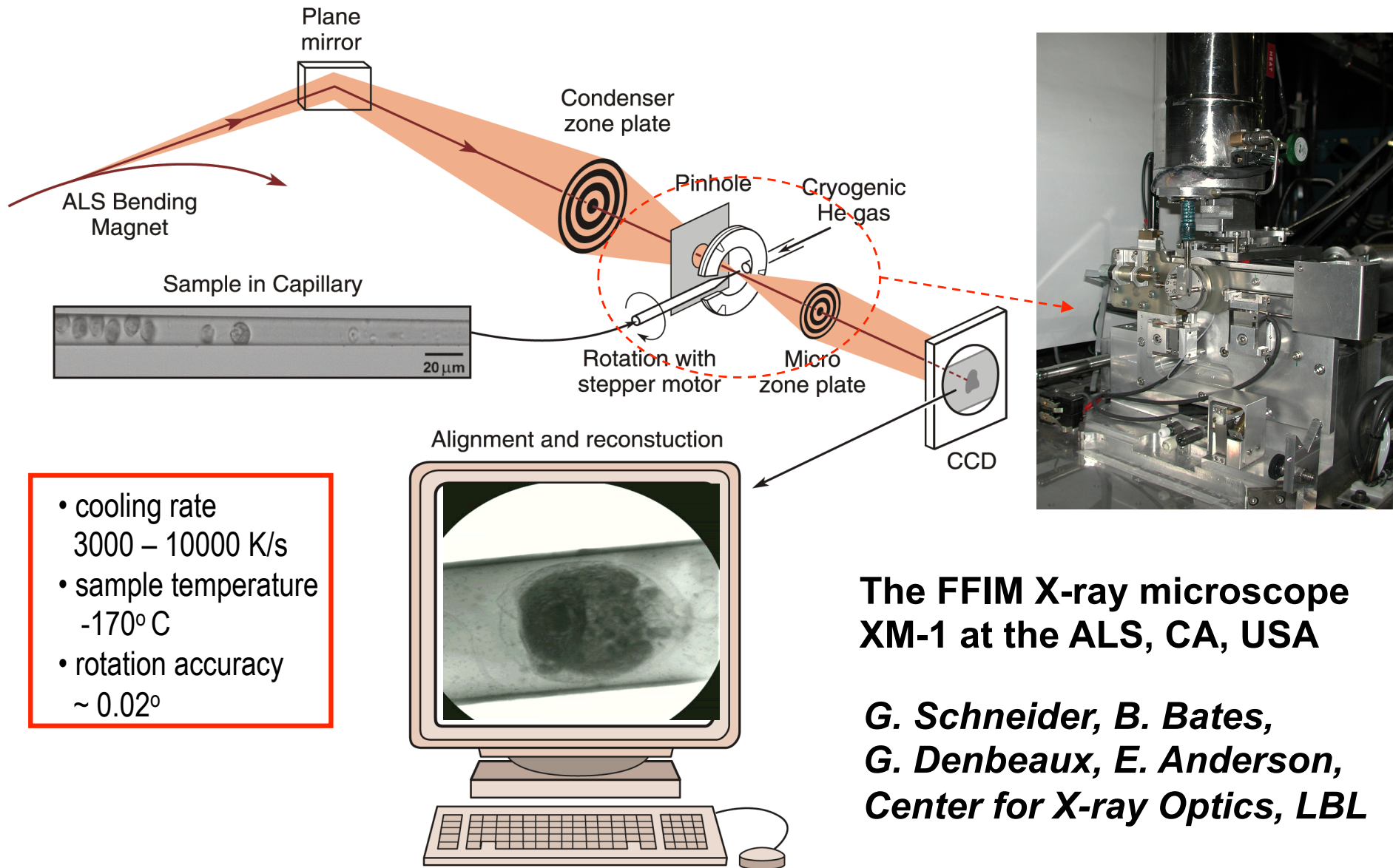
Cell nucleus and nucleoli,  
moderately absorbing,  
coded orange.

Less absorbing aqueous  
regions coded black.

W. Meyer-Ilse et al.

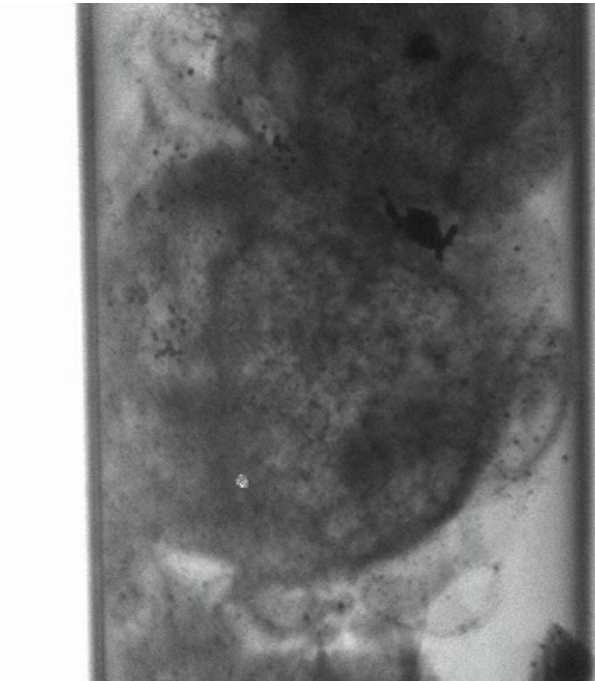
J. Microsc. 201, 395 (2001)

# Cryogenic 3D imaging of biological cells

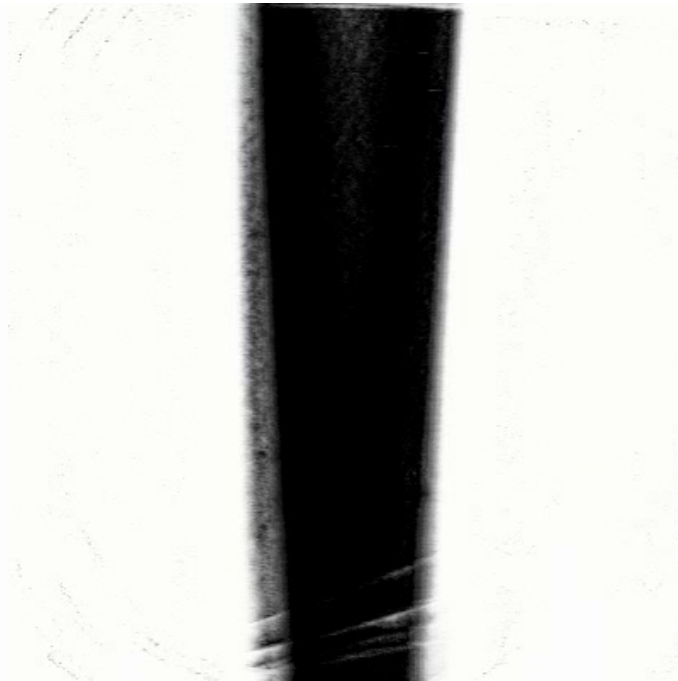


## Cryogenic 3D imaging of biological cells

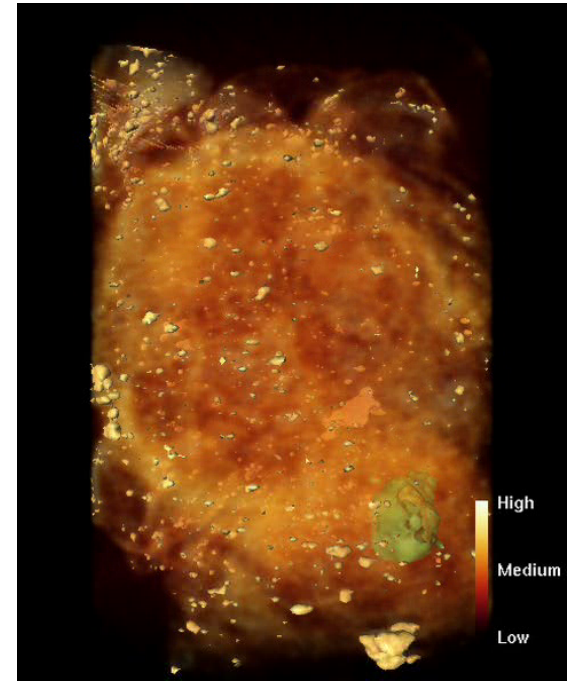
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**FFIM micrographs of  
a specimen in the  
capillary**



**Reconstructed sections  
through the volume**



**Reconstruction of  
the absorption coefficient**

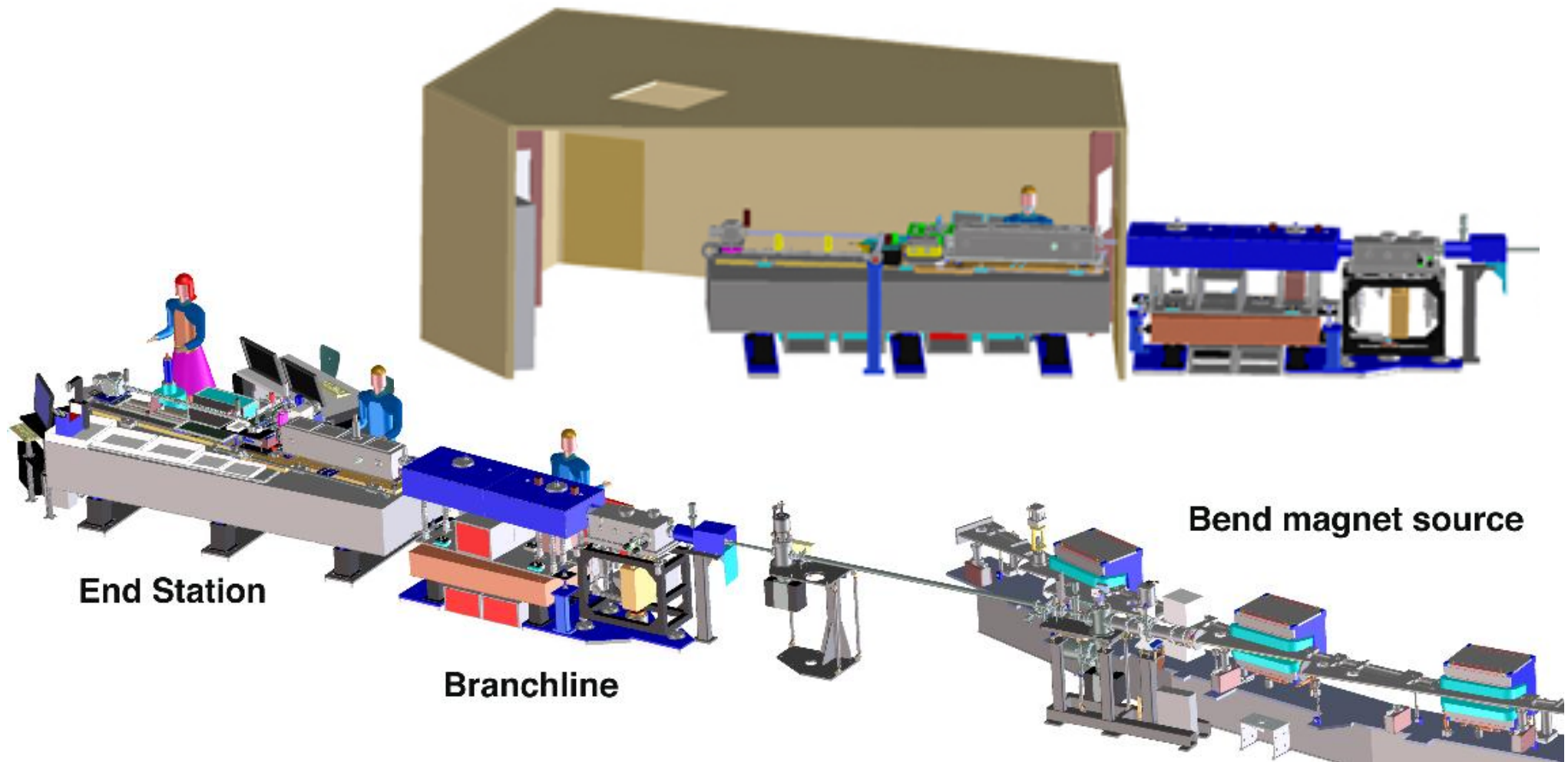
**Work performed with the XM1 microscope at the ALS, US  
(G. Schneider, G. Denbeaux, B. Bates and E. Anderson)**

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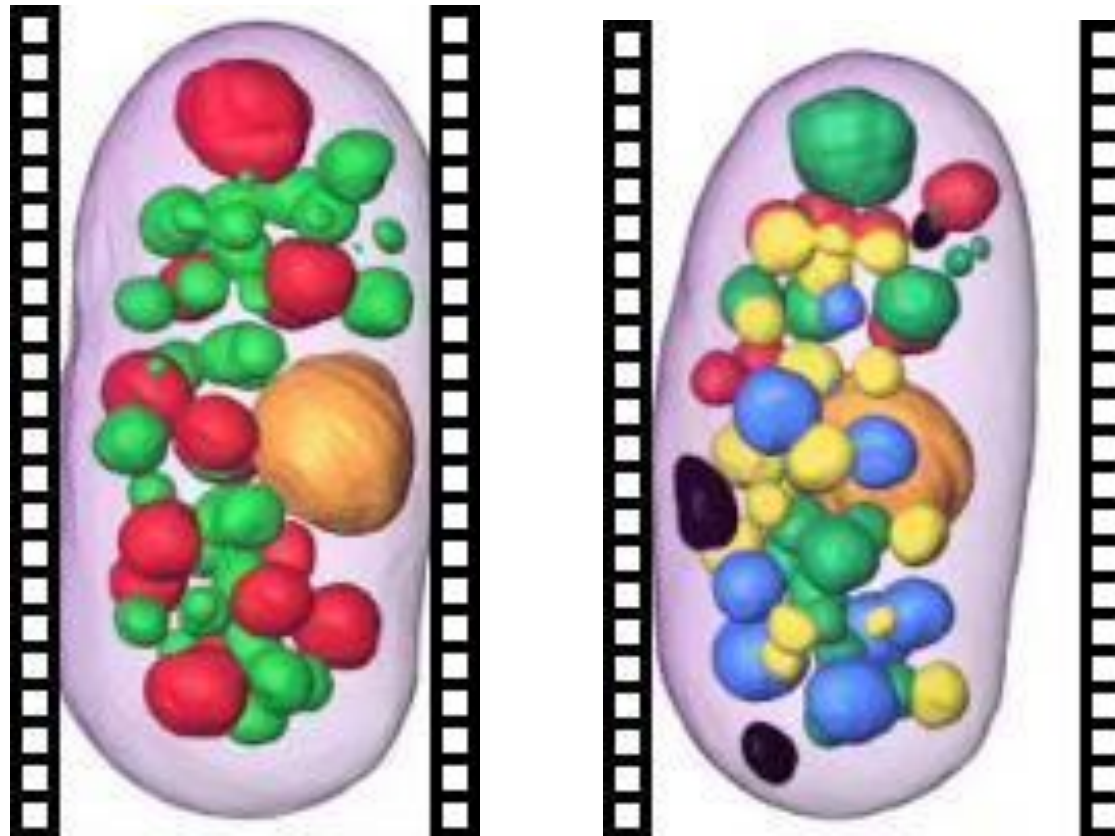
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## The XM-2 microscope at the ALS, LBL, CA, US National Center for X-ray Tomography:



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## The XM-2 microscope at the ALS, LBL, CA, US National Center for X-ray Tomography:



**3D reconstructions of  
S. pombe cells; early  
stage cell segmentation  
(left) and early stage  
mitochondria and  
vacuoles (right)**

**Visit  
<http://www.ncxt.lbl.gov>  
to see the movies**

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## The full-field imaging microscope at BESSY II, Berlin, Germany:



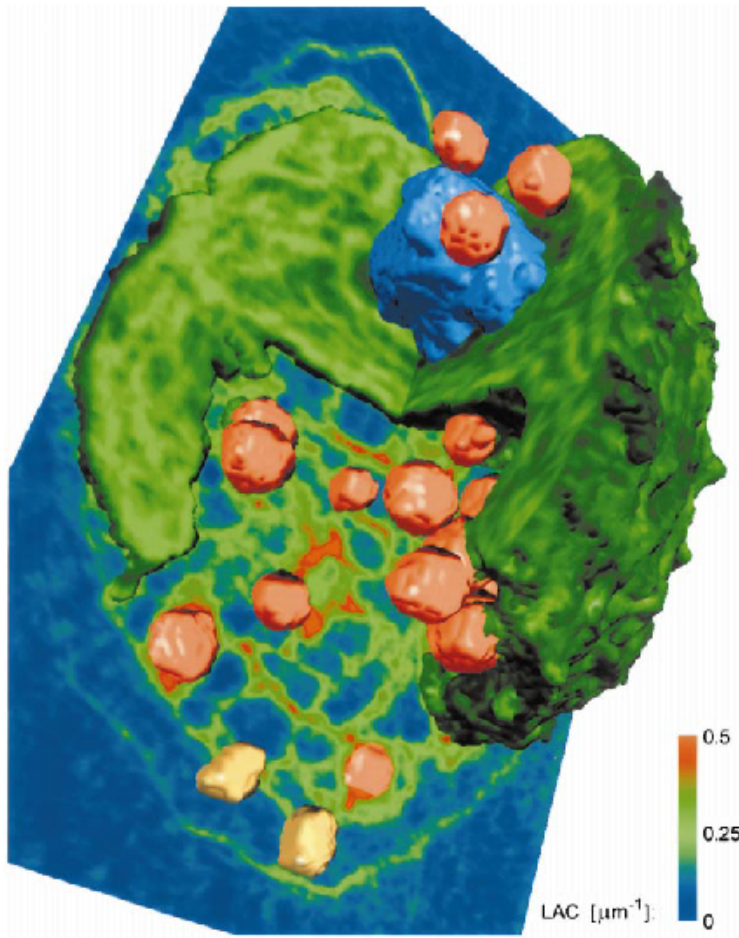
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<http://www.bessy.de>



# Cryogenic 3D imaging of biological cells

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X-ray tomography of hydrated specimen “close to their living state”

Alga: *Chlamydomonas reinhardtii*

Acquired with the full-field imaging Microscope at BESSY I

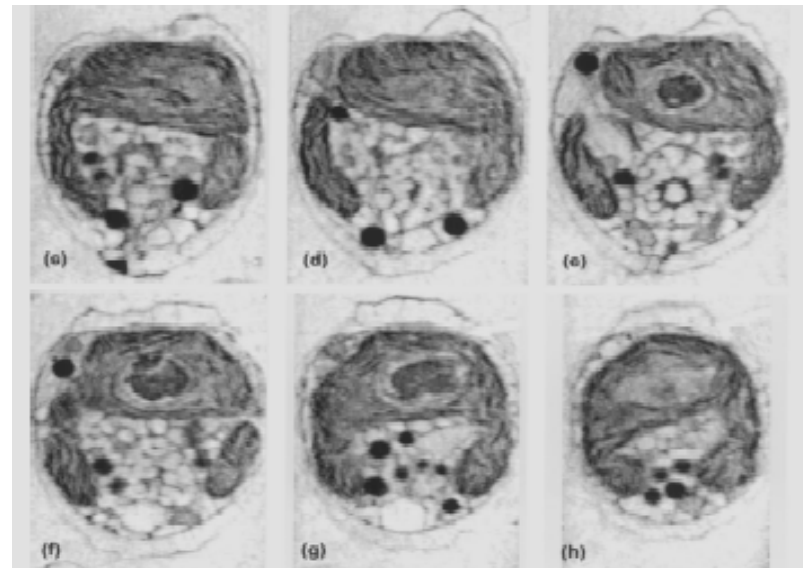
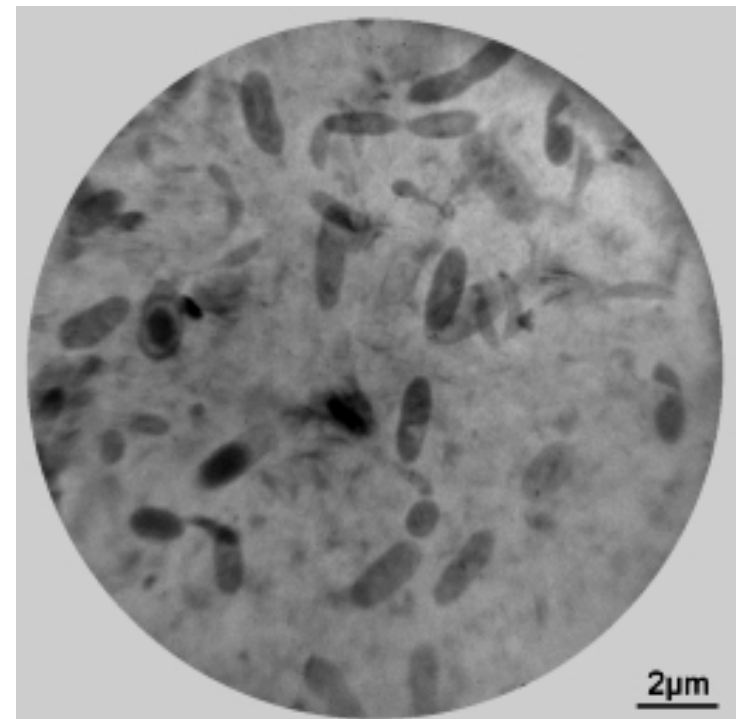
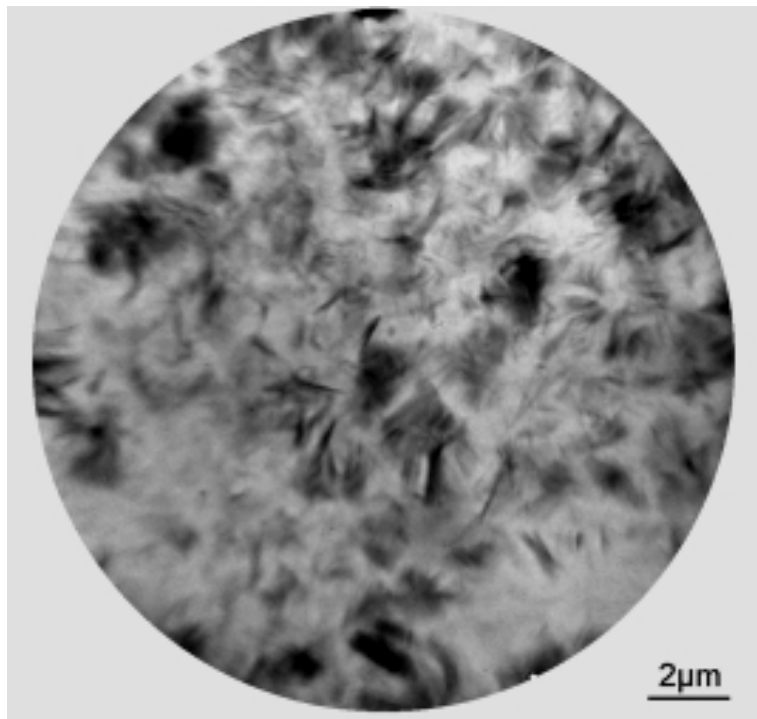


Image courtesy: D. Weiss et al., BESSY, D

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### *Bacteria and clay dispersion: Destruction of associations of clay particles by soil microbes*



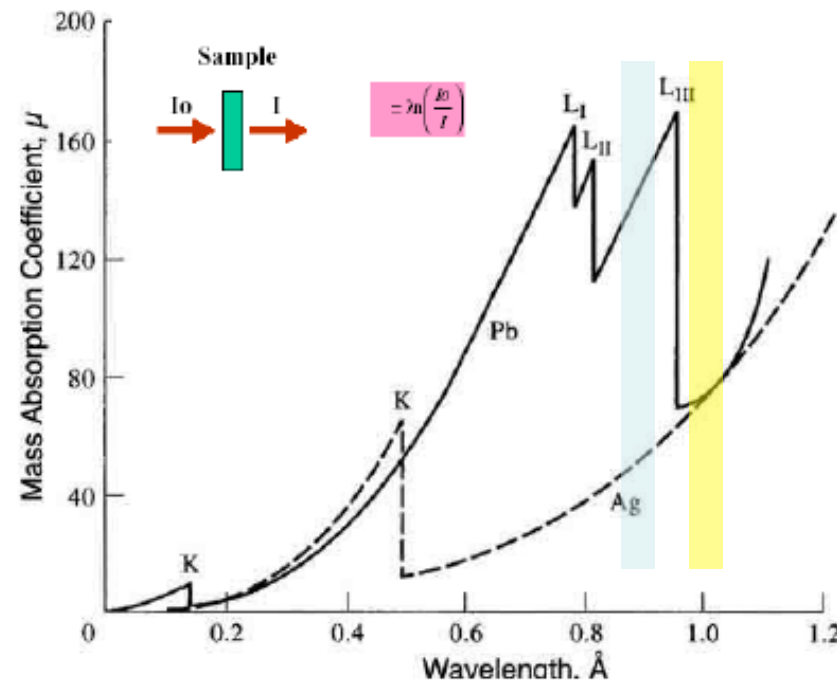
X-ray images acquired with the full-field imaging microscope at BESSY I @ 520 eV

**Samples analysed in the natural hydrated state:**  
→ no alteration of the environment of the sample

*J. Thieme et al., IRP, Uni Goettingen / G. Machulla, Uni Halle, D*

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# Across edge imaging



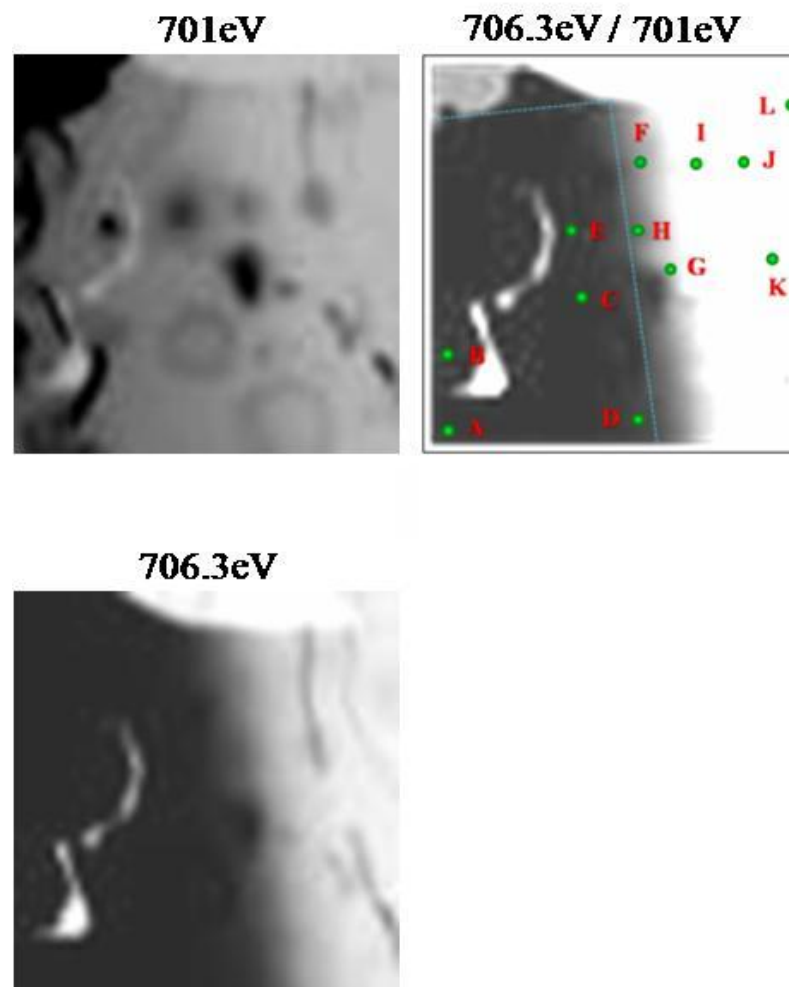
Discontinuities due to absorption

The absorption occurs when the incoming X-rays are matching the electron binding energies

**Absorption edges are fingerprints  $\Rightarrow$  they can be used to identify the chemical elements**

By taking two images, one above and one below a specific absorption edge, the correspondent chemical element will give a high contrast difference in the two images

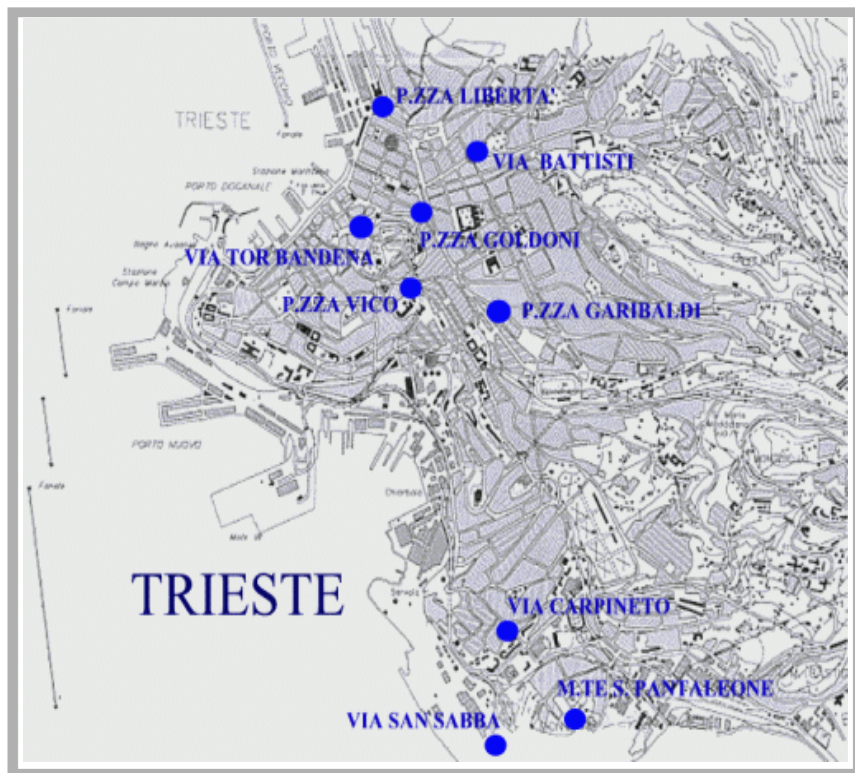
## Across edge imaging



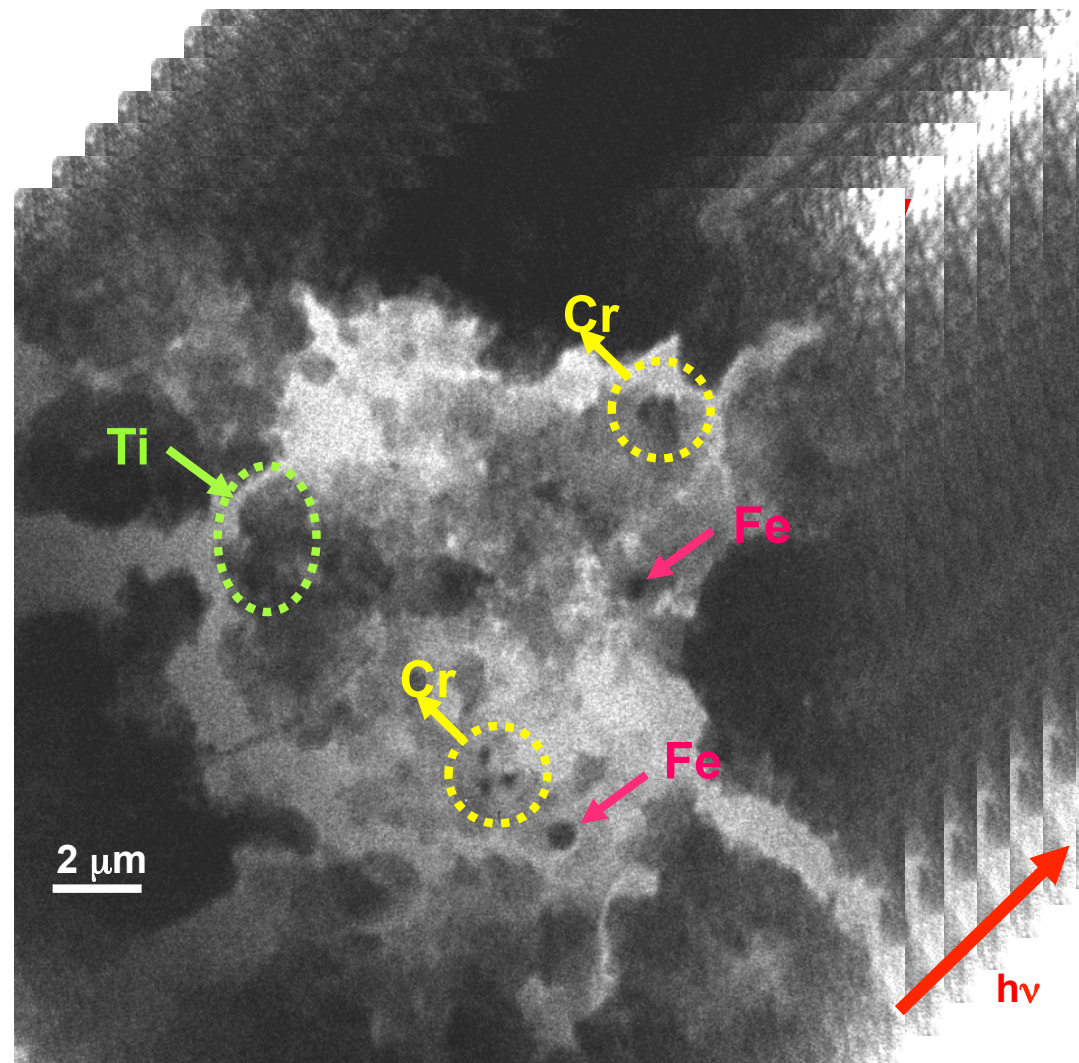




## Environmental science: Analysis of air particulate matter



*P. Barbieri et al.,  
Dept. of Chem., Univ. Trieste, I*

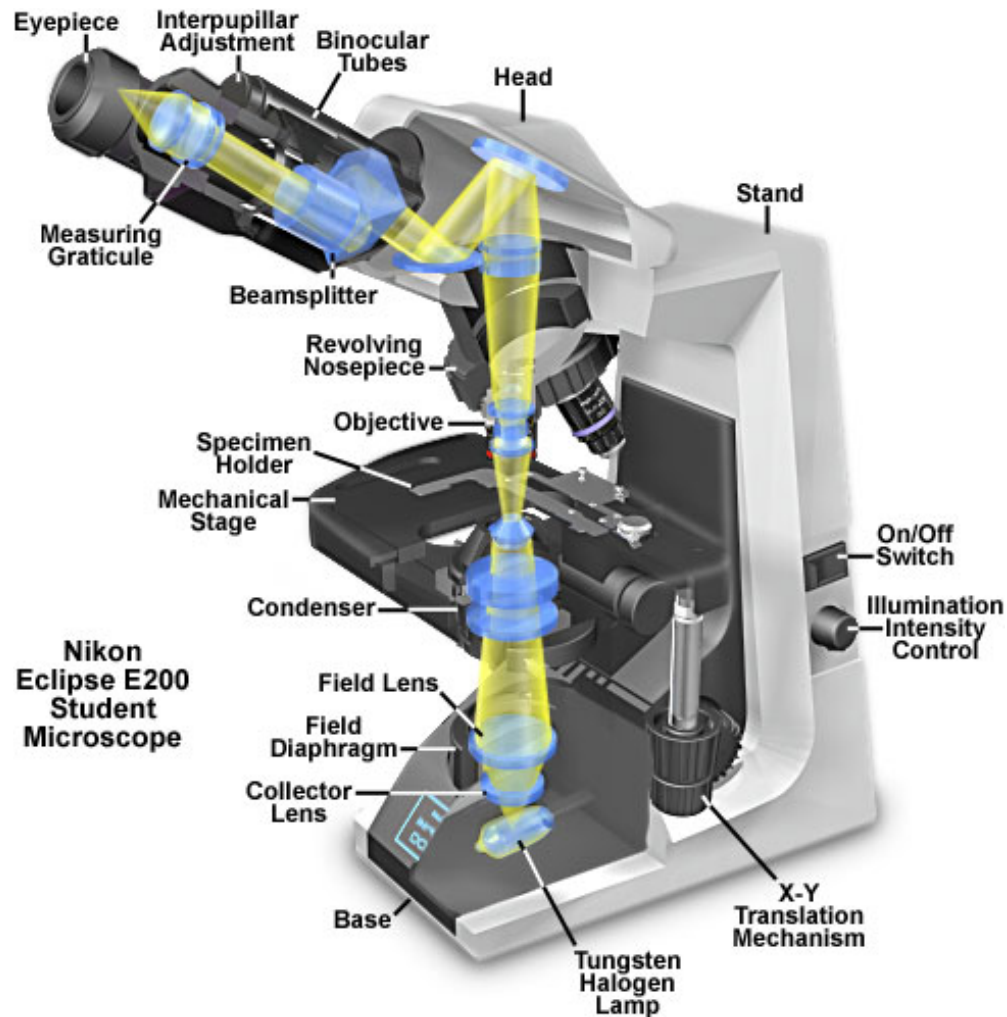


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**Phase contrast techniques are well  
established in microscopies,  
especially for low-absorbing specimen  
(as in life sciences)**

**Can we apply phase-sensitive imaging  
techniques in transmission X-ray  
microscopy?**

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**Cutaway diagram of a visible light microscope**

**Full-field X-ray imaging or “one shot” X-ray image acquisition can be considered as the optical analogon to a visible light transmission microscope**

**BUT**

**Refractive index  $n$  is very close to unity and smaller than unity!!!**

$$n = 1 - \delta(\lambda) - i\beta(\lambda) < 1$$

# The complex refractive index

$$n = 1 - \frac{n_a r_e \lambda^2}{\pi} (f_1 + i f_2) \equiv 1 - \delta - i\beta \leq 1$$

**“Conventional refractive index”  
describing phase change:**

$$\varphi(z) = \frac{2\pi}{\lambda} \delta z$$

**Exploitation of phase contrasts  
possible using X-rays ?  
Lower radiation damage ?**

**Describing photoelectric  
absorption with coefficient:**

$$\mu = \frac{4\pi}{\lambda} \beta$$

**Consequence:  
Emission of Auger, photo-electrons  
and fluorescence photons, but also  
causes radiation damage  
(energetic secondary electrons!)**



# Refractive index and X-ray contrast techniques

**X-ray contrast is generated by *differences* in the complex scattering factor per unit volume**

$$n(\lambda) = 1 - \delta(\lambda) - i\beta(\lambda) = 1 - \frac{n_a r_e \lambda^2}{2\pi} f_1(\lambda) - f_2(\lambda)$$

$$\delta(\lambda) = \frac{n_a r_e \lambda^2}{2\pi} f_1(\lambda)$$



## Scattering, refraction:

- Zernike phase contrast
- Differential phase contrast
- Differential interference contrast
- Dark-field imaging
- Magnetic phase contrast

$$\beta(\lambda) = \frac{n_a r_e \lambda^2}{2\pi} f_2(\lambda)$$



## Absorption:

- Bright-field imaging
- Chemical contrast techniques
- Magnetic absorption contrast

$\delta(\lambda)$ : Phase sensitive

$\beta(\lambda)$ : Absorption ...

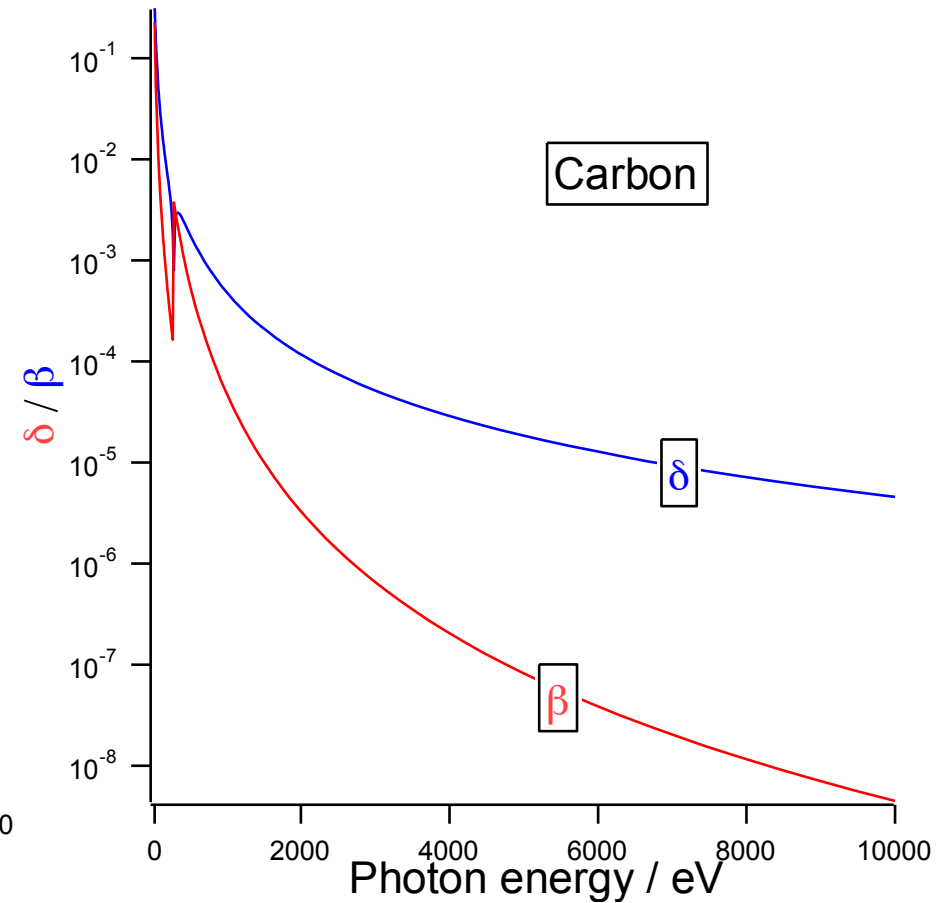
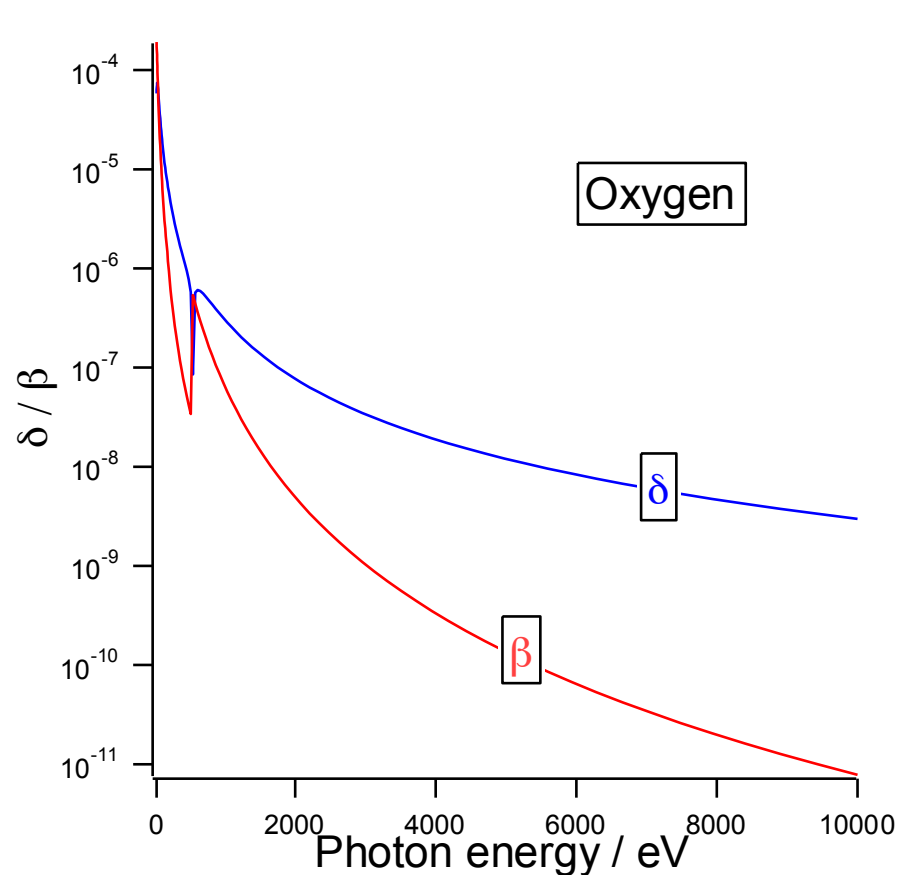
$n_a$ : average atom density

$r_e$ : classical electron radius

$f_1, f_2$ : atomic form factors

# Delta versus beta

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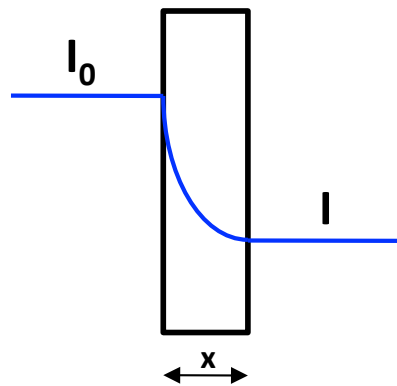
**Delta is orders of magnitude larger !!!**

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## Absorption mode

X-ray photons are selectively absorbed by the material according to its density and thickness  
(ex. radiography)



**Beer - Lambert' s law:**

$$I = I_0 e^{-mx}$$

## Phase contrast mode

Absorption can produce little contrast for light (transparent) materials or for materials with similar atomic number (similar attenuation factors).

Moreover as the energy increases the contrast diminishes (absorption coefficient  $\propto 1/E^3$ )

Phase contrast is more sensitive to edges and borders in the sample

Contrast techniques using the real, phase-shifting part of the complex refractive index are in many cases superior to absorption contrast because:

- (i) the x-ray dose can be reduced dramatically
- (ii) the throughput is higher (the phase shift dominates the absorption in the x-ray regime)

# Definition of contrast

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## Often applied definition:

Contrast is defined as the difference in light intensity between the image and the adjacent background relative to the overall background intensity

$$C = 100 \cdot \frac{(I_S - I_B)}{I_B}$$

$I_s$ : Specimen intensity  
 $I_b$ : Background intensity

## Definition used for XRM:

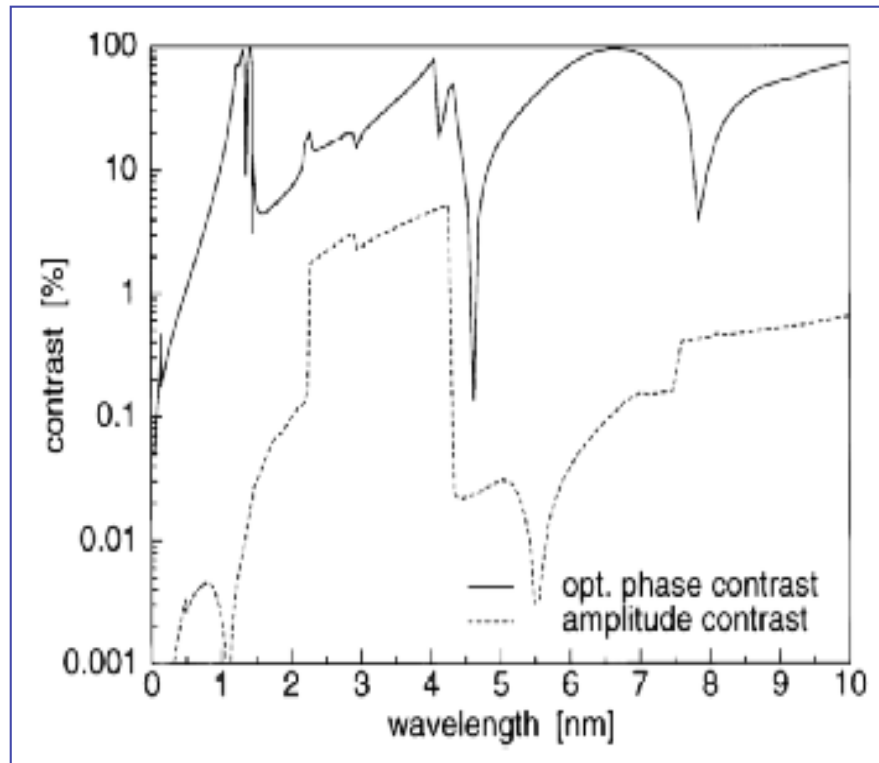
Contrast is defined as the difference in maximum and minimum light intensity normalized to the sum of maximum and minimum light intensity

$$C = \frac{(I_{\max} - I_{\min})}{I_{\max} + I_{\min}}$$

$I_{\max}$ : Max. image intensity  
 $I_{\min}$ : Min. image intensity

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# Absorption versa phase contrast techniques



Amplitude and phase contrast  
for a model protein  $C_{94}H_{139}N_{24}O_{31}$

## Absorption contrast

Mostly used for chemical studies in  
combination with XANES and XRF

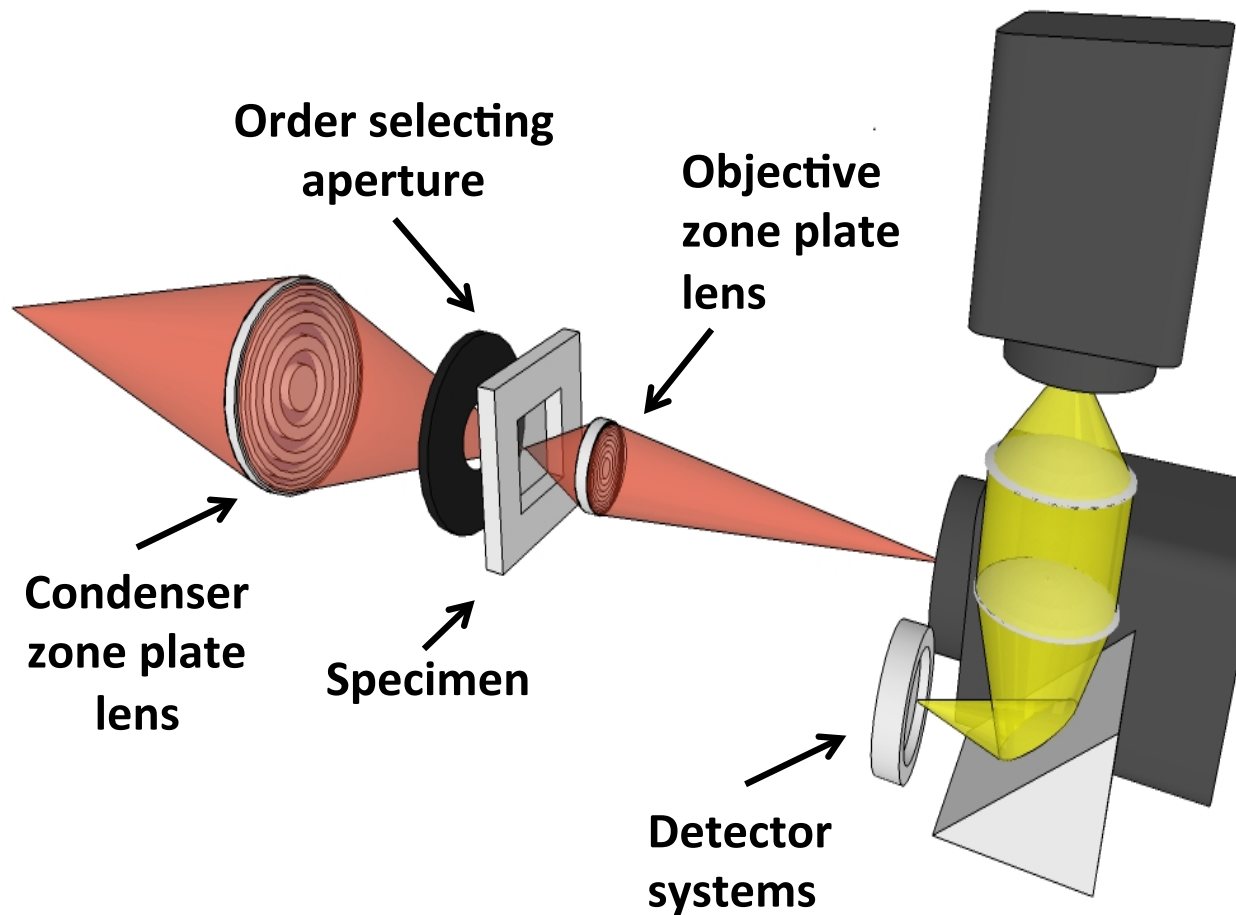
## Phase contrast techniques

- tremendous reduction of dose applied to object (dose  $\sim t^{-4}$  with spat. resolution  $t$ )
- additional transmission information on low side of absorption edges (XANES, XRF !)

*Courtesy of G. Schneider et al. BESSY, D*

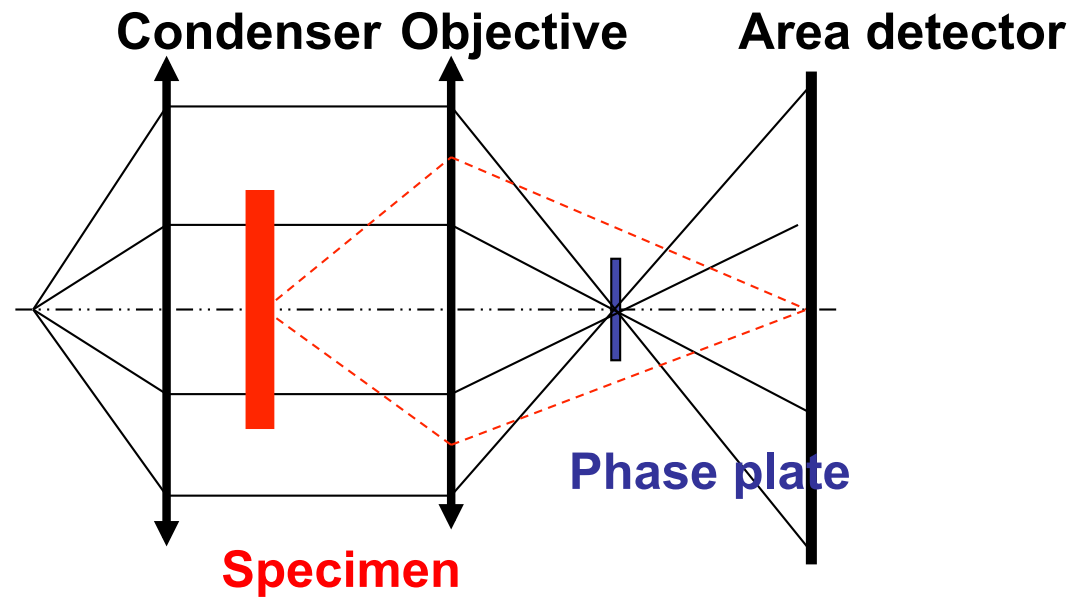
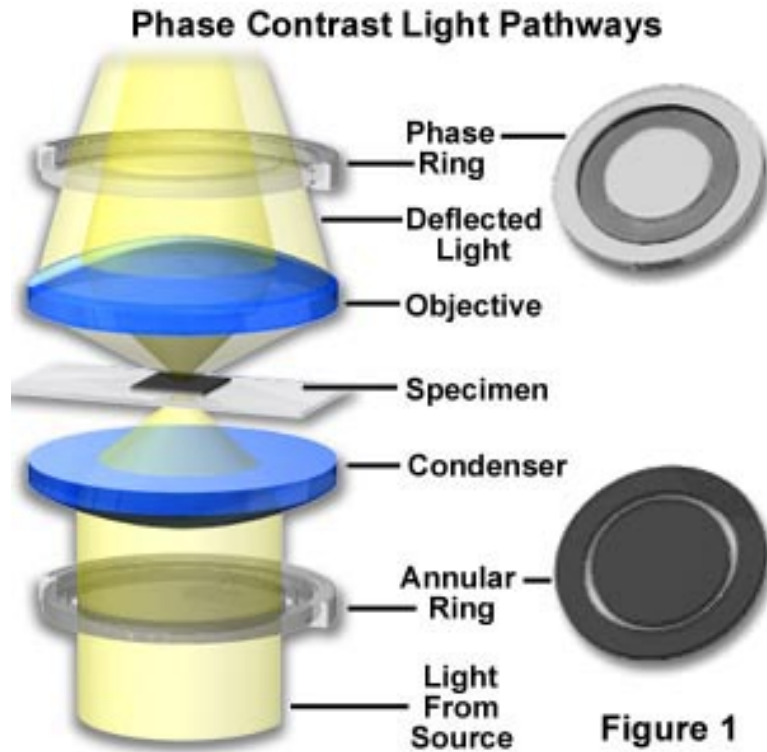


## Full field Imaging mode



- Similar to conventional visible light microscope
- Analysis of morphology in transmission
- Fast imaging, dynamics, microtomography

# Basics of Zernike phase contrast



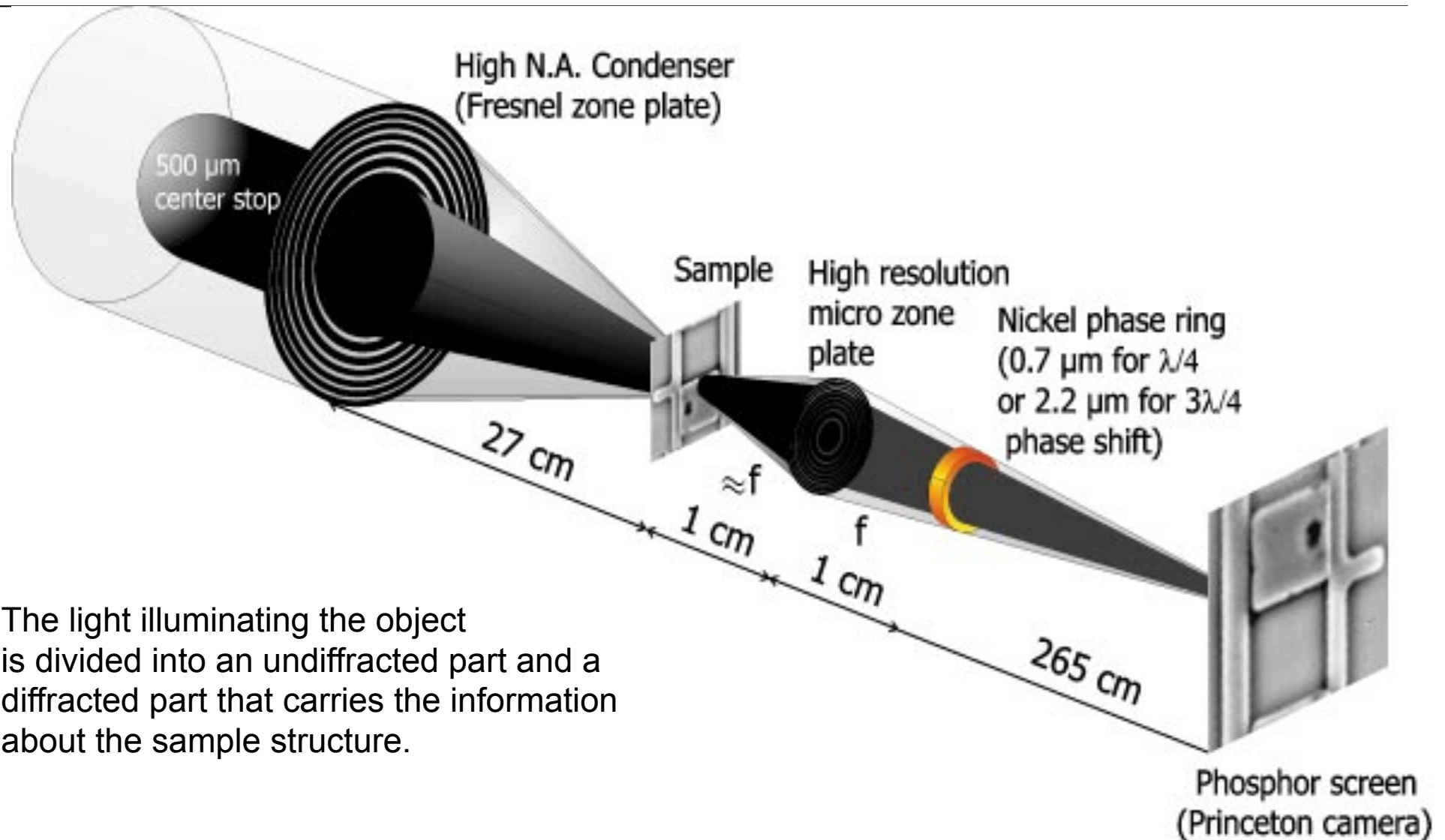
$$A_{specimen} = A_{surr} e^{i\Phi} = A_{surr} e^{i \frac{2\pi}{\lambda} \Delta t} \approx A_{surr} (1 + i\Phi) \quad \Phi \ll 1$$

For imaging weakly absorbing samples

Phase plate in “back-focal” plane: Phase of  $A_{surr}$  can be shifted by  $\pm \pi/2$  !!!

Phase differences are converted in amplitude differences !!!

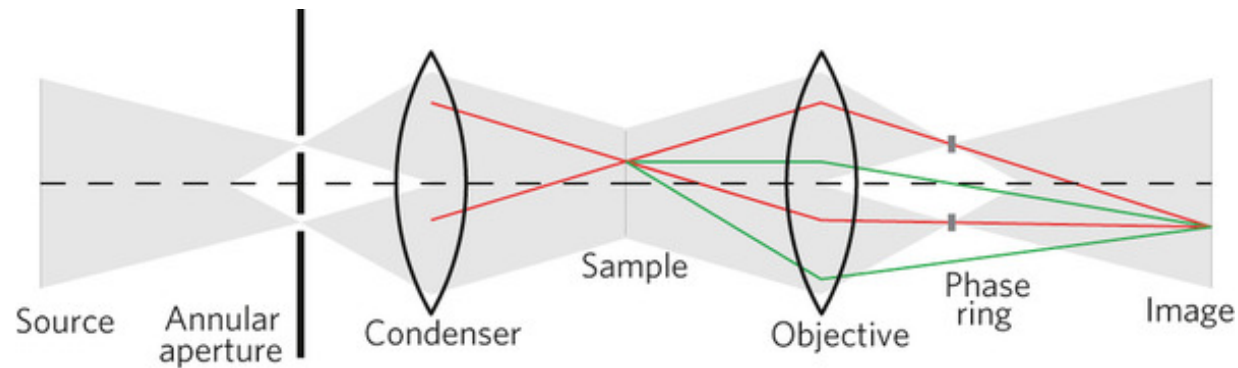
## Zernike phase contrast in full-field imaging X-ray microscopy





# Zernike phase contrast in full-field imaging X-ray microscopy

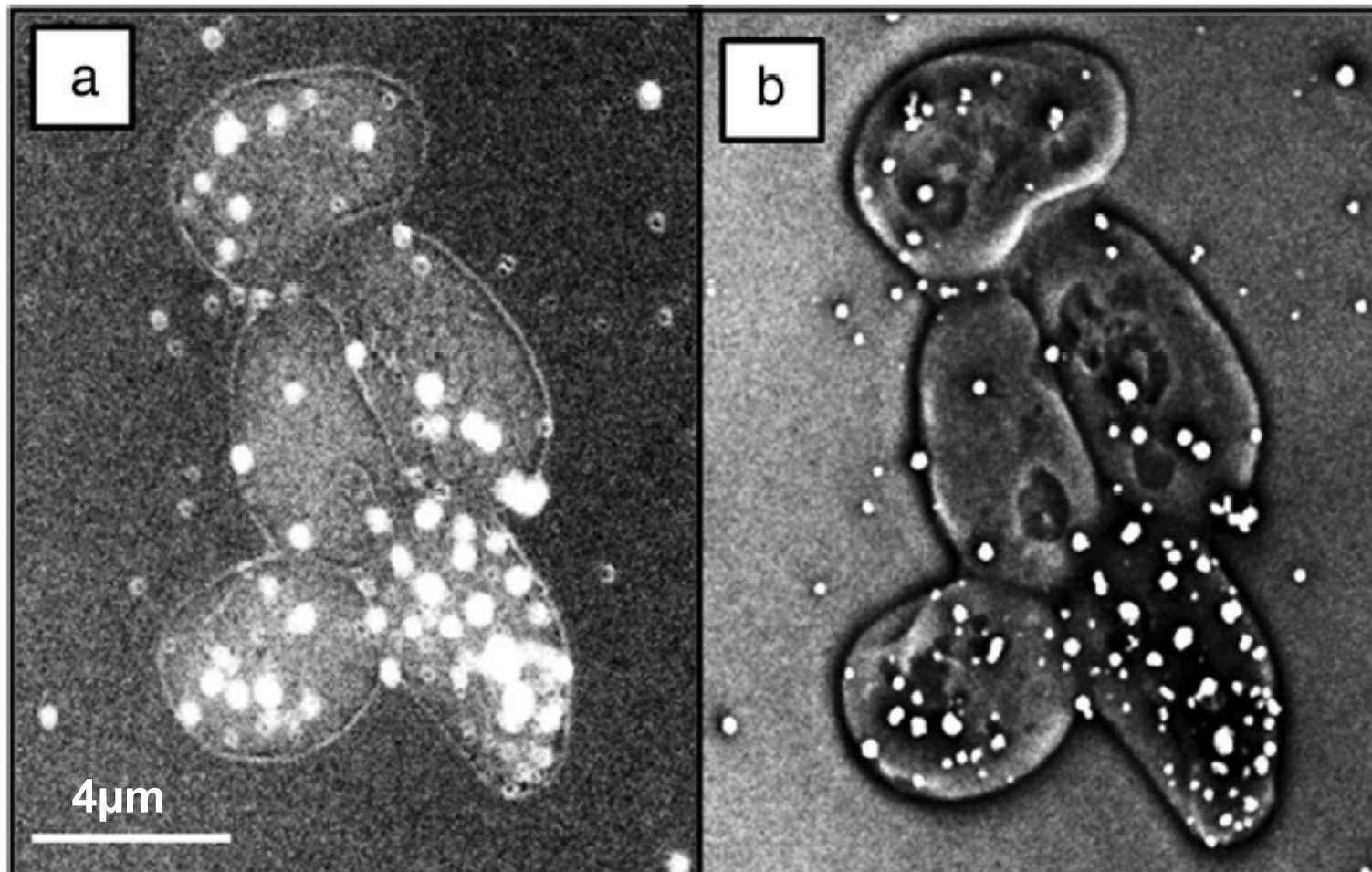
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- The light illuminating the object is divided into an undiffracted part and a diffracted part that carries the information about the sample structure.
- A spatial separation of these two components is achieved in the back-focal plane of the objective lens, where a phase-shifting ring imparts a predetermined phase shift ( $90^\circ$  for positive contrast or  $270^\circ$  for negative contrast) onto the undiffracted part.
- The phase-contrast image is formed by the interference of the phase-shifted undiffracted component with the undisturbed diffracted component, translating phase modulations of the sample into intensity modulations in the image plane.
- For small phase shifts, these modulations are due to the differences in the real part of the object's index of refraction, whereas the imaginary part leading to absorption contrast is usually small and can often be neglected.

## TXM images of *S. cerevisiae* at 5.4 keV

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a) in absorption contrast

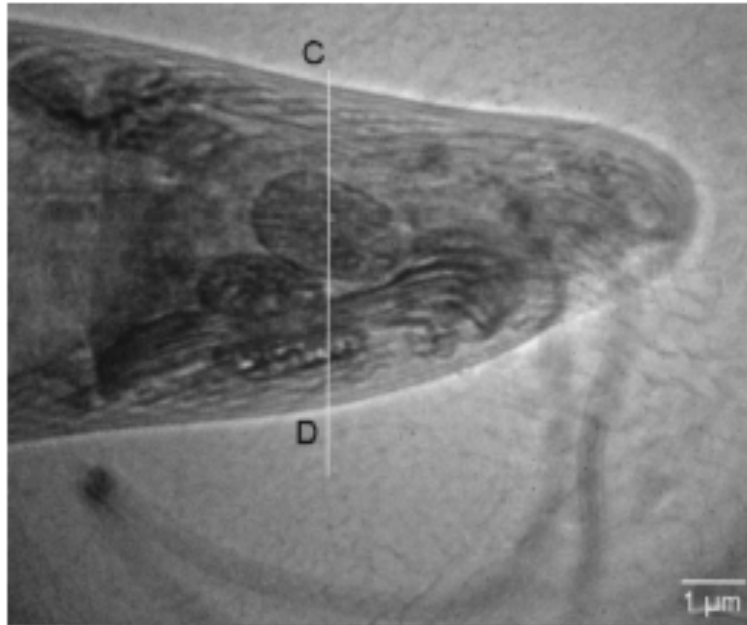
(b) in Zernike phase contrast

J. C. Andrews et al. *Microscopy Research and Technique* 74: 671-681 (2011)

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# Zernike phase contrast in X-ray microscopy

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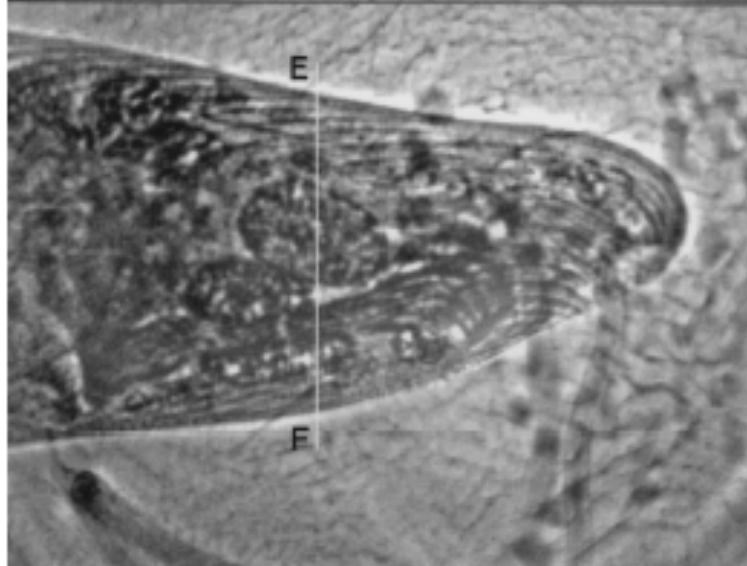


**Amplitude and Zernike phase contrast images of an alga *Euglena gracilis***

**E = 500 eV, accumulated dose is  $3 \times 10^6$  Gray**

**Amplitude: 3 s**

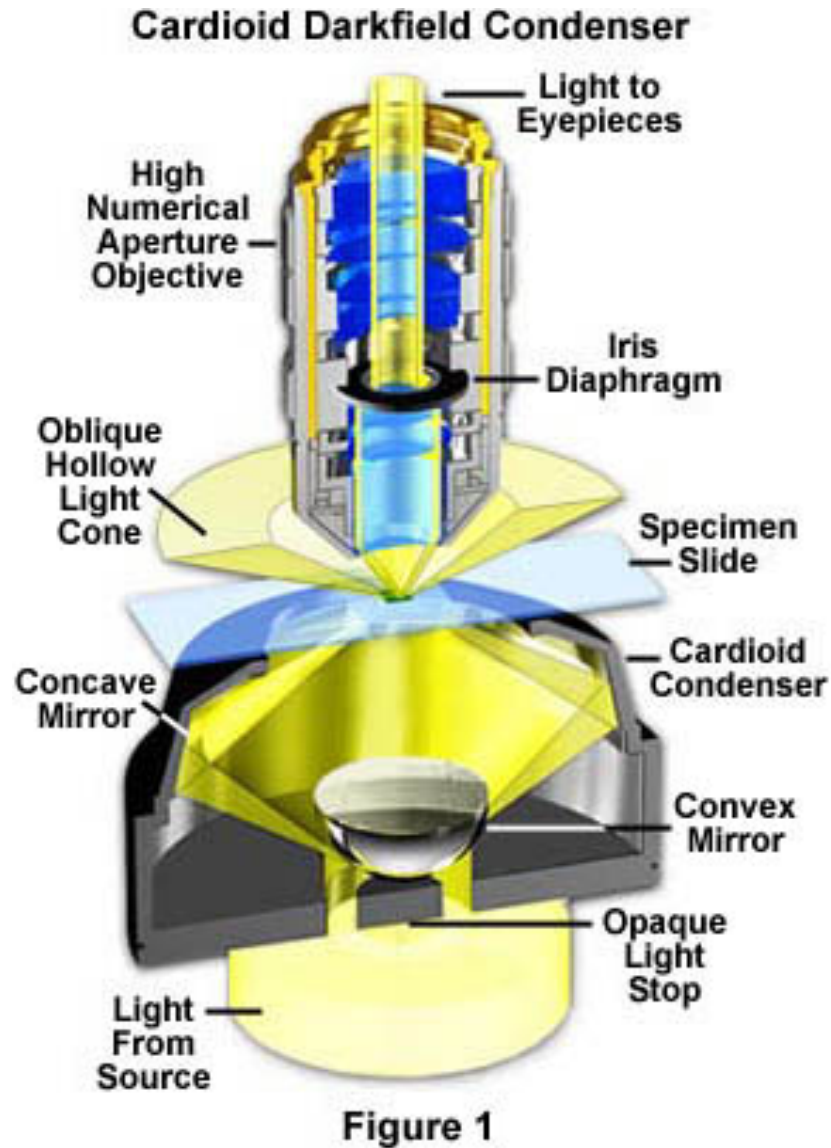
**Phase contrast: 15 s**



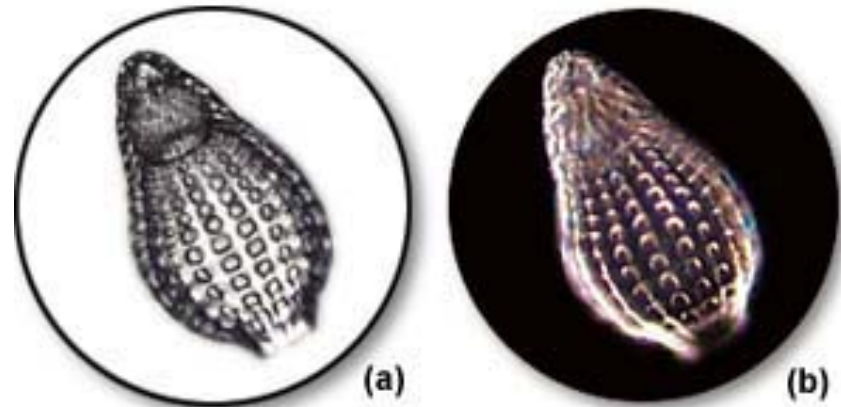
## Drawbacks of Zernike phase contrast:

- Halos around structures
- Quantitative analysis difficult
- Limitation in spatial resolution
- Not all spatial frequencies are treated equally

# Darkfield or darkground imaging



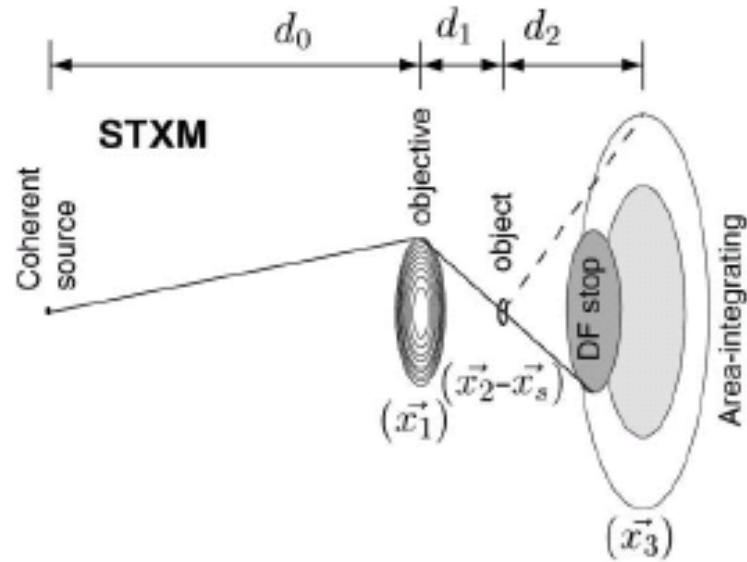
Darkfield illumination requires blocking out of the central light which ordinarily passes through and around (surrounding) the specimen, allowing only oblique rays from every azimuth to "strike" the specimen.



Visible light micrographs of silica skeletons from a small marine protozoan (radiolarian)



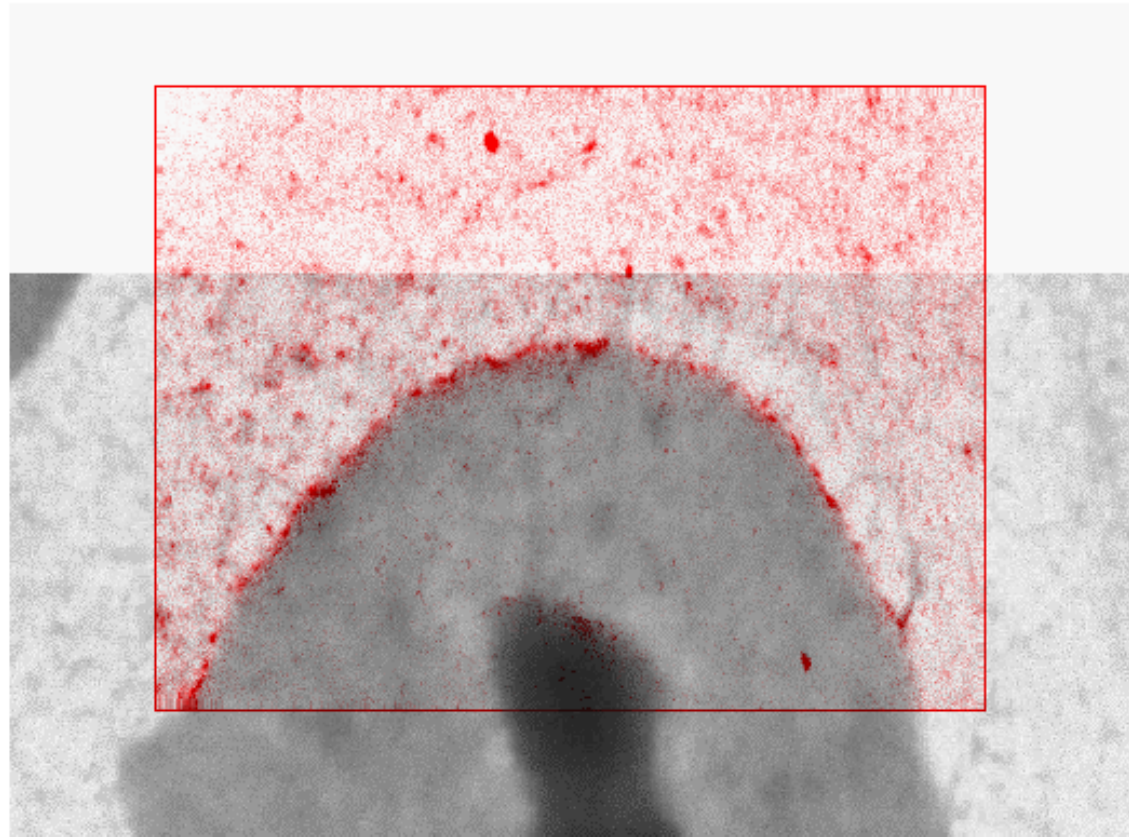
# Darkfield imaging in scanning X-ray microscopy



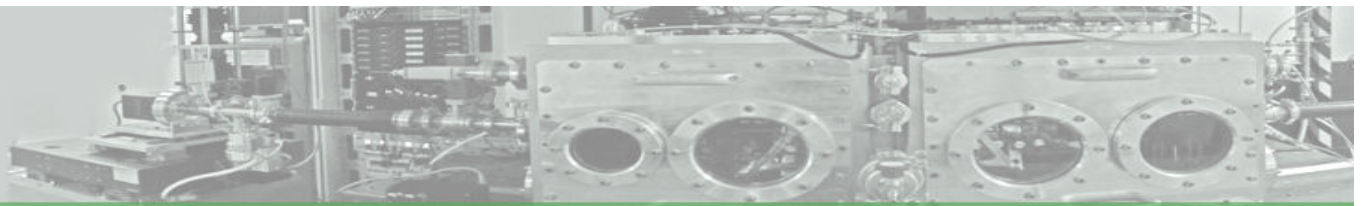
Technique is especially suited for small, strongly scattering particles as for example a few 10nm diameter labelling spheres

Brightfield image of a cell with Au labelling spheres overlayed with a darkfield image

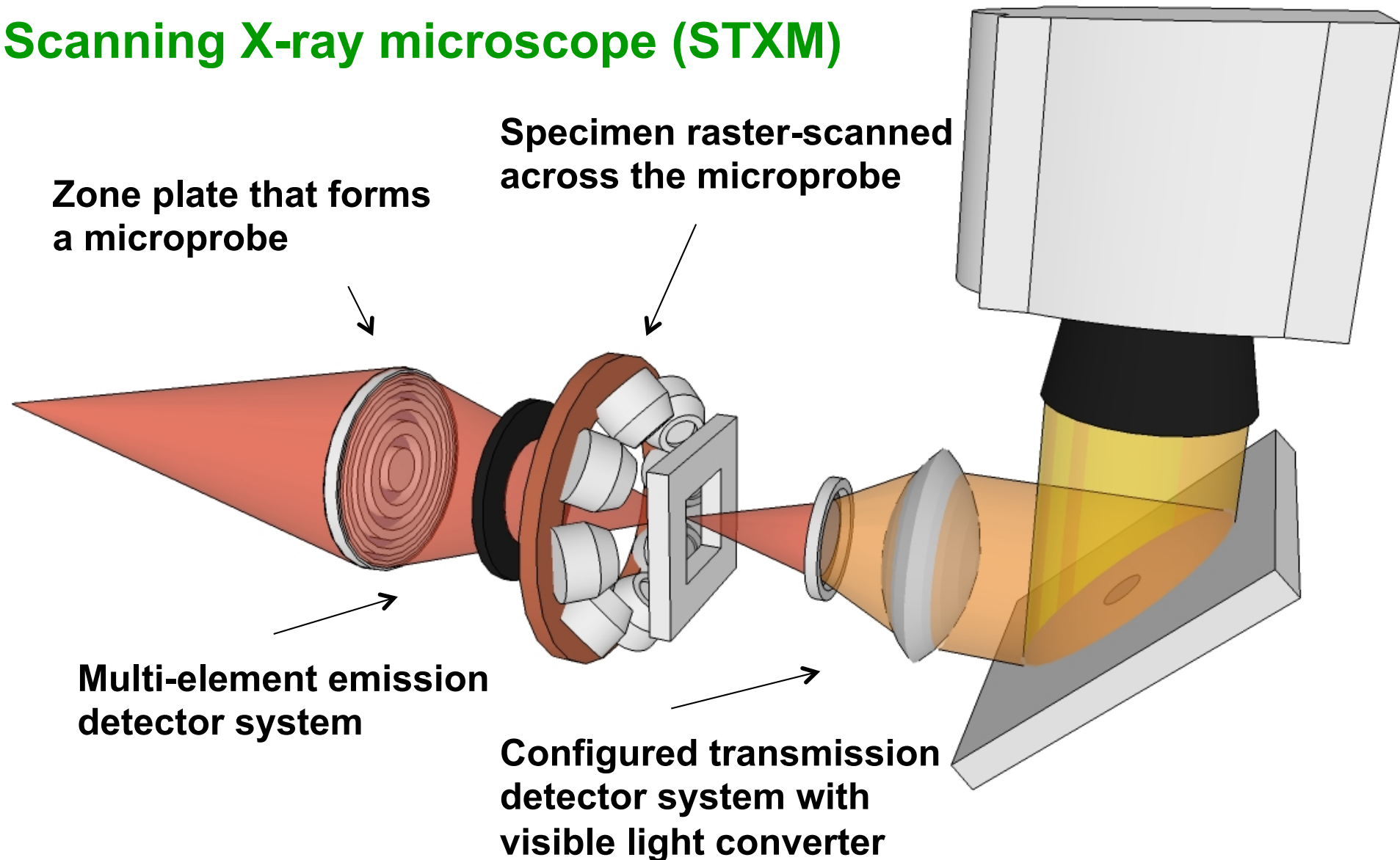
Images acquired with STXM at the NSLS



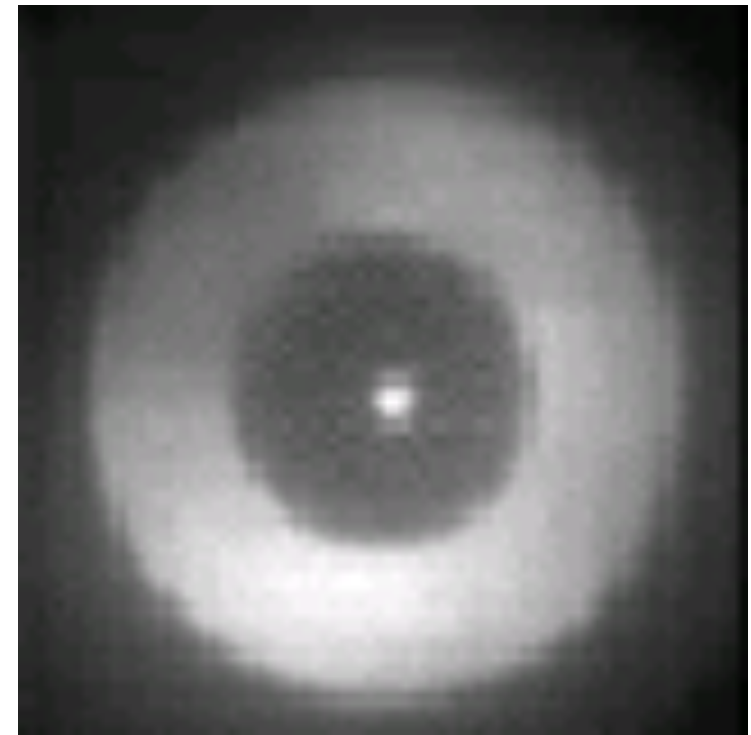
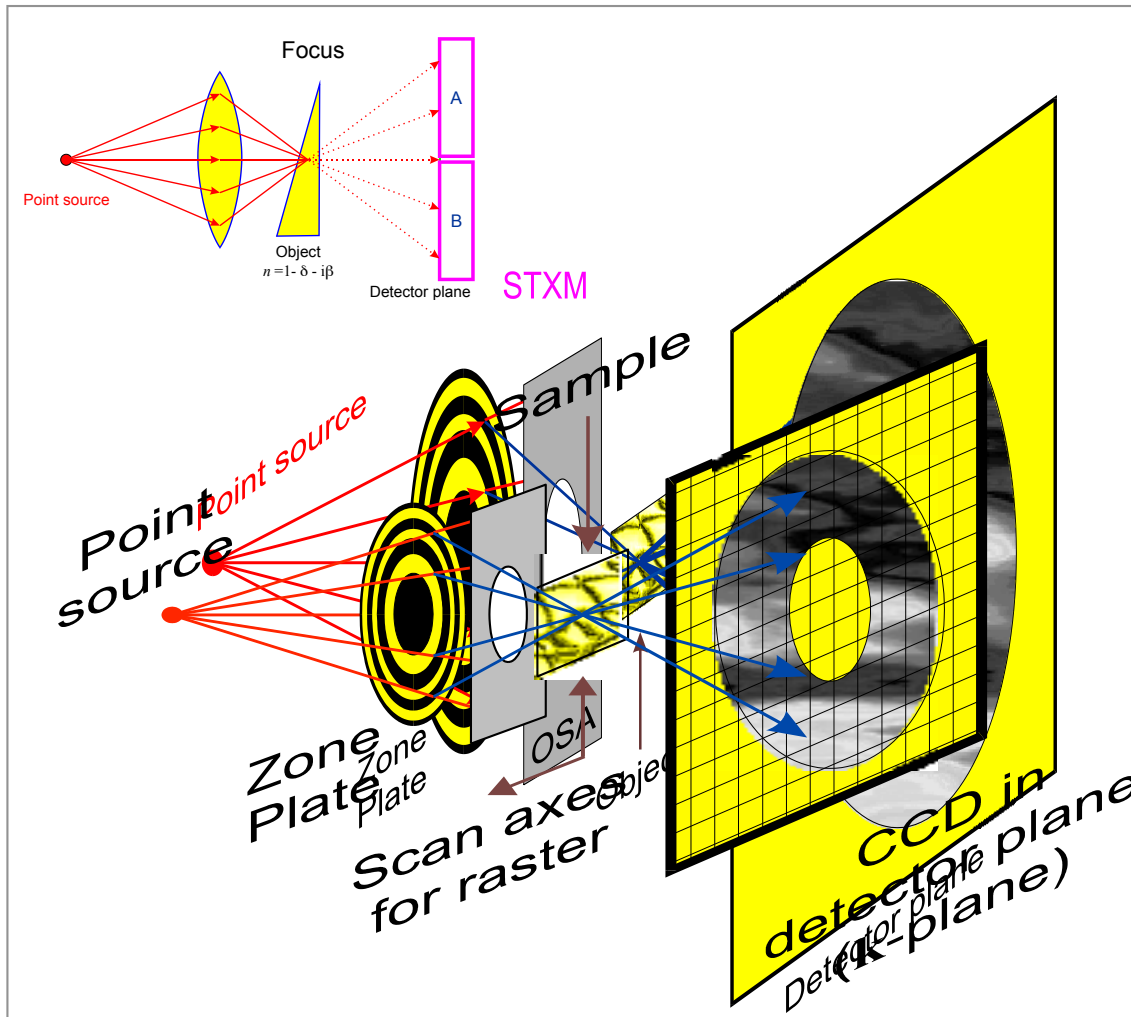
*S. Vogt, M.A. thesis, SUNY Stony Brook (1997).*



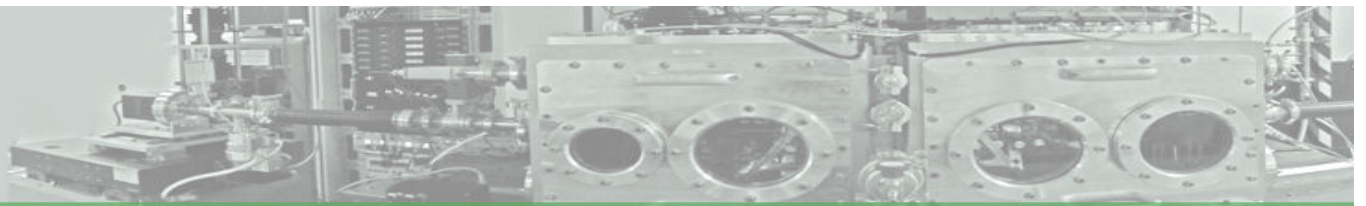
## Scanning X-ray microscope (STXM)



# Detector based contrast technologies in scanning X-ray microscopy:

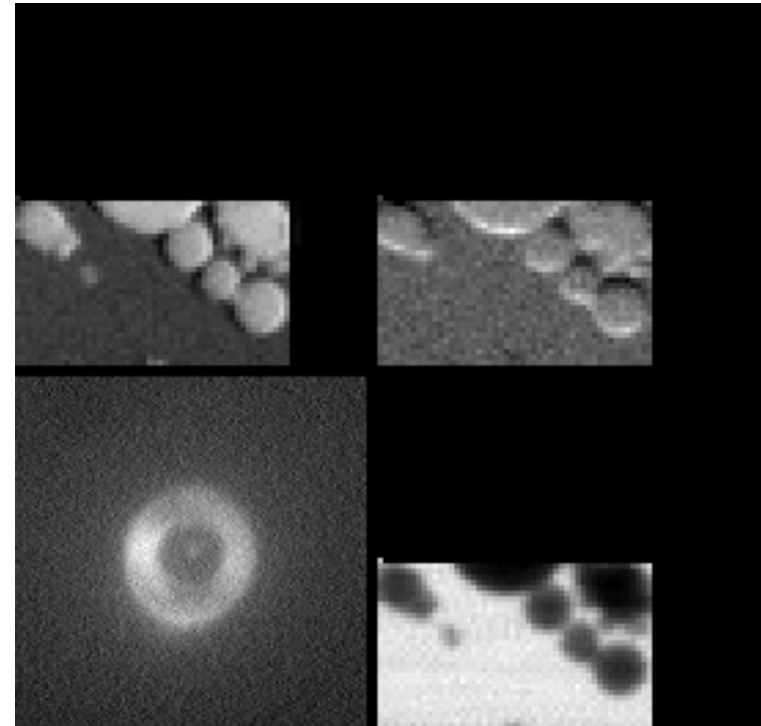
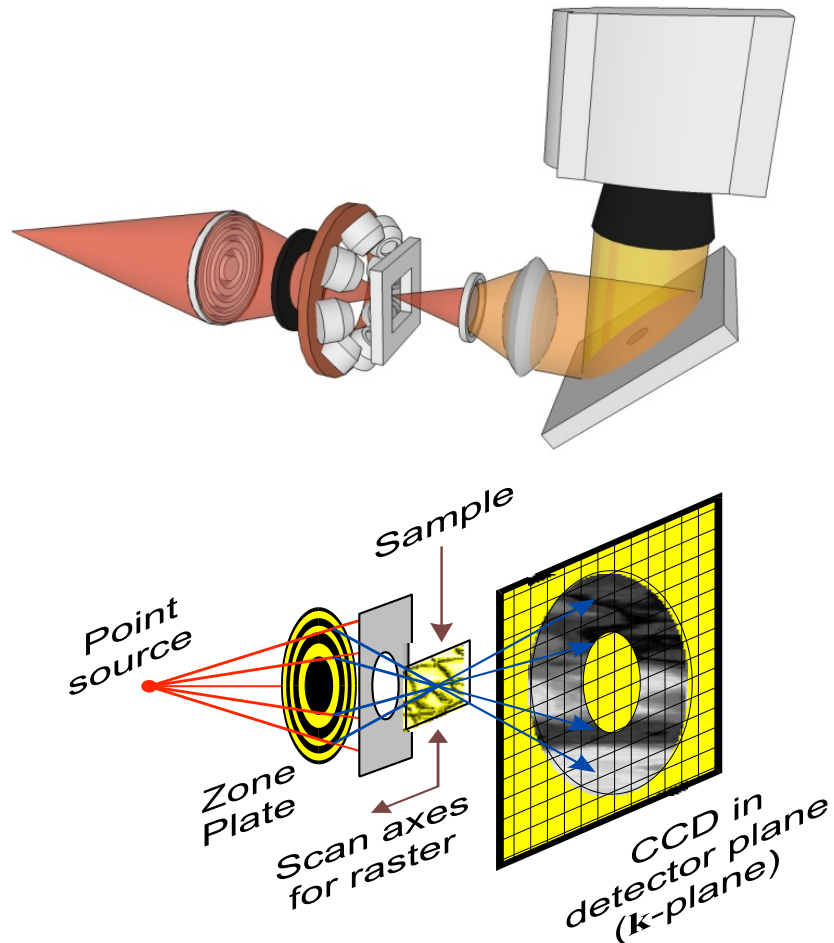


Acquired with Andor Ixon DV860A  
Frame transfer back-illuminated  
Electron Multiplying CCD with shutter  
and light converting system  
(128x128px, 5 Mhz, 110f/s)



# Scanning transmission mode

Differential phase contrast with a fast read-out CCD camera



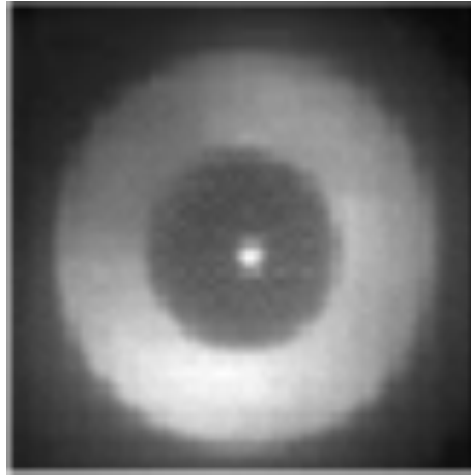
Simultaneous acquisition of:

- Absorption or transmission
- Differential phase contrast
- Darkfield images



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***Computational  
extraction of  
contrasts  
by masking:***

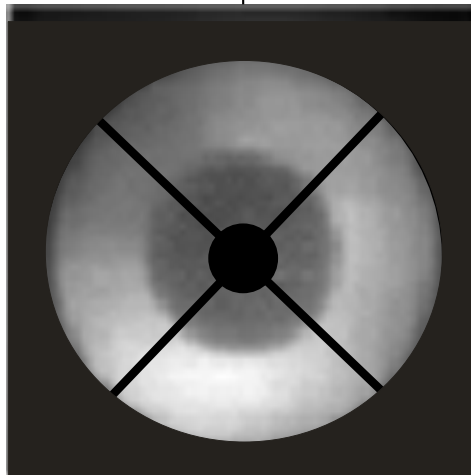


Raw data acquisition of  
first diffraction order image  
for each pixel of the raster  
scan

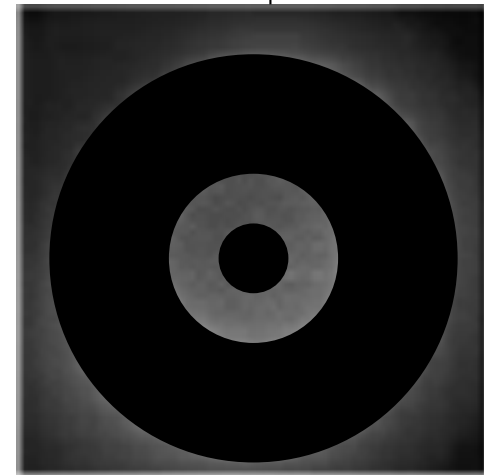
Applying different masks



**Bright field**

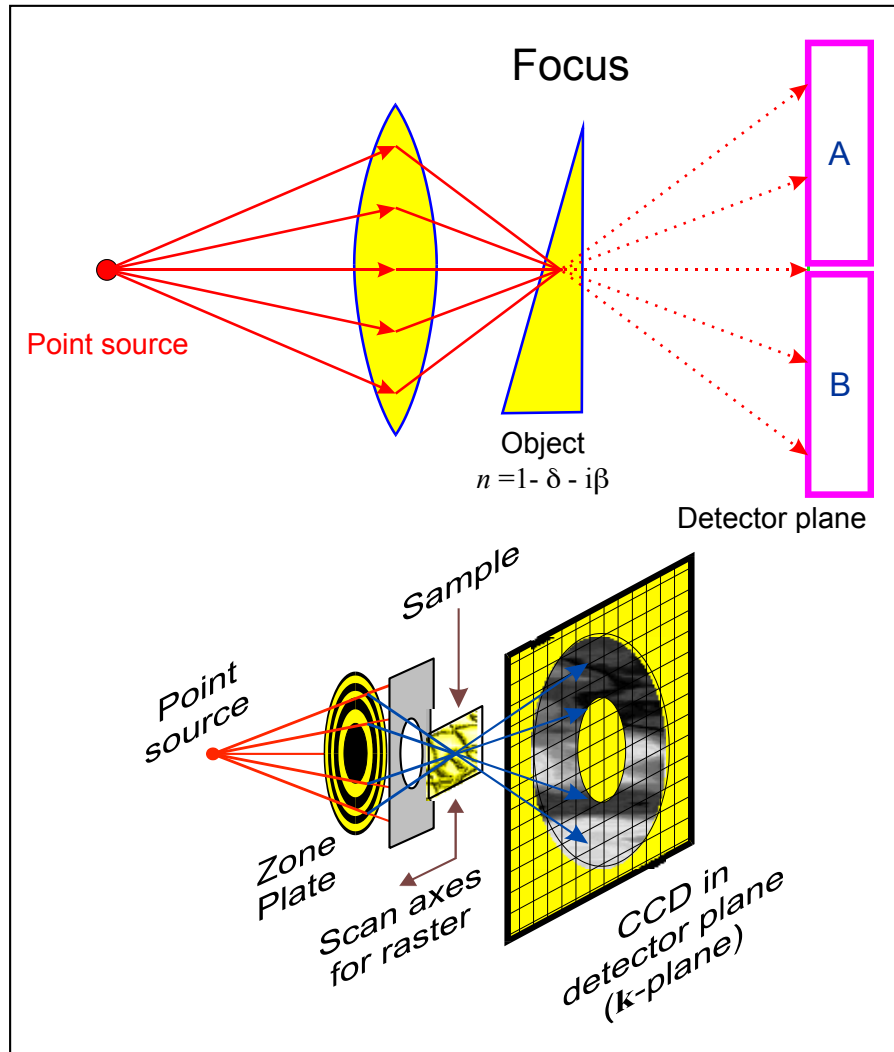


**Differential phase  
and absorption**

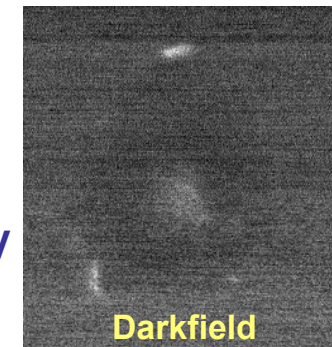
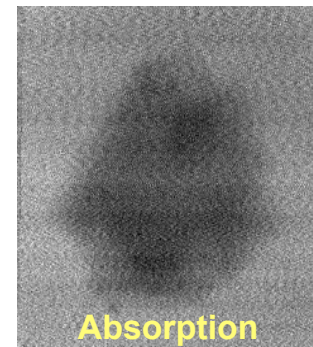
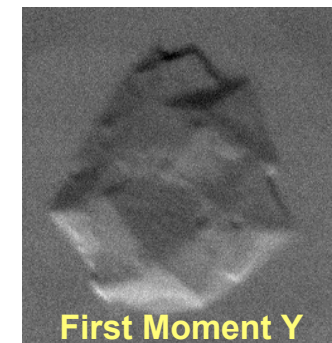


**Darkfield**

# Principle: Differential phase contrast

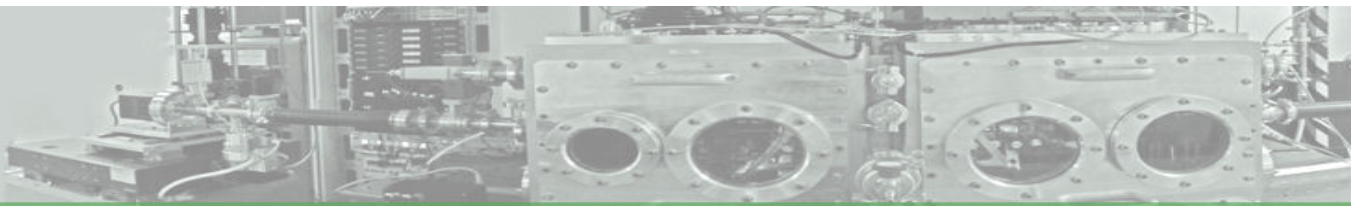


- The detector can be split into several elements
- The sum signal gives the incoherent bright-field signal
- Anti-symmetric signal combinations relate to the *phase gradient* of the object transmittance.

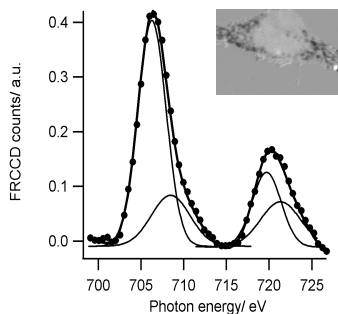
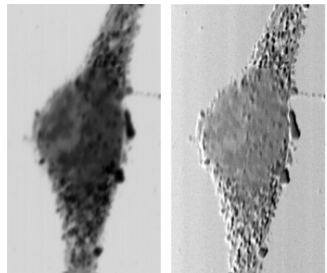
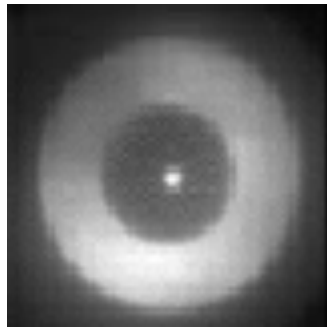


10  $\mu\text{m}$

3.3 keV



## The benefit of a fast read-out, electron-multiplied CCD as configured detector in STXM

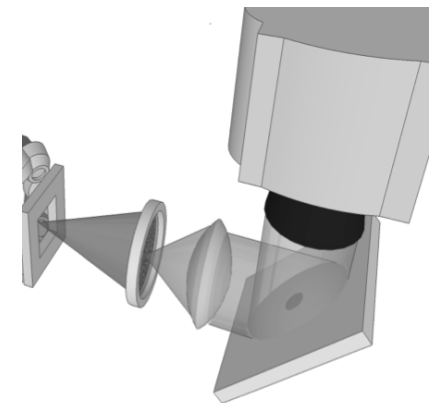


- Projection imaging for alignment purposes
- Simultaneous acquisition of absorption, differential phase contrast and darkfield imaging
- Diffraction imaging as ptychography
- XANES by across-edge imaging

Morrison, G. et al., *IPAP Conf. Series* 7, 377-379 (2006)  
Gianoncelli A. et al., *Appl Phys Lett* 89, 251117 (2006)

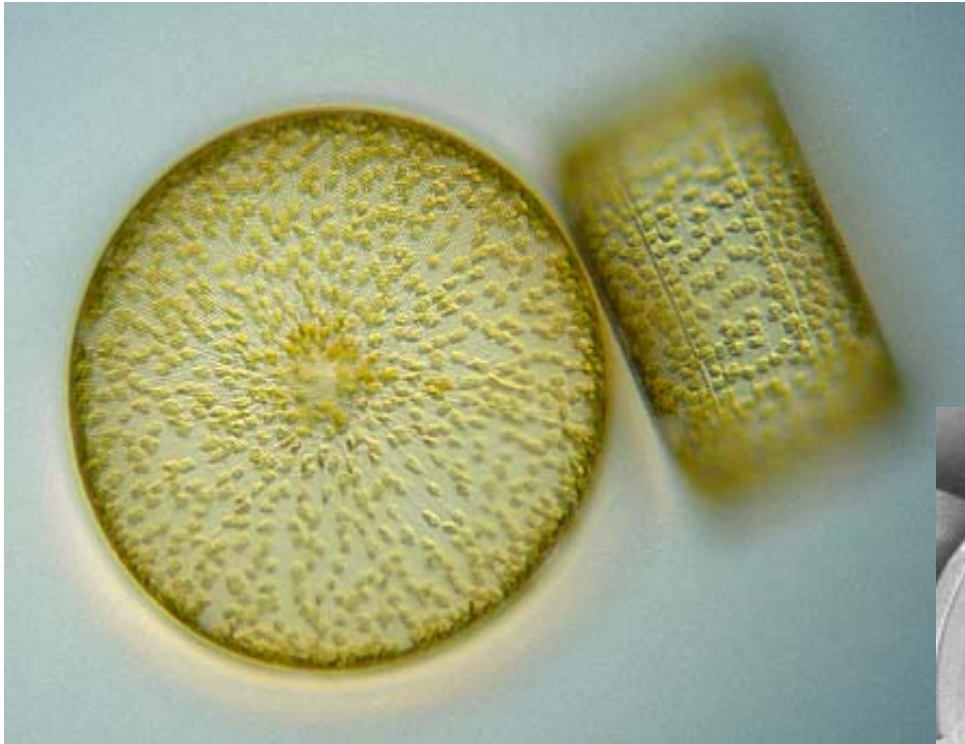


**G. R. Morrison,  
A. Gianoncelli**  
*King's College London*

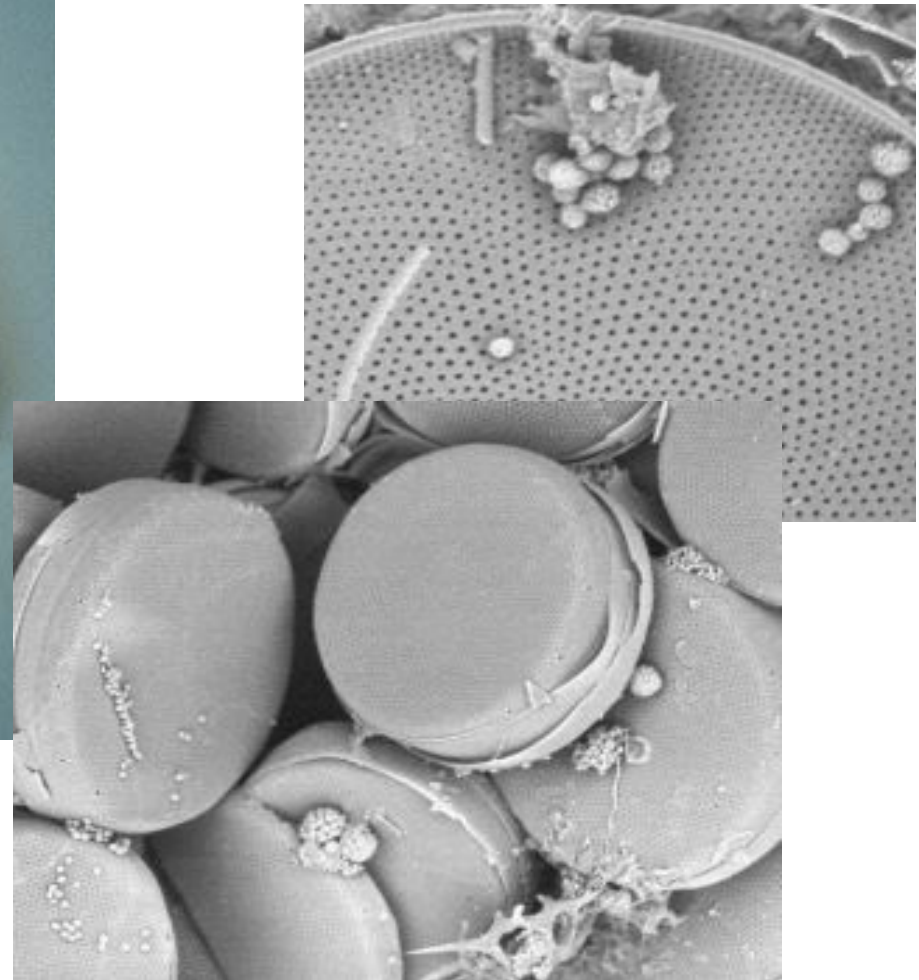


## Marine biology: Imaging of giant diatoms

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**Planktonic diatom *Coscinodiscus* sp.  
(A. Beran, Laboratory for Marine  
Biology, Trieste, I)**

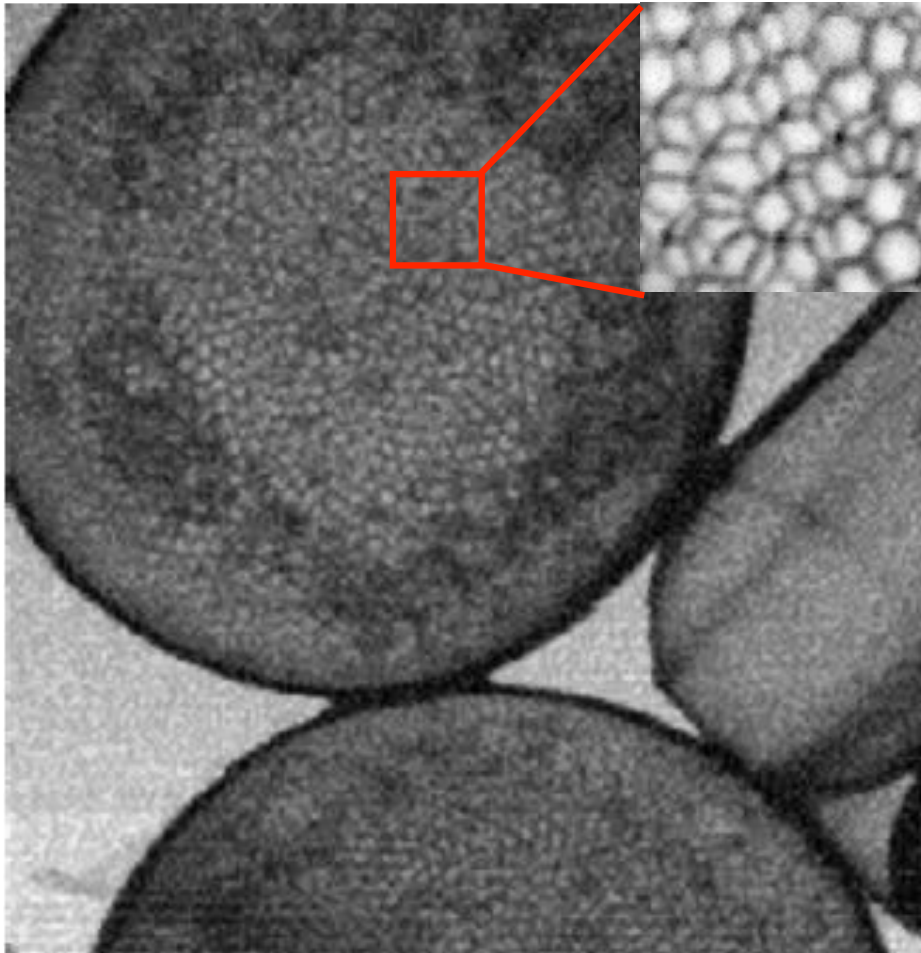




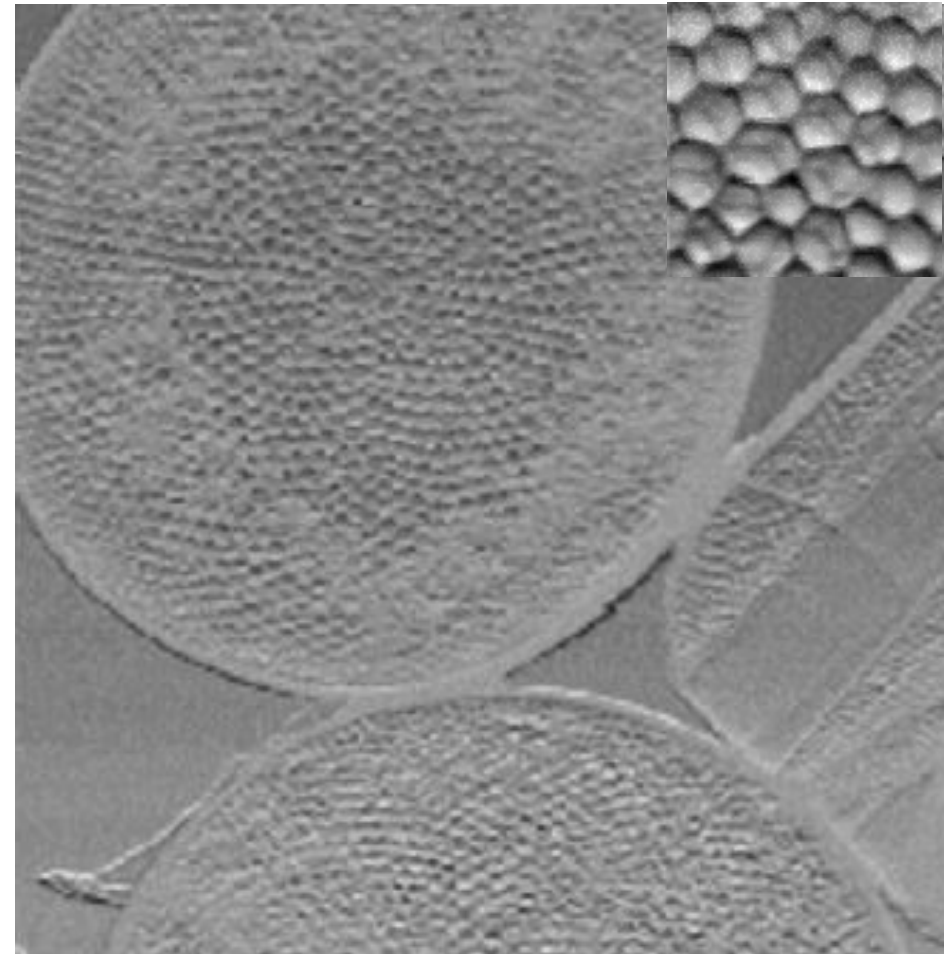
**Brightfield and differential phase contrast images acquired simultaneously  
with configured detector**

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***Planktonic diatom “Casciodiscus sp.” (provided by LBM, Trieste, I)***



***Bright field image***



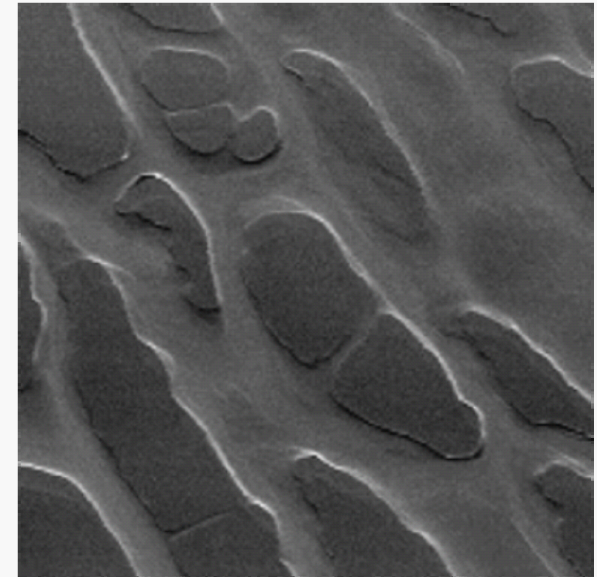
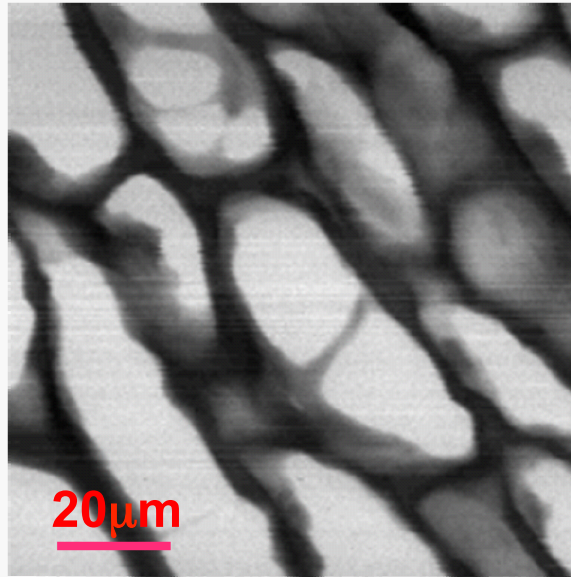
***DPC mode – X-moment***

Images acquired in STXM mode (TwinMic microscope) with FRCCD camera; E=1320 eV, 200x190 px, 50ms dwell/px

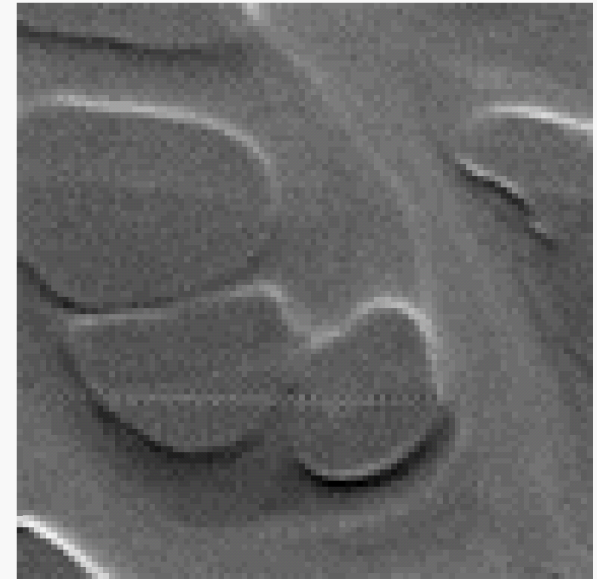
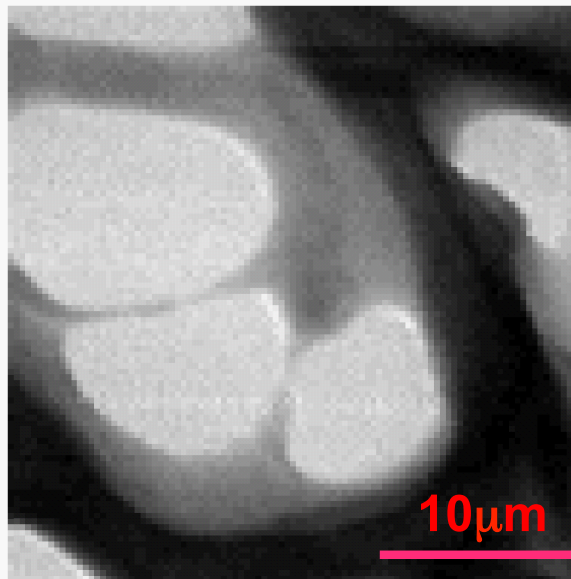
---



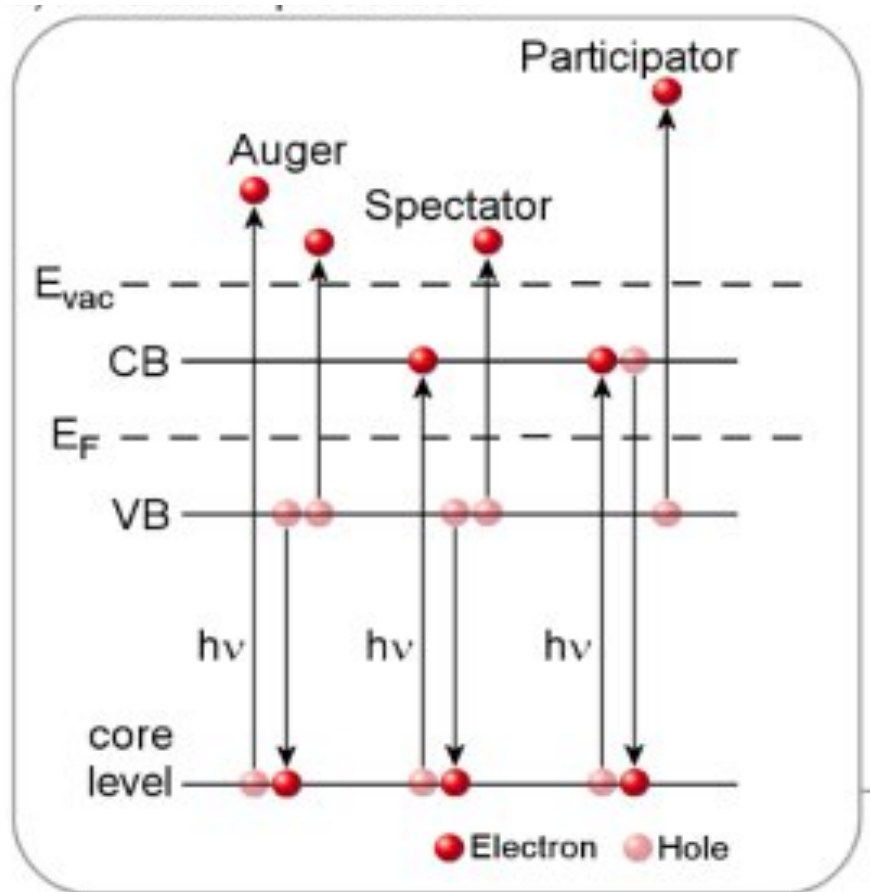
# Brightfield and differential phase contrast images acquired simultaneously with configured detector



**Coffee bean cell membranes**

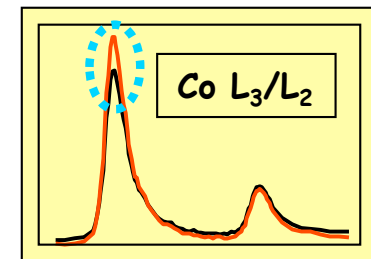
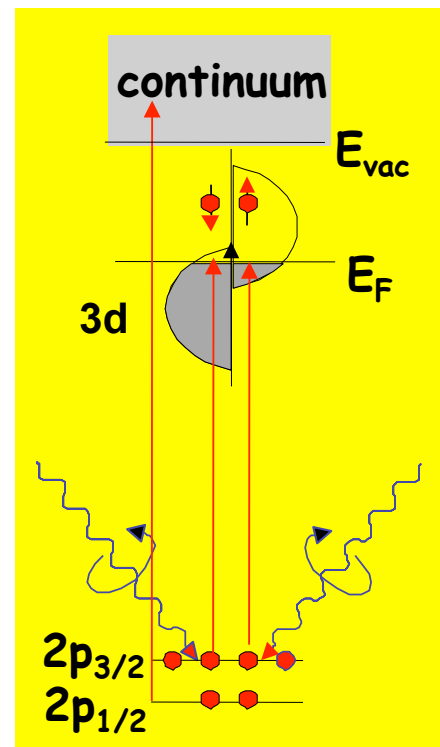


# Chemical/ Magnetic contrast



$$I \propto \exp(-\mu d) , \quad \mu \propto \frac{Z^2}{(h\nu)^3}$$

**XANES = X-ray Absorption Near Edge Spectroscopy**



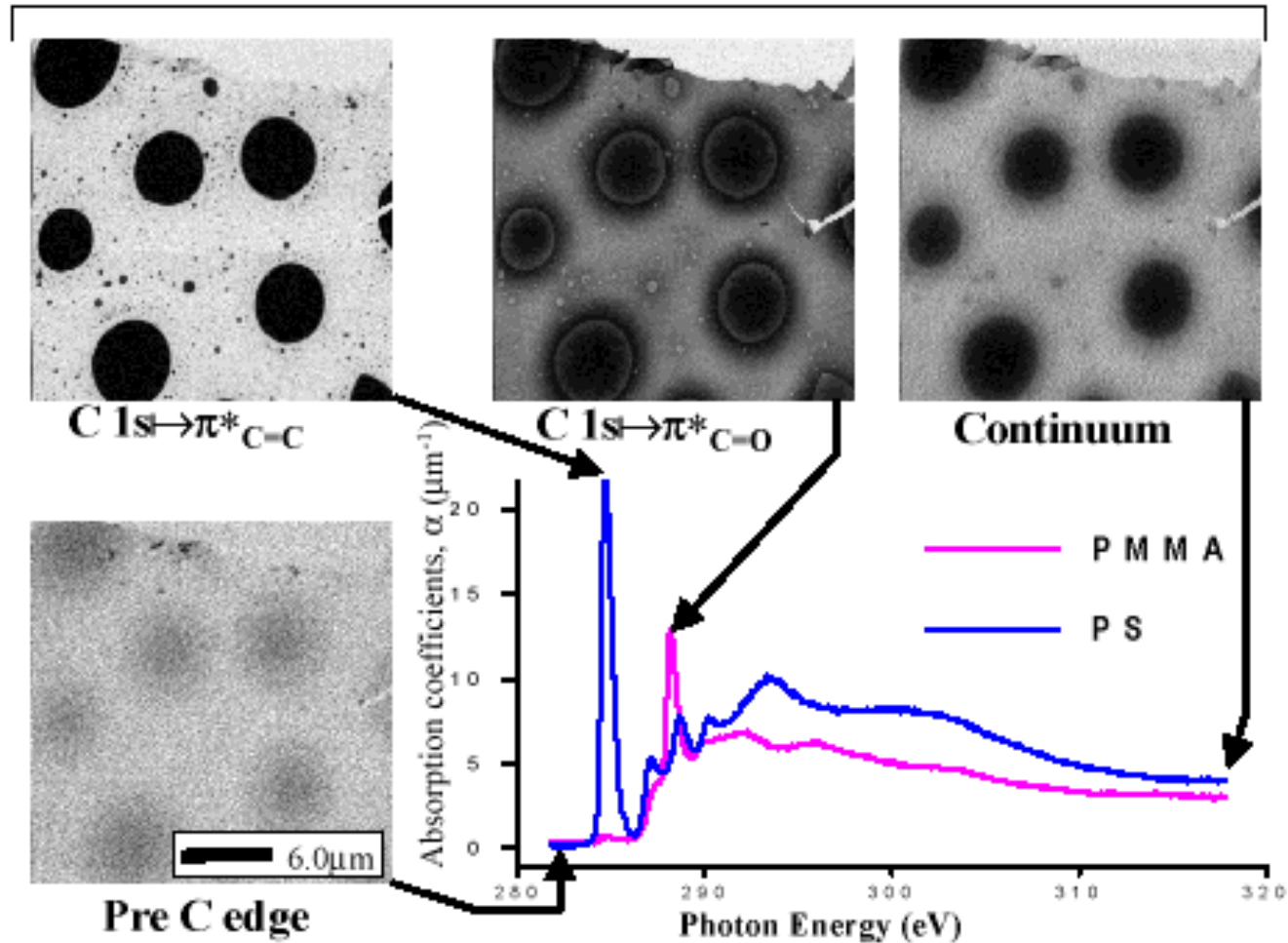
Resonances with unfilled states.

**XANES:** tuning on molecular orbitals  
**XMLD:** imaging antiferromagnets,  
**XMCD:** imaging ferromagnets

# Chemical contrast

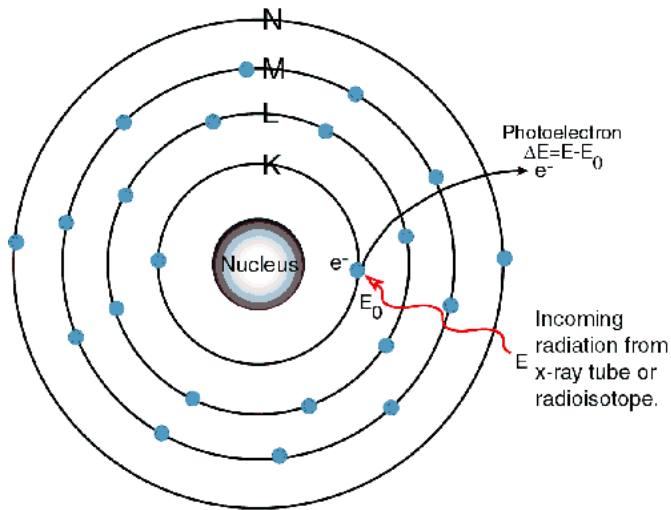
## Outlining the lateral distribution of PS/ PMMA

### Transmission x-ray micrographs



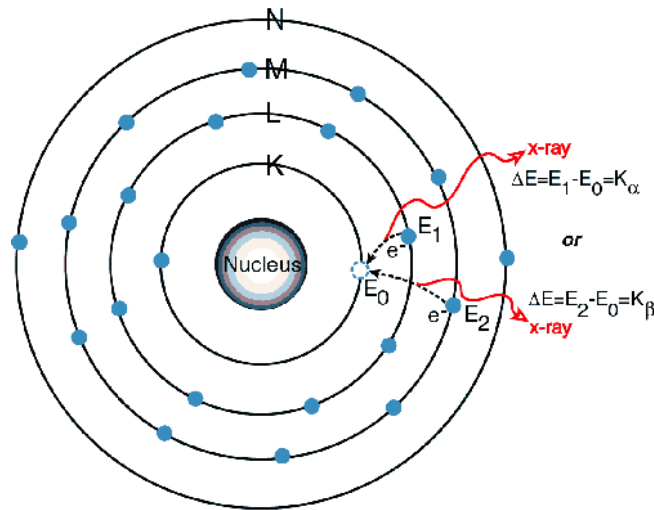
*H. Ade, SUNY-SB STXM at the NSLS*

# Photoionization



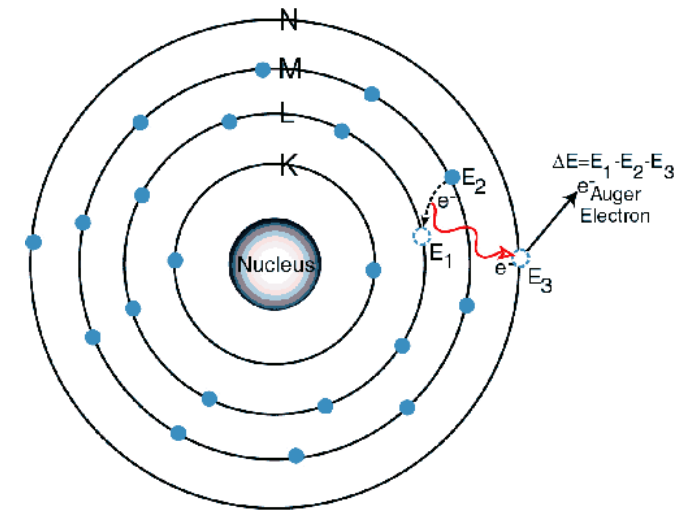
## X-ray absorption (through photoelectric effect)

The primary X-ray photon causes the ejection of electrons from the inner shells, creating vacancies



## X-ray Fluorescence

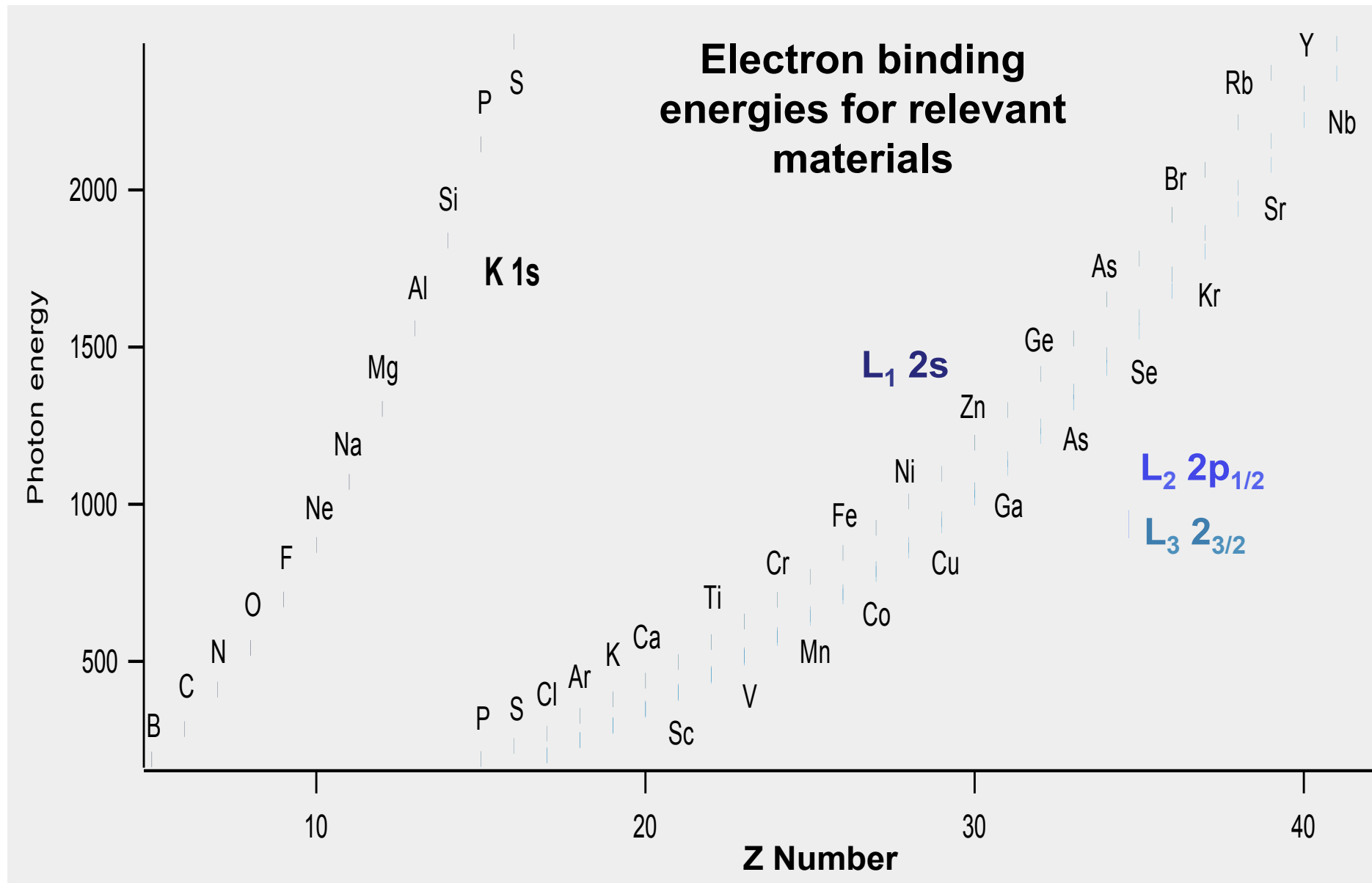
The vacancy created by the primary X-ray photon is filled by an electron coming from an outer shell causing the emission of a characteristic X-ray photon whose energy is the difference between the two binding energies of the corresponding shells



## Auger effect

The vacancy created by the primary X-ray photon is filled by an electron coming from an outer shell and the energy is transferred directly to one of the outer electrons, causing it to be ejected from the atom.

# Chemical sensitivity of X-rays: Elemental mapping

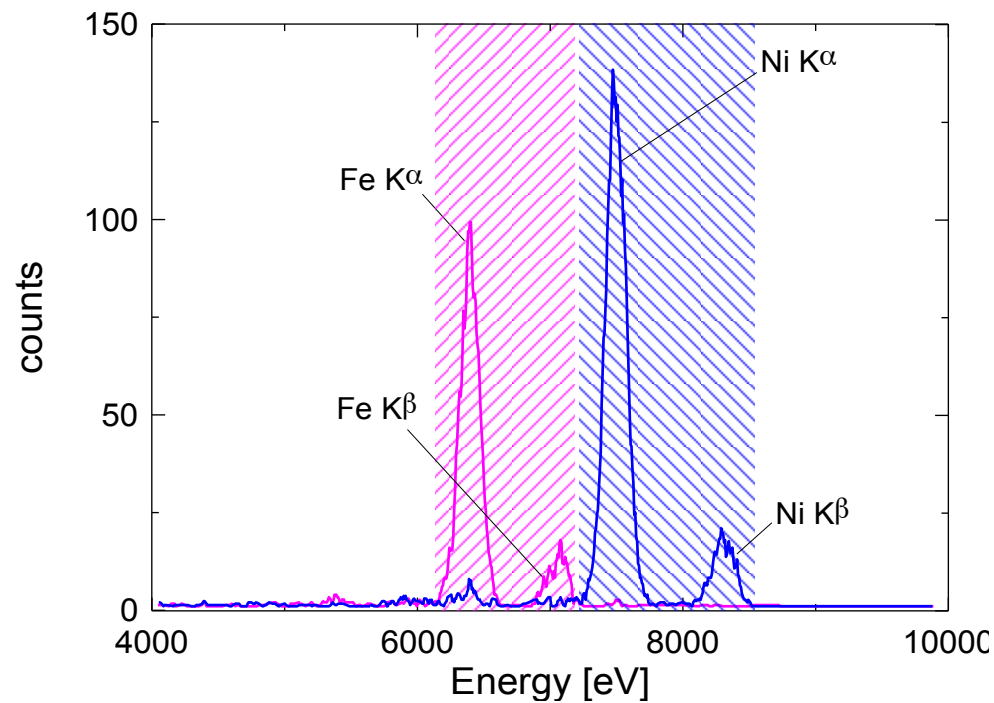


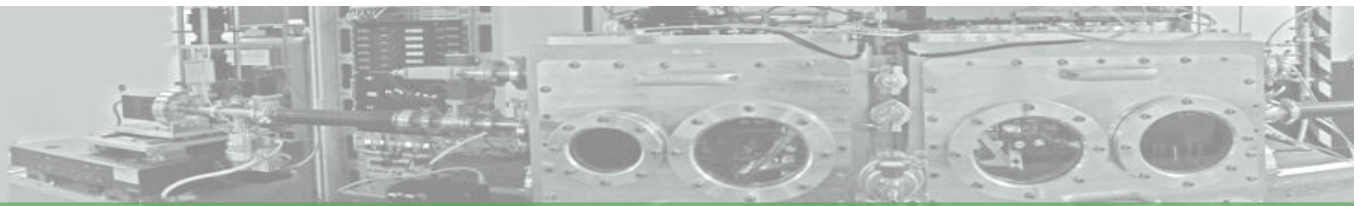


# Fluorescence contrast

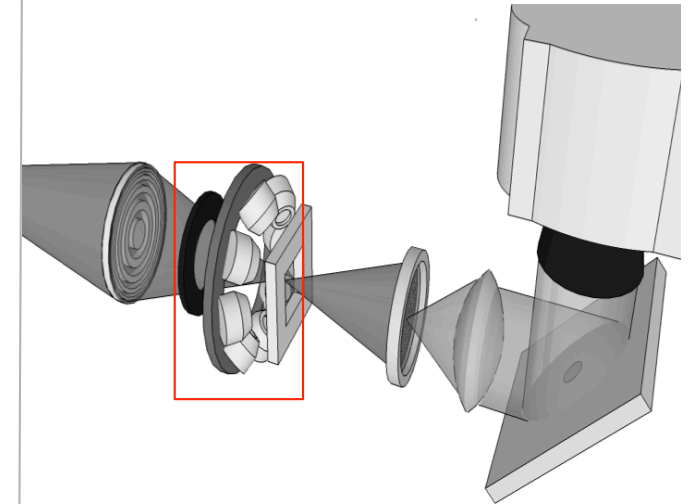
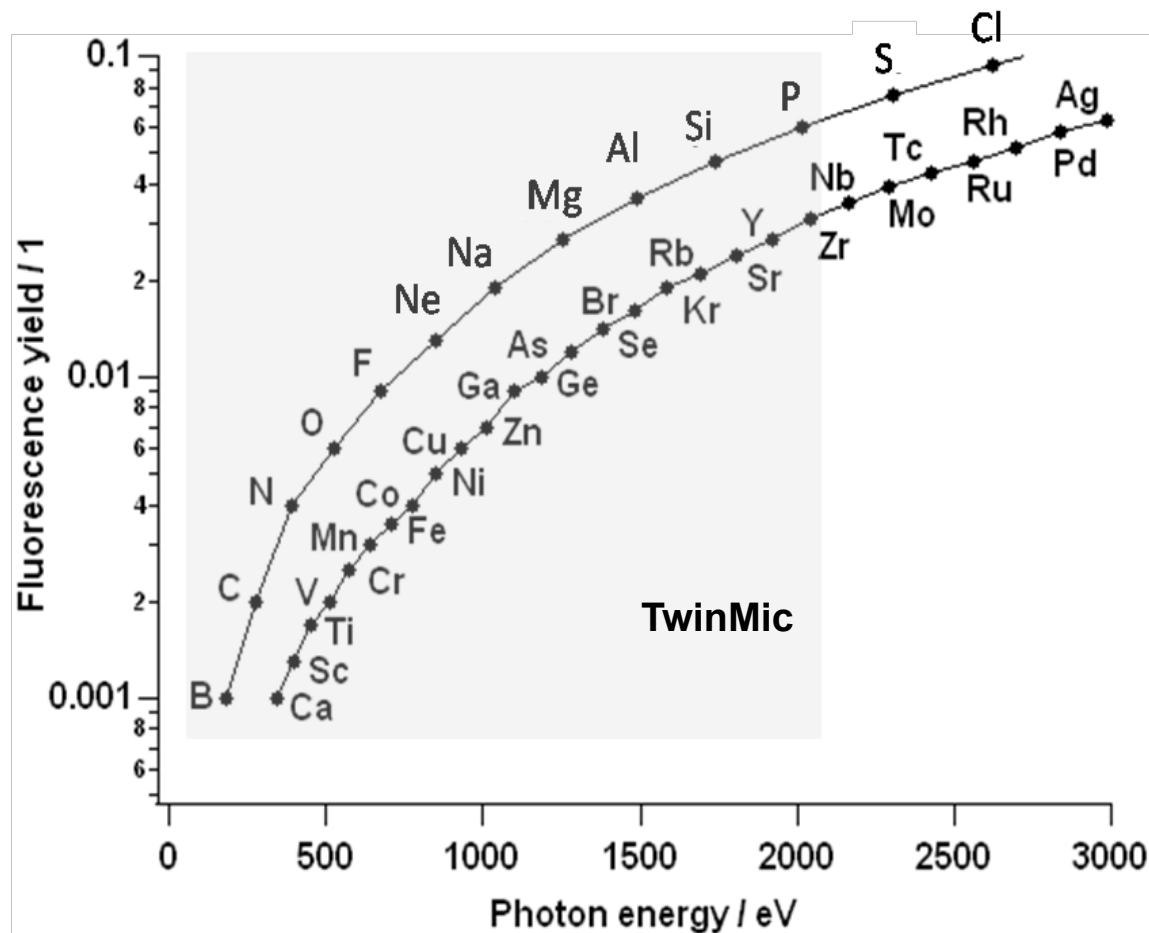
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- Microprobe focused on the sample
- Sample raster scanned
- Analysis of each spectrum in the raster scan
- Construction of an X,Y map for each chemical elements present in the spectrum





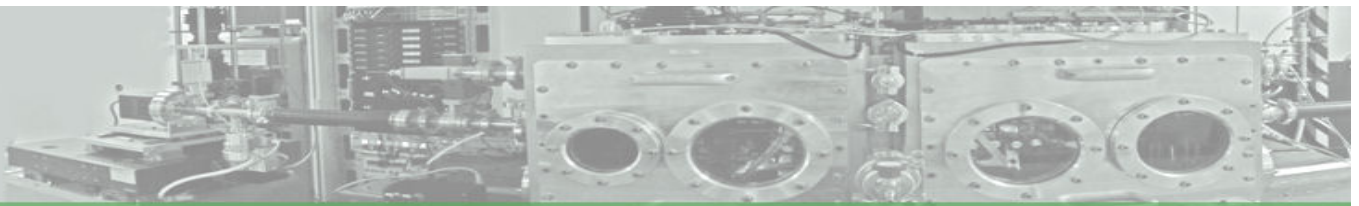
## Low-energy X-ray fluorescence for elemental analysis:



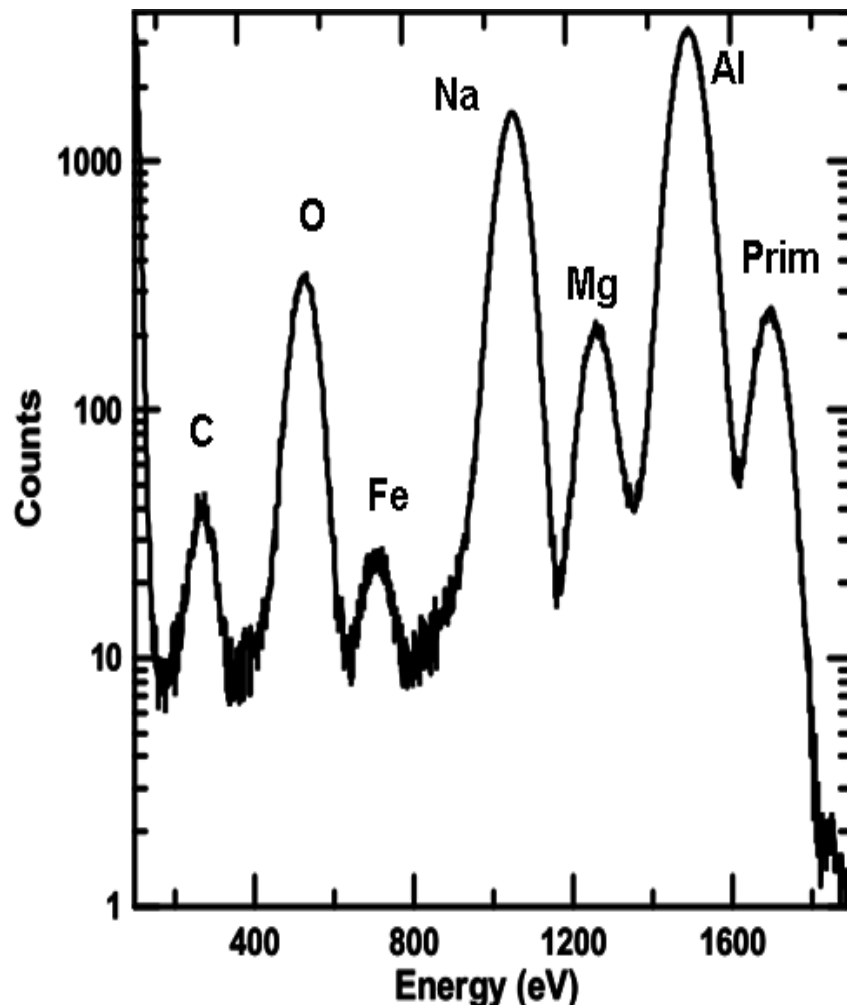
**Detecting trace elements:**

**X-ray fluorescence:**  
**~1000x better sensitivity**  
**than electrons for trace**  
**elemental mapping (ion**  
**concentrations etc.).**

**Low fluorescence yields for**  
**soft X-rays! !!**



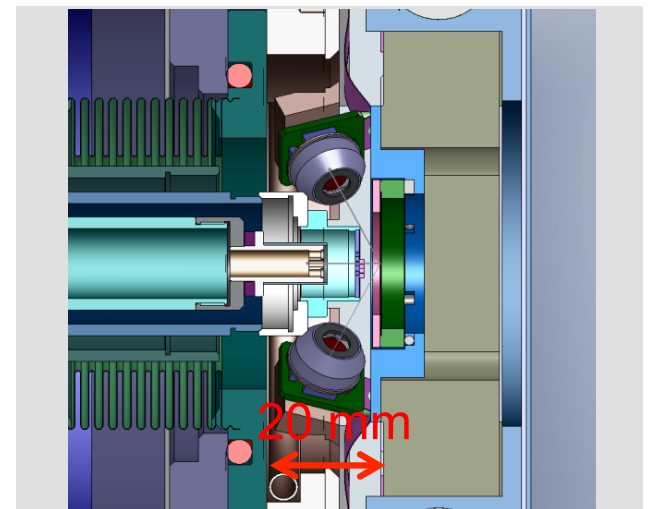
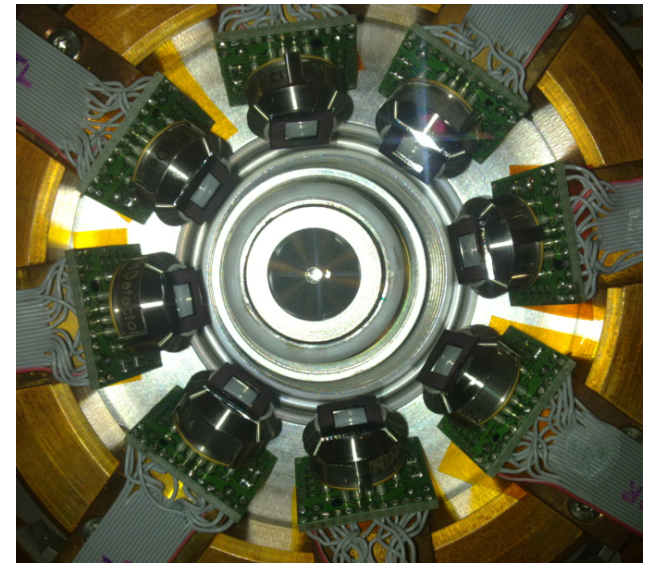
# Low-energy X-ray fluorescence

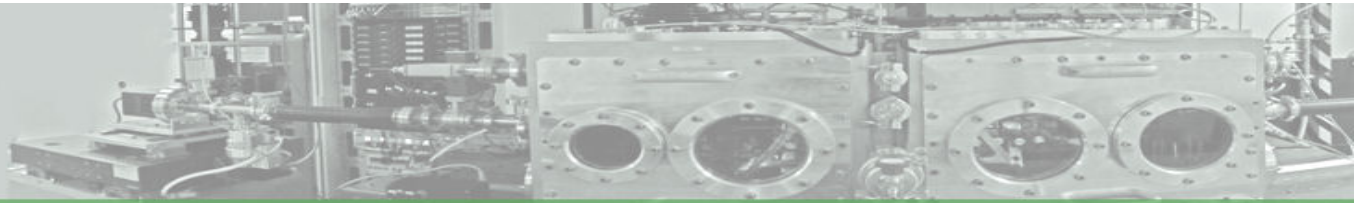


**TwinMic LEXRF spectrum with unfocused beam of a test organic matrix on a metal shim**

**Dynamic range: up to 30 kcounts/s**

**Average FWHM energy resolution @ C- K edge: 69 eV**



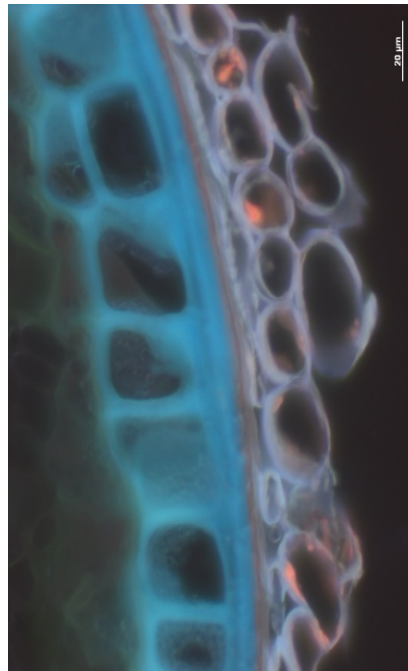
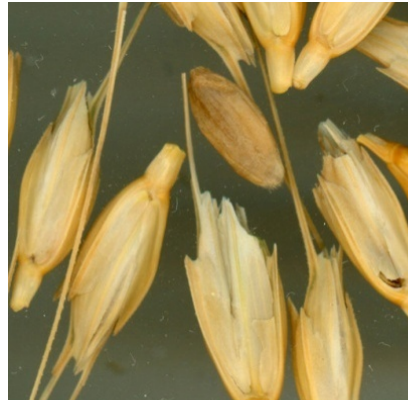


# Food Science: Inside the wheat

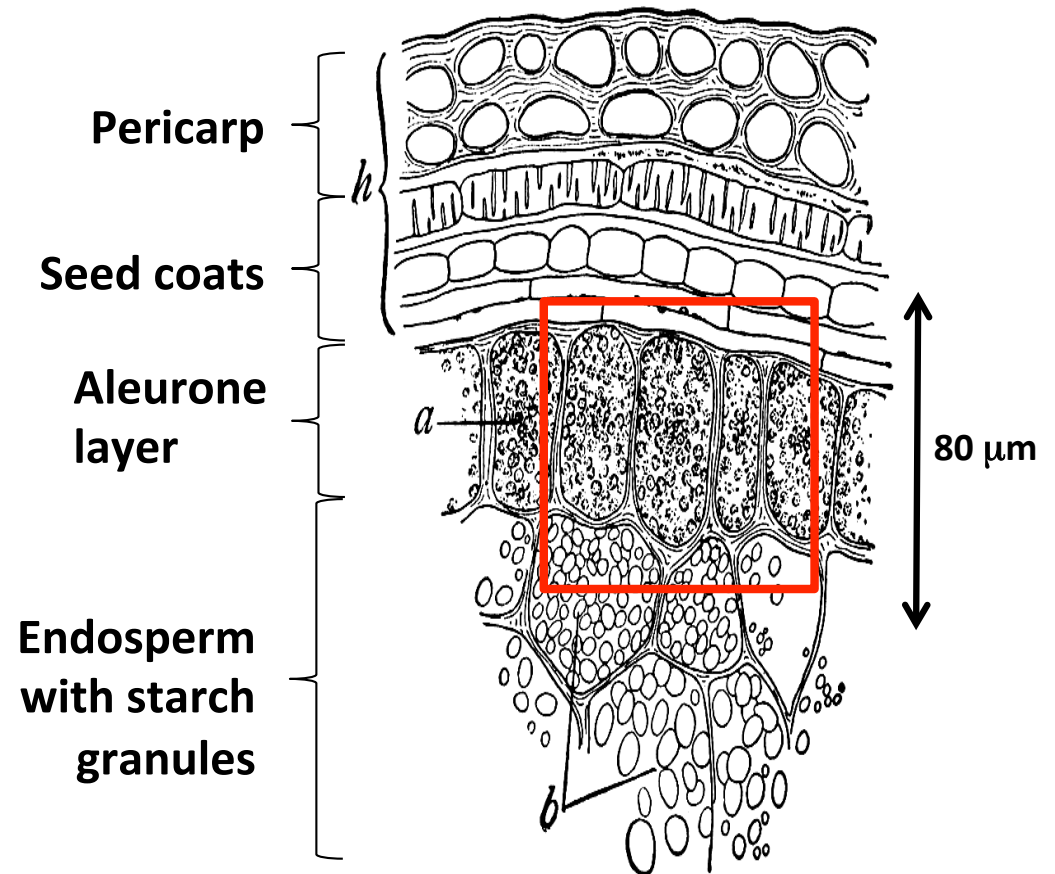


***Ivan Kreft,  
University Ljubljana***

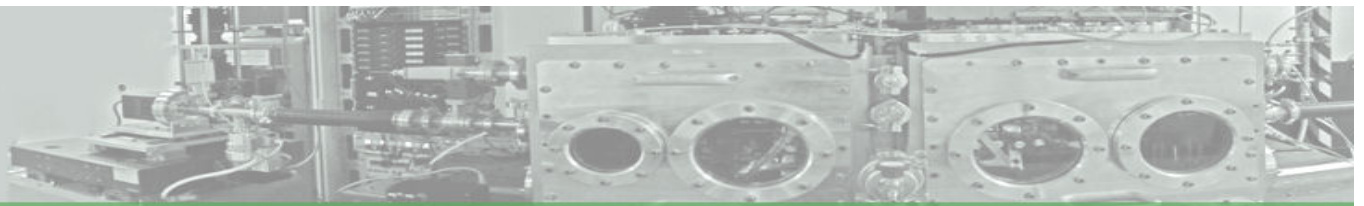
Functionality and toxicity of Zn in wheat and buckwheat analyzed on sub-cellular level



**Structure of a wheat grain**





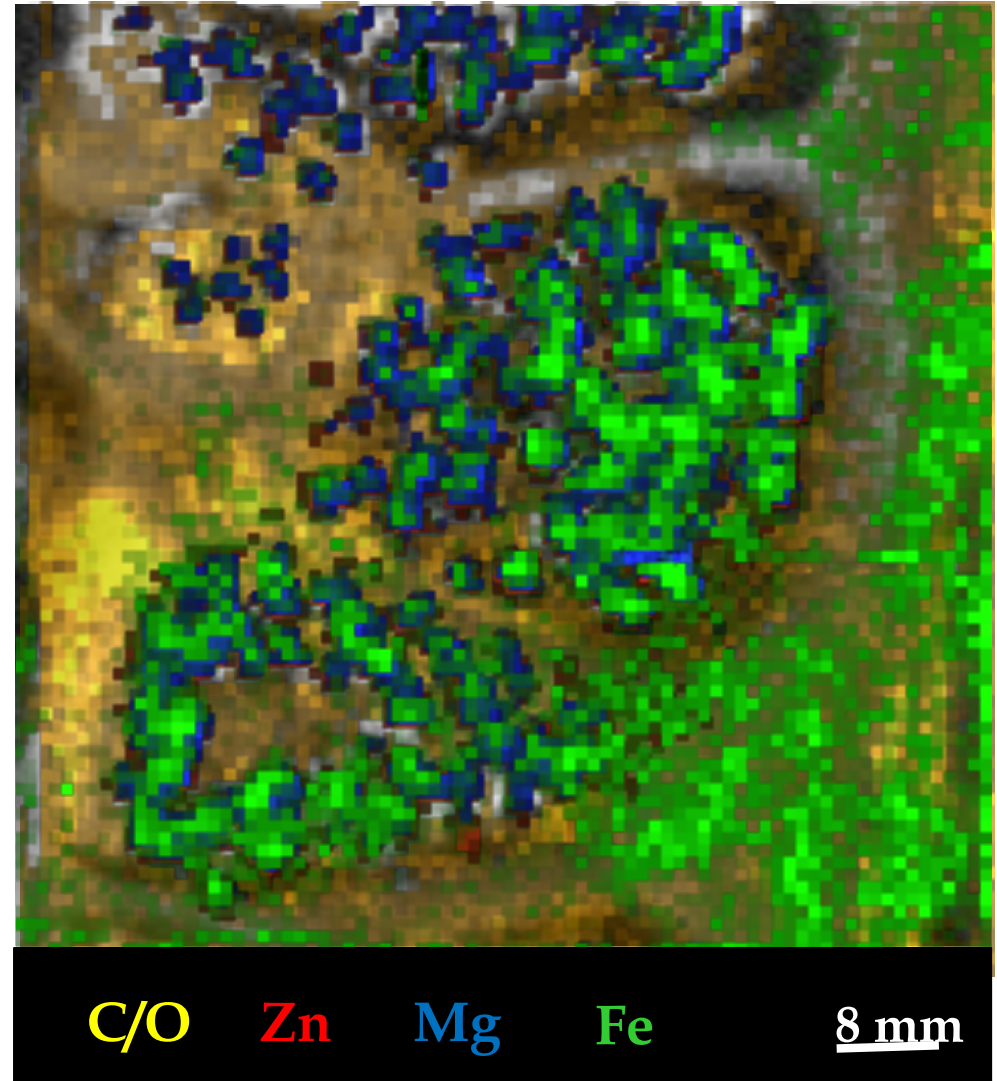


*Ivan Kreft,  
Fac. of Biotechnology,  
University Ljubljana*

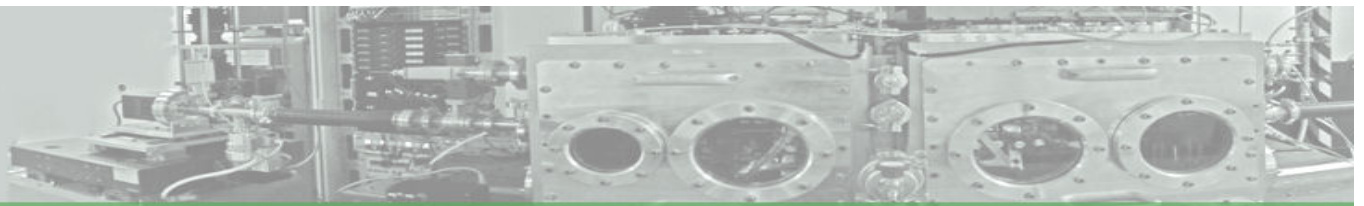
**Functionality and  
toxicity of Zn in  
wheat and buckwheat  
analyzed on sub-  
cellular level**

**Healthy control  
wheat**

**E=1686 eV  
80 x 80 mm<sup>2</sup>  
80 x 80 px  
8 s dwell/ px  
1 mm  
resolution  
4 detectors**

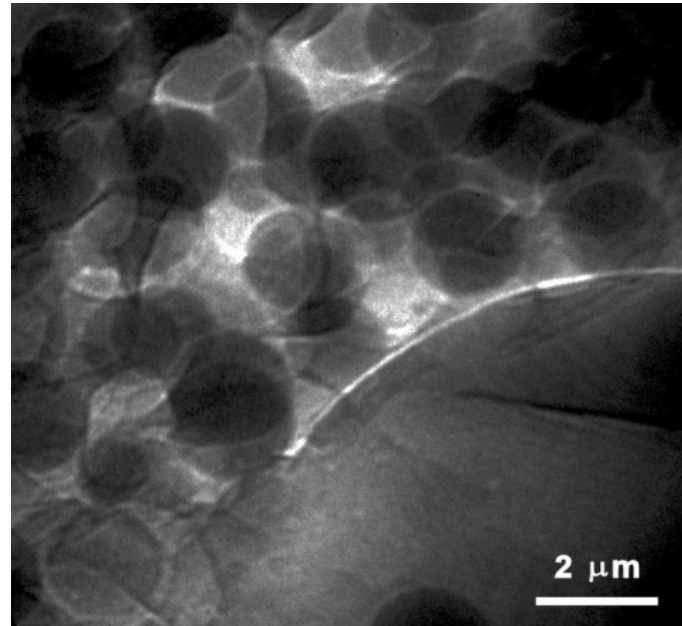




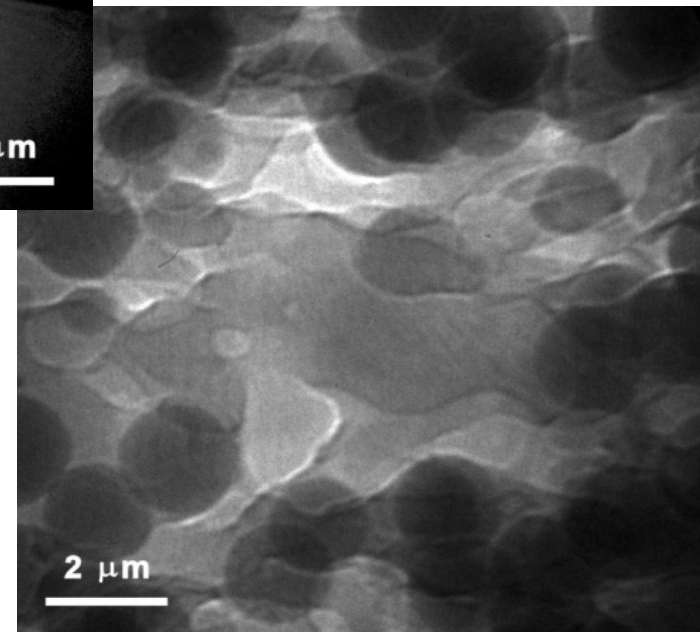


*Ivan Kreft,  
Fac. of Biotechnology,  
University Ljubljana*

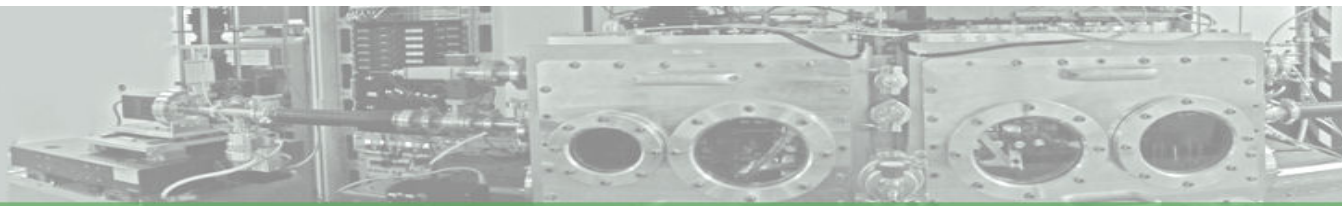
**Functionality and  
toxicity of Zn in  
wheat and buckwheat  
analyzed on sub-  
cellular level**



**1s dwell, 740 eV photon  
energy**



**TXM images  
acquired with a  
double-frequency ZP  
(15nm outermost  
zone width from J.  
Vila-Comamala (PSI))**

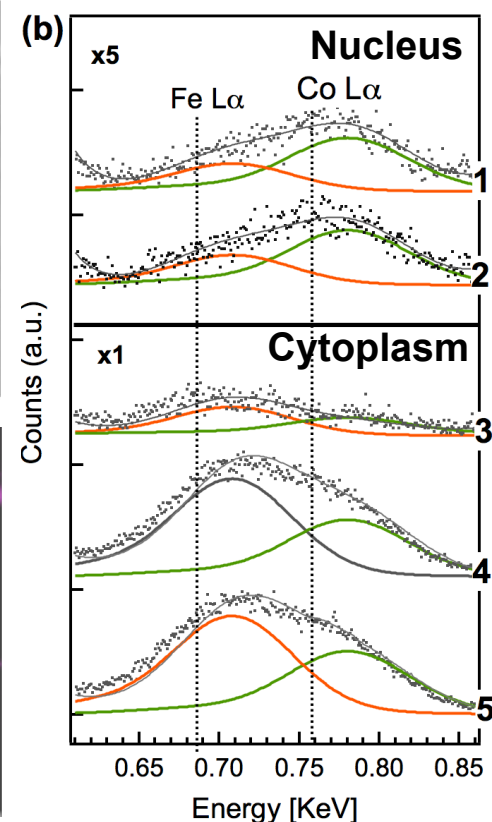
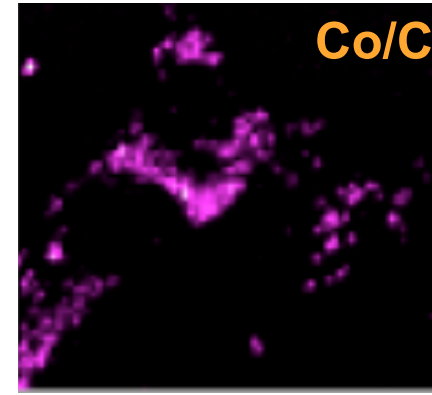
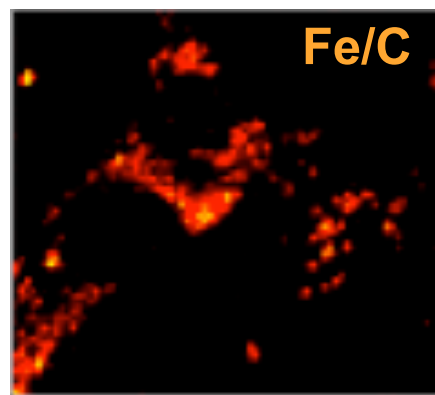
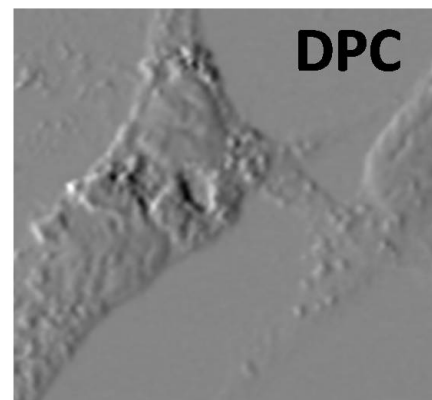
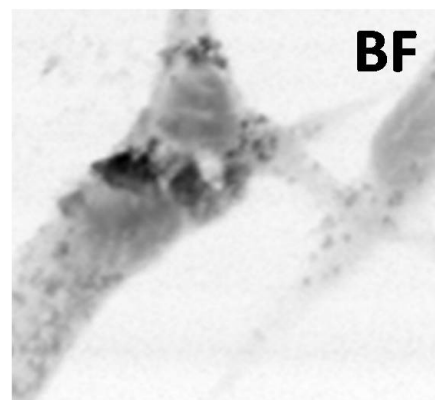


## Nanotoxicology: $\text{CoFe}_2\text{O}_4$ ENPs

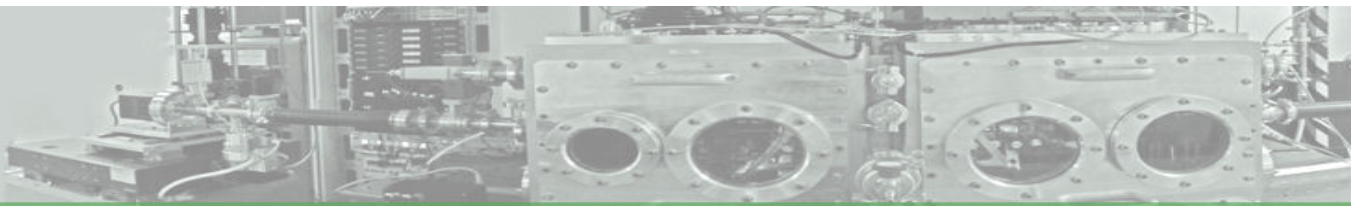


**G. Ceccone,  
P. Marmorato et al.,  
EC Joint Research  
Center, Ispra, I**

**Localization of  
engineered  
nanoparticles (ENPs)  
inside a cell and on  
the possible effects  
on the cell metabolic  
behaviour**

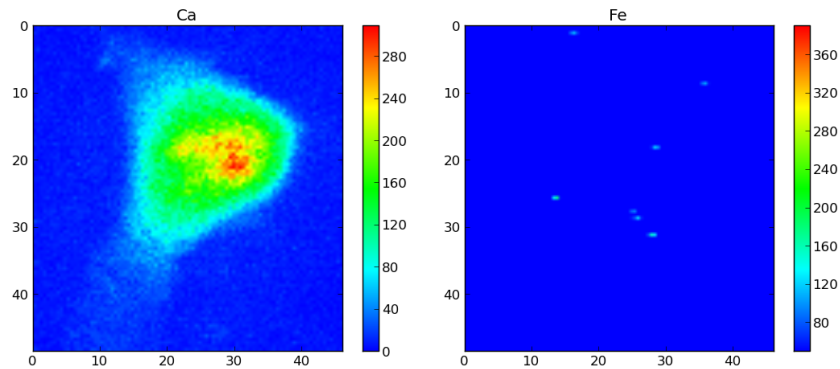


**$\text{CoFe}_2\text{O}_4$  in mouse 3T3 fibroblast cells,  $E=2019$  eV,  $60\mu\text{m} \times 60 \mu\text{m}$ ,  $80 \times 80$  pixels,  $15\text{s/pixel}$**

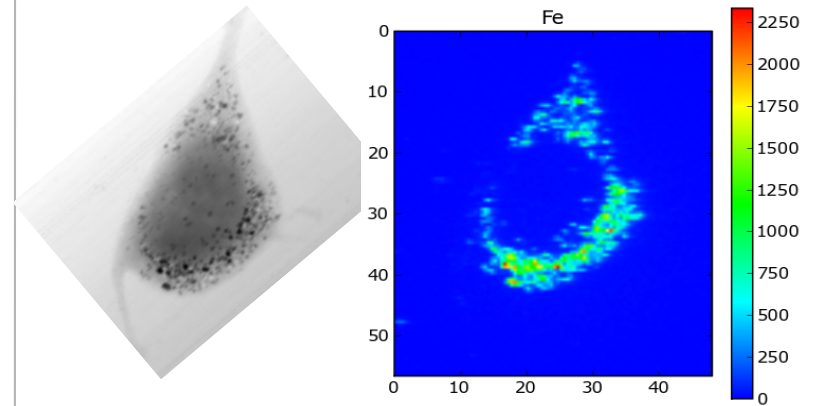


# Nanotoxicology: $\text{CoFe}_2\text{O}_4$ ENPs

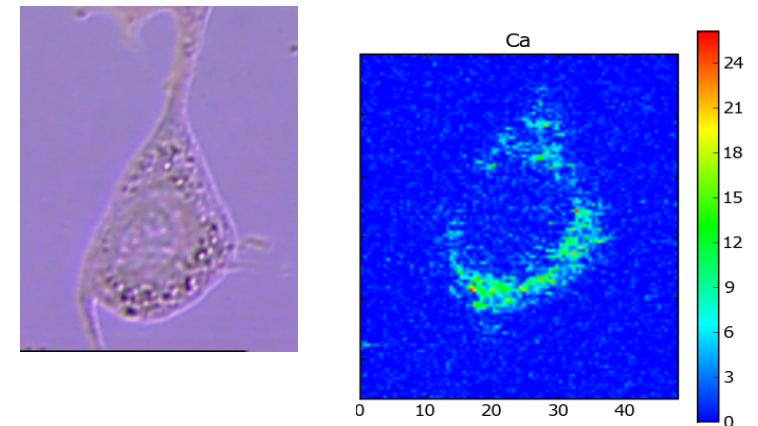
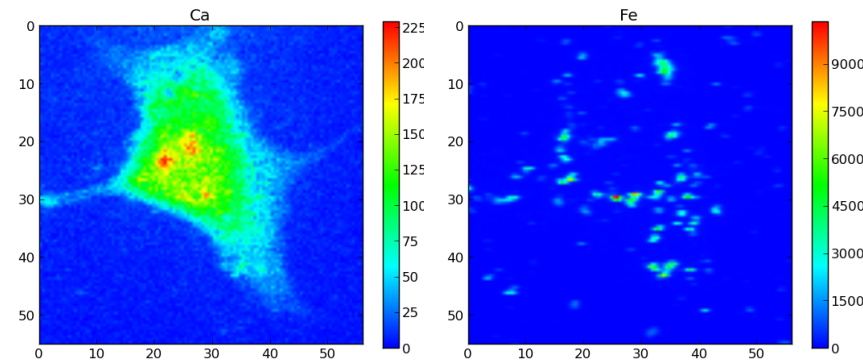
**Control**



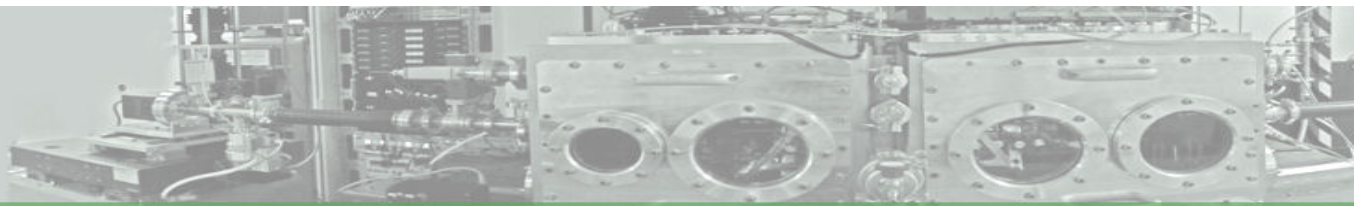
**Exposed to 500 $\mu\text{M}$**



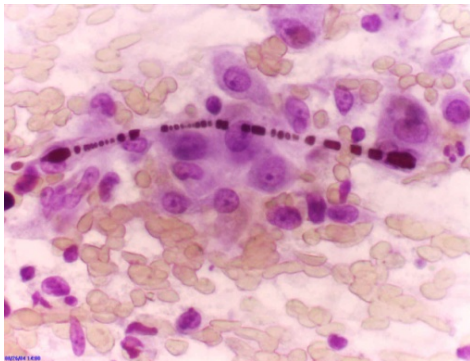
**Exposed to 40 $\mu\text{M}$**





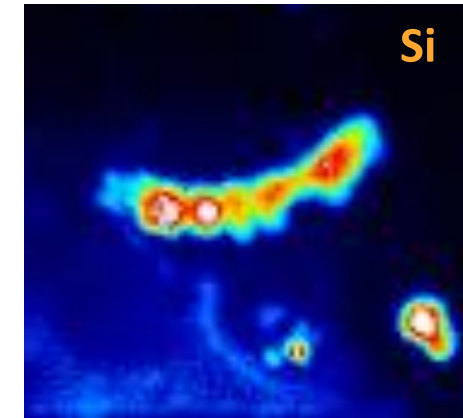
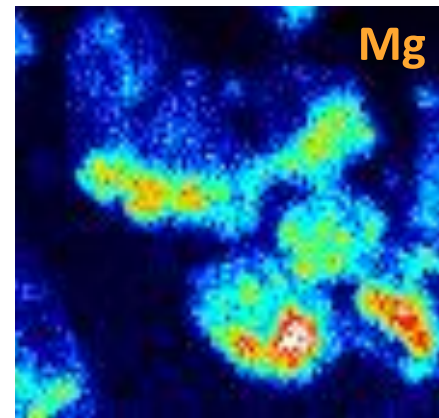
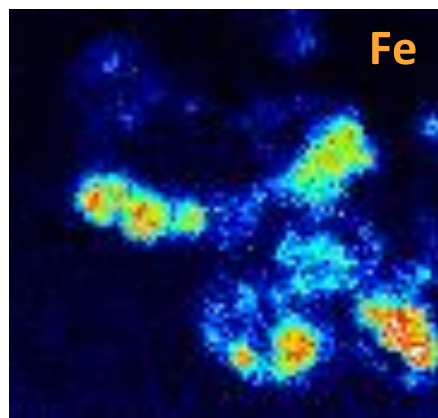
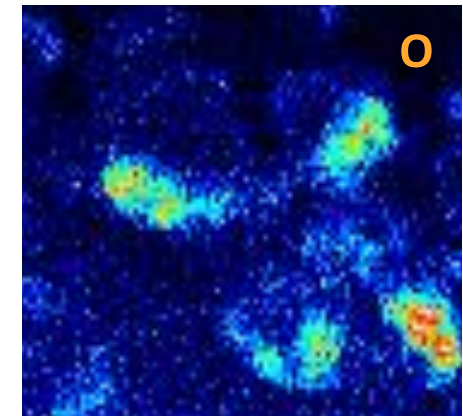
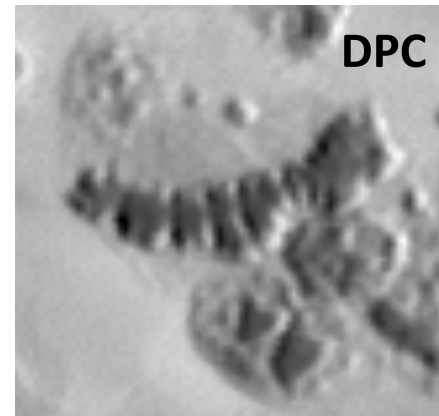
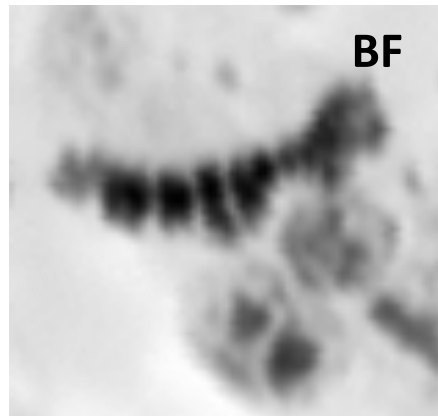


# Clinical medicine: Asbestos in lung tissue

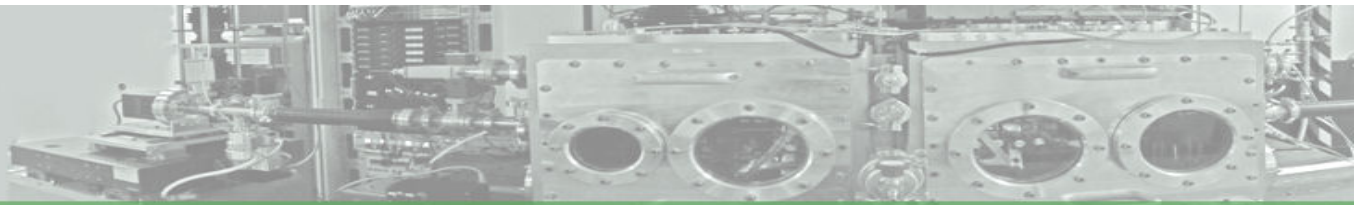


**M. Melato,  
Monfalcone Hospital  
L. Pascolo,  
Sincrotrone Trieste**

**Mesothelioma and  
differentiation of  
lung tissue due to  
asbestos;  
the role of Mg**



E=2019 eV, 50mm x 50 mm, 100 x 100 pixels, 15s/pixel  
LEXRF



# Biotechnology: Al in tea leaves

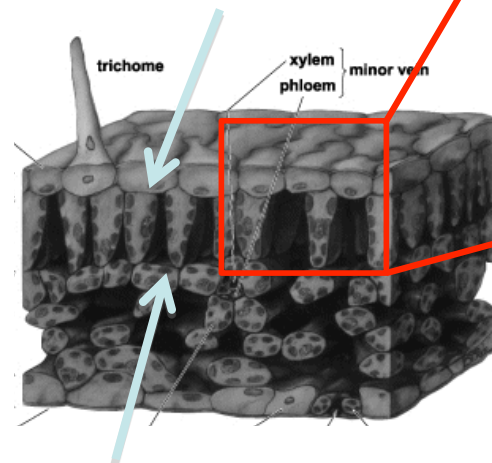


**Charlotte Poschenrieder,**  
*Uni Barcelona, ES*  
**Katharina Vogel,**  
*Uni Ljubljana, SI*

**Functionality and  
toxicity of Al in tea  
leaves analyzed on  
sub-cellular level**

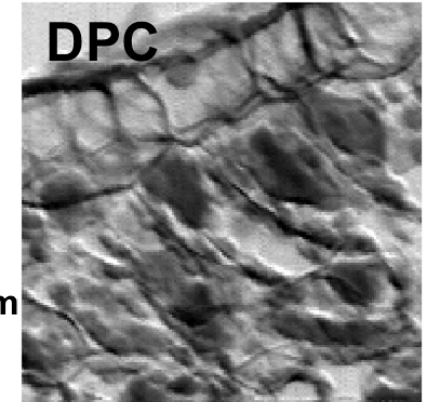
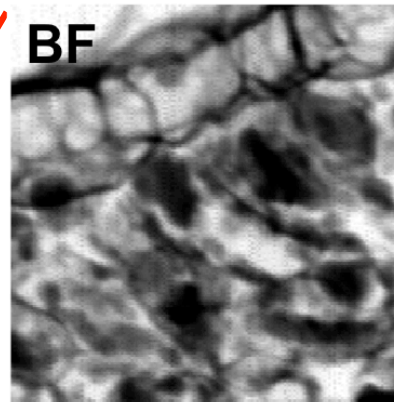
**Cross-section of a leaf**

**Upper epidermis**

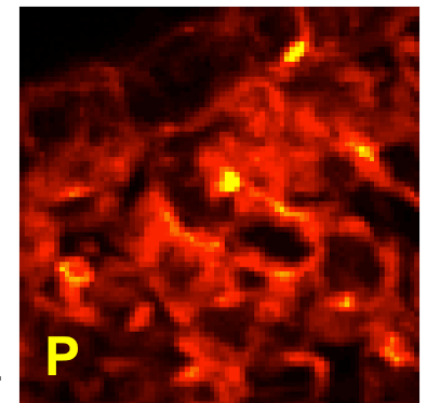
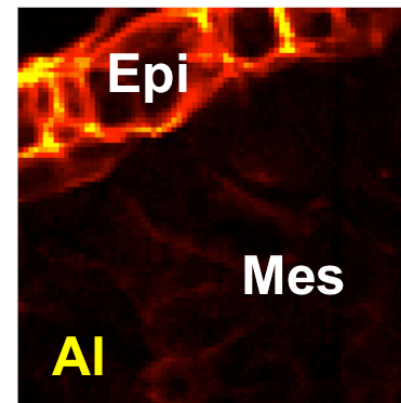


**Mesophyll**

**E=2.19 keV**  
**80 x 80  $\mu\text{m}^2$ , 80 x 80 px**  
**12s /px**



**5  $\mu\text{m}$**



3.0  
2.5  
2.0  
1.5  
1.0  
0.5  
0.0  
a.u.

In young tea leaves the preferential accumulation of Al occurs at the end of the transpiration stream, in the epidermal cell walls



# Conclusions

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- Synchrotron radiation facilities provide state of the art techniques
  - Synchrotron radiation X-ray microscopy techniques offer high spatial resolution (through absorption and phase contrast imaging) and high chemical sensitivity (through XRF contrast)
  - Different contrast techniques are available, suitable to specific applications
  - Wide range of applications
  - Complementarity to other techniques (laboratory, SR...)
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