The Abdus Salam International Centre for Theoretical Physics

United Nations Educational, Scientific and Cultural Organization

IAEA International Atomic Energy Agency

2139-26

School on Synchrotron and Free-Electron-Laser Sources and their Multidisciplinary Applications

26 April - 7 May, 2010

Transmission X-ray microscopy

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Transmission X-ray microscopy

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Topics of this lecture:

- Why using X-rays for microscopy (XRM)
- Scanning and full-field imaging transmission XRM
- Different contrast modes based on absorption, scattering and refraction
- The benefits of simultaneous transmission and emission XRM
- Typical applications of XRM
- Radiation damage in XRM

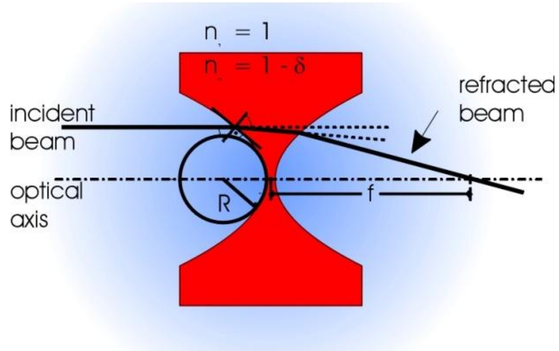
What you learnt before and what you wish to remember:

- **X-rays have a very specific interaction with matter (chemical sensitivity).**
- **Synchrotron radiation and free electron laser sources are energy-tunabie necessary to access the chemical sensitivity of X-rays.**
- **There exists a manifold of different optics to focus X-rays, in some cases down to the diffraction limit of a few 10 nm.**
- **The diffraction limited lateral (or optical) resolution scales with photon energy or inverse with the wavelengths of the light used.**

Conrad Wilhelm Roentgen

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

Why? …



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

For example: Al lens with R=500μm, E=10keV, δ=5.46 10^{-6}: **f = 92m**

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

C. W. Roentgen

“Wilhelm Conrad Roentgen was not the typical physicist. He never received his high school diploma, was a notoriously scatter-brained professor, and lived most of his life in seclusion. However, his unique background did not hinder him from making one of the most important discoveries of the 19th century, even if it was by accident...

...In his personal life, he was also a sort of eccentric. His wife was chronically ill and Roentgen, who took care of her, also remained isolated from an active social life. Nevertheless, he lived a quiet life at home, where he was an avid photographer, and at the University of Wuerzburg where he was the head of the physics department. Although his teaching style was unpopular, he was especially gifted at creating effective lab experiments with astounding results...”

Extracted from http://www.umw.edu.

Transmission X-ray microscopy

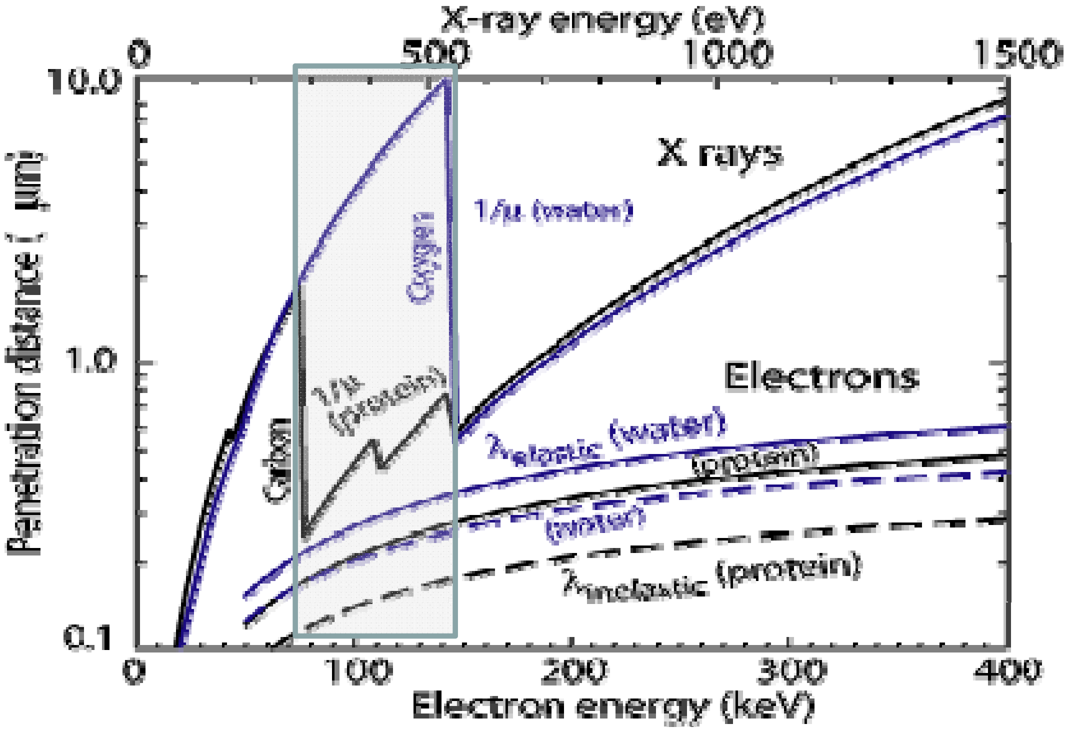
"It would be a big improvement on microscopes using light or electrons, for X-rays combine short wavelengths, giving fine resolution, and penetration. The main problems standing in the way have now been solved."

P. Kirkpatrick, 1948

- Orders of magnitude higher penetration power of X-rays compared to charged particles
- Elemental, chemical and magnetic sensitivity
- Imaging contrasts as for other microscopies
- Imaging in solid or liquid environment

Transmission X-ray microscopy

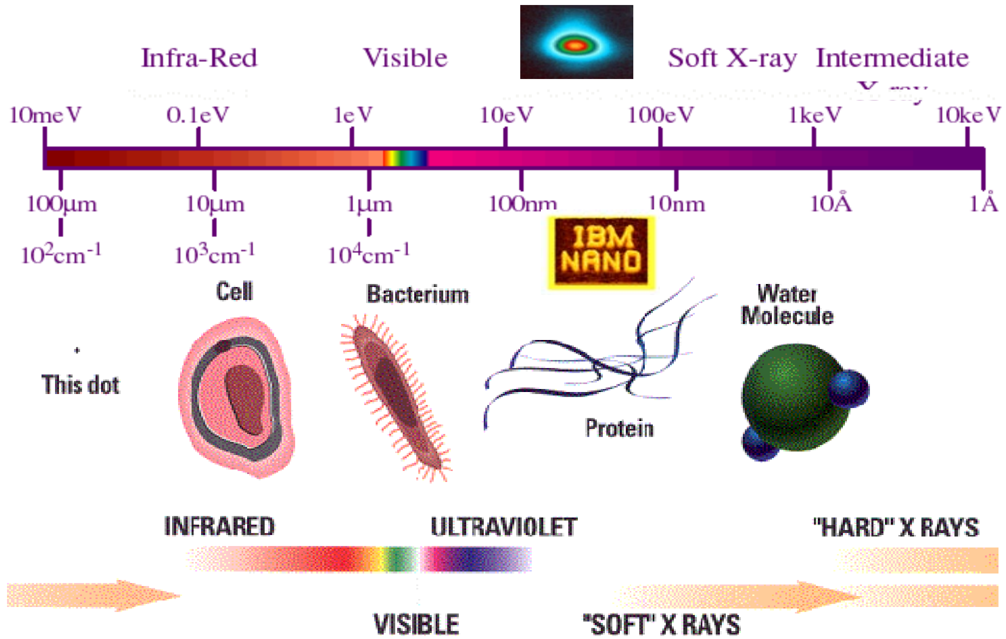
The "Wolter window": A natural contrast for high-resolution imaging



H. Wolter, Annal. Phys. 10, 94-114 (1952)

- **Orders of magnitude higher penetration power of X-rays compared to charged particles**
- **Elemental, chemical and magnetic sensitivity**
- **Imaging contrasts as for other microscopies**
- **Imaging in solid or liquid environment**

Optical resolution scales with the light wavelength



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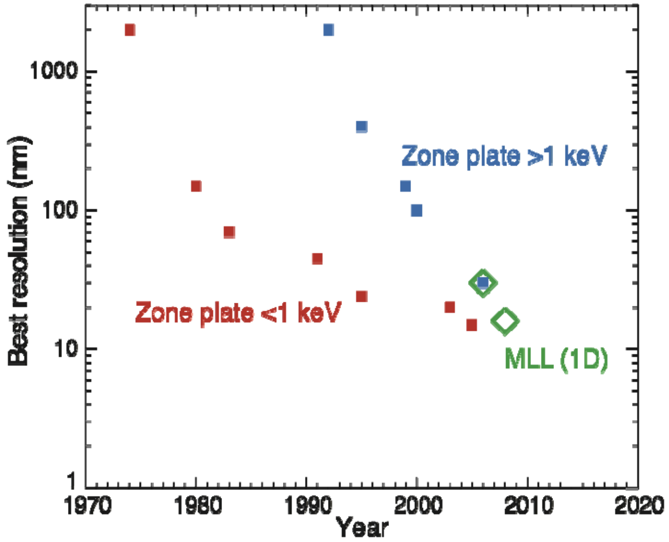
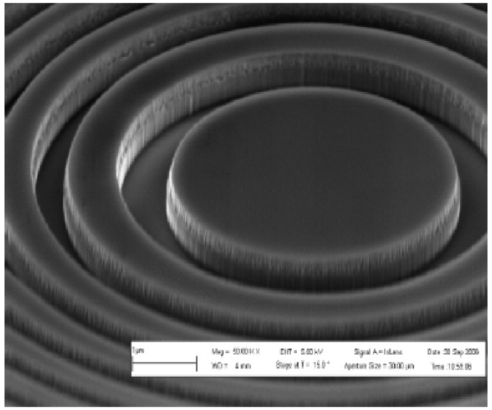
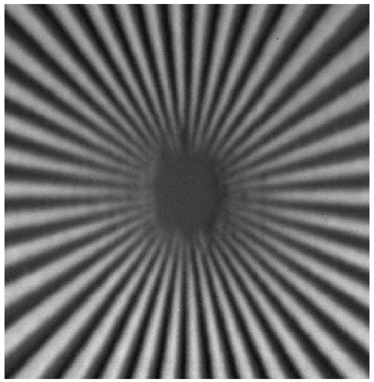
Development in optical resolution of focusing diffractive optics (zone plates)







- **Orders of magnitude higher penetration power of X-rays compared to charged particles**
- **Elemental, chemical and magnetic sensitivity**
- **Imaging contrasts as for other microscopies**

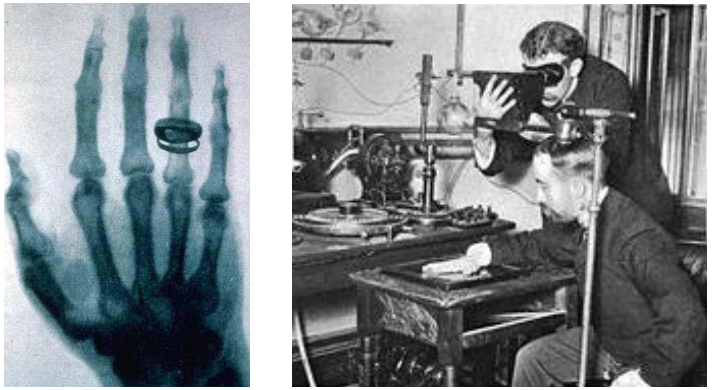
- **Imaging in solid or liquid environment**
- **Realistic optical resolution achievable by XRM is not better than 30 nm**

Different X-ray microscopy techniques:

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

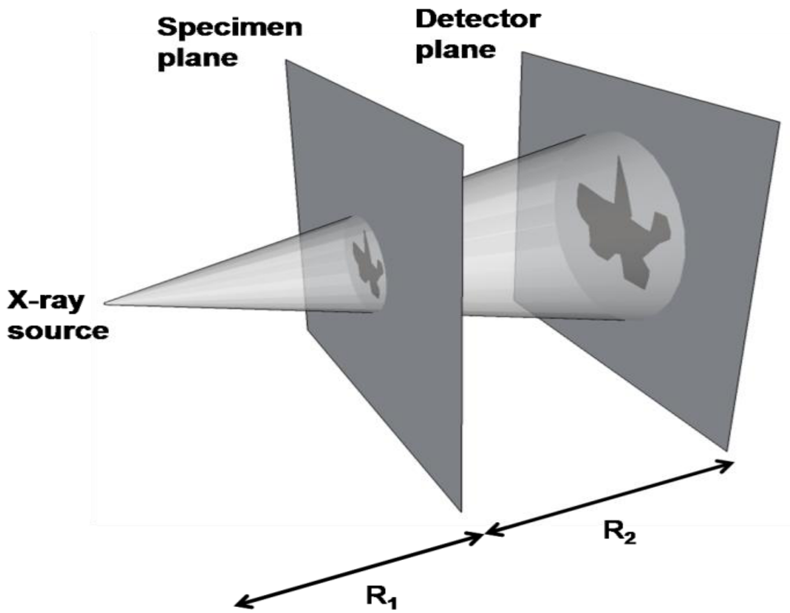
We will try to explore now different transmission XRM techniques...

The classic and most common: X-ray projection imaging



Old Crooke's apparatus

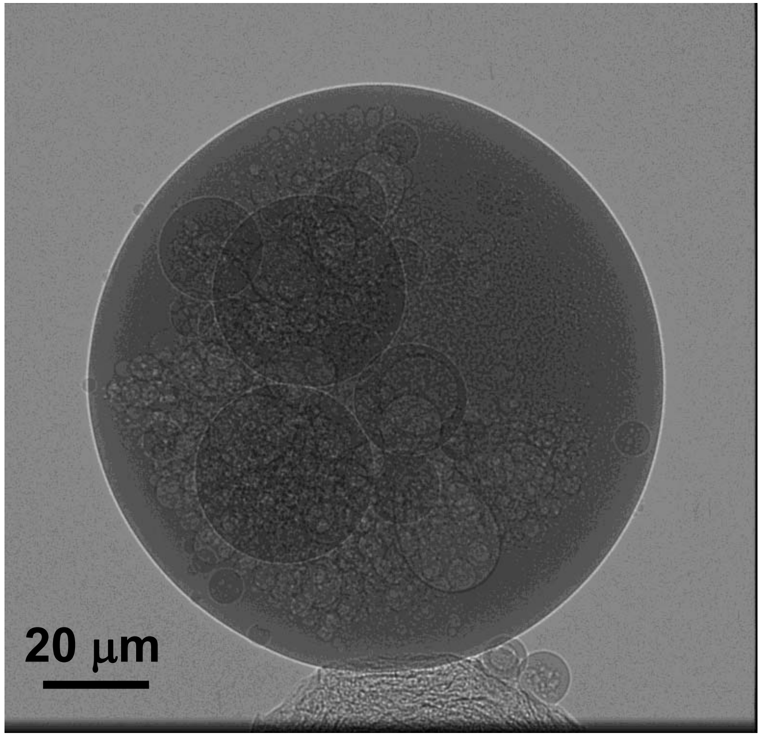
Modern digital radiograph

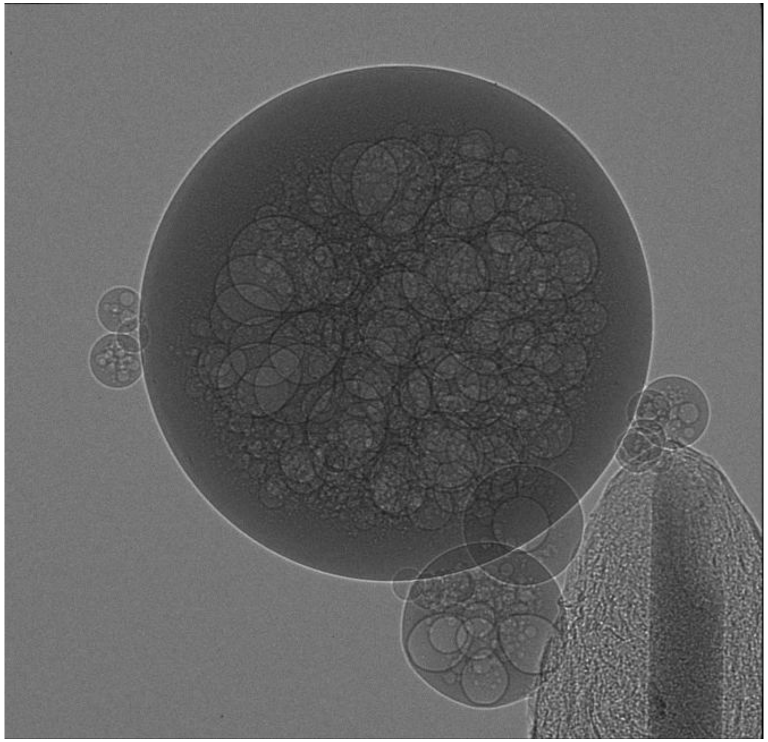


The magnification M is given by M=R1/R2, the optical resolution is determined by $\Delta=(\lambda a)^{1/2}$

Problem: Fresnel diffraction

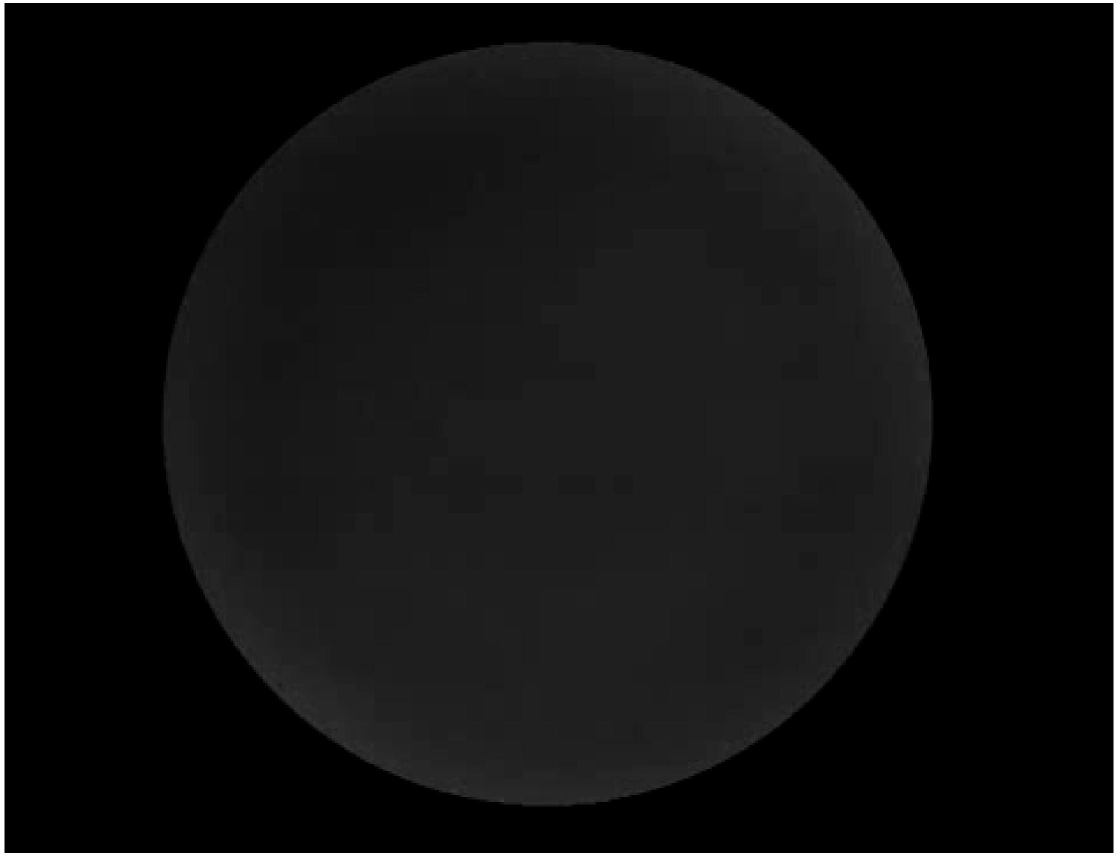
The classic and most common: X-ray projection imaging





3D phase contrast dataset view, reconstructed cross section of part of a ceramisphere, acquired with the projection x-ray microscope at the CSIRO, Australia. The total collection time of the microtomogram was 10 h . Mayo,S.C., Miller,P.R., Wilkins,S.W., Gao,D., and Gureyev,T.E. Laboratory based x-ray microtomography with sub-micron resolution. SPIE Proceedings - Optics & Photonics Conference. Developments in x-ray tomography V, San Diego, 2006.

The classic and most common: X-ray projection imaging



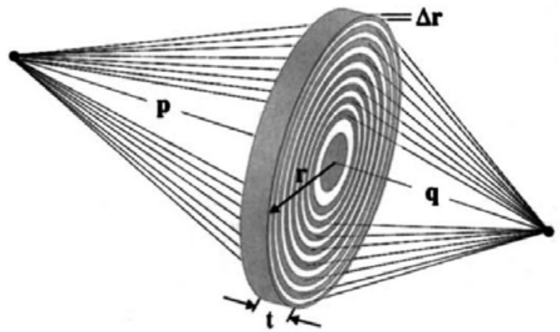
“... phat to flat in a snowboard comp, cause L2 vertebrate to "explode"(medical term). The good Doctor reconstructed it with bone from my pelvis...”

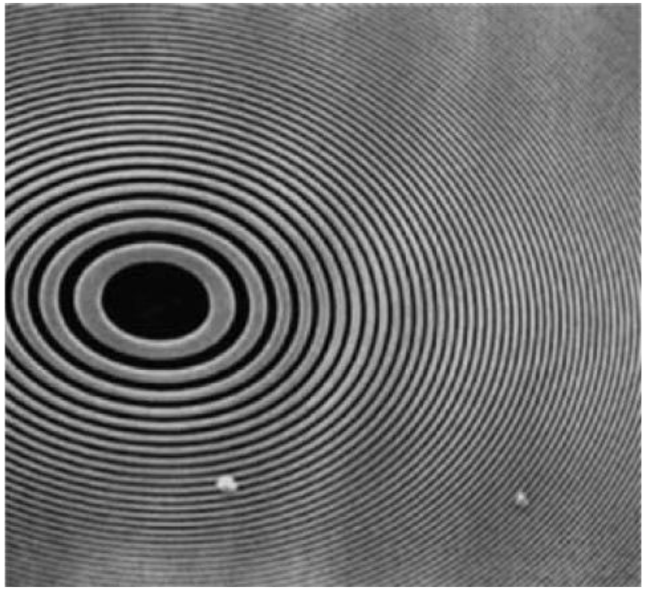
YouTube source:

http://www.youtube.com/watch?v=tkj5ZIAF4SY

Now:
Transmission X-ray microscopy techniques with focusing optics

A reminder towards X-ray microscopy: The optics?





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

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

Lateral resolution of a ZP (Rayleigh):

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

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

Diffraction efficiency of a ZP is the portion of the light diffracted in one diffraction order normalized by the incident light, here 1st order

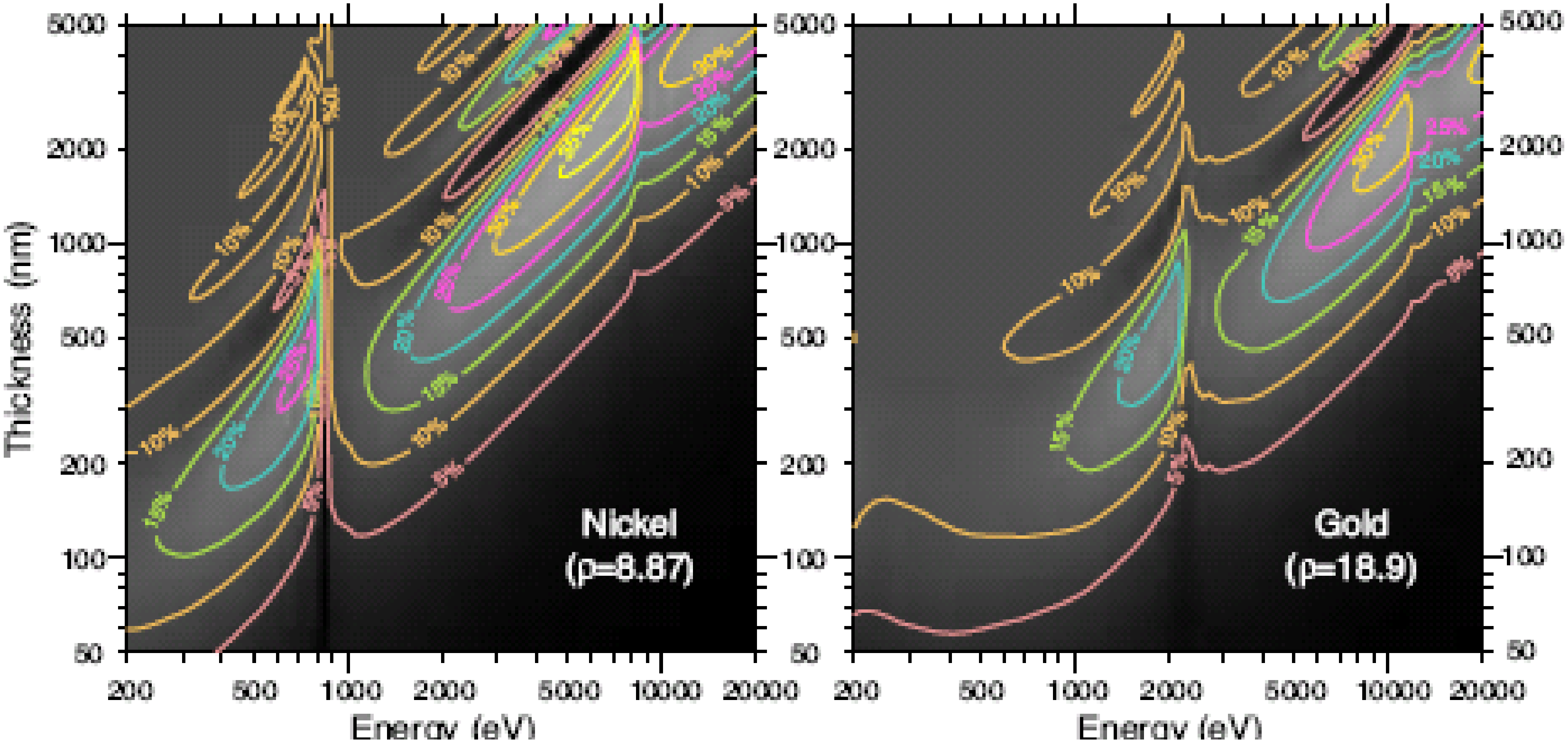
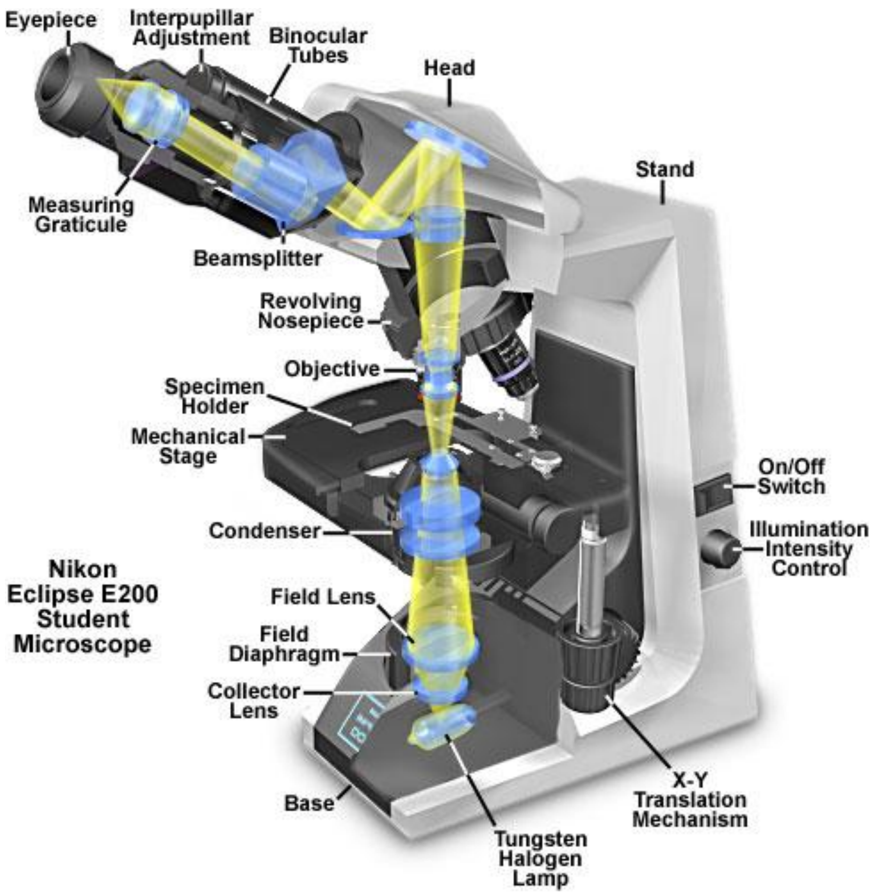


Image courtesy: M. Howells, ALS, LBL, Berkeley, US

X-ray full-field imaging ?!!!



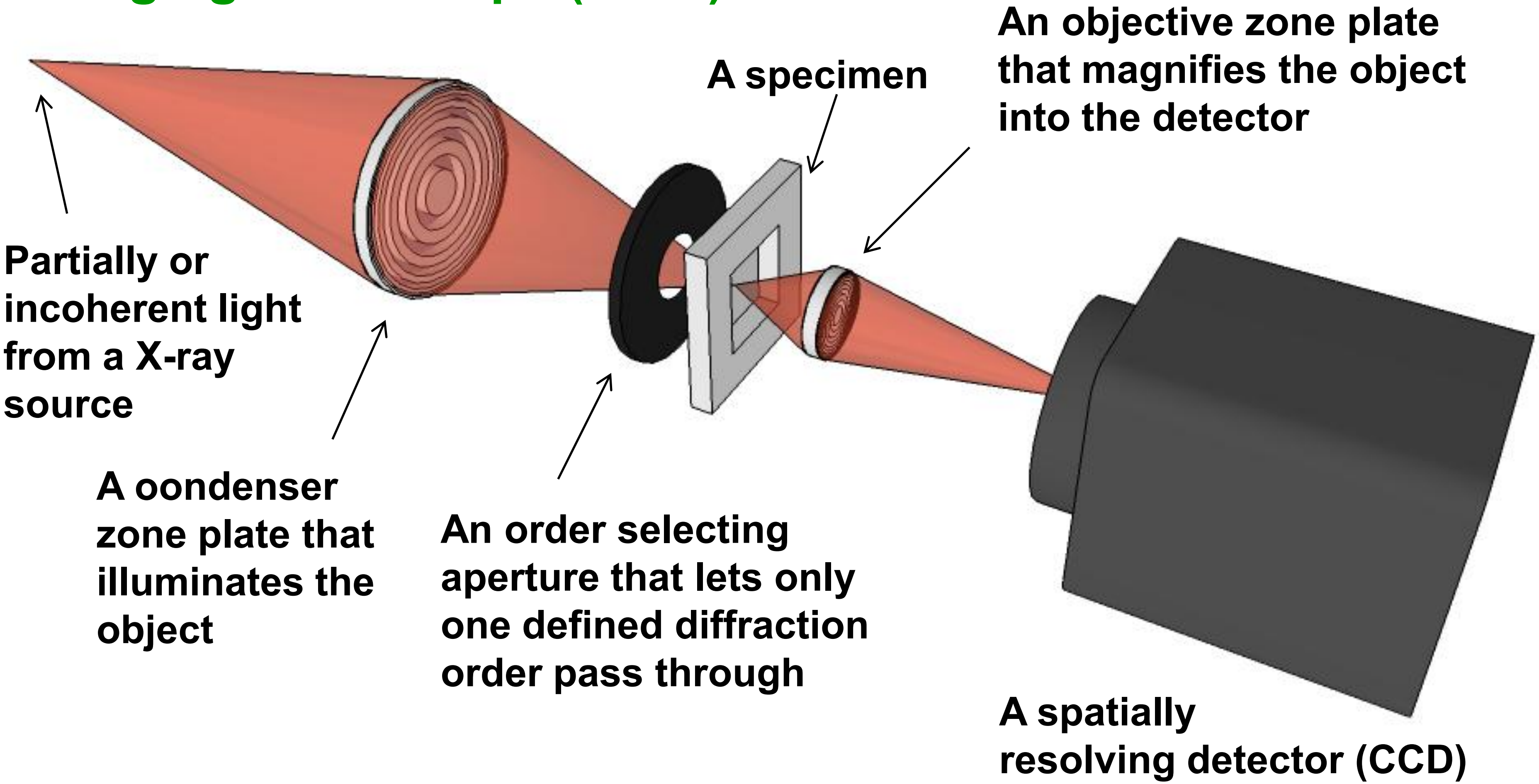
Cutaway diagram of a visible light microscope. From: http://micro.magnet.fsu.edu/primer/

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

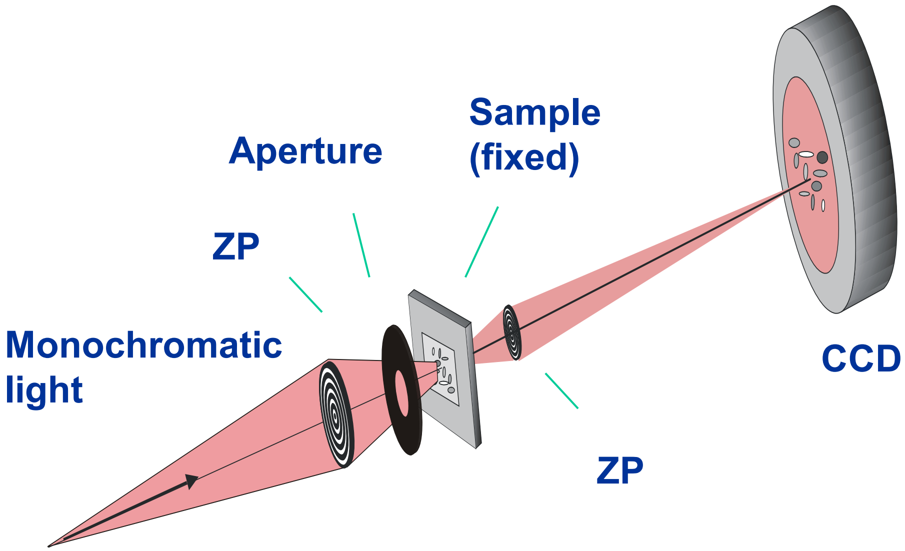
We do need ?:

- a suited condenser system
- a specimen stage (automated)
- an eyepiece/ camera

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



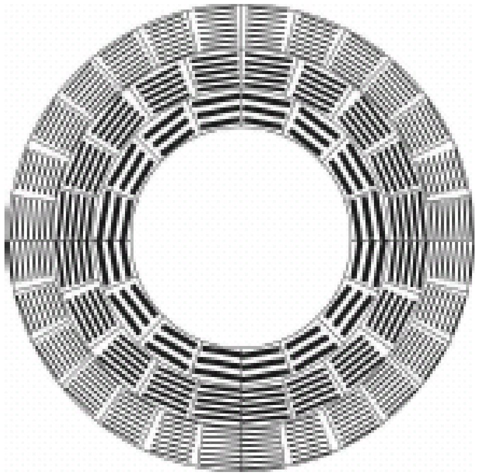
The transmission X-ray microscope (TXM) or full-field imaging microscope (FFIM)



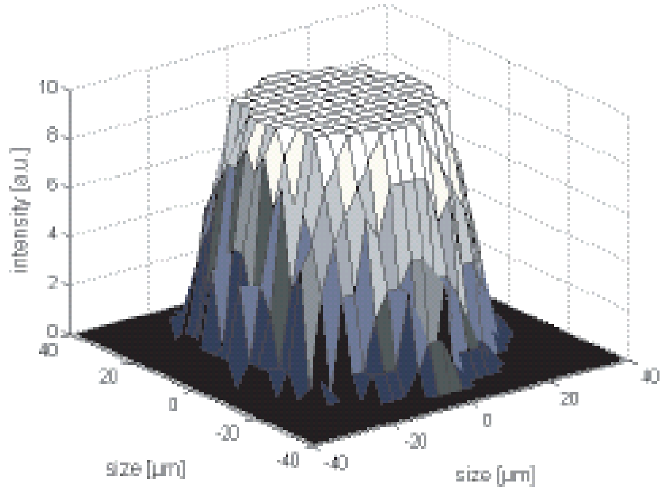
- Best spatial resolution
- Modest spectral resolution
- Shortest exposure time
- Bending magnet radiation
- Higher radiation dose
- Flexible sample environment (wet, cryo, labeled magnetic fields, electric fields, cement, ...)

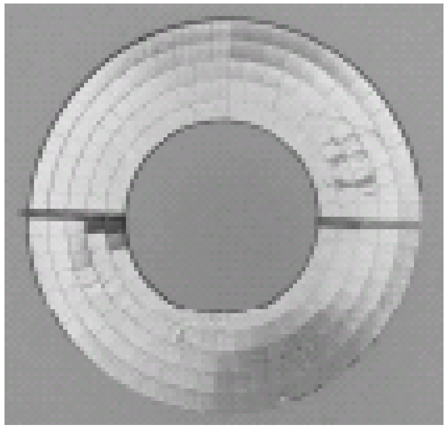
When the detector is matched to the magnification, the lateral or optical resolution is given by the diffraction limit of the objective lens

The condenser illumination in FFIM imaging



A condenser ZP for Koehler-like illumination



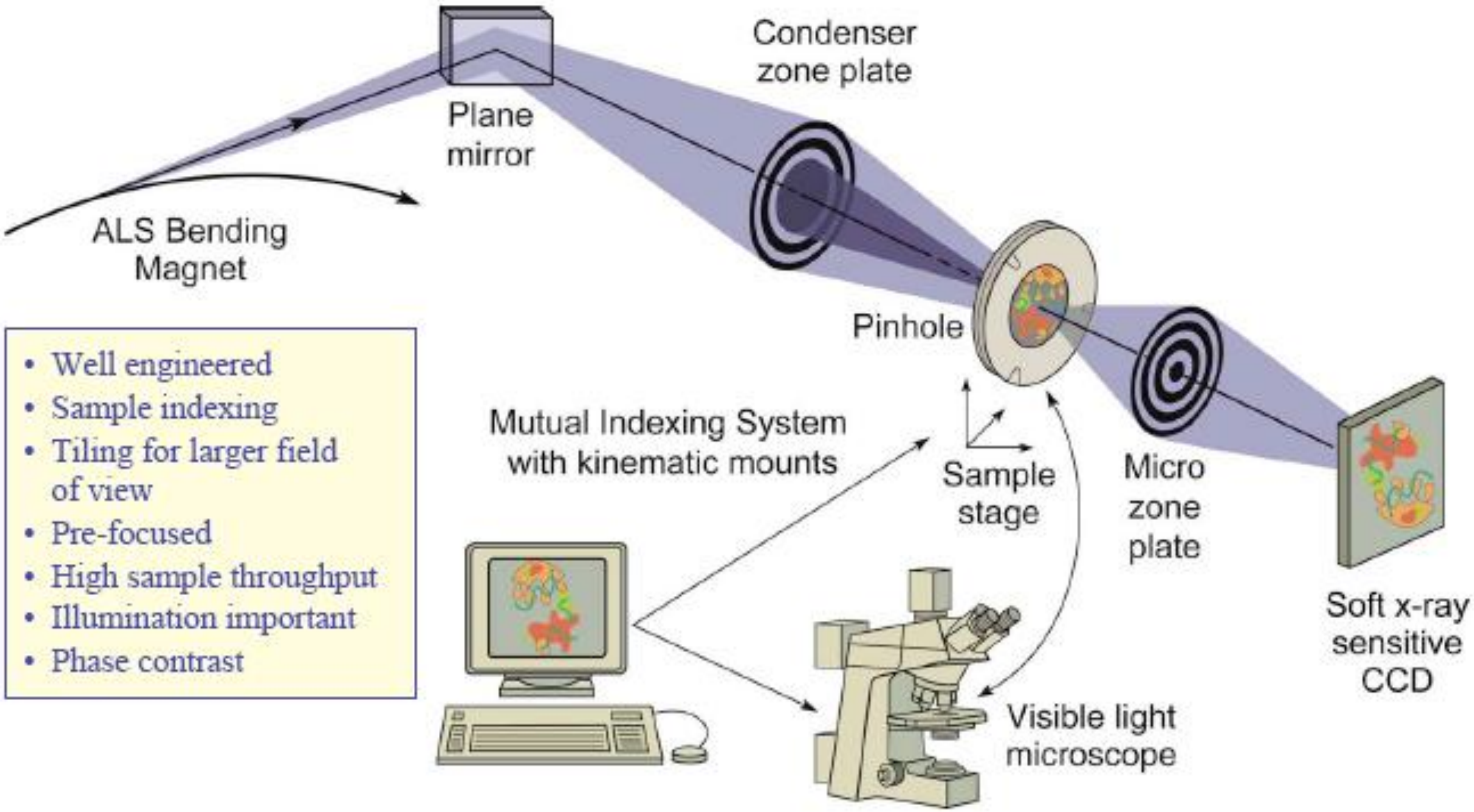


Optic fabricated by ZonePlates.com

Conventional ZP with zones is replaced by concentric array of gratings; each grating diffracts light into the object plane and decreases the coherence

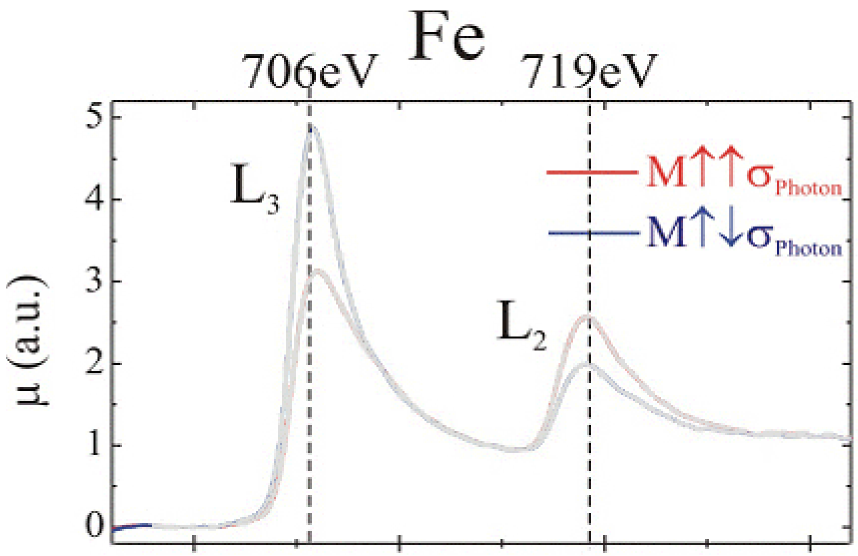
U. Vogt et al., Optics Letters 31, 1465-1467 (2006)

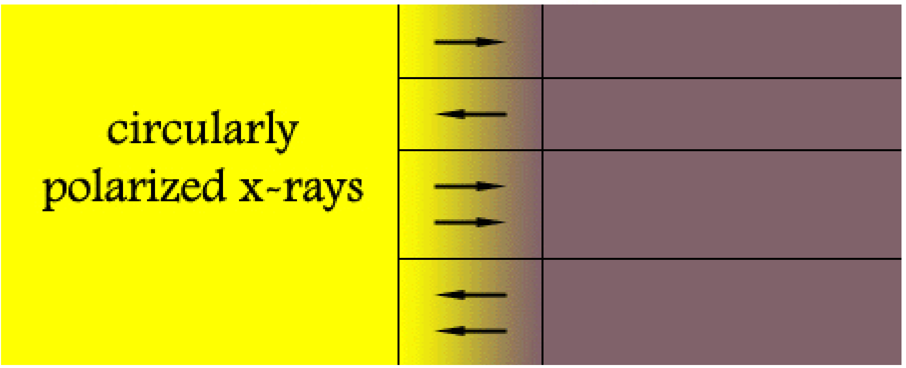
The XM-1 microscope at the ALS, LBL, CA, US:



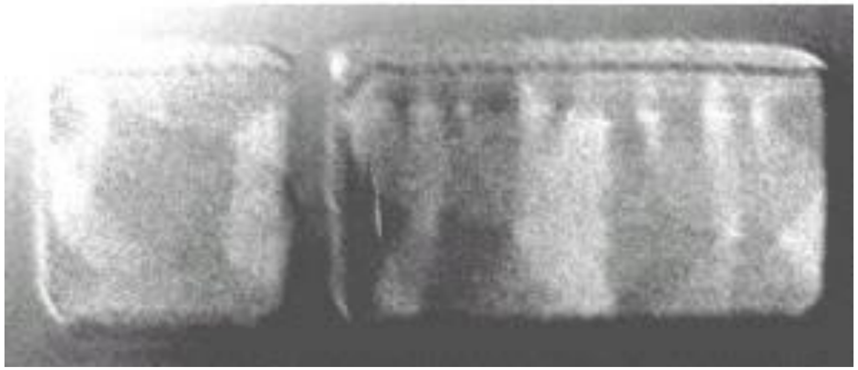
- Well engineered
- Sample indexing
- Tiling for larger field of view
- Pre-focused
- High sample throughput
- Illumination important
- Phase contrast

Magnetic absorption contrast



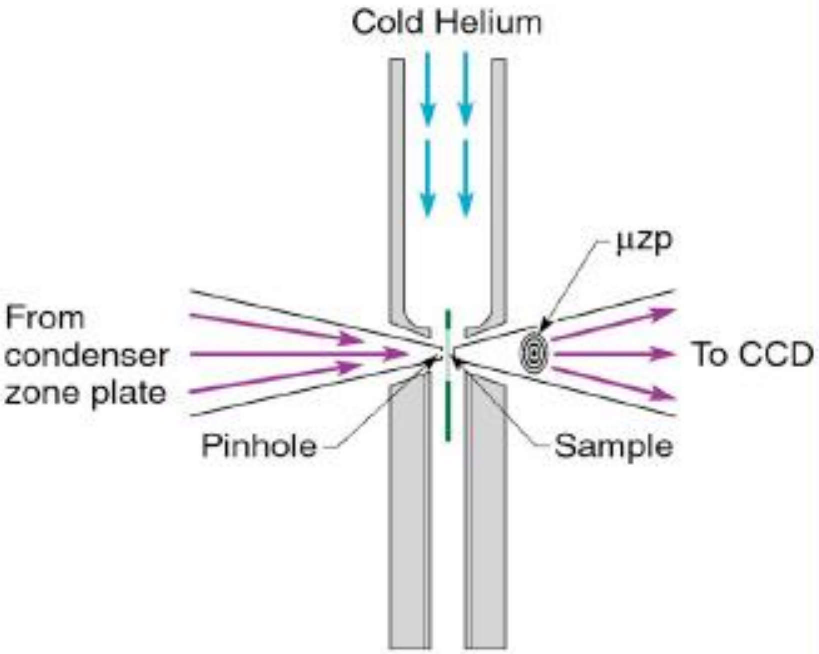


The X-ray absorption coefficient depends strongly on the relative orientation between the helicity of the photons and the projection of the local magnetization onto the photon propagation direction.

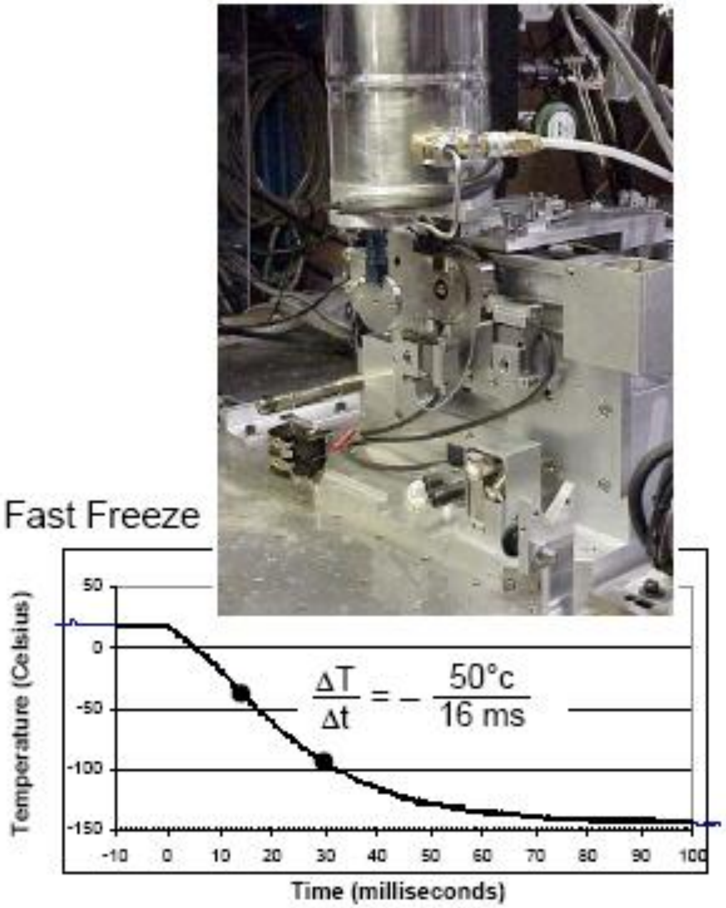


P. Fischer et al., CXRO, Berkeley, US
G. Meier, *et al.* Appl. Phys. Lett. 85(7) (2004) 1193-1195

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



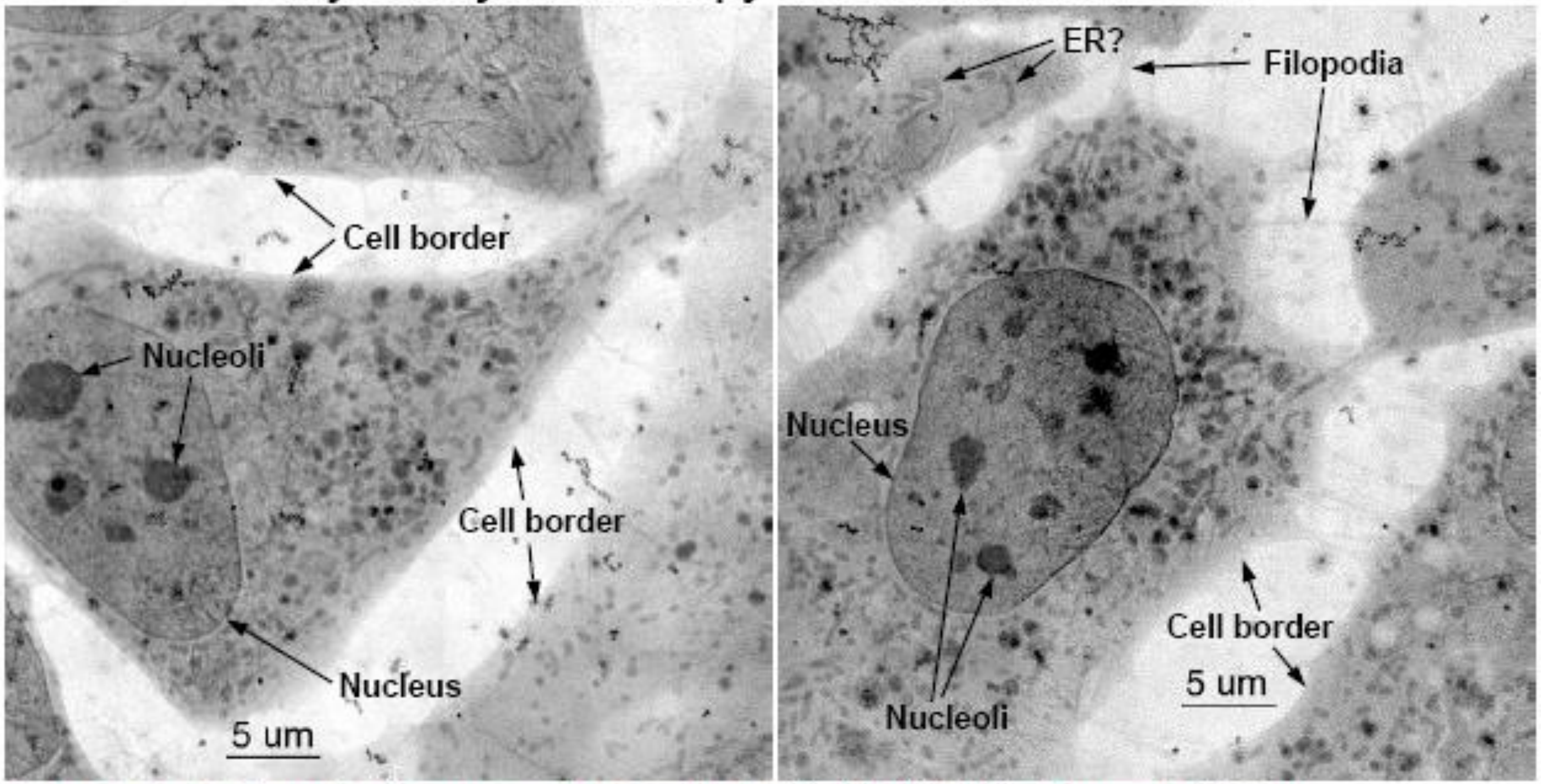
Helium passes through LN, is cooled, and directed onto sample windows



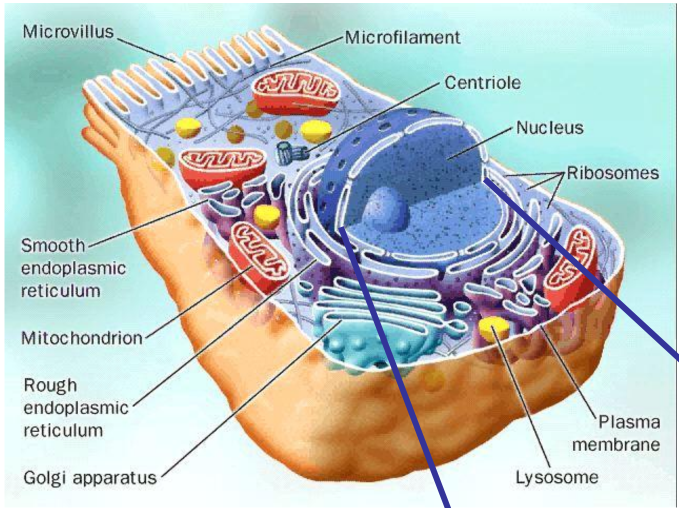
W. Meyer-Ilse, G. Denbeaux, L. Johnson, A. Pearson (CXRO-LBNL)

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

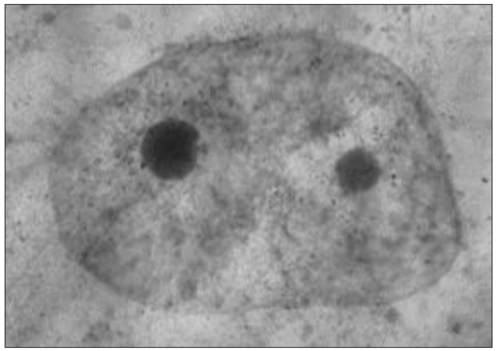
Cryo x-ray microscopy of 3T3 fibroblast cells



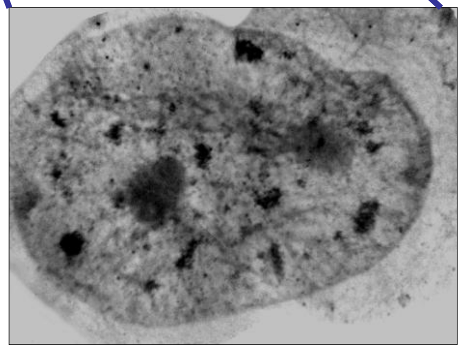
C. Larabell, D. Yager, D. Hamamoto, M. Bissell, T. Shin (LBNL Life Sciences Division)
W. Meyer-Ilse, G. Denbeaux, L. Johnson, A. Pearson (CXRO-LBNL)



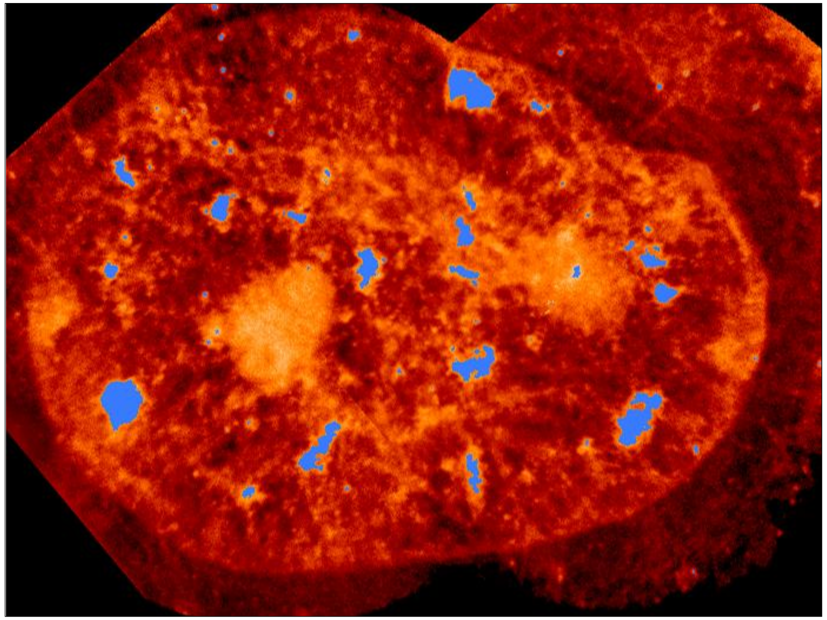
Location of Splicing Factors in whole, hydrated human mammary epithelial cells (ALS, TXM XM1)



Control nucleus, no primary antibody



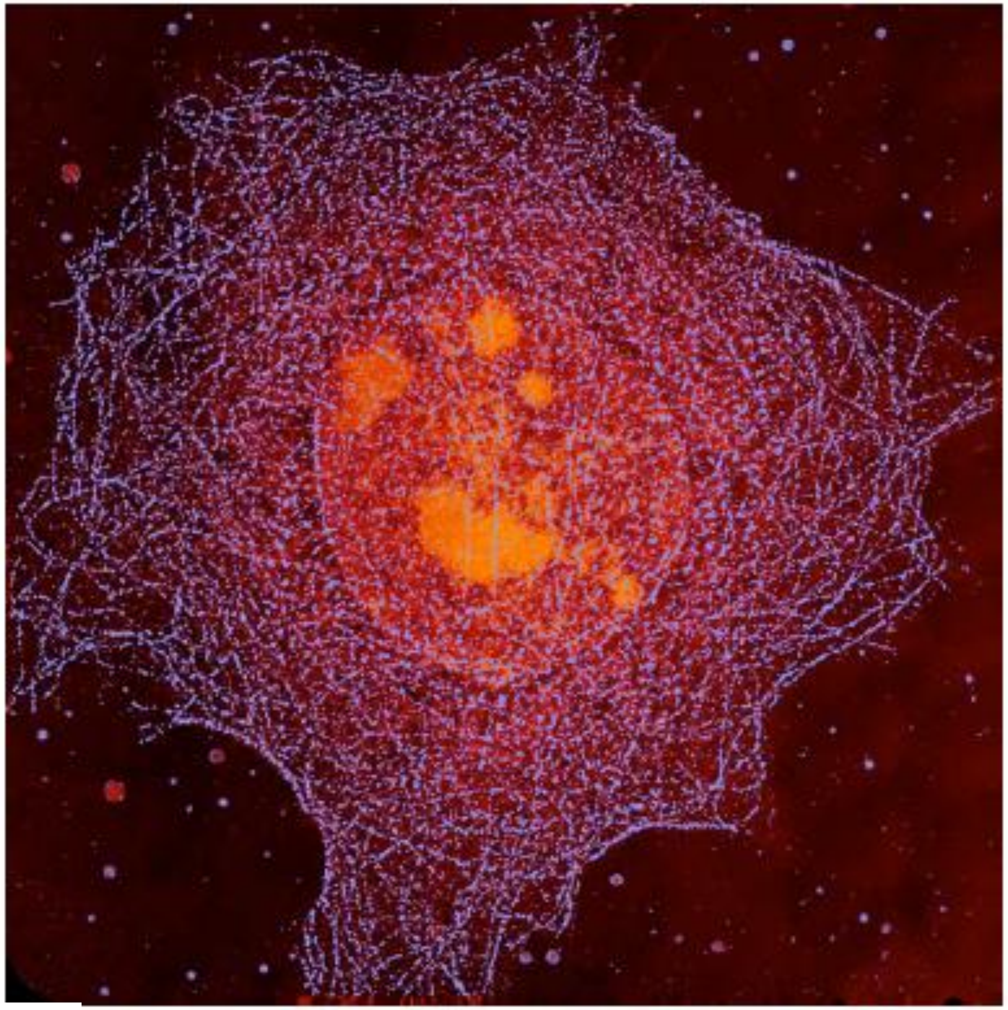
Single nucleus labeled using antibodies specific for splicing factors



Same nucleus, splicing factors colored blue

C. Larabell et al., Live Science Division, LBL, USA

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



Courtesy of C. Larabell and W. Meyer-Ilse (LBNL)

$\hbar w$ = 520 eV

32 µm x 32 µm

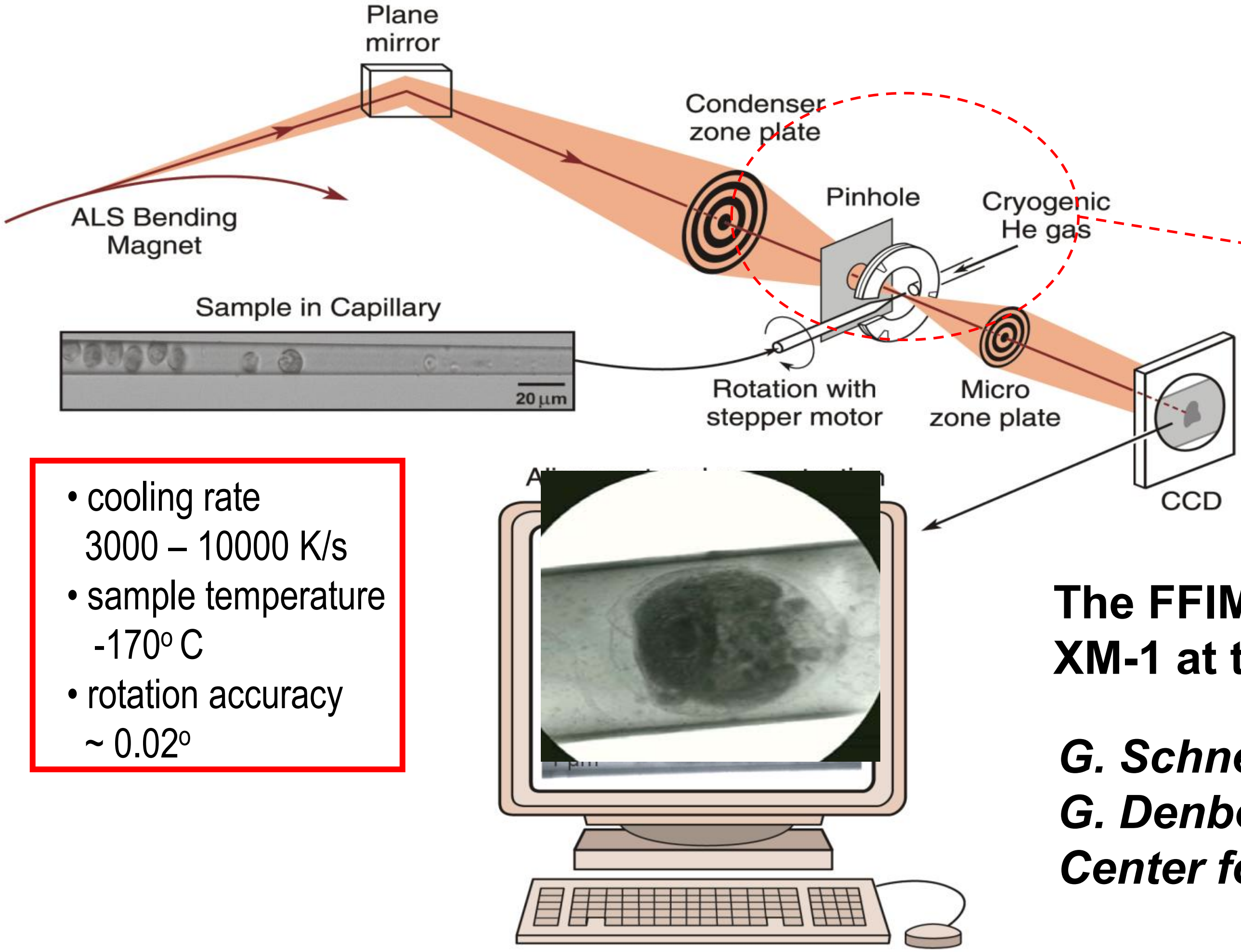
Ag enhanced Au labeling of the microtubule network, color coded blue.

Cell nucleus and nucleoli, moderately absorbing, coded orange.

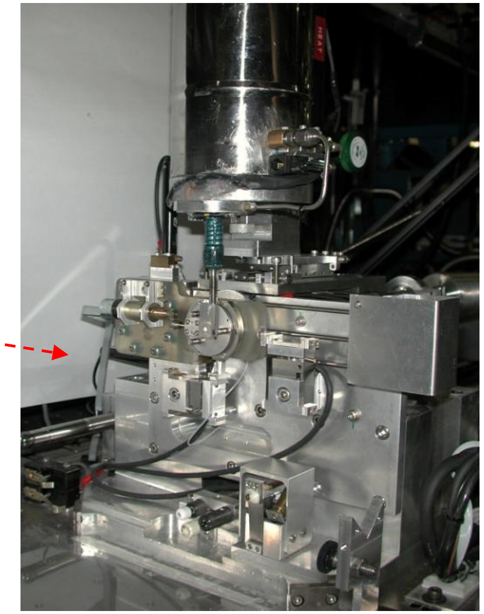
Less absorbing aqueous regions coded black.

W. Meyer-Ilse et al. J. Microsc. 201, 395 (2001)

Tomography with a FFIM



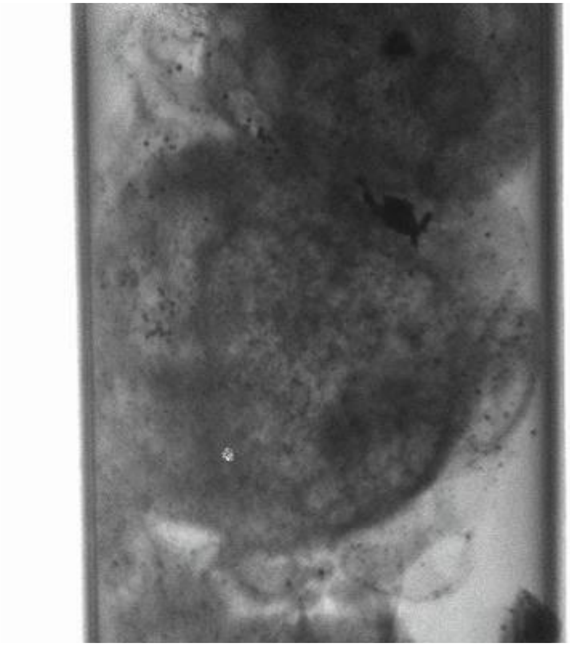
- cooling rate 3000 – 10000 K/s
- sample temperature -170° C
- rotation accuracy ~ 0.02°

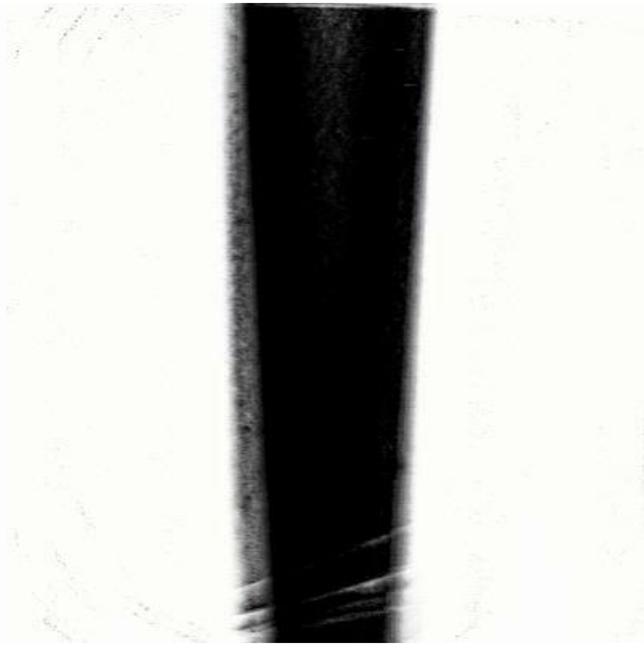


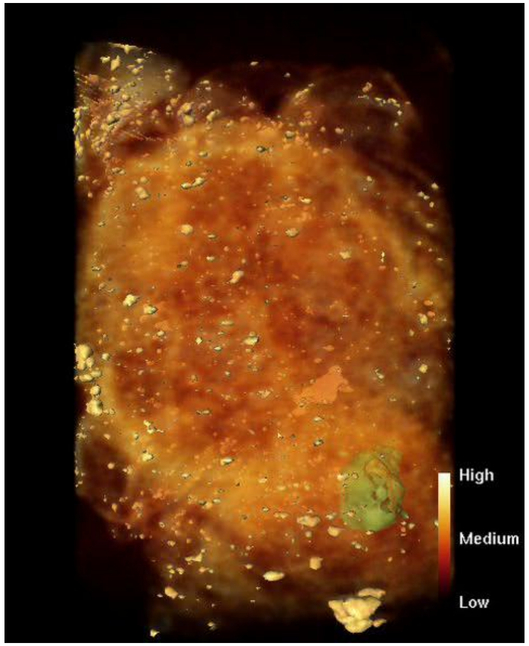
The FFIM X-ray microscope XM-1 at the ALS, CA, USA

G. Schneider, B. Bates, G. Denbeaux, E. Anderson, Center for X-ray Optics, LBL

Tomography with a FFIM







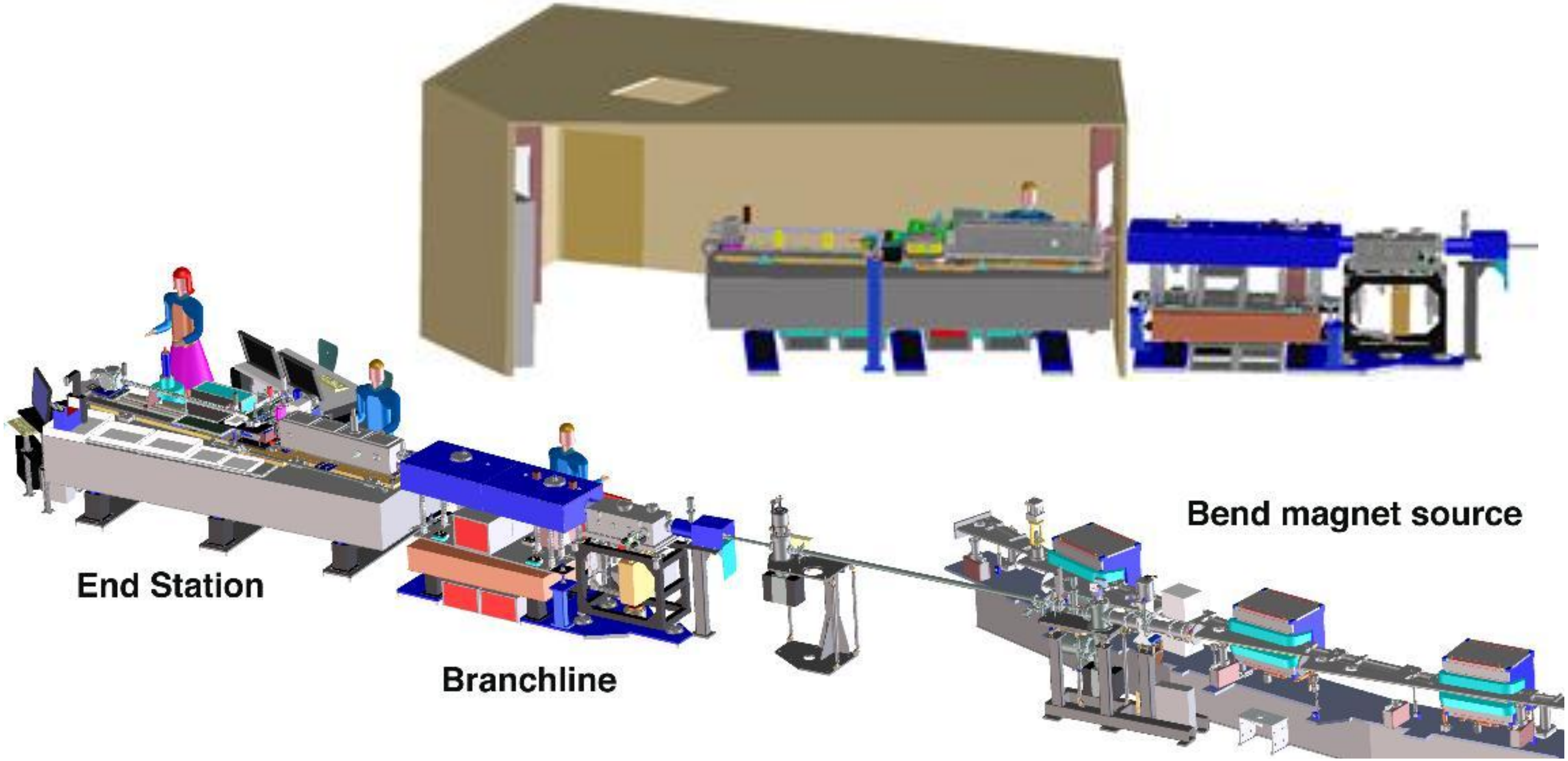
FFIM micrographs of a specimen in the capillary

Reconstructed sections through the volume

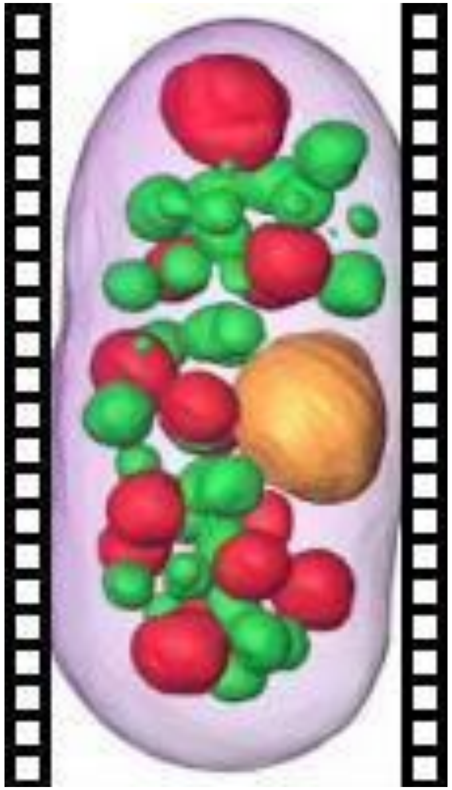
Reconstruction of the absorption coefficient

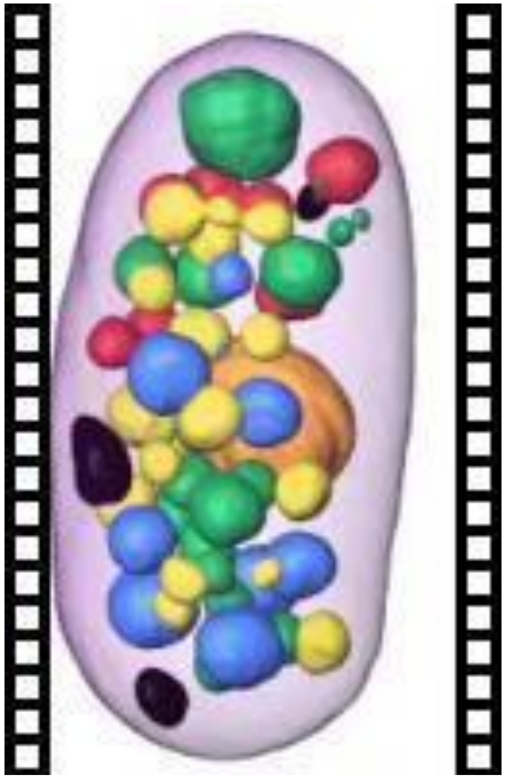
Work performed with the XM1 microscope at the ALS, US (G. Schneider, G. Denbeaux, B. Bates and E. Anderson)

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



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





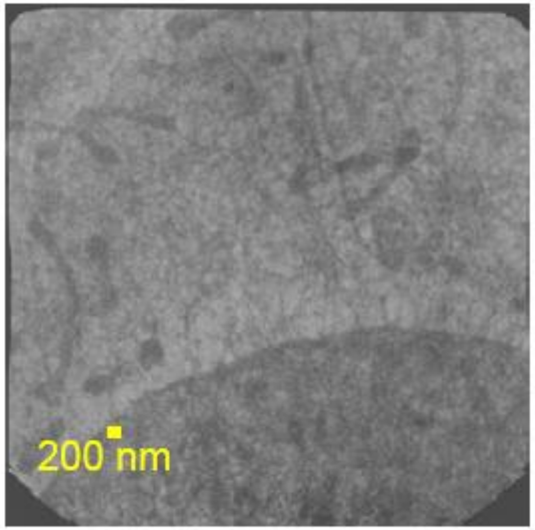
3D reconstructions of S. pombe cells; early stage cell segmentation (left) and early stage mitochondria and vacuoles (right)

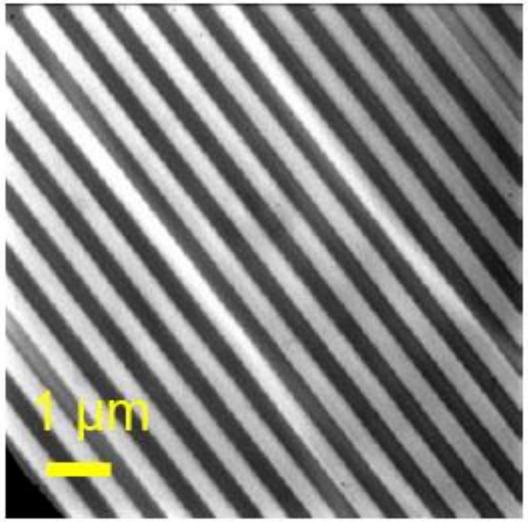
Visit http://ncxt.lbl.gov to see the movies

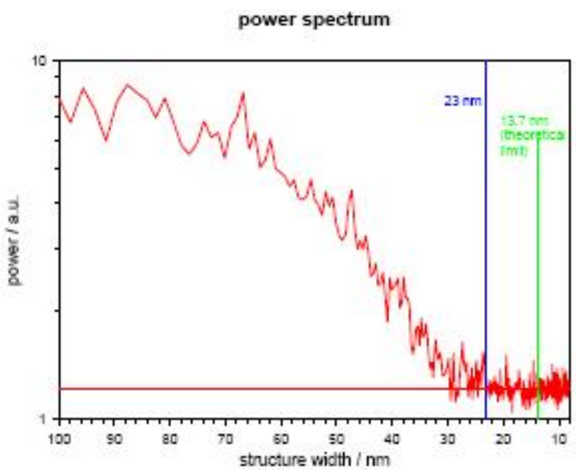
The full-field imaging microscope at BESSY II, Berlin, Germany:

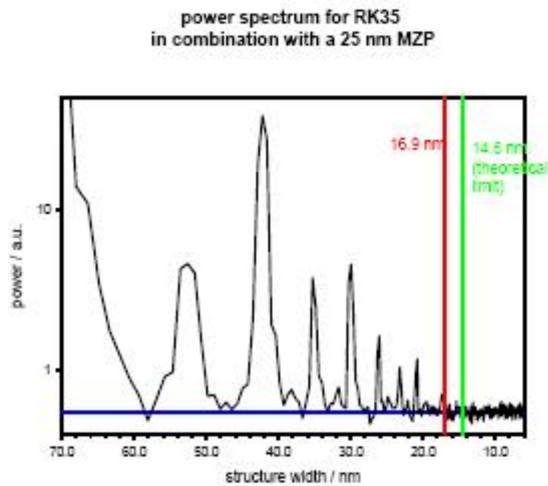
http://www.bessy.de

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

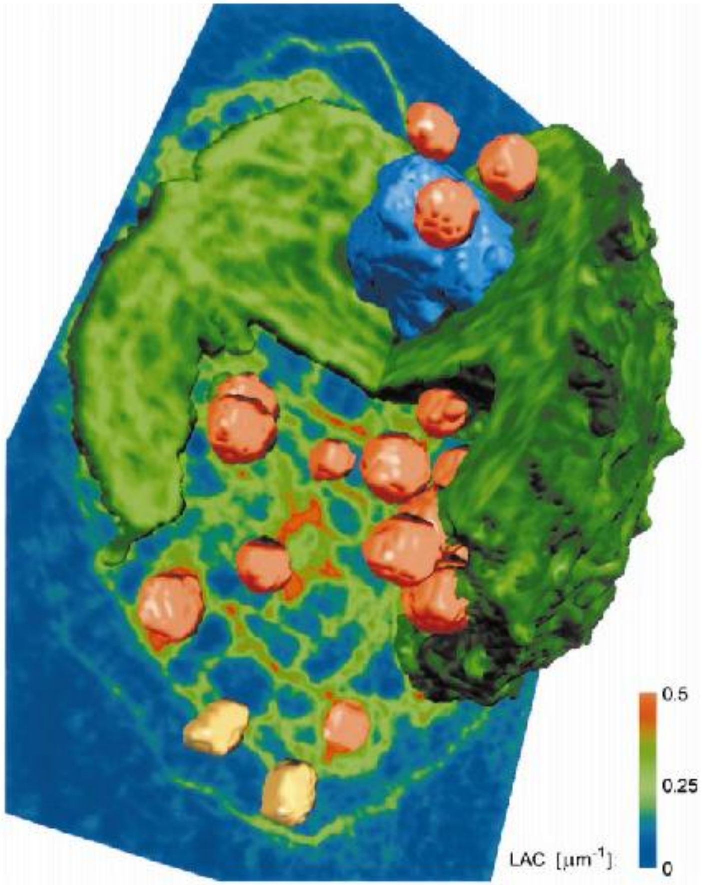








Transmission X-ray microscopy



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

Alga: Chlamydomonas reinhardtii

Acquired with the full-field imaging Microscope at BESSY I

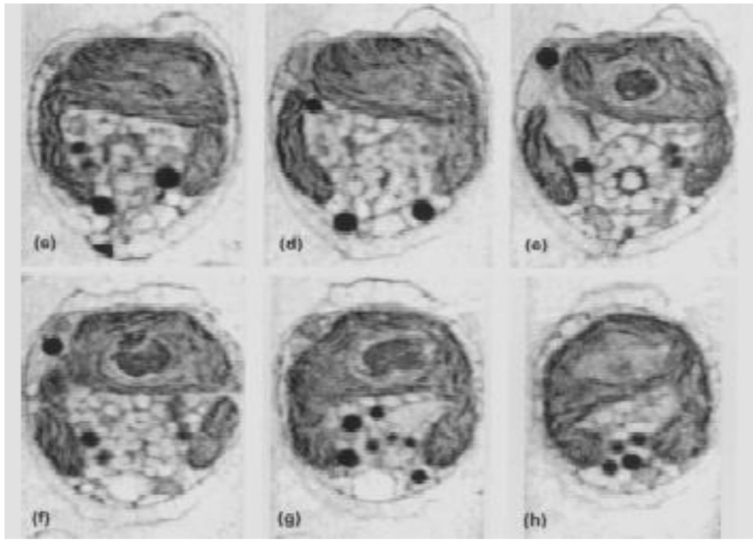
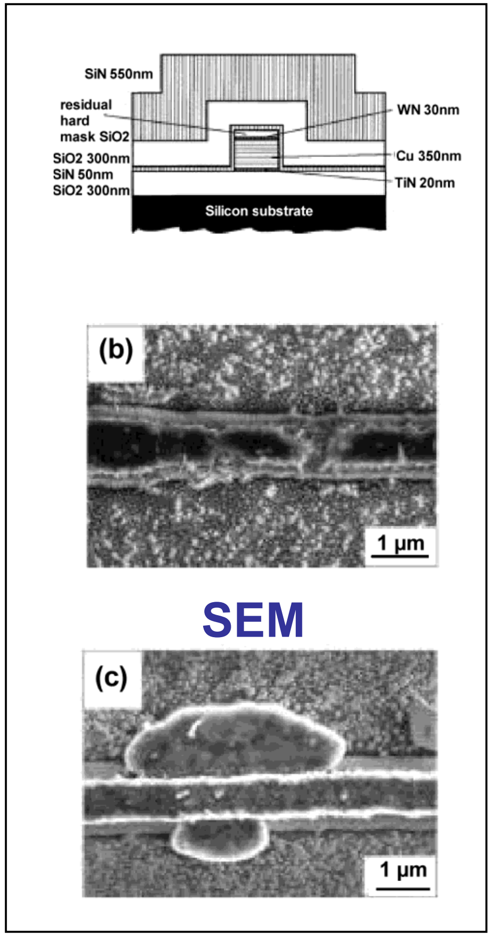
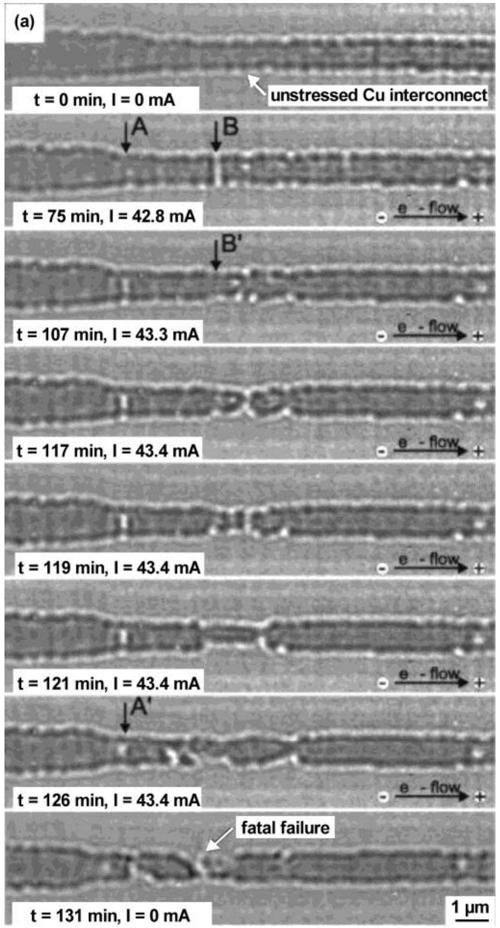


Image courtesy: D. Weiss et al., BESSY, D

Material sciences with a TXM: Electromigration in modern Cu interconnects





Non-destructive study of the mass transport with a full-field imaging microscope @ 4 keV (ESRF ID21)

NIST test structure ASTM F 1259M-96

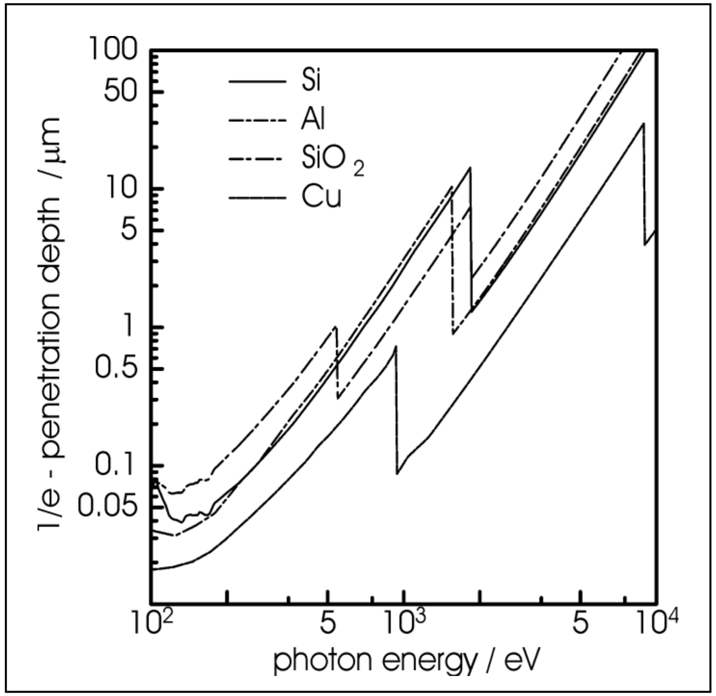
Current density up to: 2 x 10^7 A/cm^2

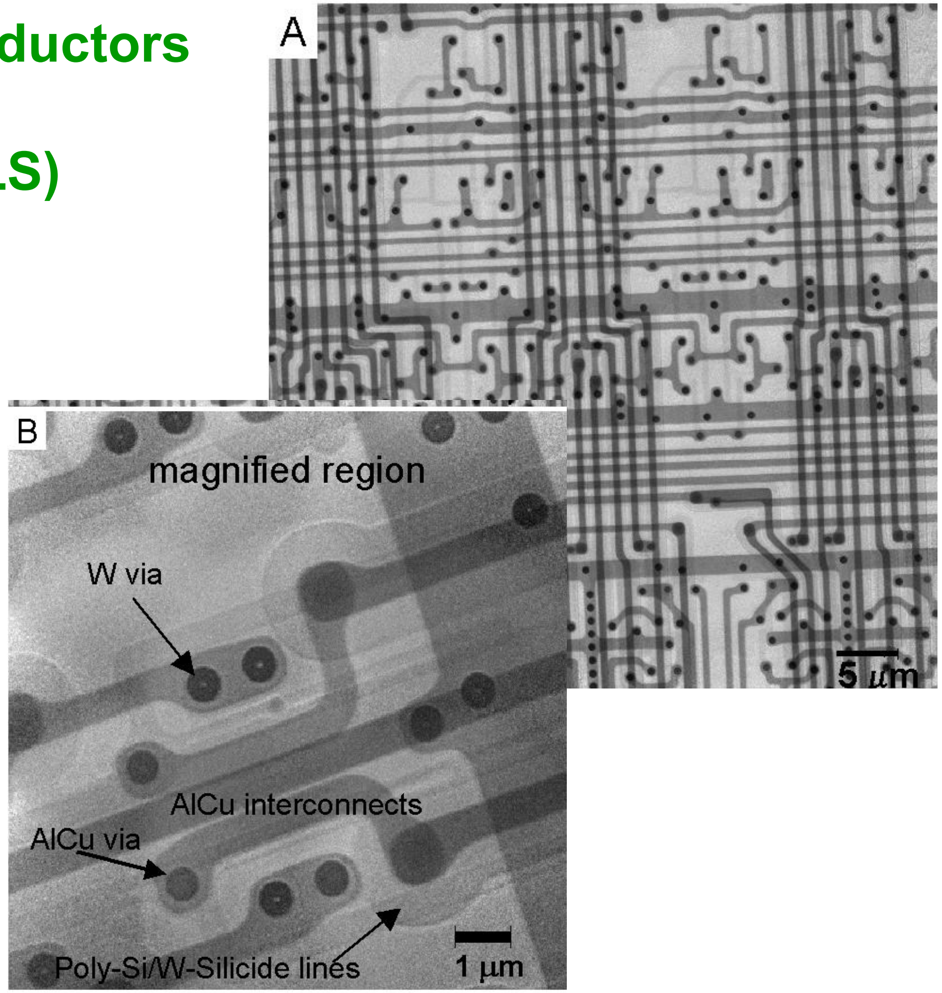
Series of 200 images during 2 h

G. Schneider et al., BESSY,D

Characterization of morphology and defects in modern semiconductors with a full-field imaging microscope (@ 1.8 keV, XM1/ ALS)

Sample preparation:
Back side thinning of Si wafer

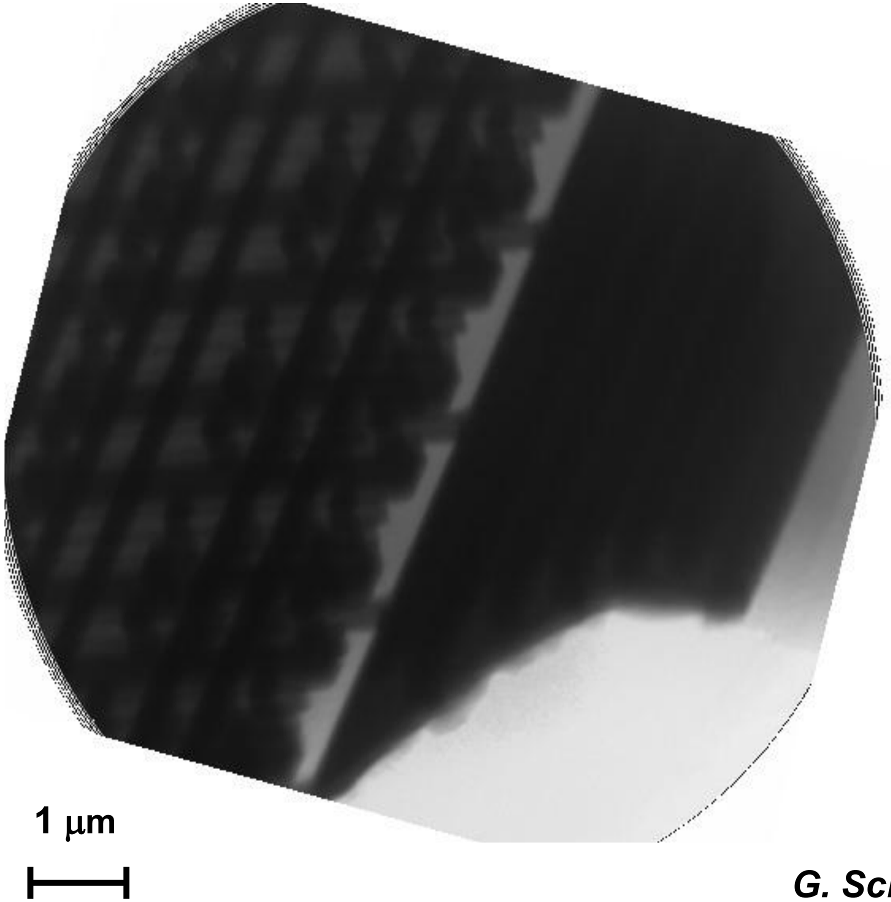




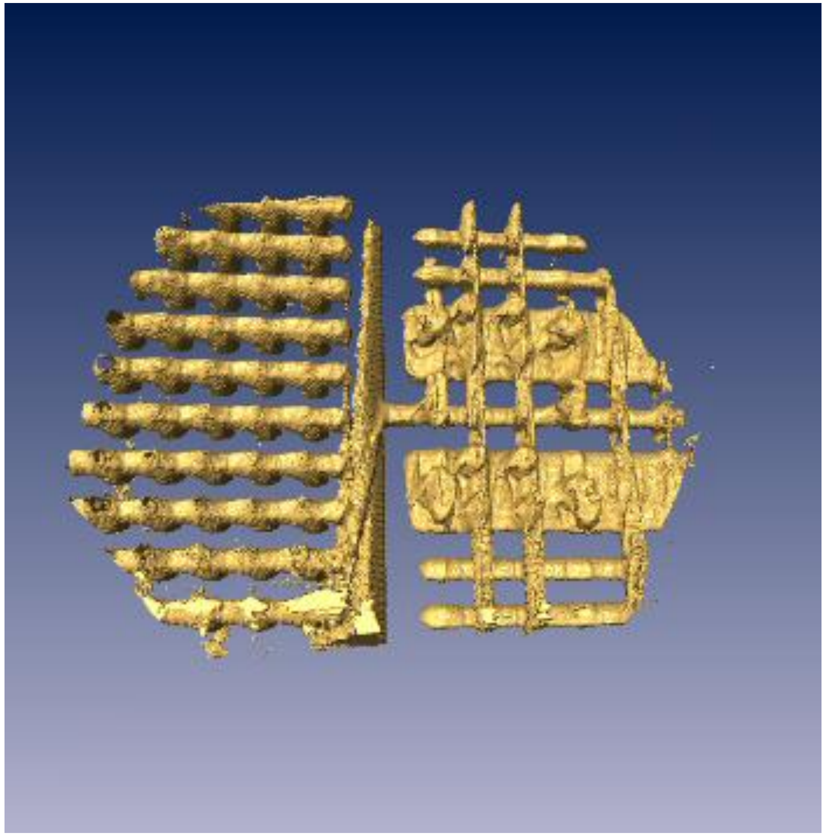
G. Schneider et al., BESSY II

Computed tomography of Cu interconnects with the BESSY TXM

High resolution images of ICs after alignment (50 projections, 140°)



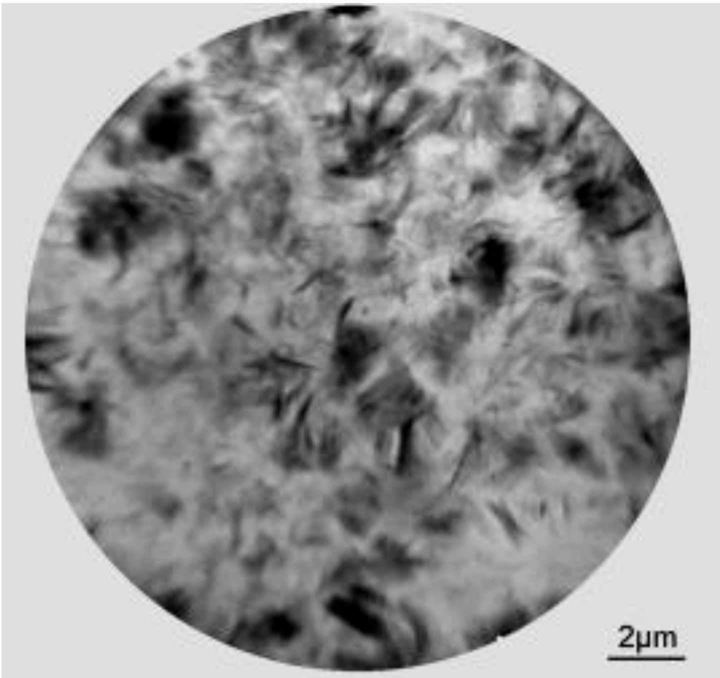
3D reconstruction

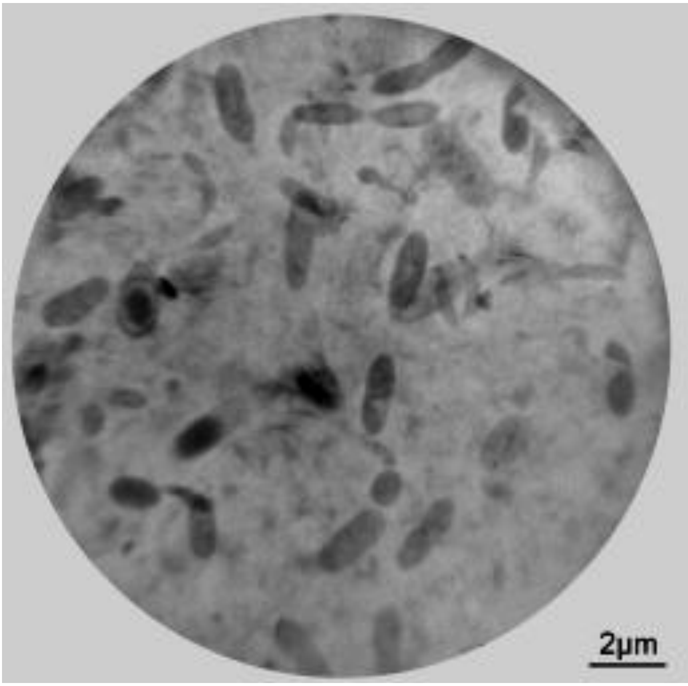


G. Schneider et al., J. Vac. Sci. Technol. B 20, 3089 (2002)

Environmental science: Imaging in liquids

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

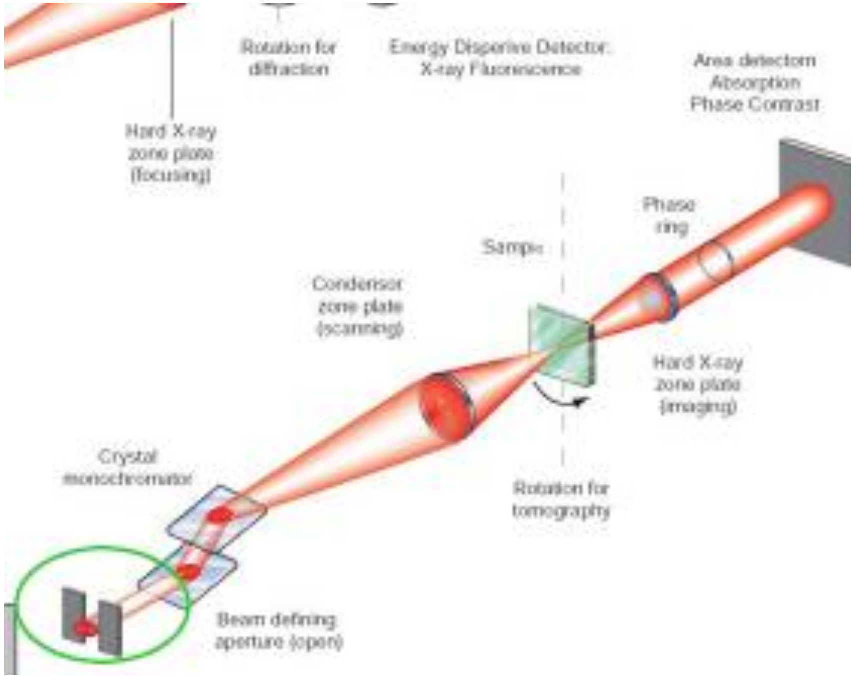




X-ray images acquired with the full-field imaging microscope at BESSY I @ 520 eV

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

Hard X-ray Nanoprobe beamline at the Center for Nanoscale Materials of the Argonne National Lab, Chicago, US



Photon energy range: 3 – 30 keV

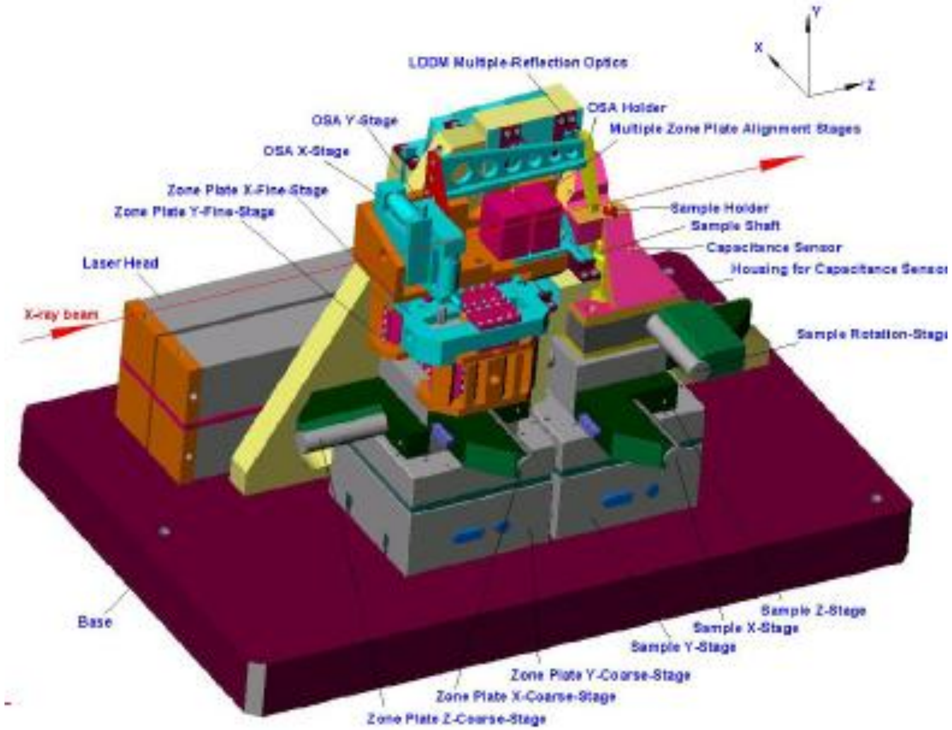


Image courtesy: J. Maser

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?

Definition of contrast

- Contrast is not an inherent property of the specimen, but is dependent upon interaction of the specimen with light AND the efficiency of the optical system to record the image to the detector

- Human eye needs at least about 2% image contrast to distinguish between image and background

- Values might vary for other detectors

- With each detector, the signal to noise ratio must be large enough to be interpreted in terms of the formation of an image

Definition of contrast

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

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

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

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

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

$\delta(\lambda)$: Phase sensitive
$\beta(\lambda)$: Absorption …
n_a: average atom density
r_e: classical electron radius
f_1, f_2: atomic form factors

⇩

Scattering, refraction:

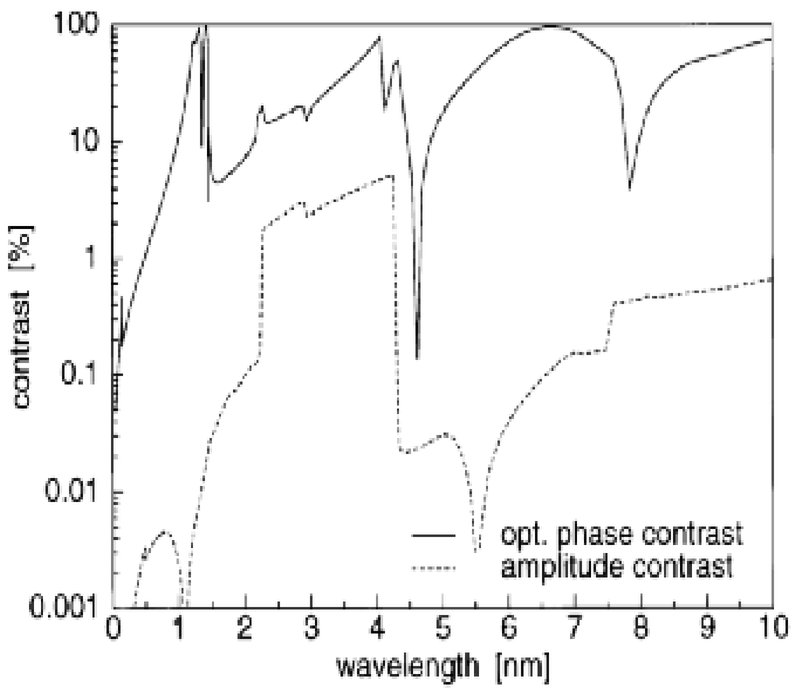
- Zernike phase contrast
- Differential phase contrast
- Differential interference contrast
- Dark-field imaging
- Magnetic phase contrast

⇩

Absorption:

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

Absorption versa phase contrast techniques



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

Courtesy of G. Schneider et al. BESSY, D

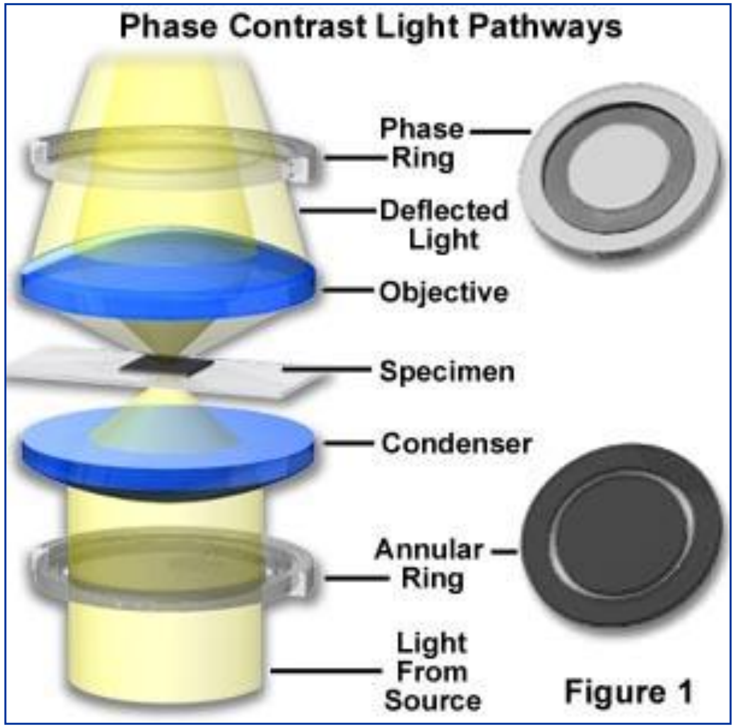
Absorption contrast

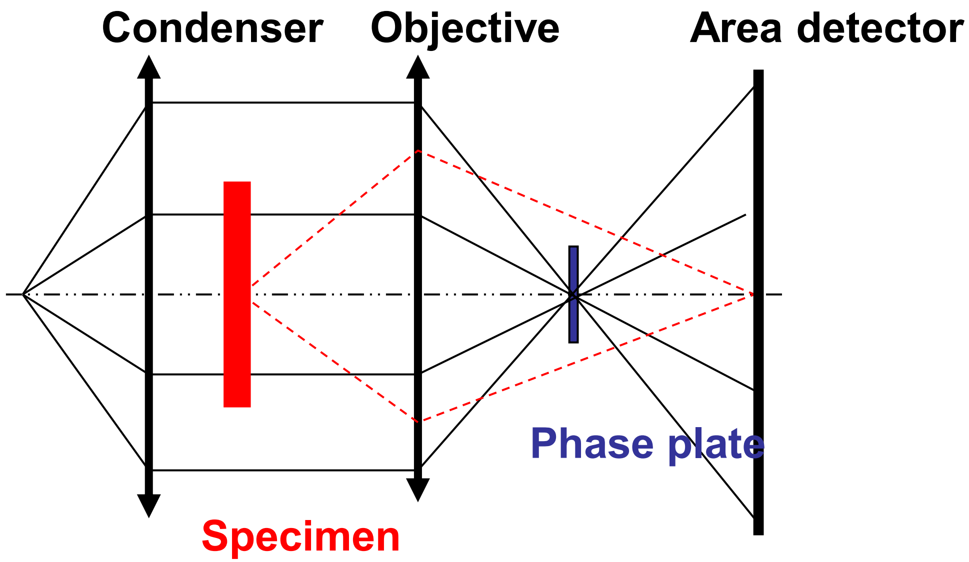
Mostly used for chemical studies in combination with XANES and XRF

Phase contrast techniques

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

Basics: Zernike phase contrast



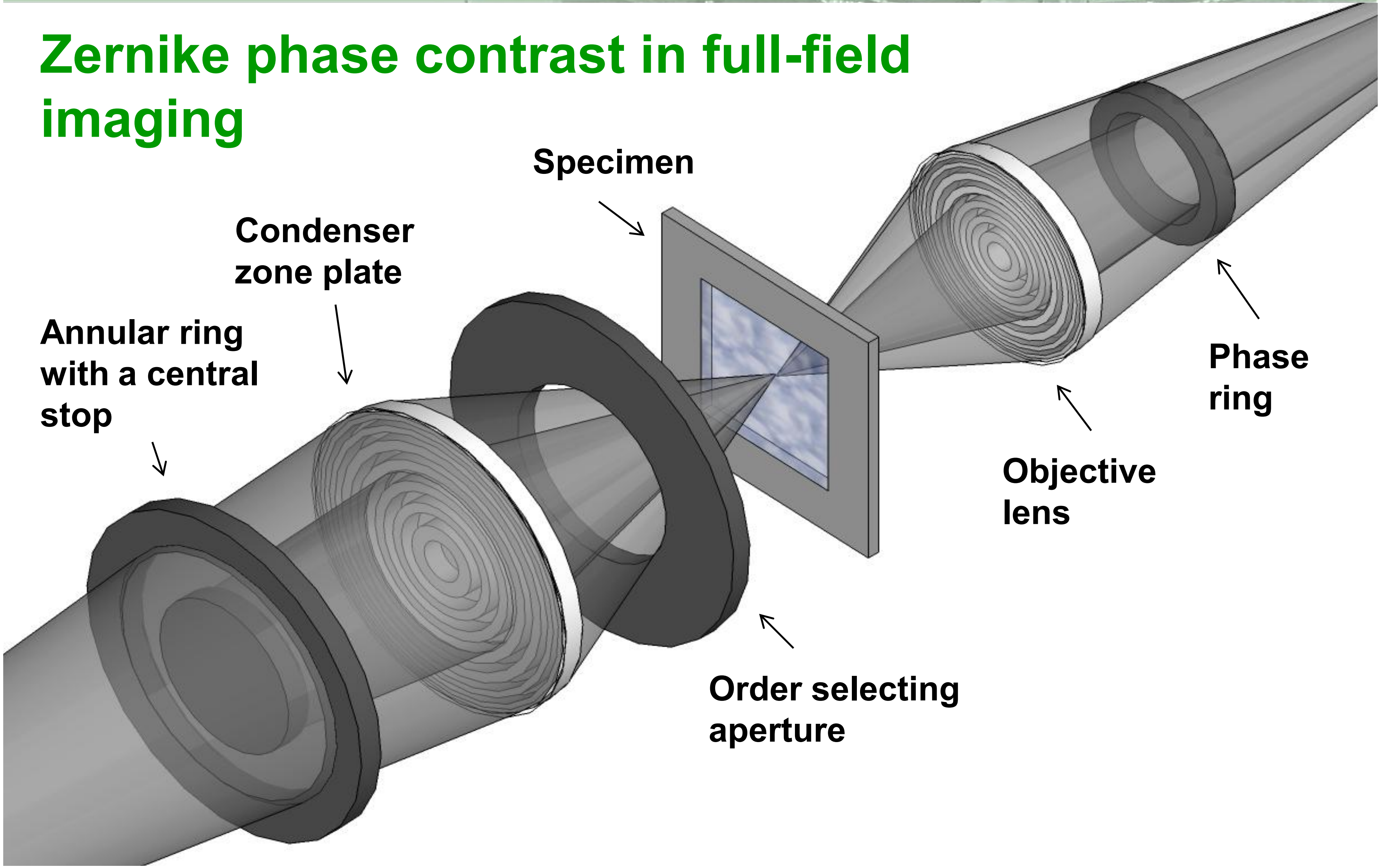


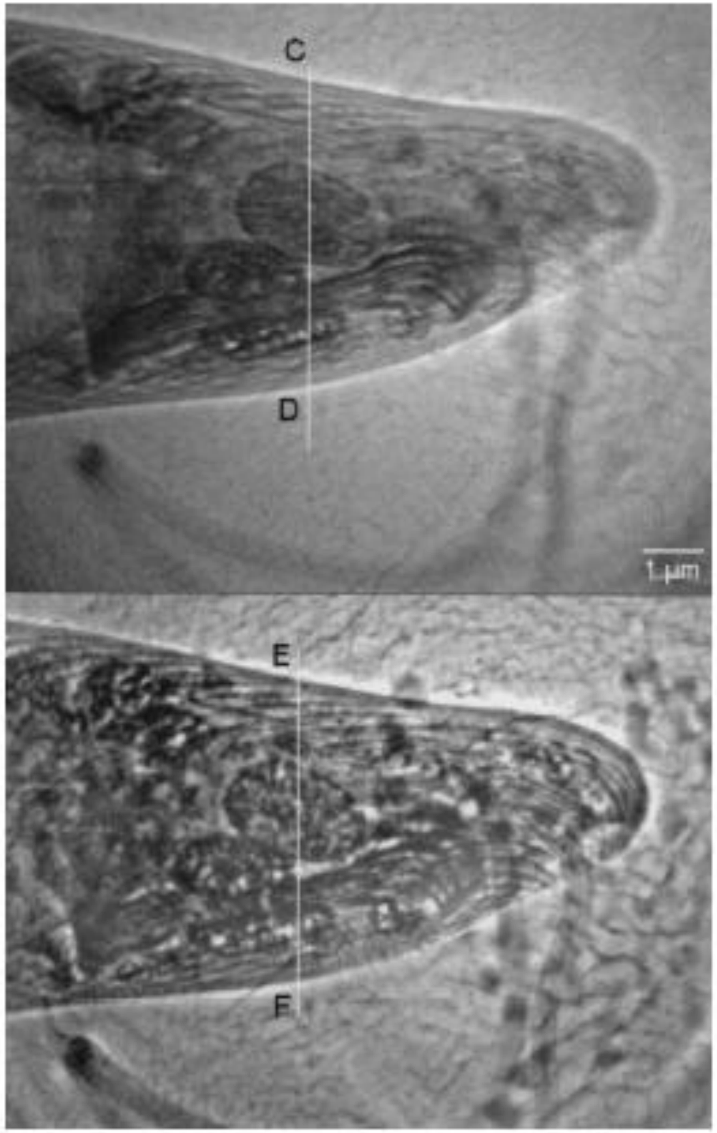
$$A_{specimen} = A_{surr} e^{i\Phi} = A_{surr} e^{i\frac{2\pi}{\lambda}\Delta t} \approx A_{surr}(1 + i\Phi) \qquad \Phi << 1$$

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

Phase differences are converted in amplitude differences !!!

Zernike phase contrast in full-field imaging





Zernike phase contrast in X-ray microscopy

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

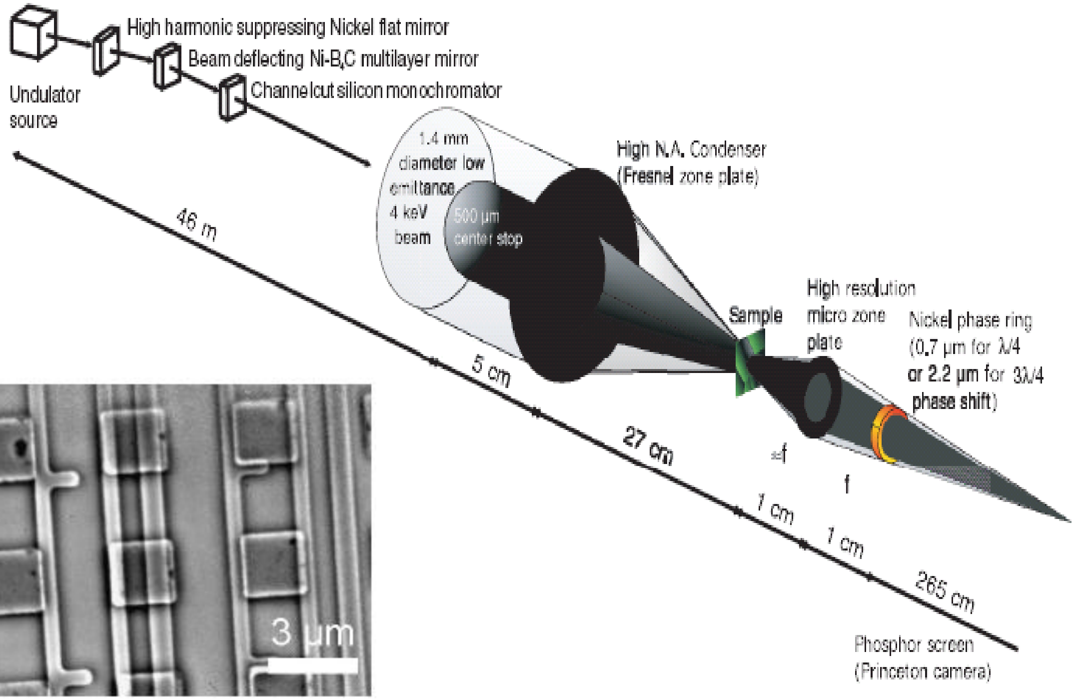
E = 500 eV, accumulated dose is $3x10^6$ Gray

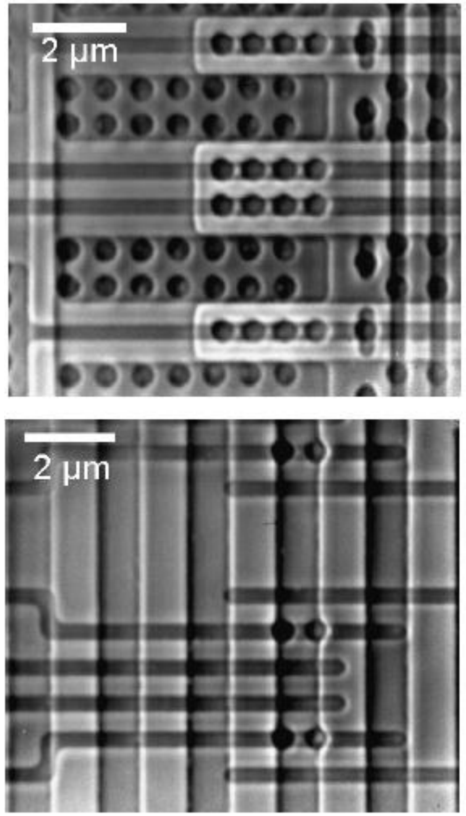
Amplitude: 3 s
Phase contrast: 15 s

Acquired with the TXM at BESSY,
Image courtesy: G. Schneider

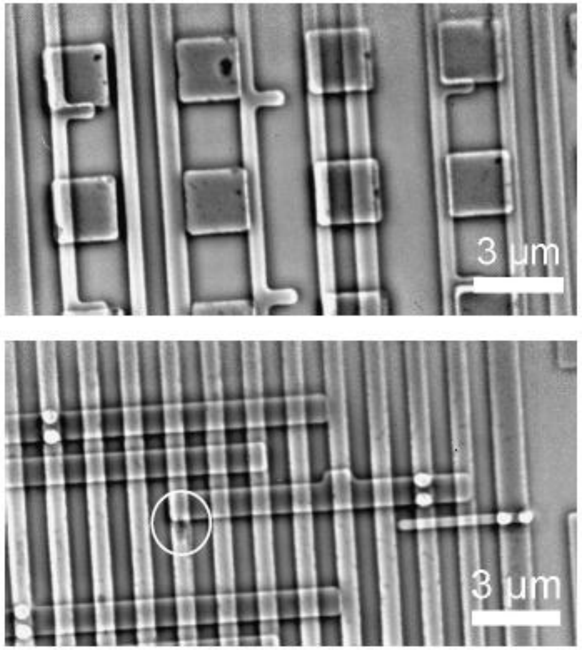
Transmission X-ray microscopy

Images acquired with the FFIM at ID21, ESRF
U. Neuhaeusler et al.





90 deg shift (pos.)



270 deg (neg.)

Cu interconnect structures imaged at 4 keV photon energy

Drawbacks of Zernike phase contrast:

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

What about differential interference contrast?

Differential Interference Contrast Schematic

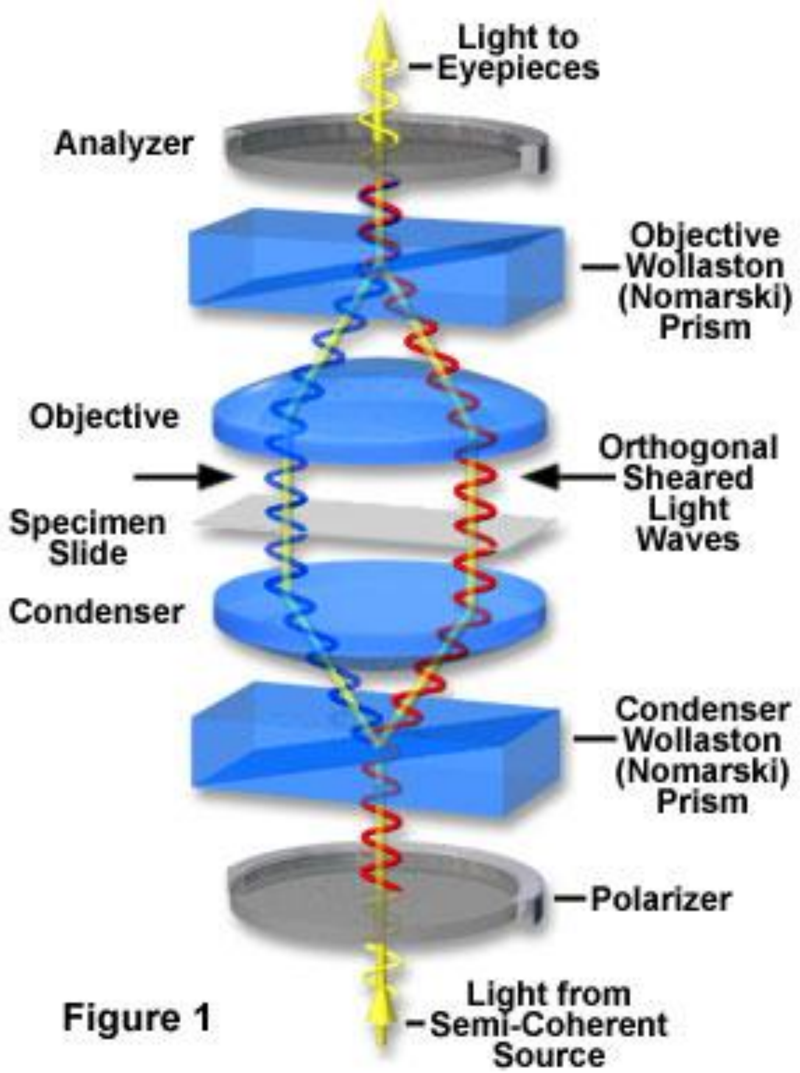


Figure 1

Principle of Differential interference contrast

- Light is polarized beneath the condenser optic
- Light pass is split by a modified Wollaston prism
- Sheared waves are recombined by a “Nomarski” prism

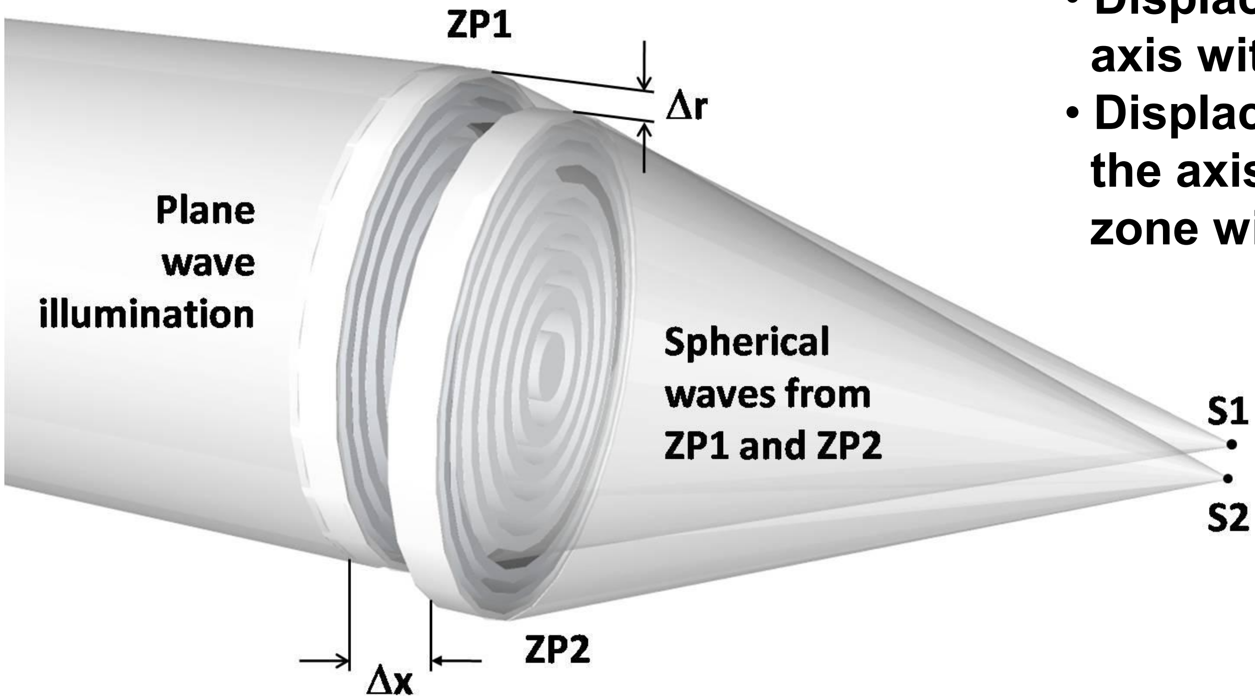
Difficulty for X-rays:

No way to create prisms necessary for beam shearing

Crystal shearers are too large to be implemented in the optical scheme and not appropriate for soft X-rays

Principle of Differential interference contrast in X-ray microscopy

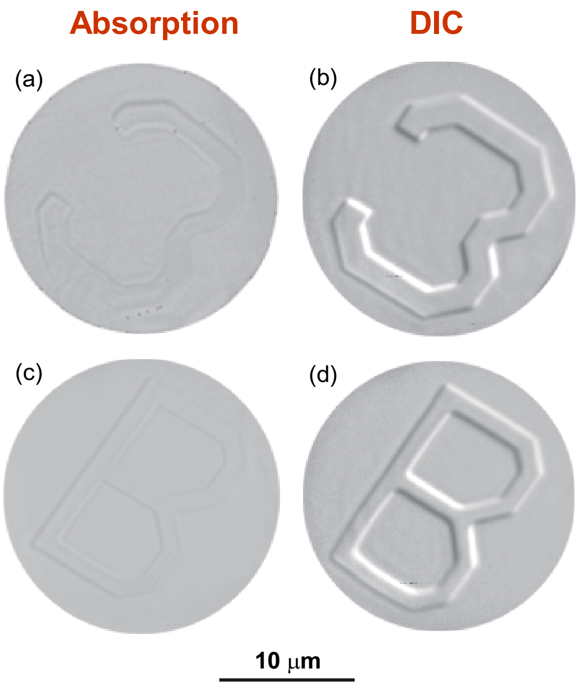
- Use two zone plates acting as beam splitter
- Displace them along the beam axis within the depth of focus
- Displace them perpendicular to the axis within the outermost zone width of the zone plates

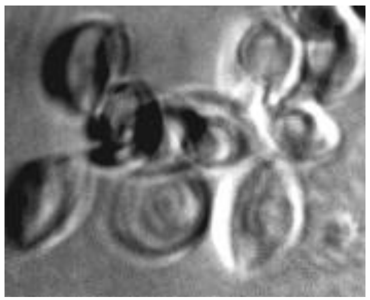


Airy disks of S1 and S2 overlap. Light in S1 and S2 can interfere but only one image is formed !

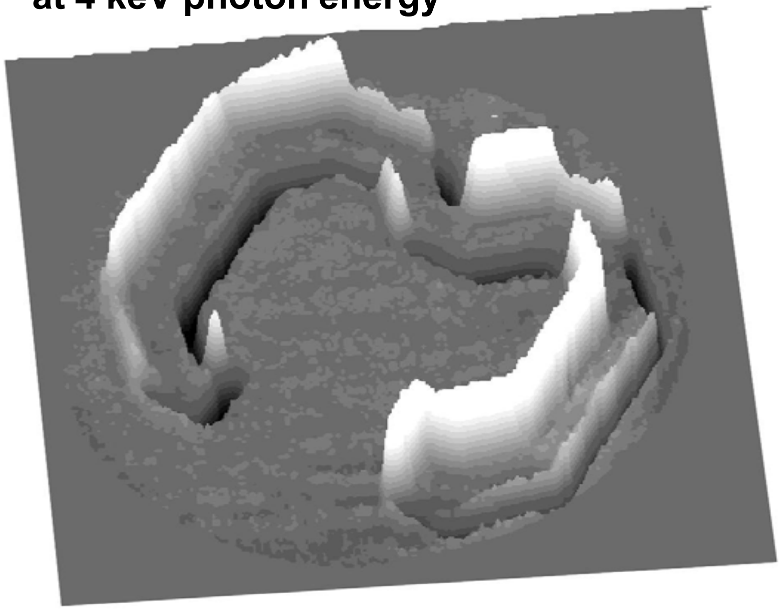
T. Wilhein, B. Kaulich et al.

DIC imaging with a FFIM microscope





DIC imaging with the FFIM at ID21, ESRF at 4 keV photon energy

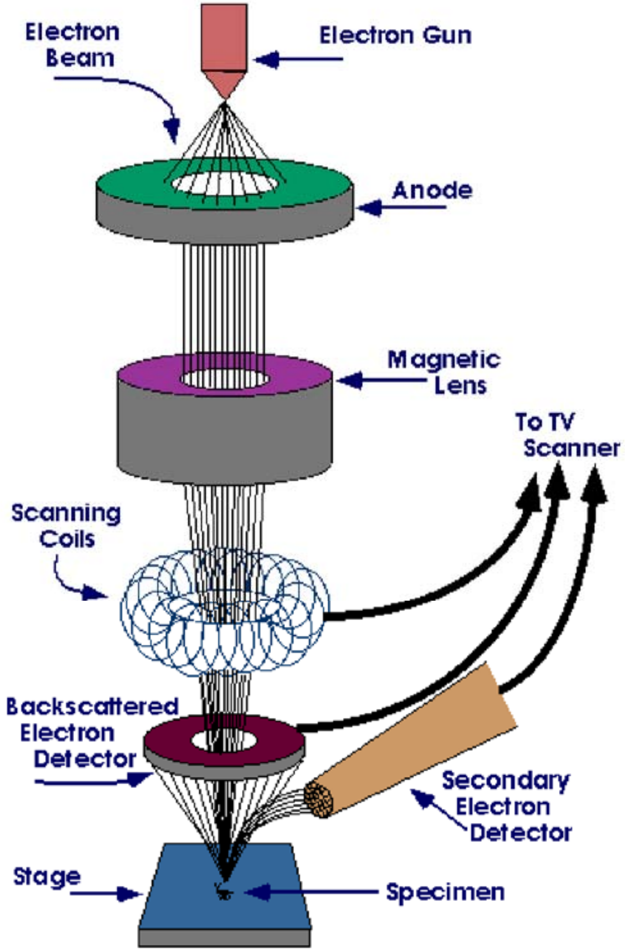


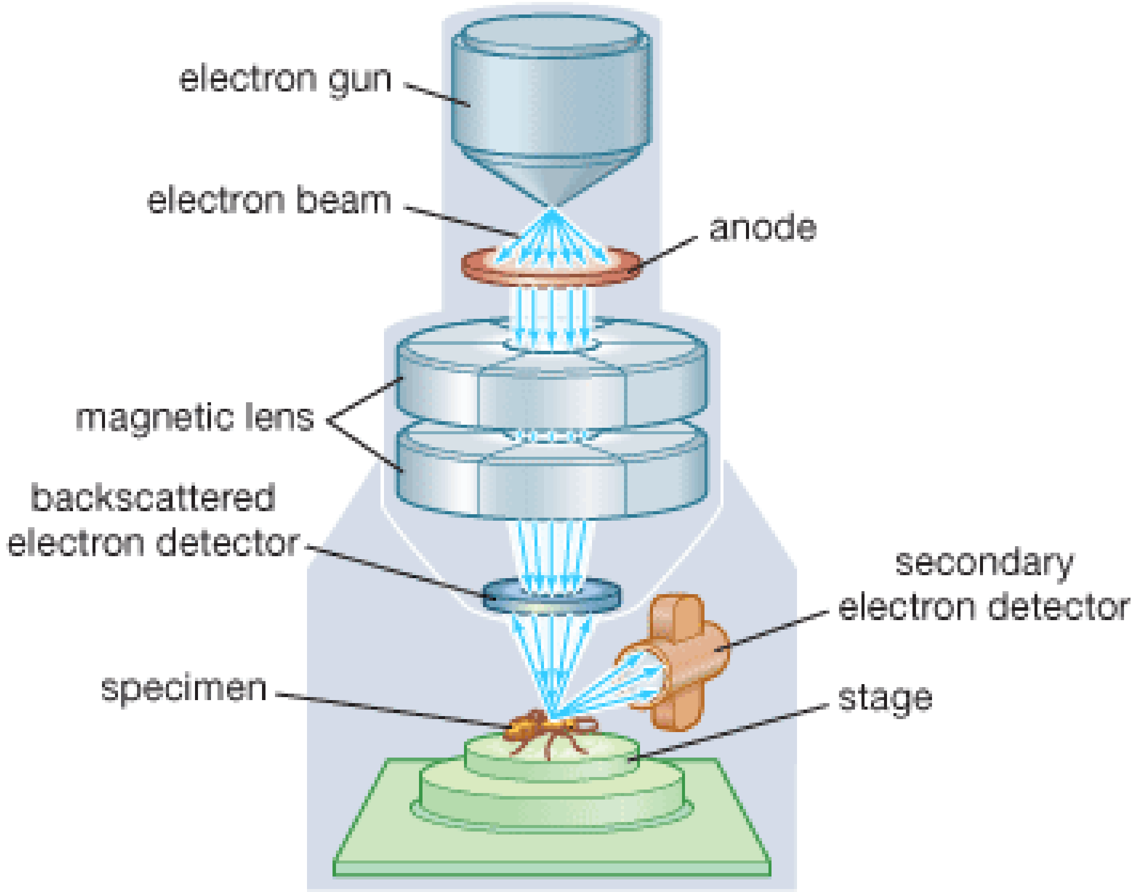
So much for full-field imaging X-ray microscopy.

What about scanning X-ray microscopy?

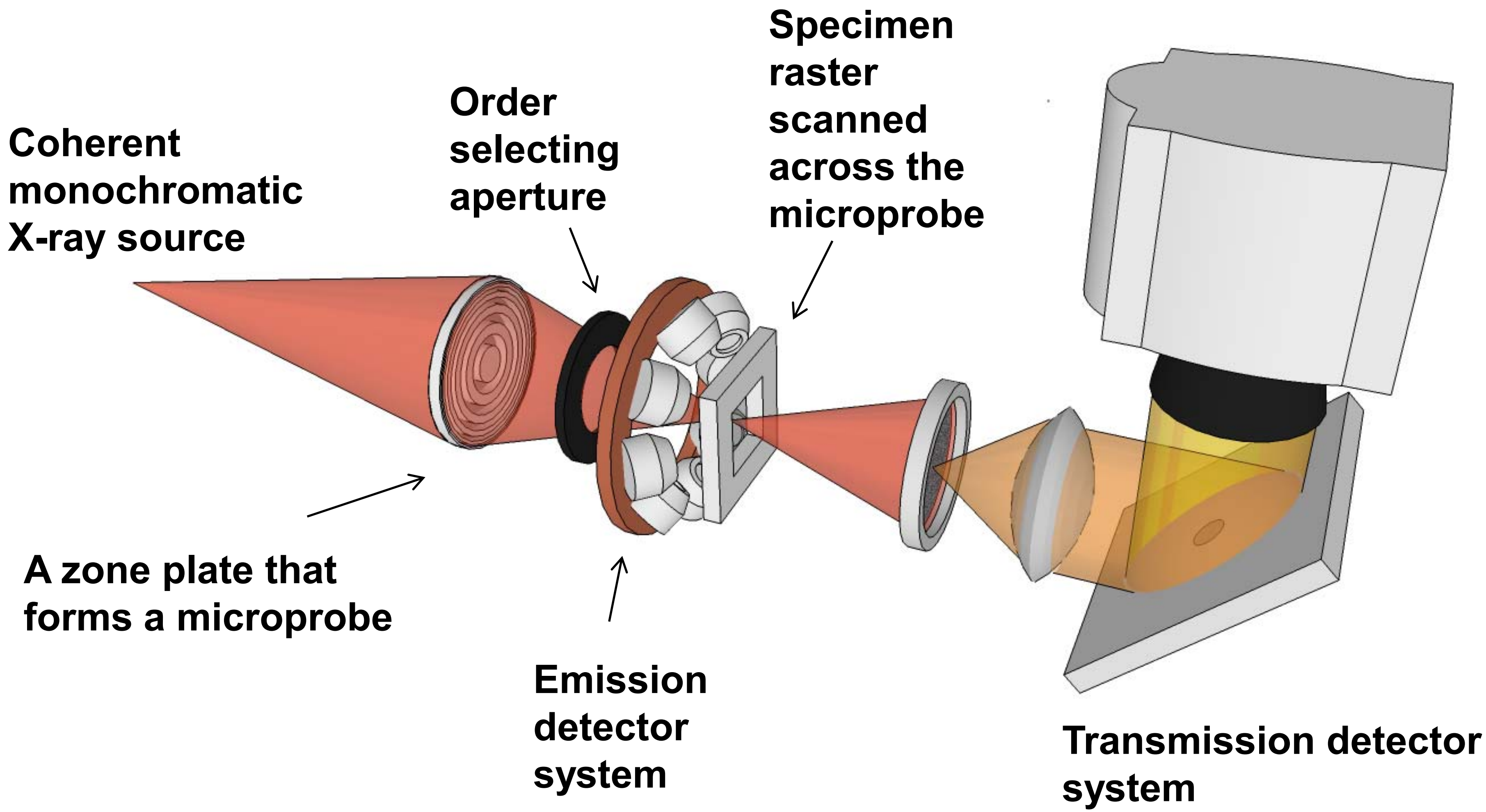
AFTER THE BREAK !

Principle of a scanning electron microscope:

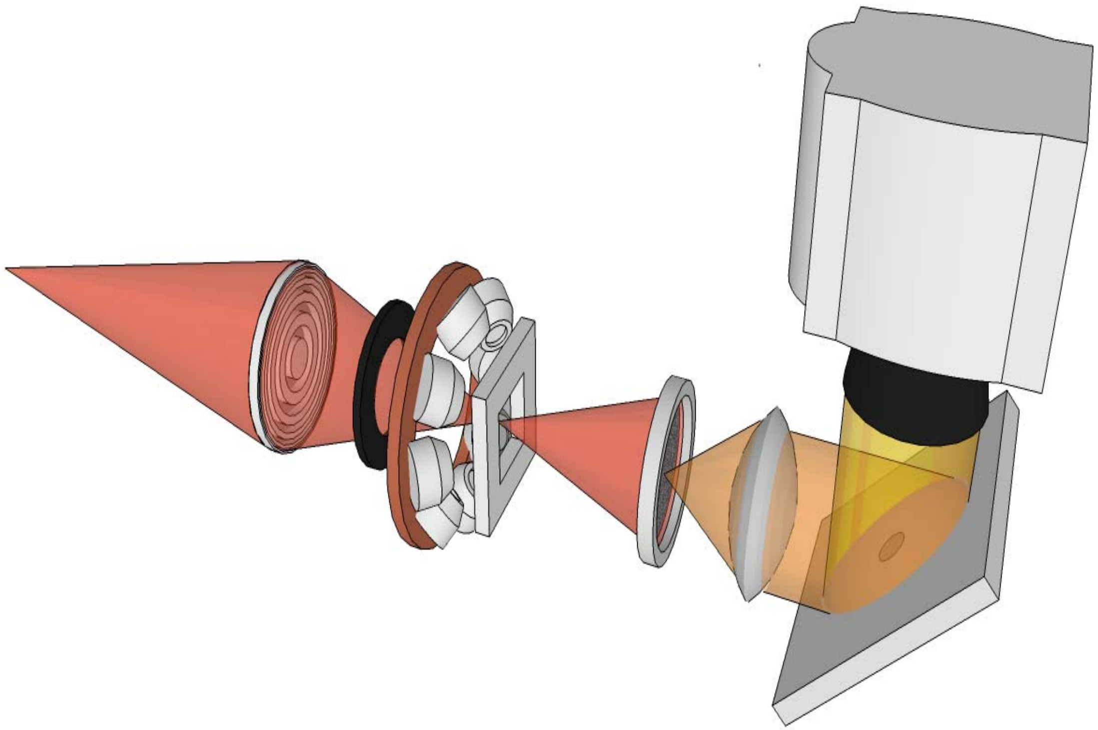




Principle of a scanning X-ray microscope:



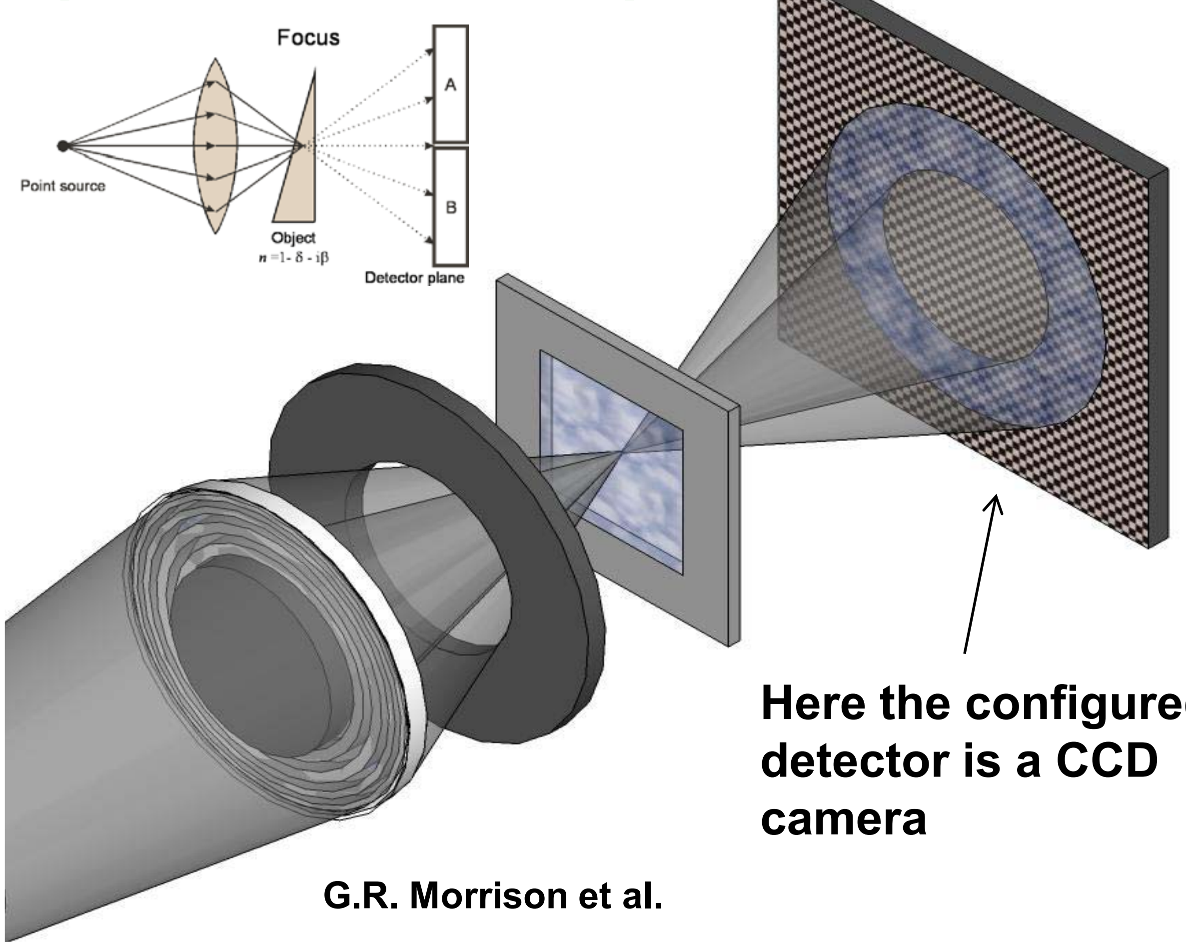
Principle of a scanning X-ray microscope:



- Least radiation dose
- Next best spatial resolution
- Best spectral resolution
- Requires spatially coherent radiation
- Long exposure time
- Flexible sample environment
- Photoemission (restricted magnetic fields), fluorescence imaging

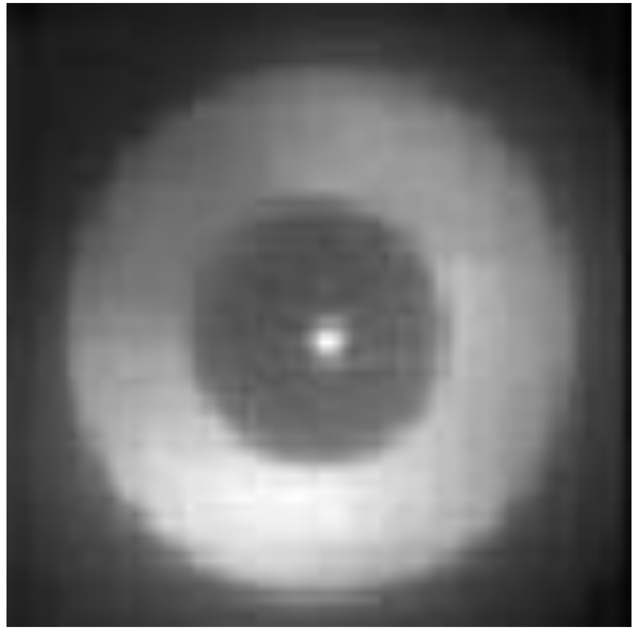
Excellent access to chemical analysis due to multiple detectors !!!

The role of a configured detector in scanning X-ray microscopy: Simultaneous acquisition of absorption and phase contrast maps:



G.R. Morrison et al.

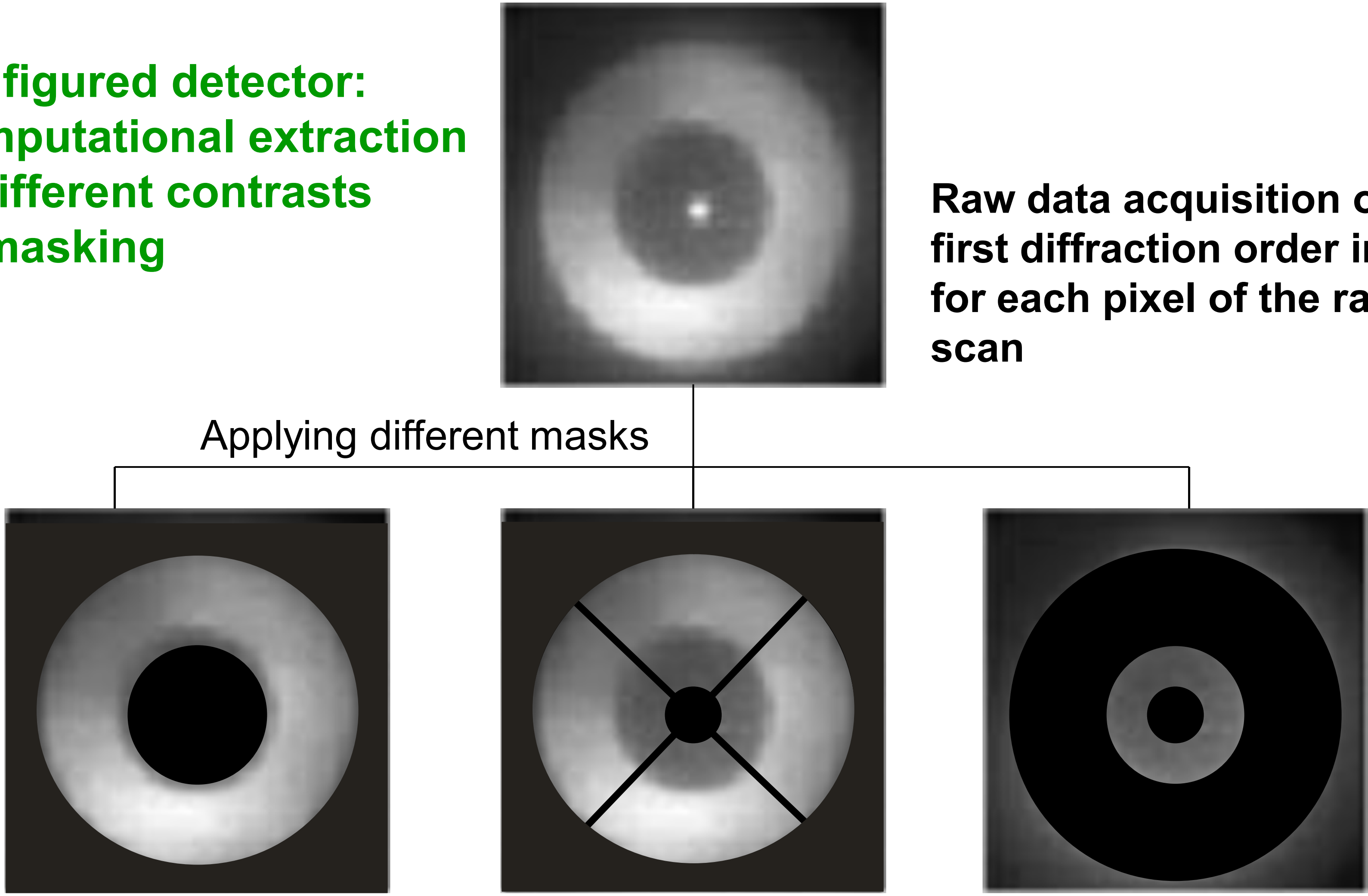
Here the configured detector is a CCD camera



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

Configured detector: Computational extraction of different contrasts by masking

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



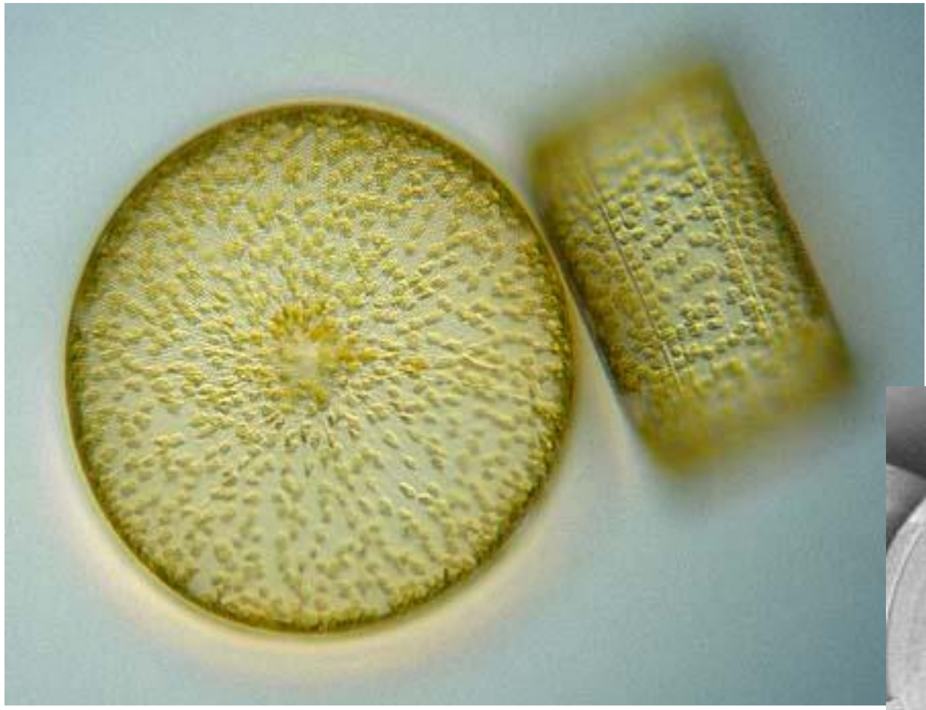
Bright field

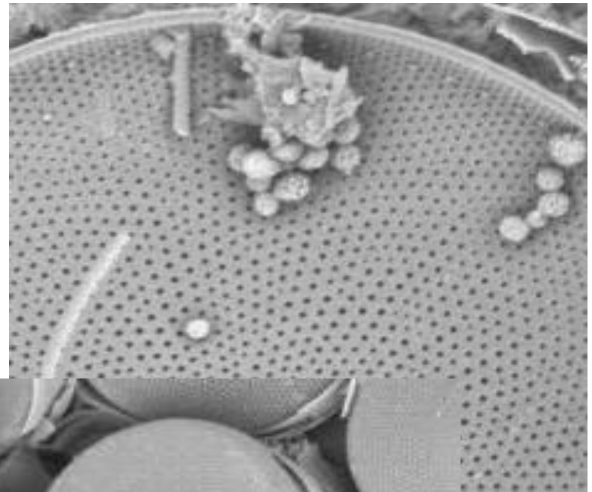
Differential phase and absorption

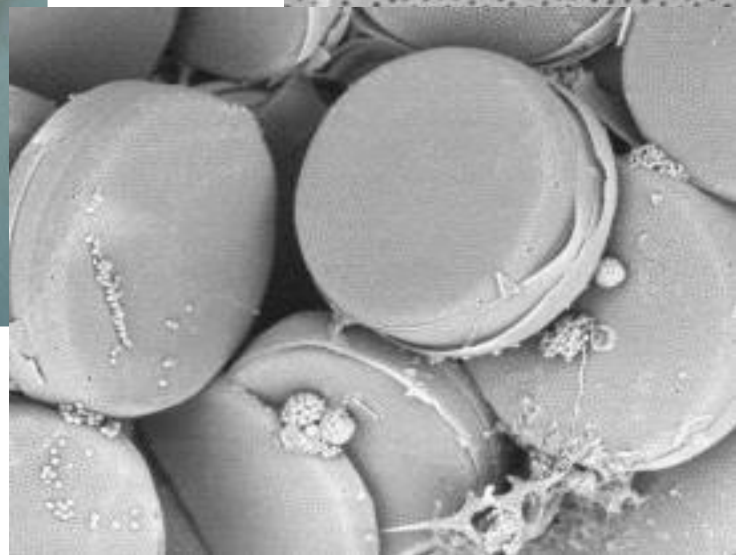
Darkfield

A. Gianoncelli et al., Appl. Phys. Lett.

Marine biology: Imaging of giant diatoms

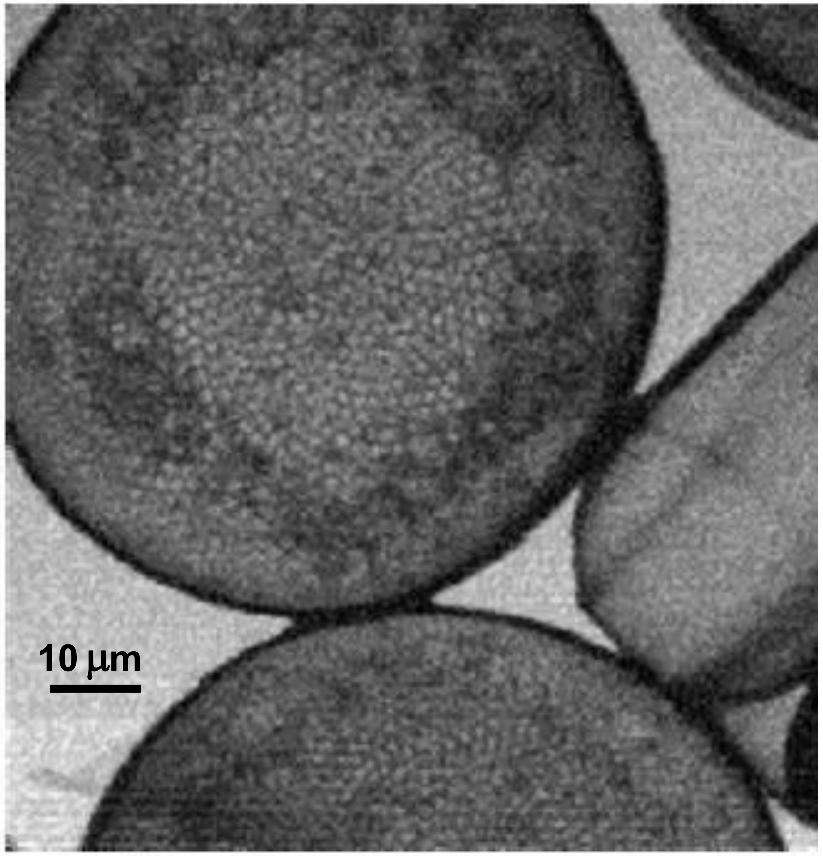




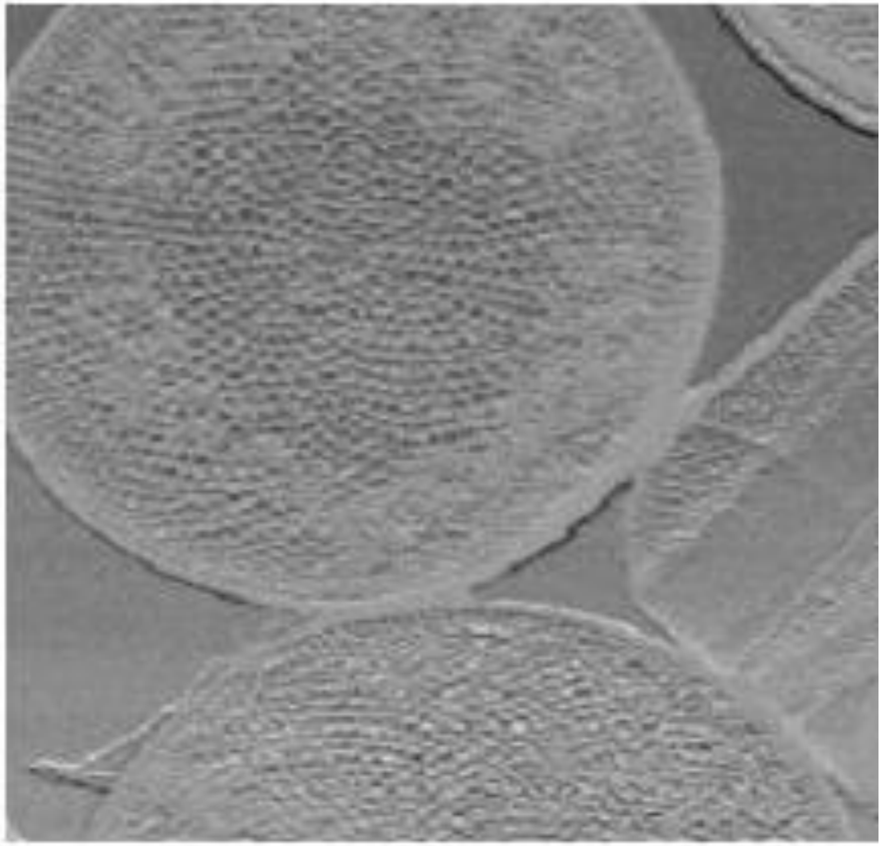


Planktonic diatom Coscinodiscus sp. (A. Beran, Laboratory for Marine Biology, Trieste, I)

Brightfield and differential phase contrast images acquired simultaneously with configured detector



Bright field image



DPC mode – X-moment

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

Optics-based phase-sensitive contrasts in STXM:

- Differential interference contrast (as for TXM/ FFIM)
- Differential phase contrast
- Dark field imaging

Darkfield imaging

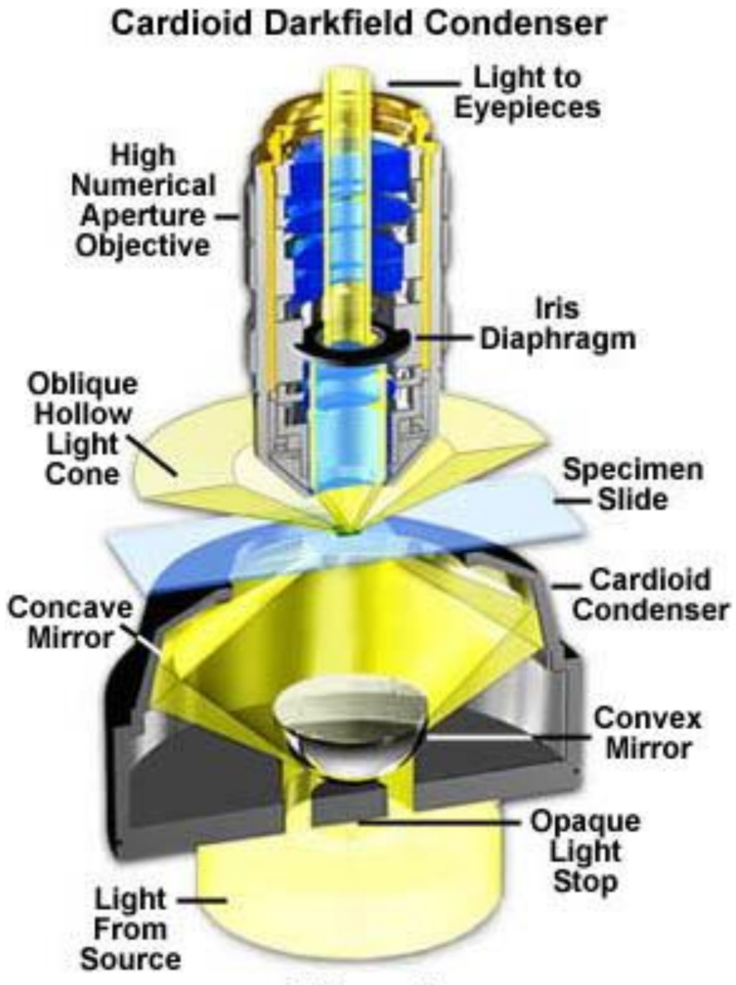
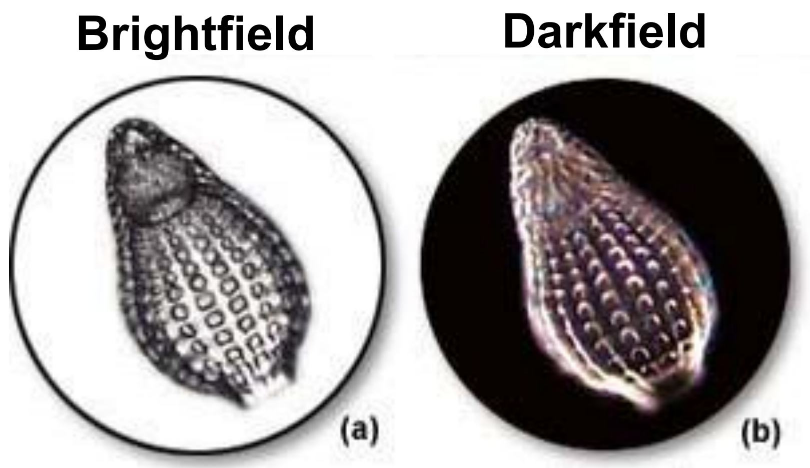


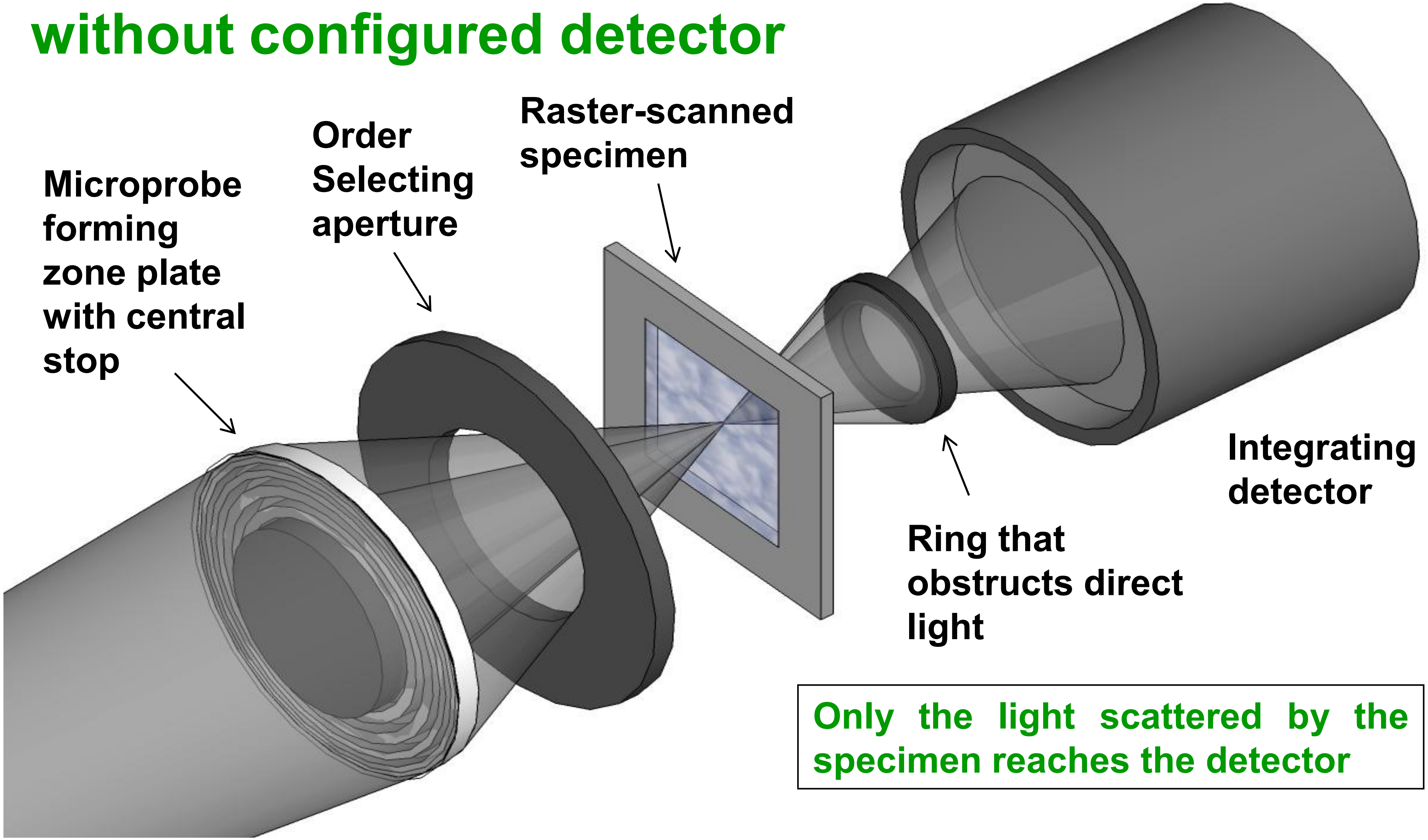
Figure 1

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.



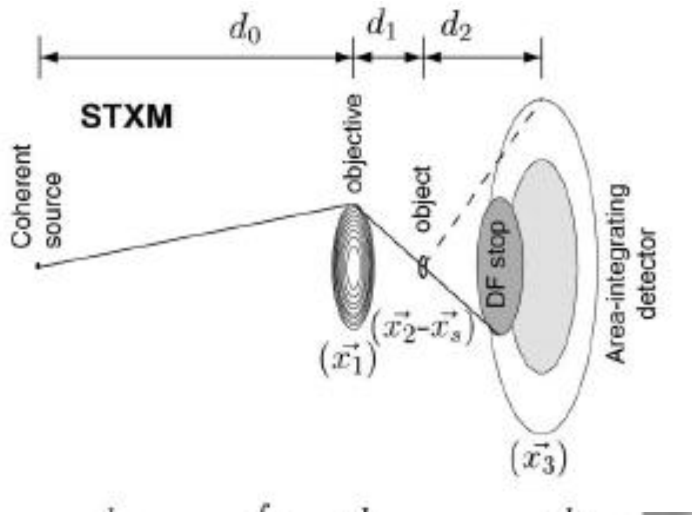
Visible light micrographs of silica skeletons from a small marine protozoan (radiolarian)

Darkfield imaging in scanning X-ray microscopy without configured detector

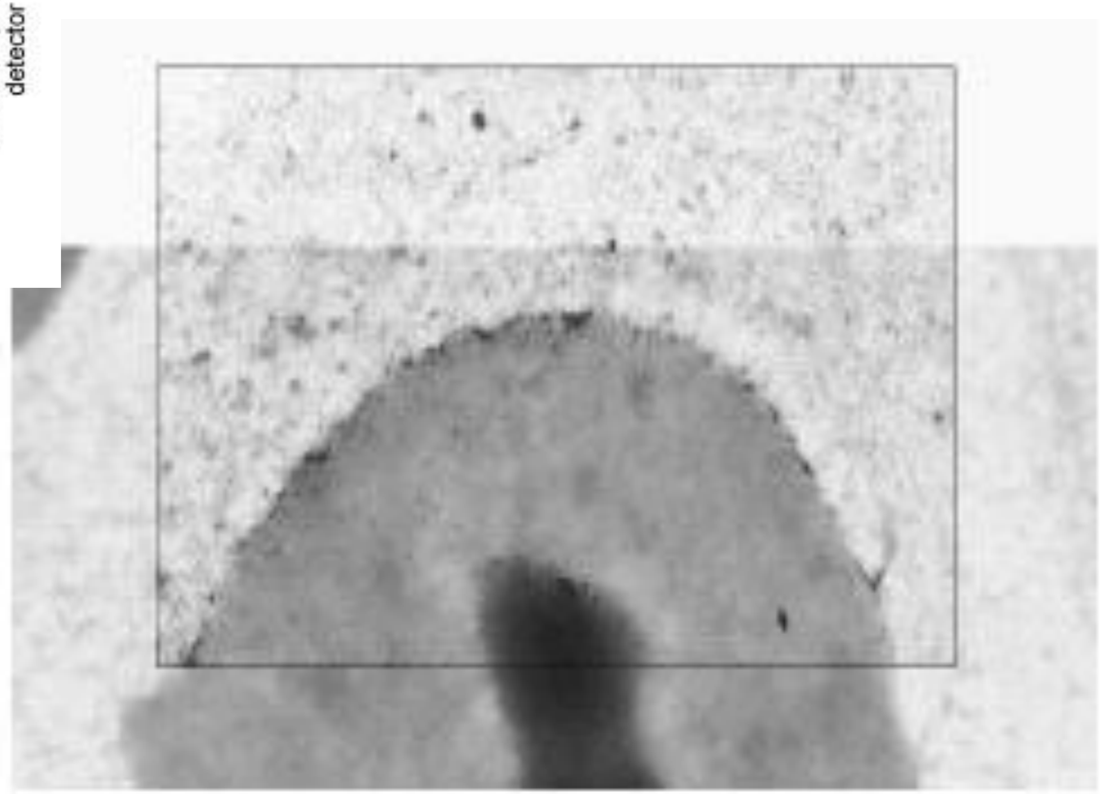


Only the light scattered by the specimen reaches the detector

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



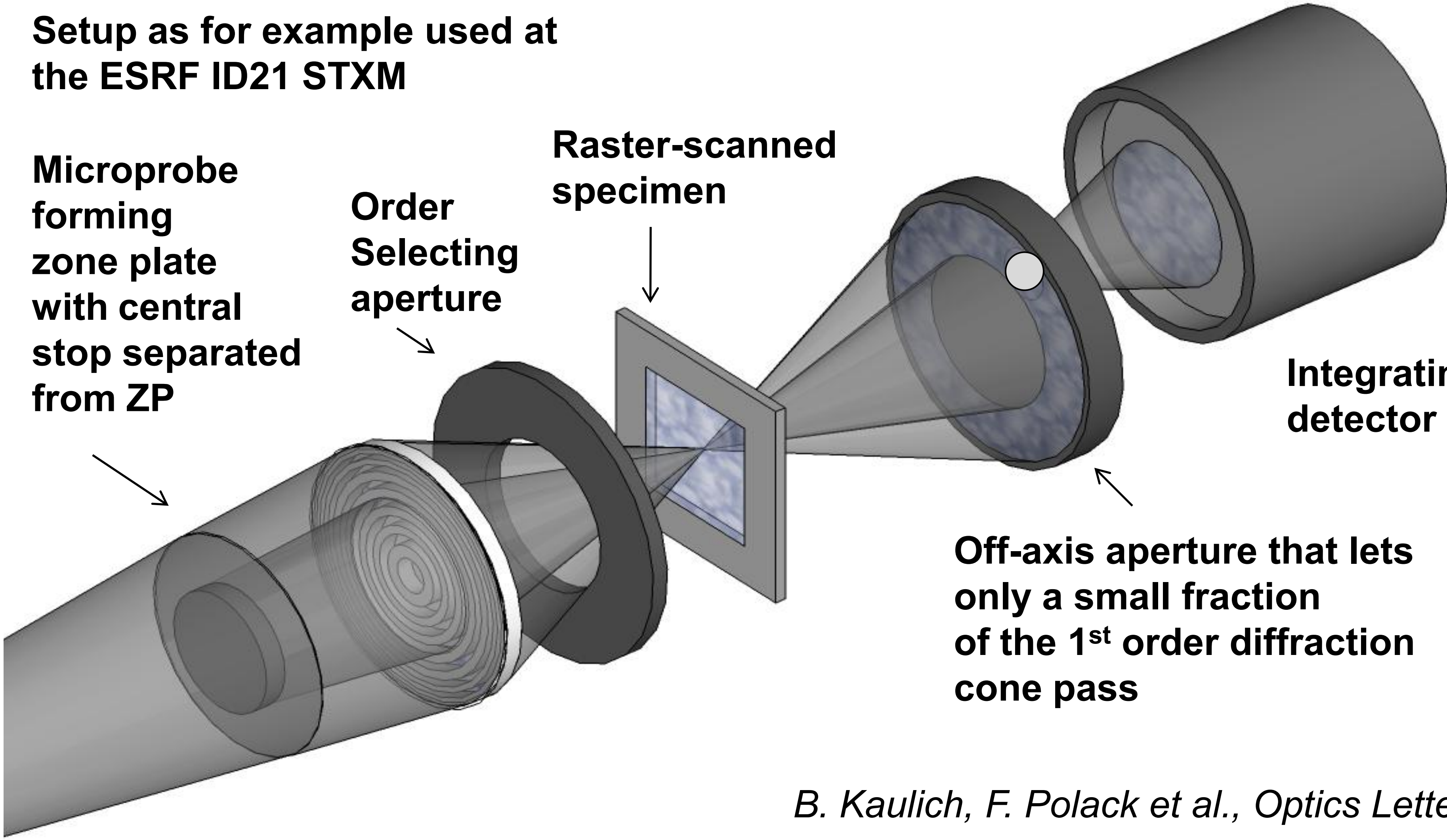
Brightfield image of a cell with Au labelling spheres overlayed with a darkfield image

Images acquired with STXM at the NSLS

S. Vogt et al.

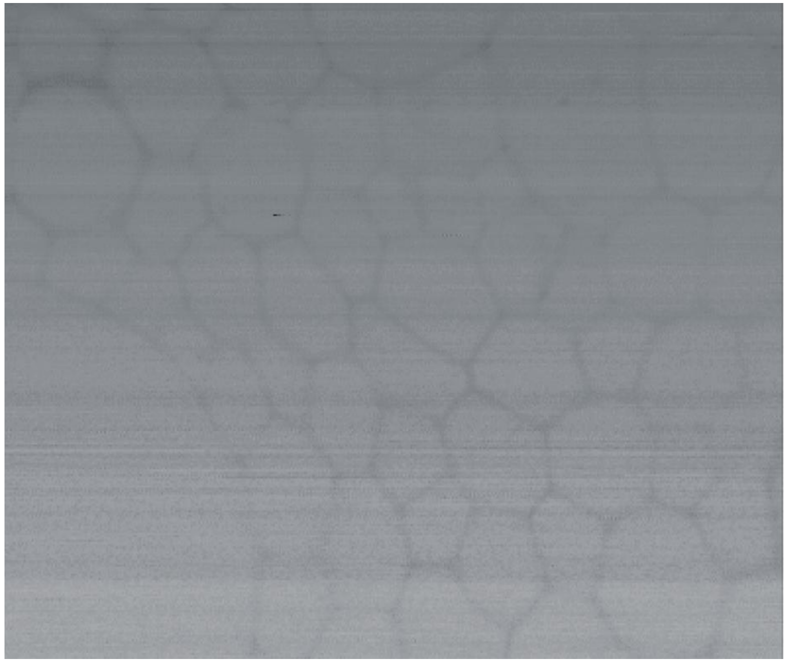
Aperture-based differential phase contrast in STXM

Setup as for example used at the ESRF ID21 STXM

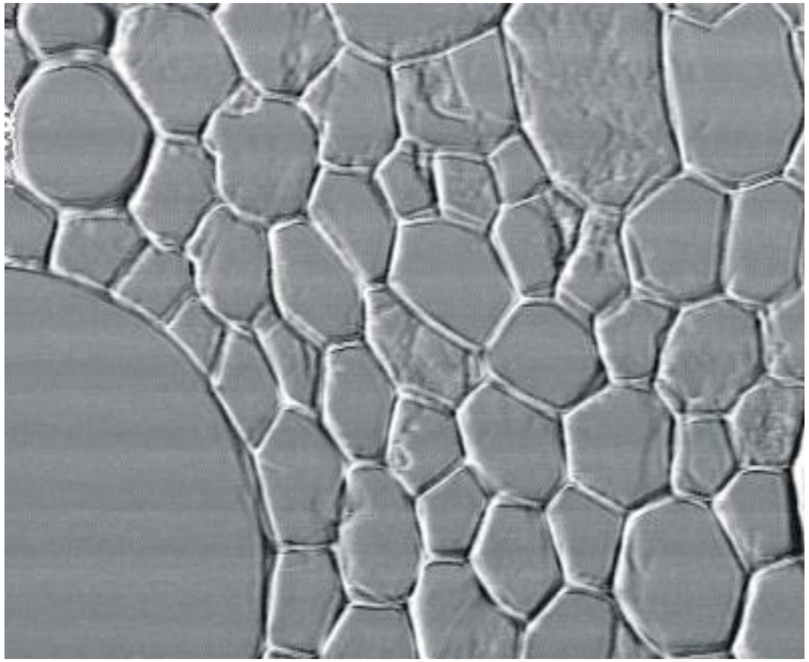


B. Kaulich, F. Polack et al., Optics Letters

Aperture-based differential phase contrast in STXM



Absorption



Differential phase contrast

Maize cell membranes imaged with the STXM at ID21, ESRF, 4keV photon energy, image width is 100 μm.

B. Kaulich, F. Polack et al., Optics Letters

Comparison of TXM/ STXM:

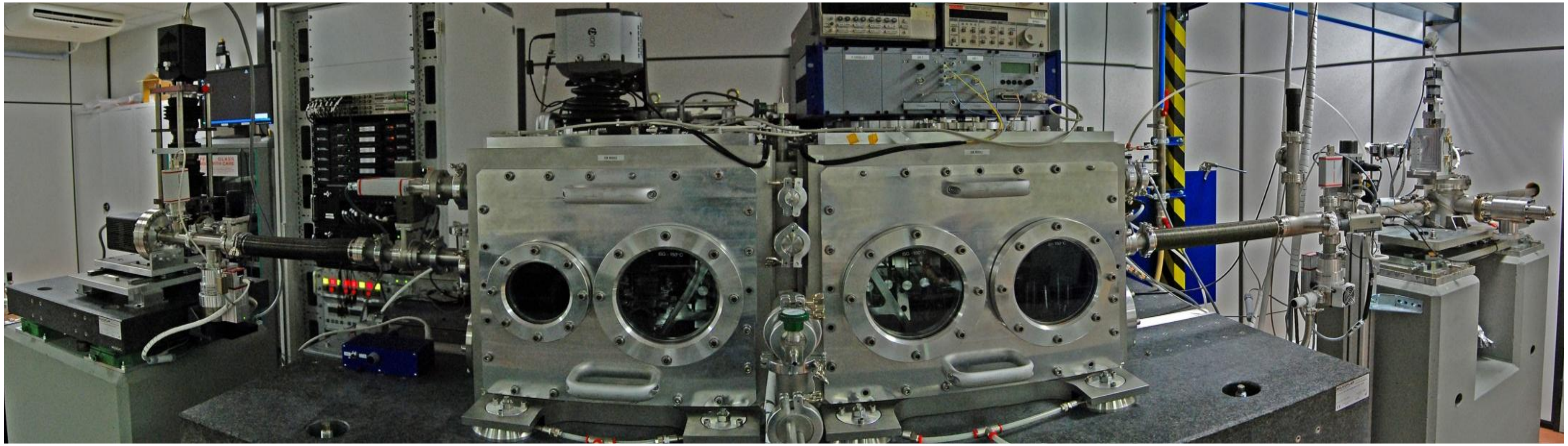
STXM

- Lowest radiation dose because one optic less in the optical path but this depends also on the detector efficiency !
- Requires fully coherent illumination
- Raster-scanning can lead to opto-mechanical instabilities and loss in lateral resolution
- Multi-detector acquisition, and therefore best suited for chemical analysis (some examples later)
- Different contrasts incl . absorption, magnetic contrast, diff. phase and interference contrasts

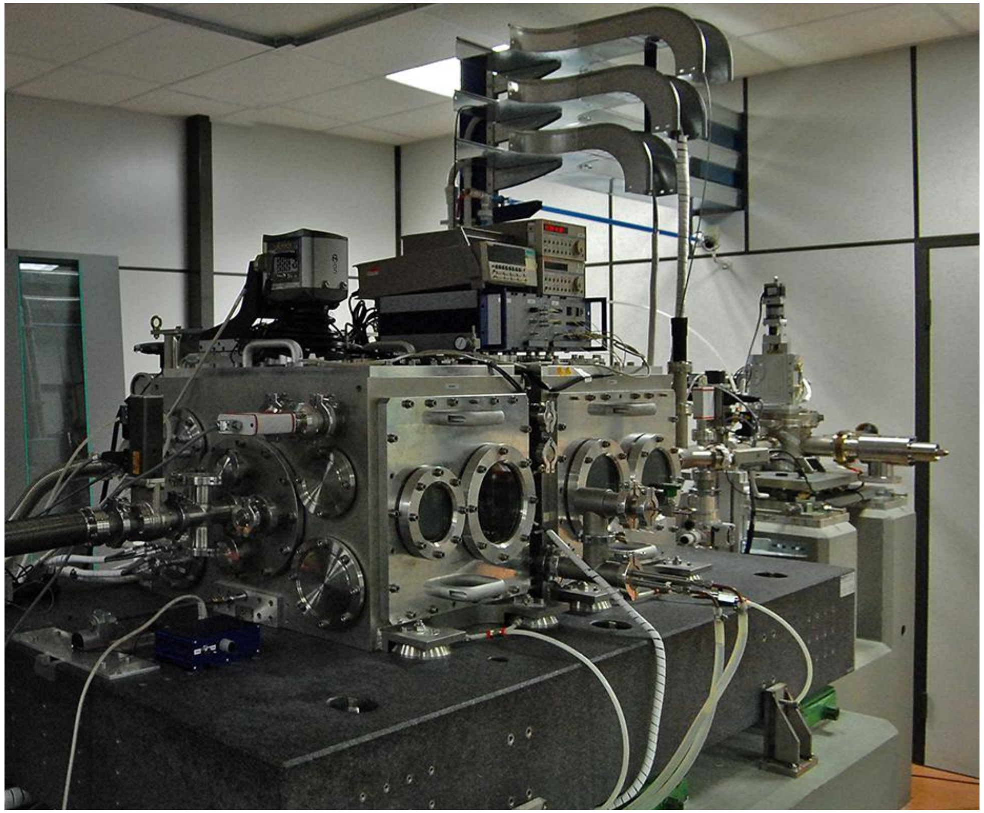
TXM

- Higher or similar radiation dose because of the objective lens
- Requires partially or incoherent illumination
- Static design and therefore highest lateral resolution (down to about 15nm)
- Single transmission detector
- Fast acquisition and therefore best suited for dynamic measurements and tomography
- Different contrasts incl . absorption, magnetic contrast, Zernike phase contrast, diff. interference contrasts

Combined scanning, full-field imaging and photoemission microscopy with the TwinMic microscope at ELETTRA



TwinMic – Combination of scanning and full-field imaging in a single instrument



The European team that initiated the project

- **Morphological analysis, XANES, LEXRF and AAEI**
- **Different contrasts incl. brightfield, differential phase and interference contrast, darkfield, etc**
- **Versatile specimen environment**

TwinMic

The twin X-ray microscopy station @ Elettra

Transmission X-ray microscopy

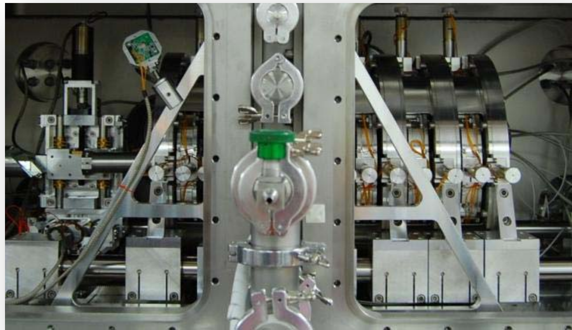
Spectromicroscopy: XANES, LEXRF, AEI

Kinetics, dynamics, 3D

STXM → **400 – 2200 eV** ← **TXM**

Brightfield
Darkfield
Differential
PC
(simult.)
Nomarski DIC

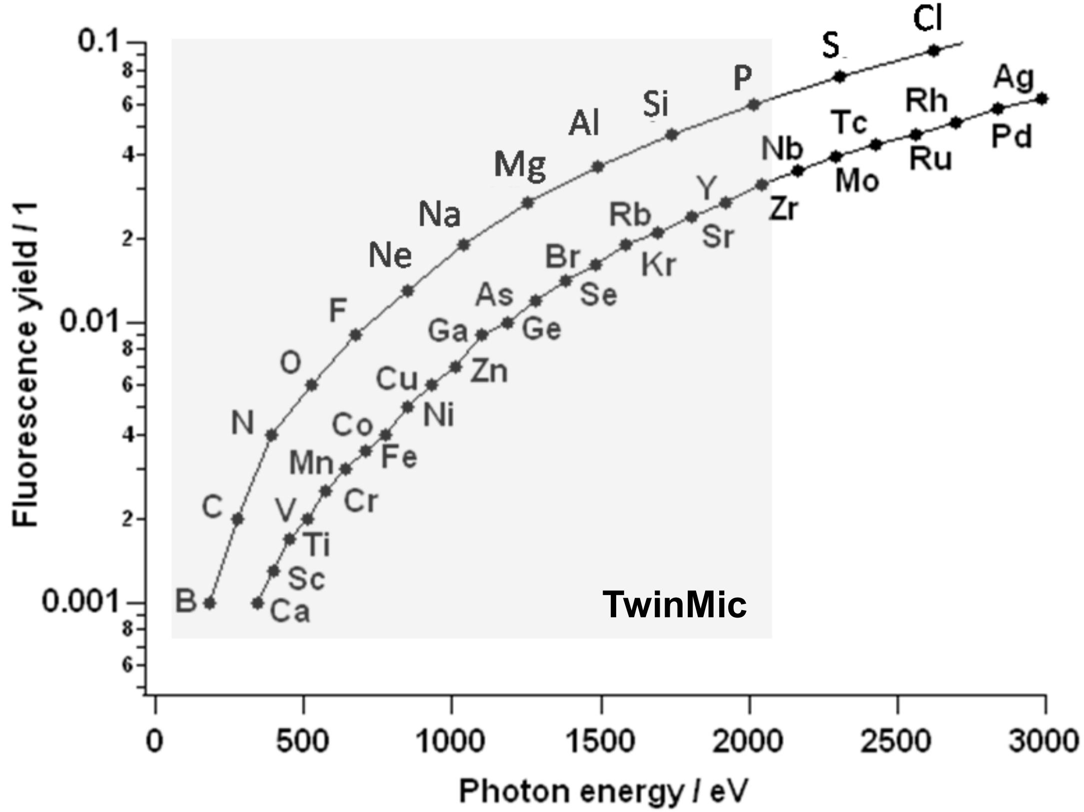
Brightfield
Darkfield
Differential
PC
Nomarski DIC

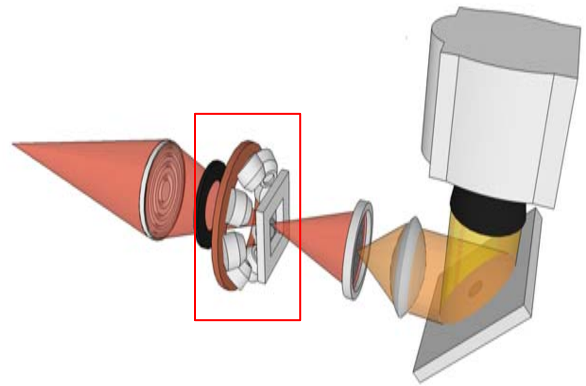


- **Biotechnology**
- **Nanotechnology**
- **Environment**
- **Geochemistry**
- **Food Science**
- **Medicine**
- **Pharmacology**
- **Cultural Heritage**
- **New Materials**

Low-energy X-ray fluorescence:

Simultaneous acquisition of absorption, differential phase contrast and LEXRF ?



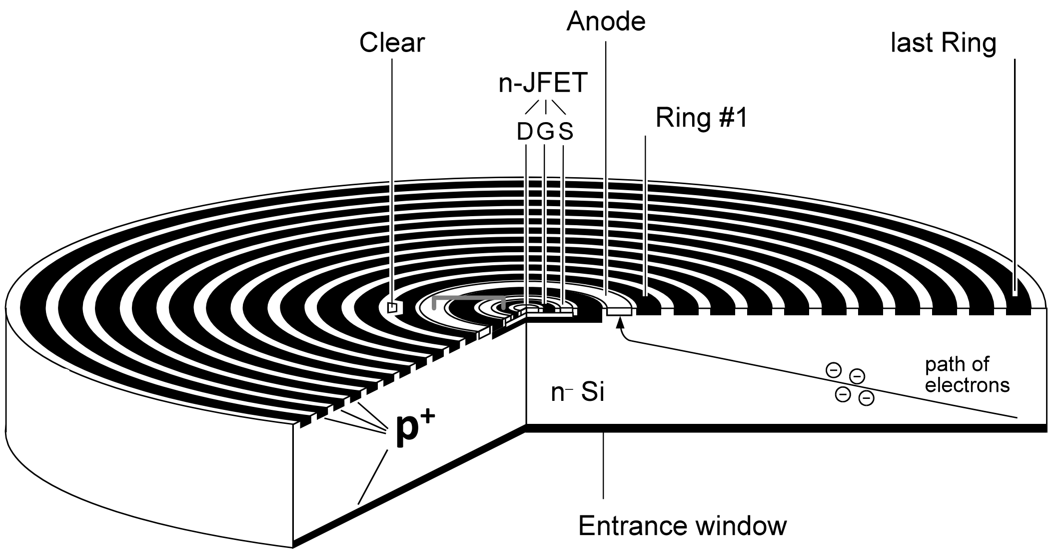


Detecting trace elements:

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

Low fluorescence yields for soft X-rays! !!

Silicon drift detectors for low-energy X-ray fluorescence:

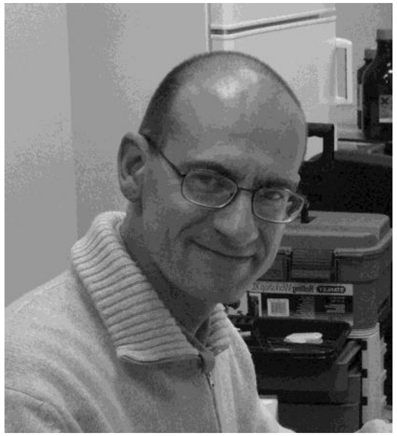


Solid state detector nvented in 1983 by E. Gatti (Politecnico di Milano) and P. Rehak (Brookhaven National Laboratory)

- **Small output capacitance (150 pF)**
- **Capacitance independent of the active area**
- **JFET integrated on the chip**

Some applications with simultaneous transmission and emission X-ray microscopy

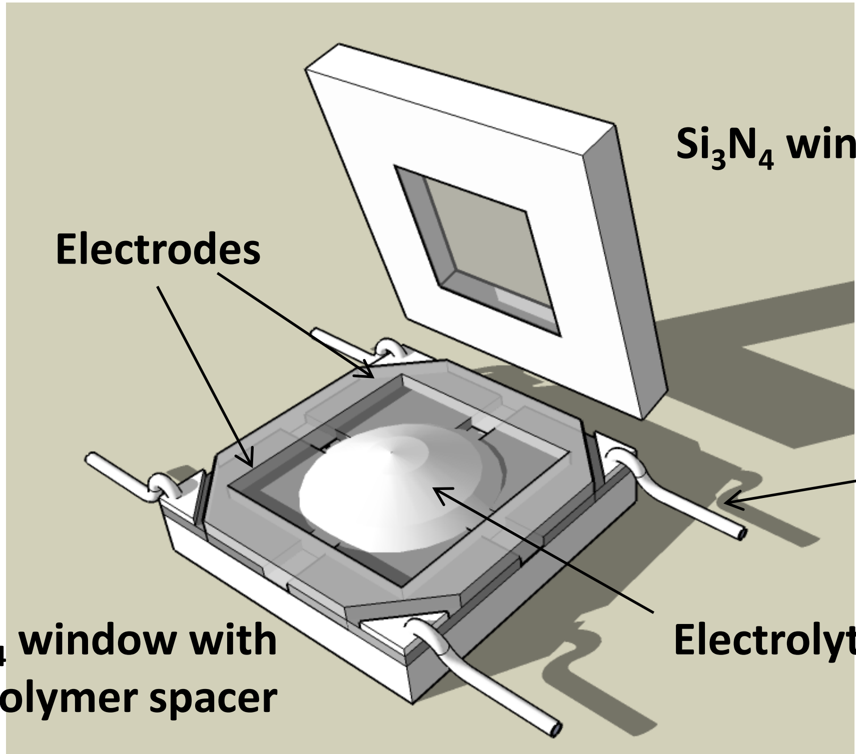
New sustainable energies: Development of fuel cells



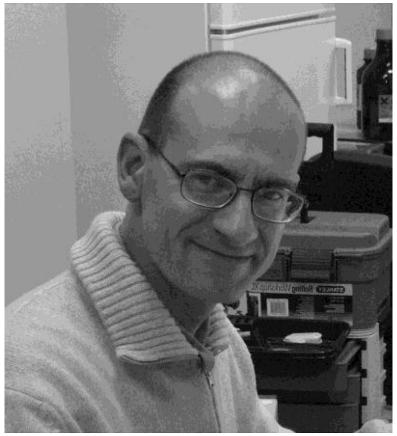
Benedetto Bozzini,
Uni Lecce, I

Understanding the electrocorrosion in fuel cells that is the main life-time limiting factor

Vacuum-compatible functional electrolytic specimen cell for in-situ studies



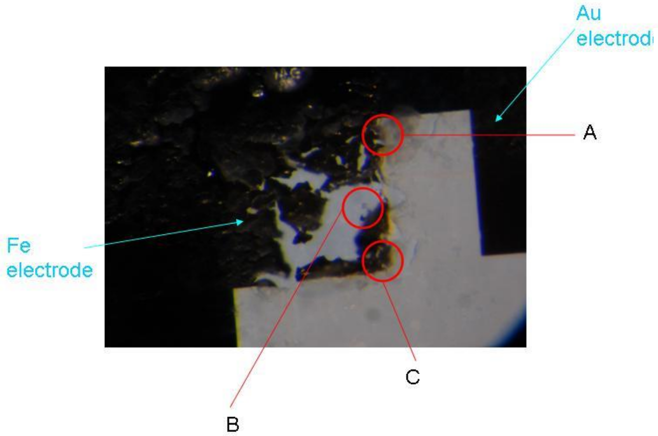
New sustainable energies: Development of fuel cells

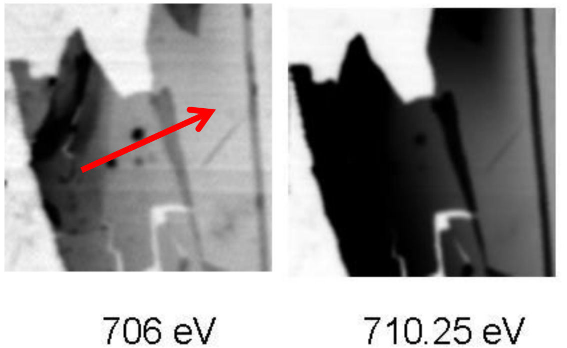


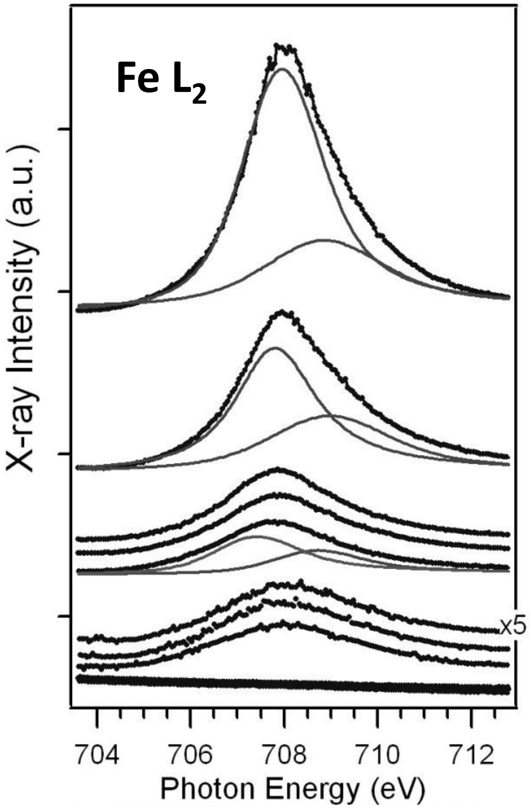
Benedetto Bozzini,
Uni Lecce, I

Understanding the electrocorrosion in fuel cells that is the main life-time limiting factor

Three different spectroscopies: AAEI, XANES, LEXRF

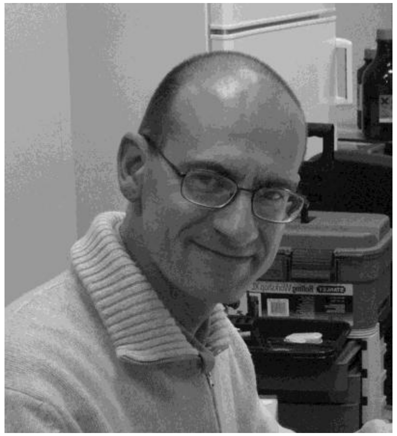






80 x 80 μm^2, 50ms dwell/px, energy stack

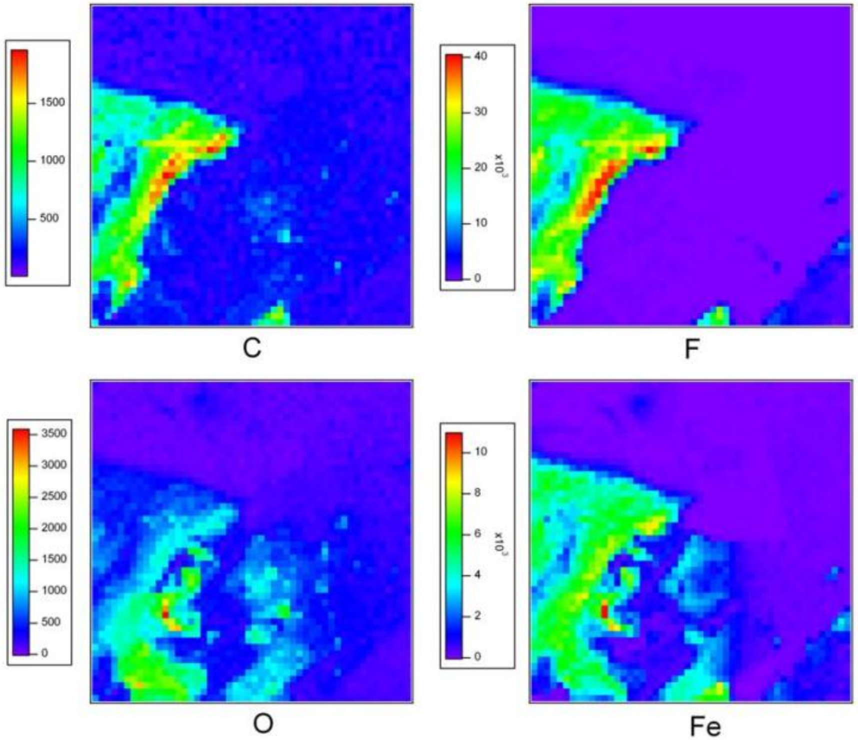
New sustainable energies: Development of fuel cells

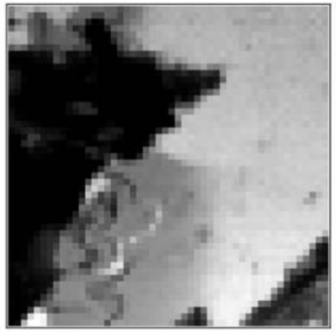


Benedetto Bozzini,
Lucia D'Urzo
Uni Lecce, I

Understanding the electrocorrosion in fuel cells that is the main life-time limiting factor

Three different spectroscopies: <u>AAEI</u>, <u>XANES</u>, LEXRF





80 x 80 μm², 50ms dwell/px, 5s dwell/ px

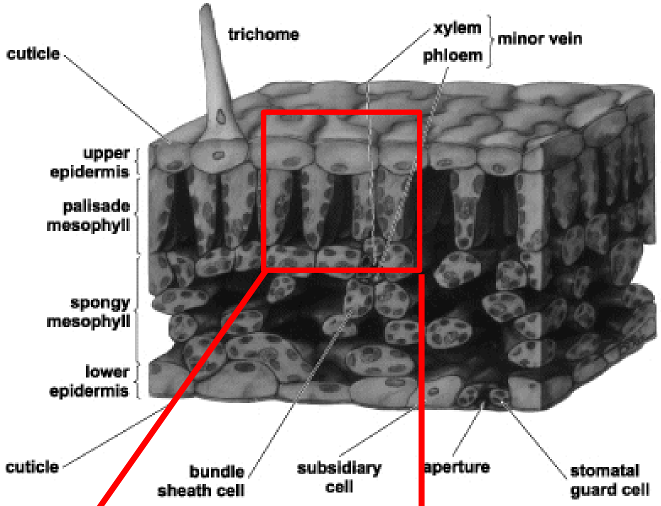
Biotechnology: Al in tea leaves

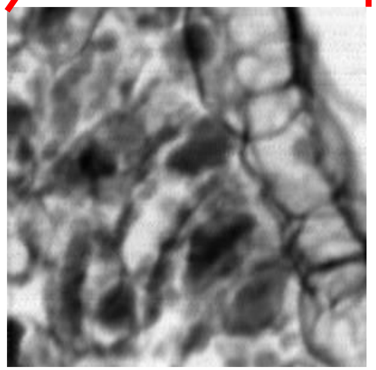
Charlotte Poschenrieder,
Uni Barcelona, ES

Katharina Vogel,
Uni Ljubljana, SI

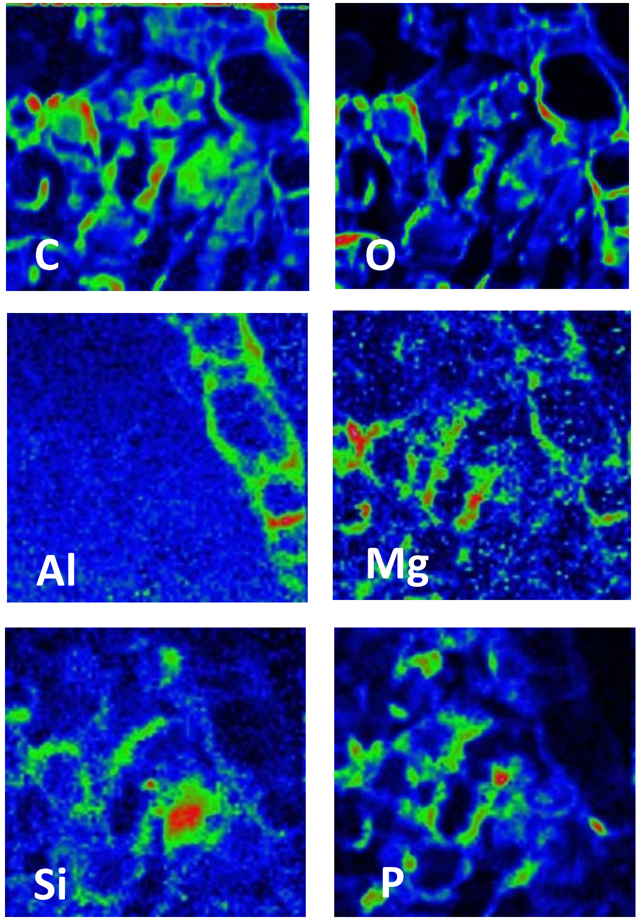
Functionality and toxicity of Al in tea leaves analyzed on sub-cellular level

Cross-section of a leaf





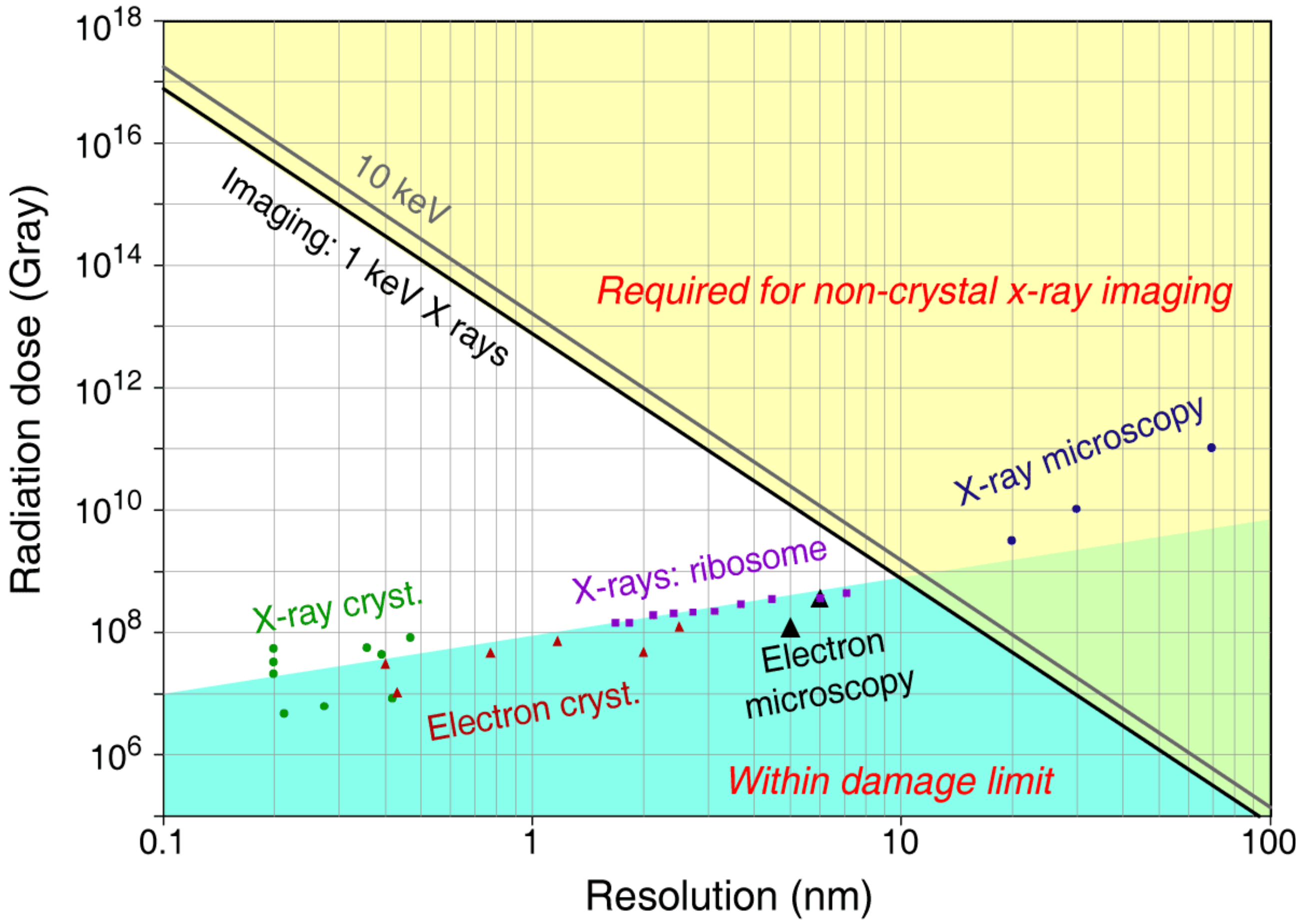
E=2.19 keV
80 x 80 μm²
80 x 80 px
12s /px



To be always considered as for other microscopies:

The radiation damage issue !!!

The issue of radiation damage



Experimental data by M. Howells, ALS, US

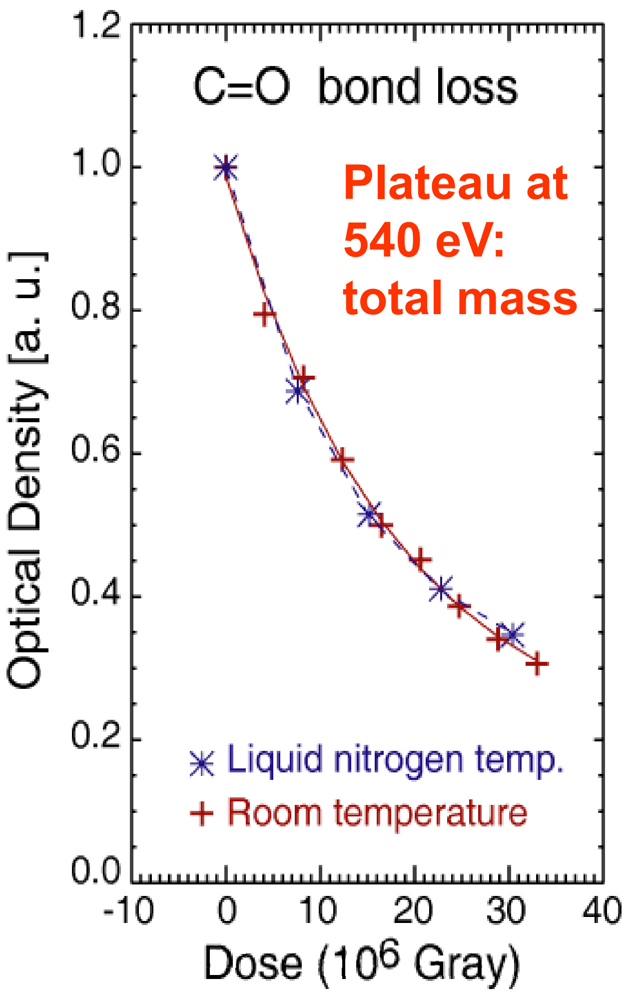
Radiation dose scales with (resolution element)^4

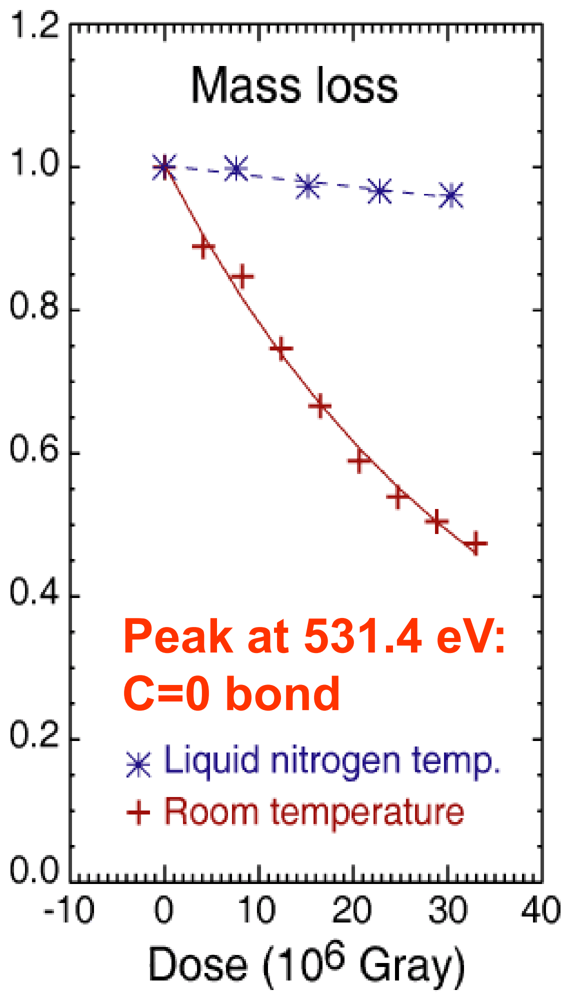
The issue of radiation damage

Cosmic ray dose on high-altitude flight	0.001 – 0.01 mGy/ h
Natural background radiation	0.01 mGy/ day
Chest X-ray	0.06 mGy
Dental X-ray	0.09 mGy
Mammogram	0.7 mGy
Head CT	2 mGy
Cernobyl reactor accident victims	up to 500-1000 mGy ?
World war II nuclear bomb victims	up to 500-1000 mGy
Lethal for humans	about 2000 mGy
Electron and X-ray microscopy	about 10^{9}-10^{16} mGy in the microspot

Approximate values are indicative and can vary with conditions

The issue of radiation damage: Cryogenic cooling





Cryogenic cooling CANNOT avoid bond loss

Cryogenic cooling CAN significantly reduce mass loss

***Beetz and Jacobsen, J. Synchrotron Radiation* 10, 280 (2003)**

Transmission X-ray microscopy

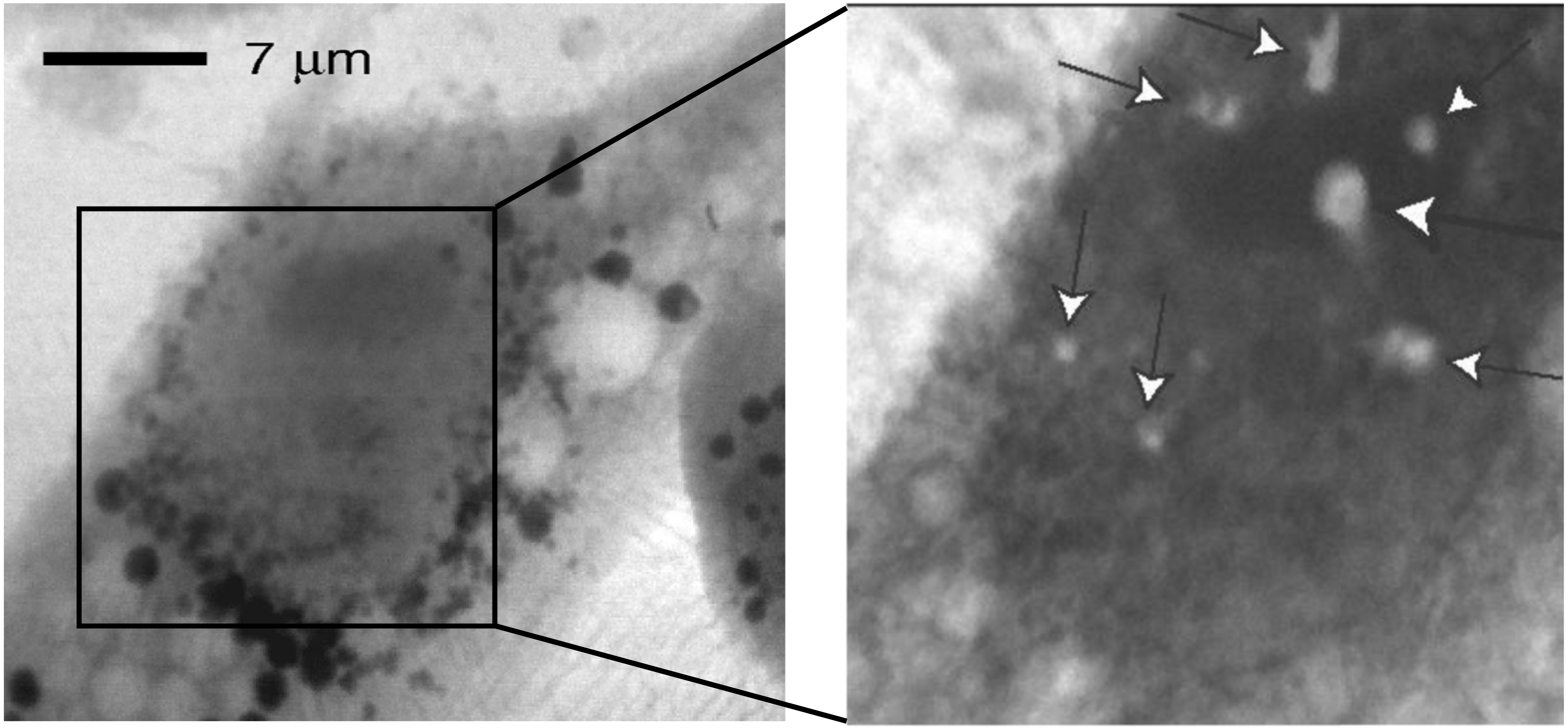


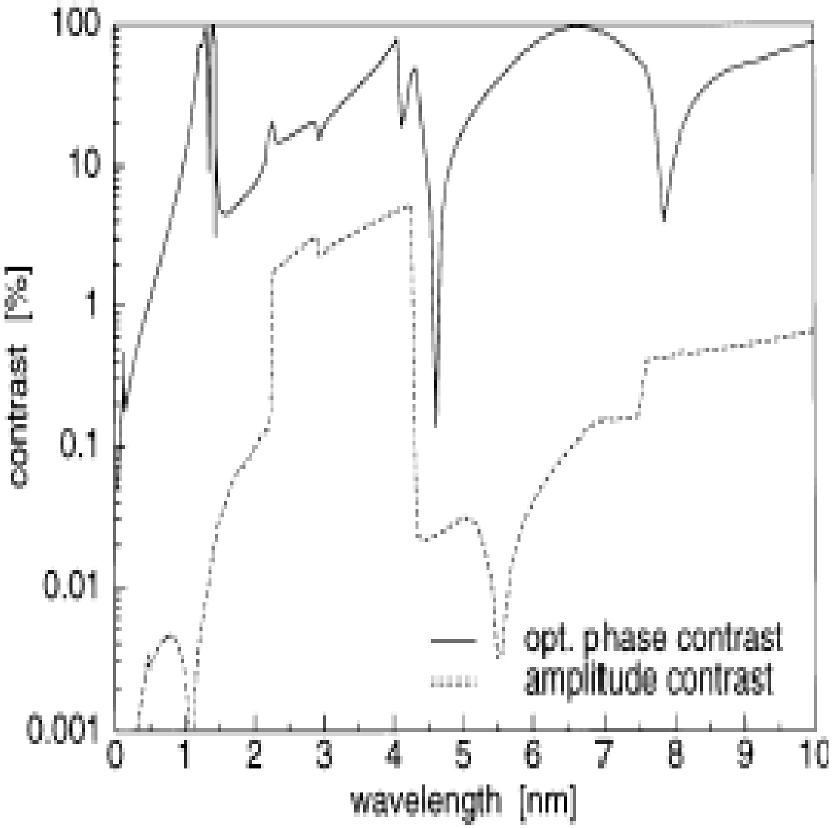
Image of a cryo-fixed cell after exposing several regions to ~10^{10} Gray

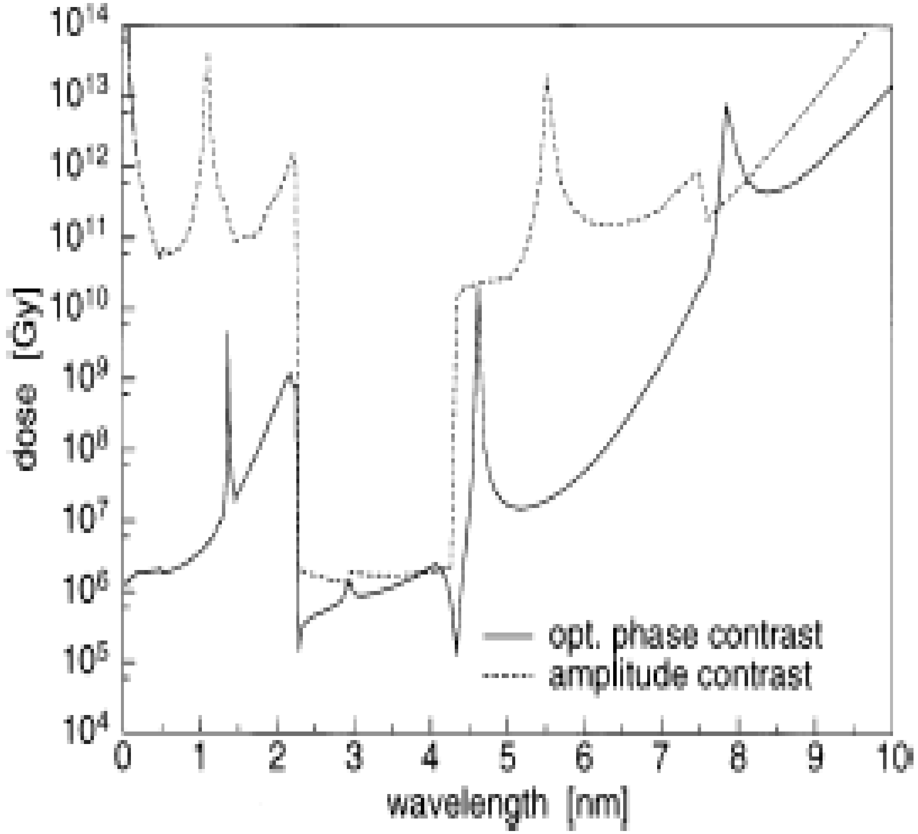
After warmup in microscope: holes indicate irradiated regions!

Image courtesy: J. Maser, NSLS, Stony Brook at this time

The issue of radiation damage: Use phase contrast

Contrast and dose for a model protein $C_{94}H_{139}N_{24}O_{31}S$





G. Schneider, Ultramicroscopy 75 (2), 85-104 (1998)

Student positions open at the X-ray Microscopy of Elettra

Interested applicants are welcome to contact for further information:

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Dr. Burkhard Kaulich
burkhard.kaulich@elettra.trieste.it