

X-RAY IMAGING AND MICROSCOPY

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With thanks to:

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A. Pacureanu, F. Peyrin, A. Rack, M. Salome, P. Tafforeau, Y. Yang

X-ray (absorption) imaging is not a new technique....



History : 1895, G.C. Röntgen

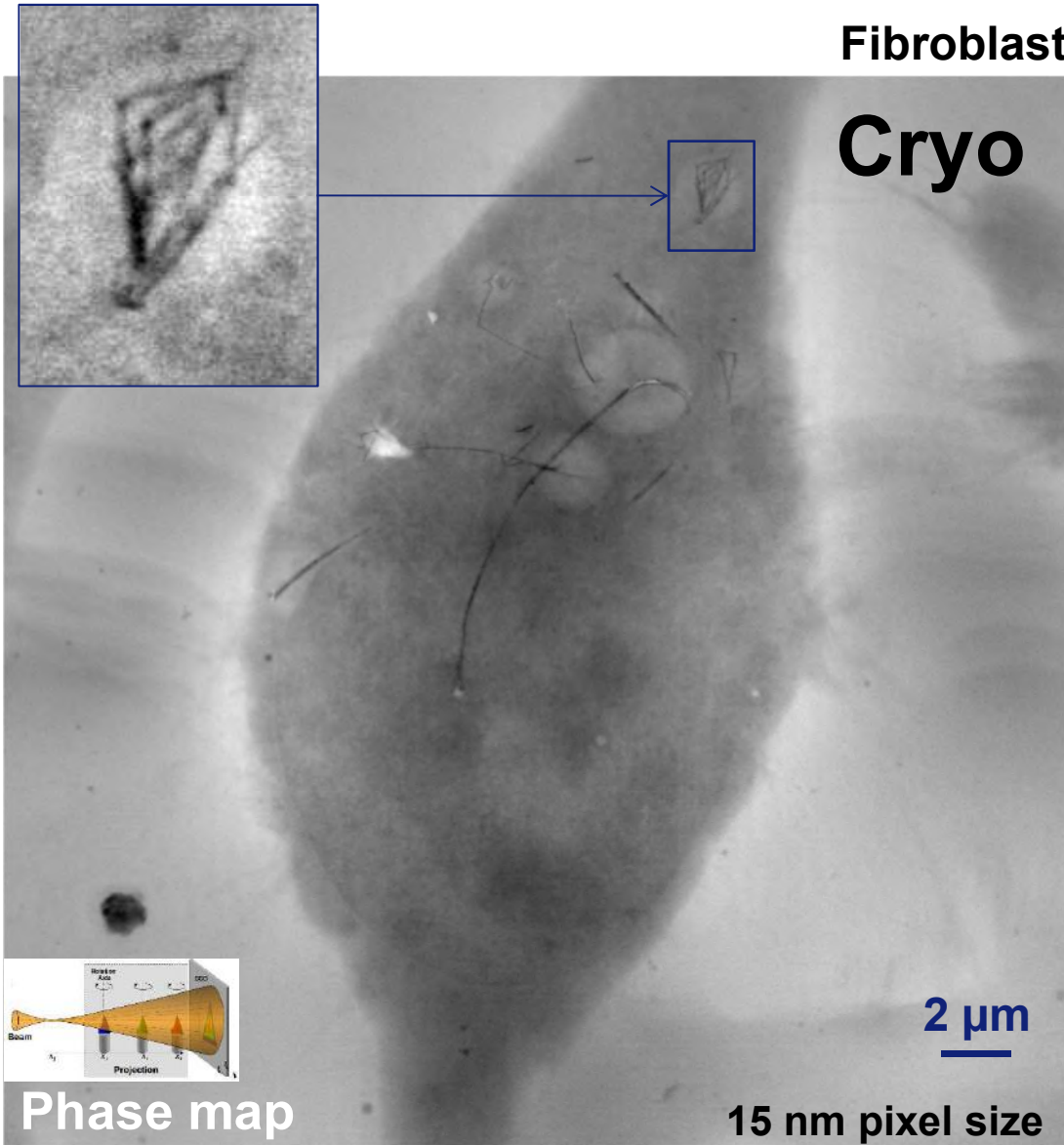


First X ray made in public. Hand of the famed anatomist, Albert von Kölliker, made during Roentgen's initial lecture before the Würzburg Physical Medical Society on January 23, 1896.

CRUMPLING OF SILVER NANOWIRES BY ENDOLYSOSOMES STRONGLY REDUCES TOXICITY

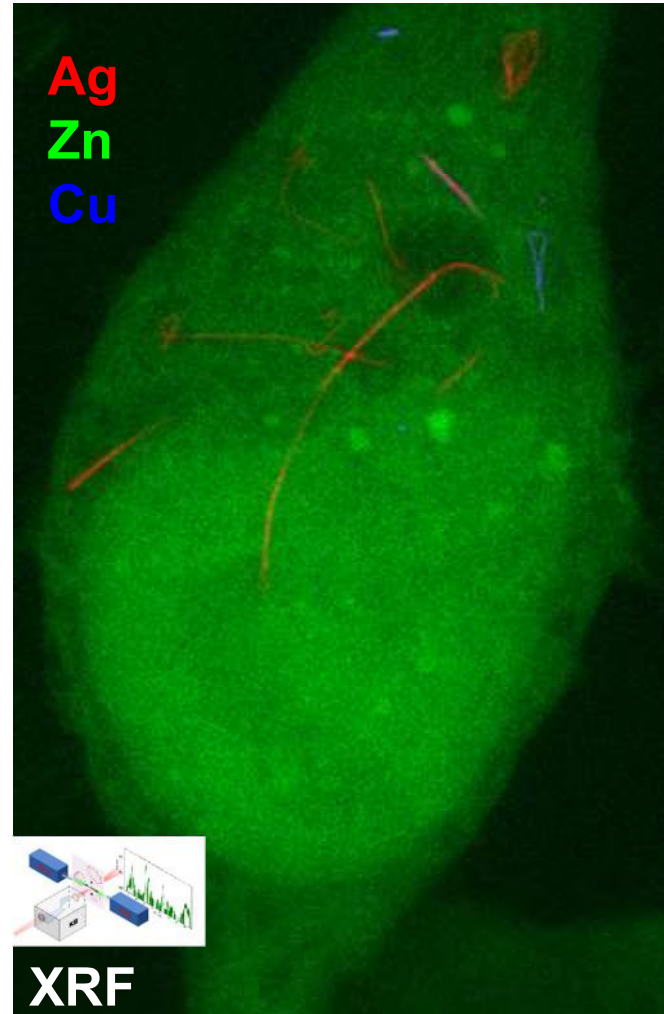
Fibroblast cell

Cryo



Phase map

33 nm Ag nanowires



XRF

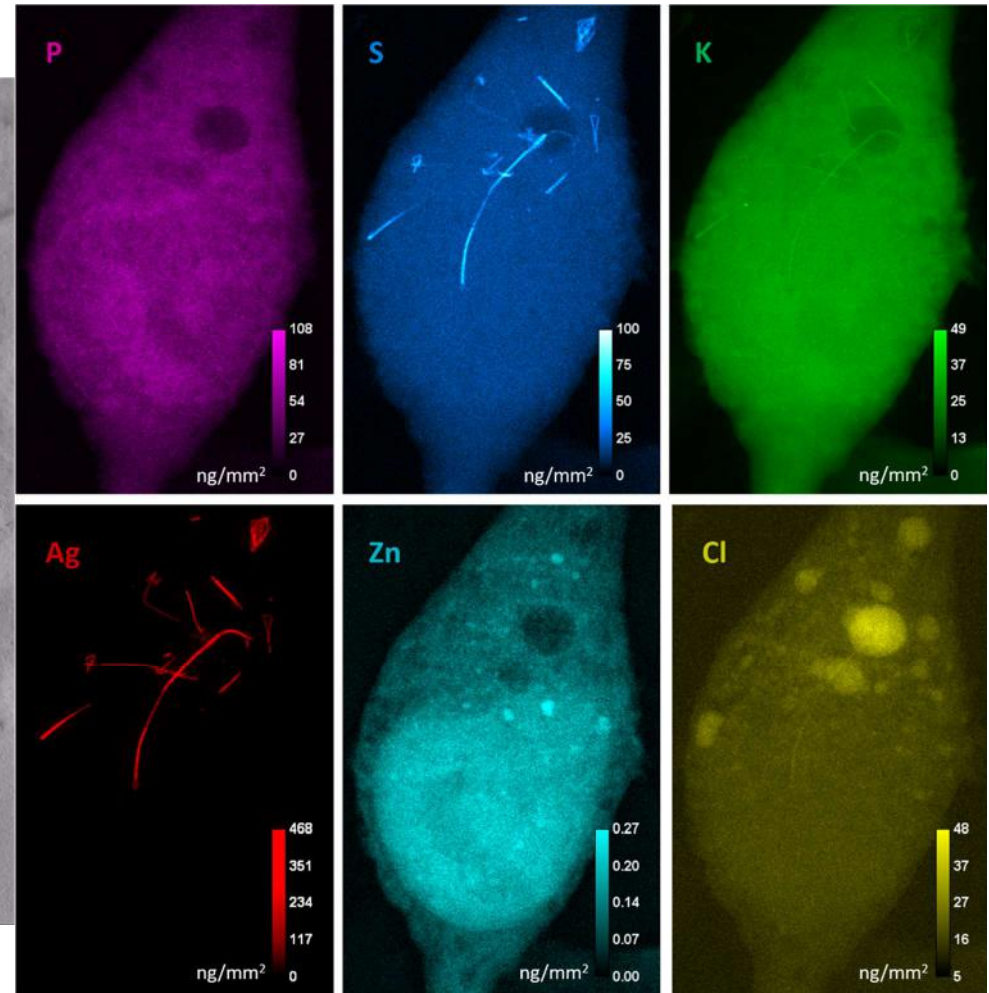
50 nm pixel size

S Lehmann, A E Prada, L Charlet, B Gilbert (LBNL) et al, *PNAS*, 116, 14893 (2019)

CRUMPLING OF SILVER NANOWIRES BY ENDOLYSOSOMES STRONGLY REDUCES TOXICITY



30 nm voxel size



S Lehmann, A E Prada, L Charlet, B Gilbert (LBNL) et al, *PNAS*, 116, 14893 (2019)

“Micron scale”

- Tomography
- (Spatial) Resolution
- Absorption / Phase Contrast

“Nano-scale”

- Techniques
- Full-field (& Dark-field Microscopy)
- (CDI / Ptychography)
- Nano-probe techniques

Generalized Tomography

Fluorescence

Diffraction

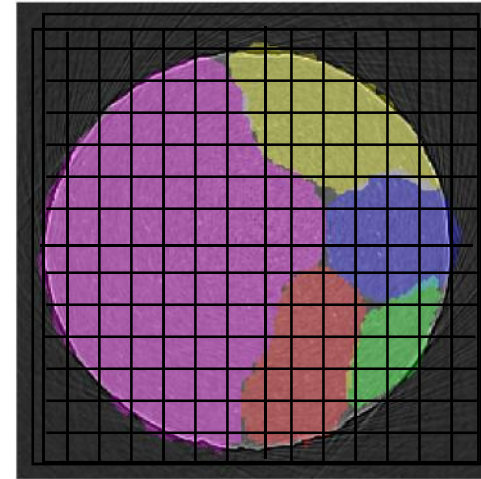
SAXS

- Holography

Inhomogeneous sample

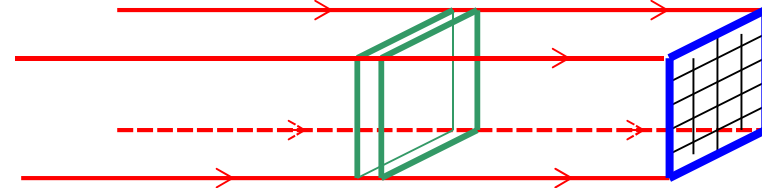
(density, composition, domains, phases, defects, ...)

→ requires a “**local**” investigation

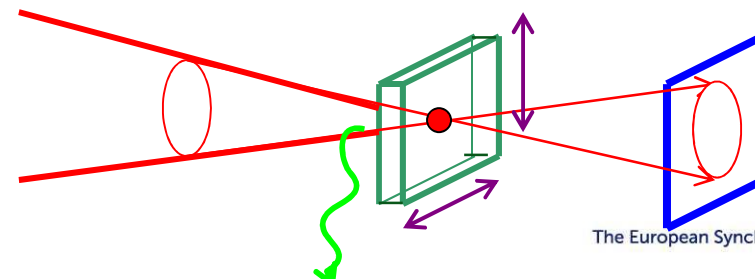


Two main techniques:

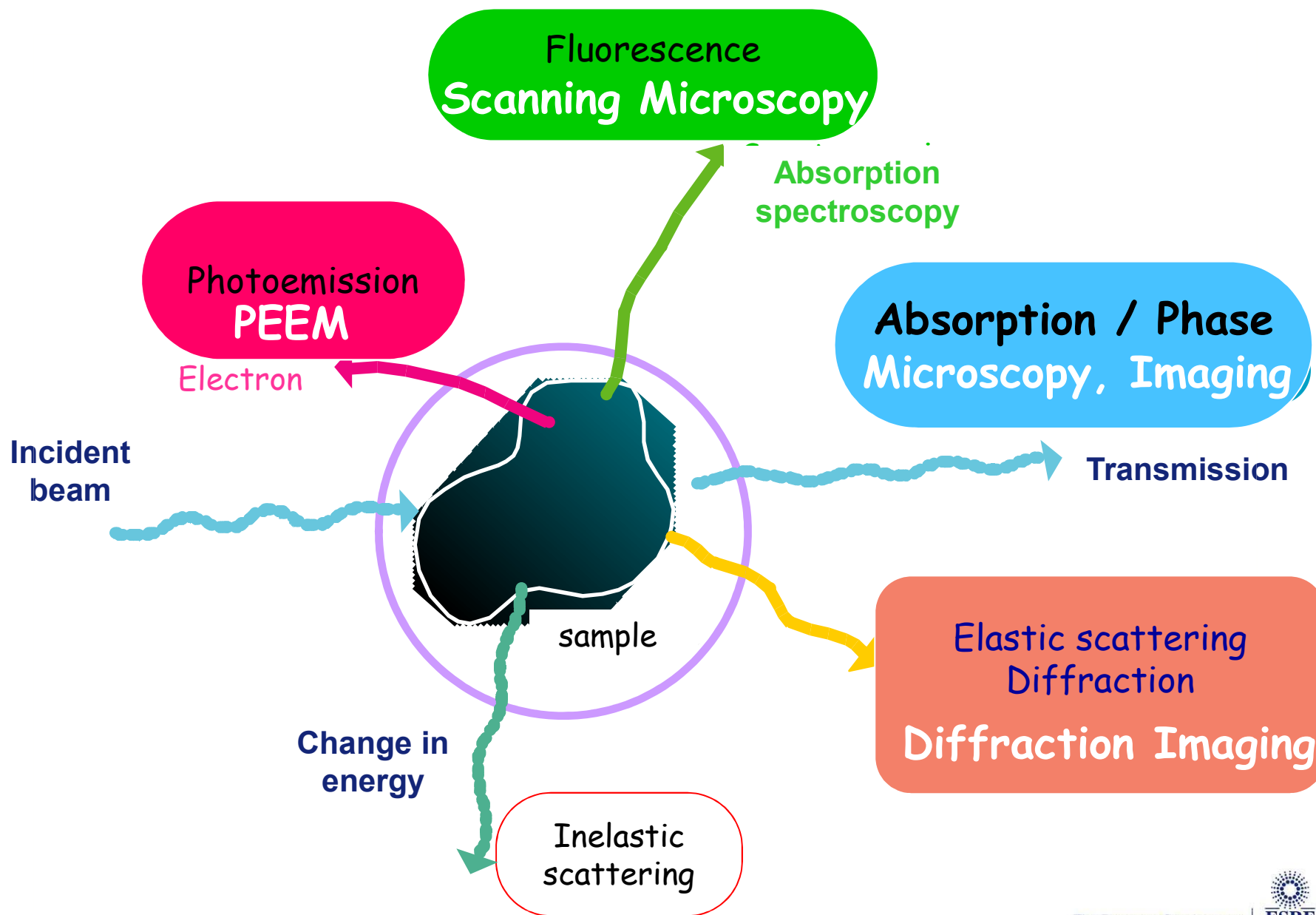
Full-field Techniques
with Parallel Beam



Scanning Techniques
with Focused Beam

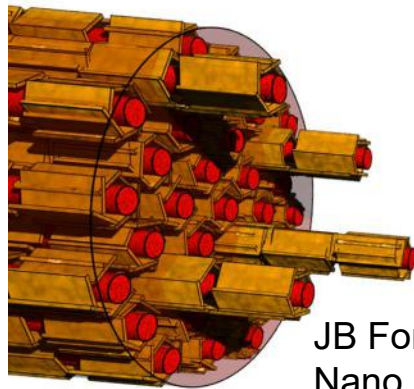


X-RAY MATTER INTERACTIONS USED FOR X-RAY IMAGING

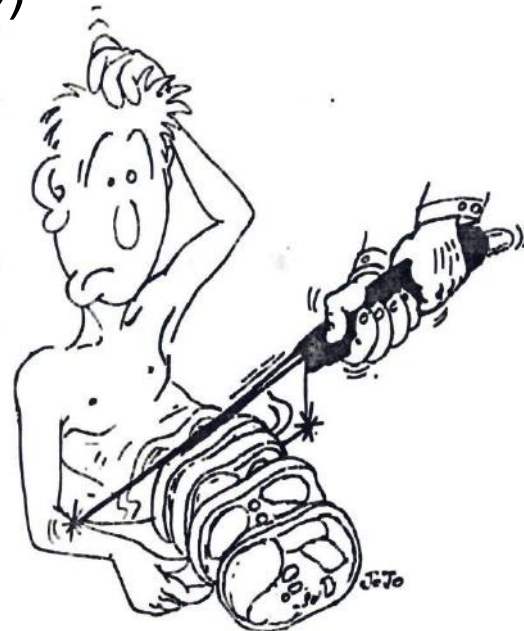
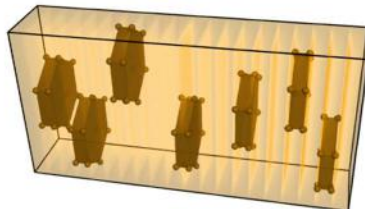


MAIN ASSETS OF HARD X-RAYS IN MICROSCOPY

- Combine **high spatial resolution** on '**thick**' samples in 3D
- **Multi-modal approach**; natural and quantitative contrast from electron density, elements, chemistry and (crystalline) structure
- **Access to large volumes** (representative elementary volume)
- **Dynamics**: follow evolving systems with fast 2D and 3D techniques
(mostly non-destructive)



JB Forien, P Zaslansky et al.
Nano Lett. 15 (2015) 3729



Goal: **quantitative imaging**:

= *measuring* a given *object* quantity as a function of *space* and *time*

$$\alpha(r,t)$$

with $r = (x,y,z)$ or (x,y)

and α can be $n = 1 - \delta + i\beta$

lattice distortion u

element concentrations

(electron) density ...

Always put a scale bar and color bar!

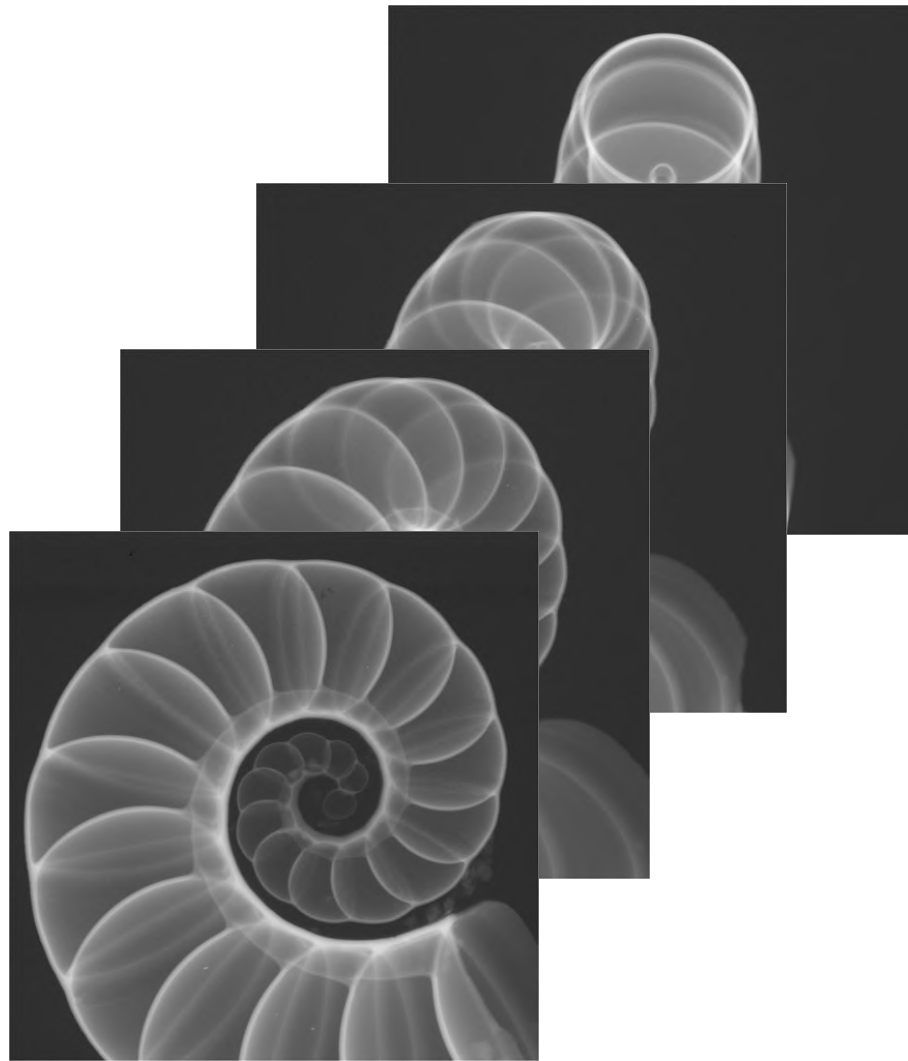
X-rays well suited:

Weak and linear interaction, but not always (e.g. dynamical diffraction)

But

'Poor' instrumentation compared to light / electron microscopy

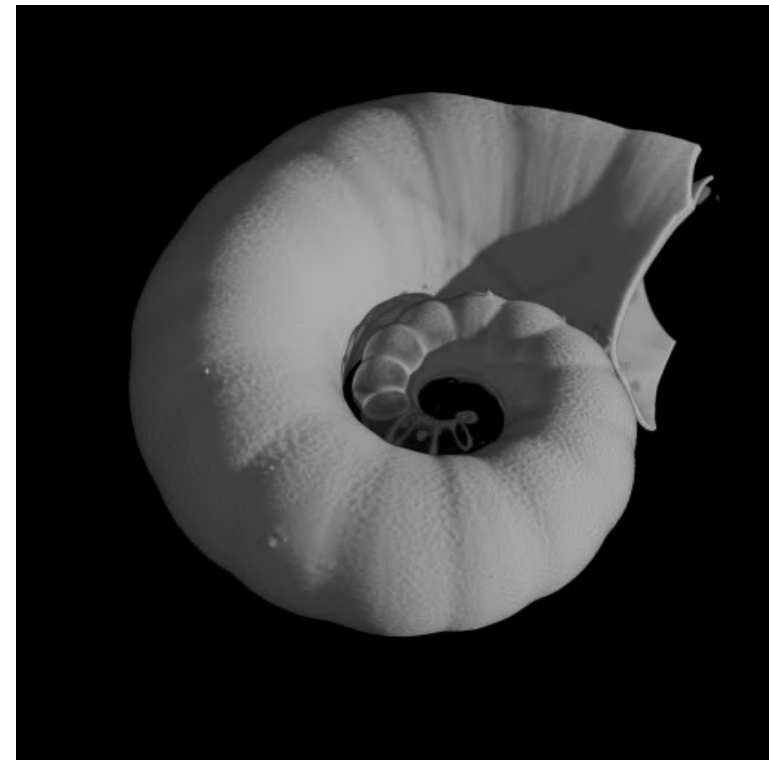
COMPUTED TOMOGRAPHY (2D → 3D)



N 2D projections

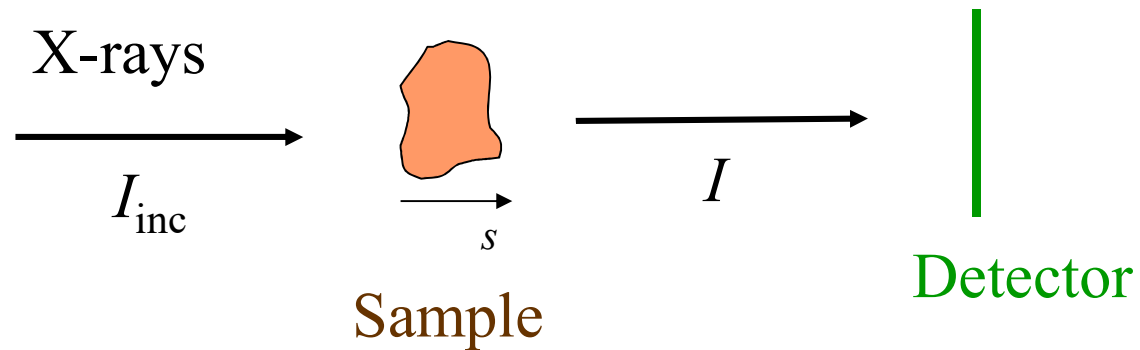


Volume Rendering



Reconstructed
3D volume

Lambert-Beer law



$$\frac{I}{I_{\text{inc}}} = \exp \int -\mu ds$$

$$\ln \frac{I_{\text{inc}}(x')}{I(x')} = \int \mu(x, y) ds$$

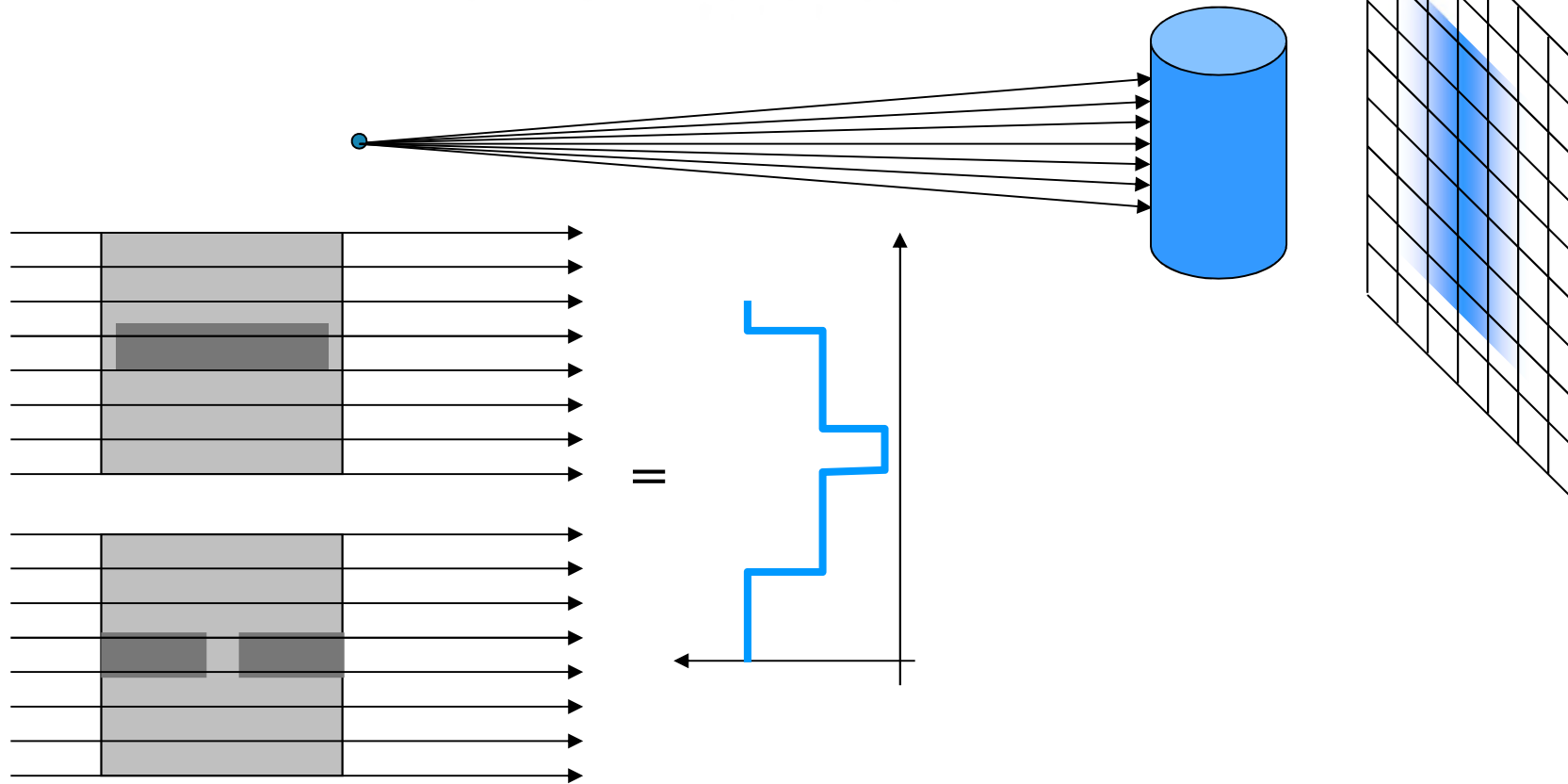
μ , linear attenuation coefficient

LATERAL AND DEPTH RESOLUTION

Radiography → Sum of the attenuation along a ray

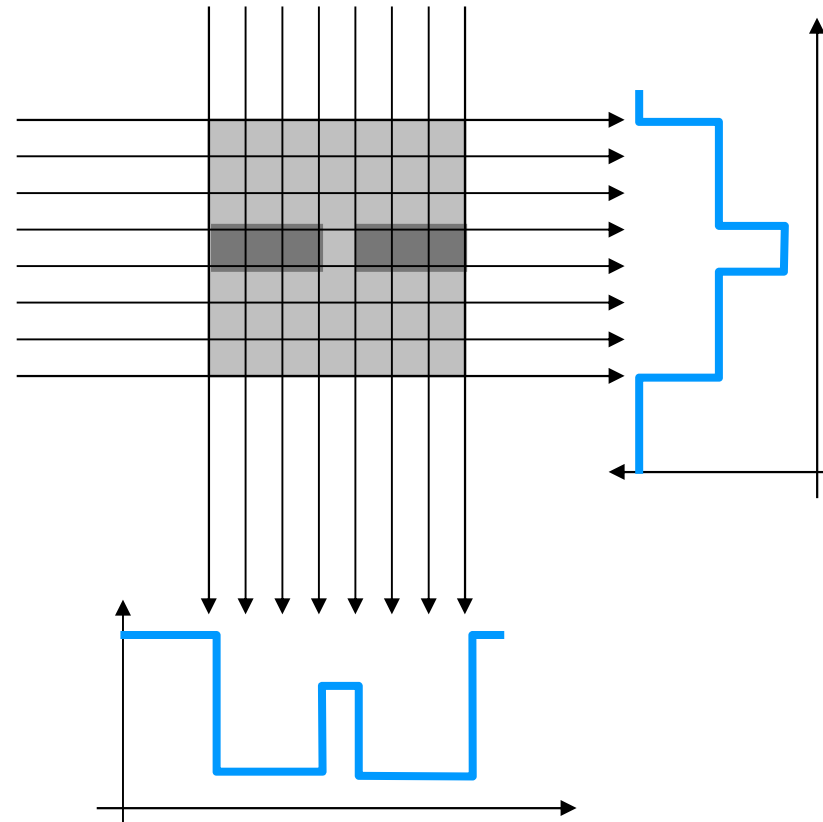
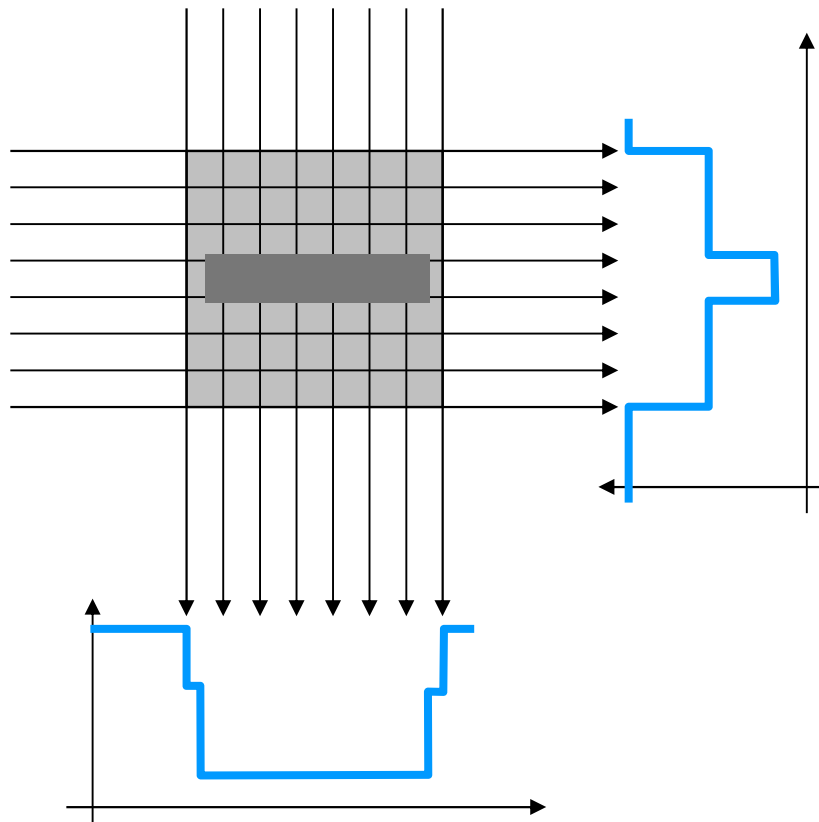
→ Good lateral resolution

→ No depth resolution



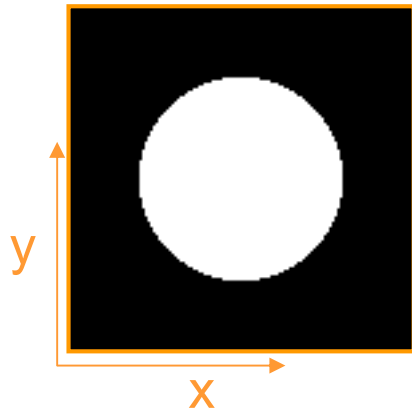
TOMOGRAPHY PRINCIPLE “WITH HANDS”

To distinguish the 2 : take 2 projections at 90 degrees



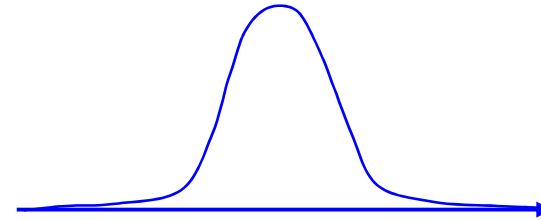
RADON TRANSFORM: EXAMPLES

Image Space



Same projection $\forall \theta$

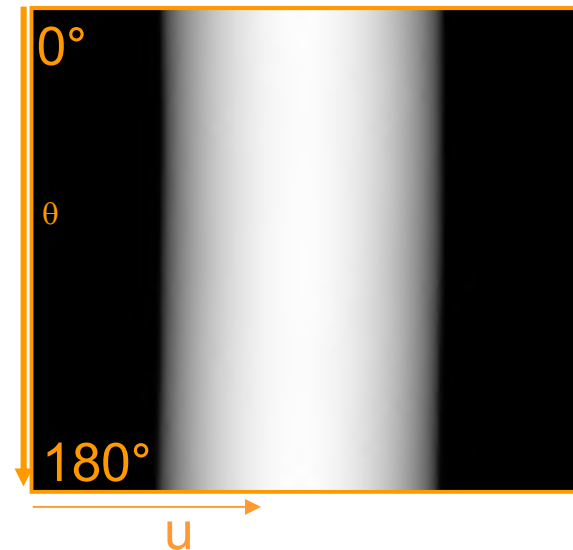
amplitude



Gray level

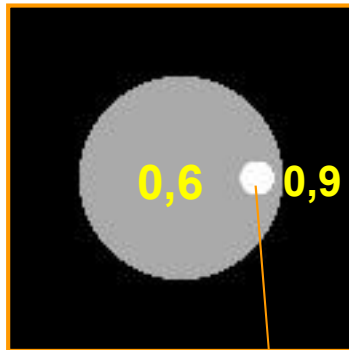


Radon Space
= **sinogram**

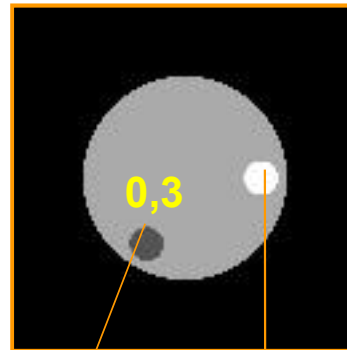


RADON TRANSFORM: EXAMPLES

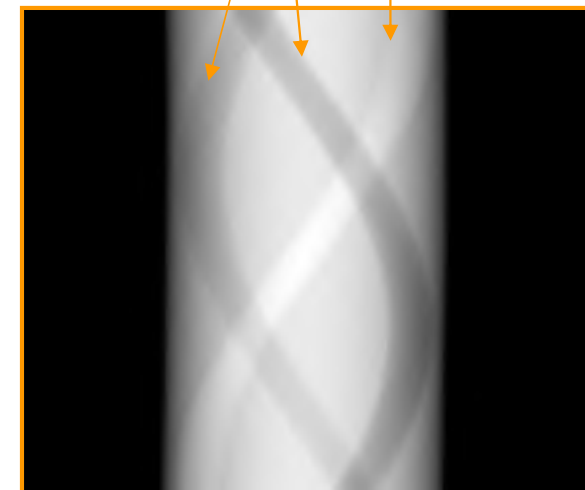
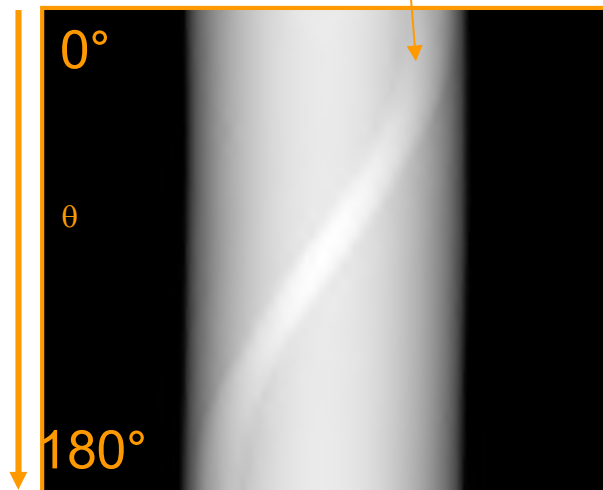
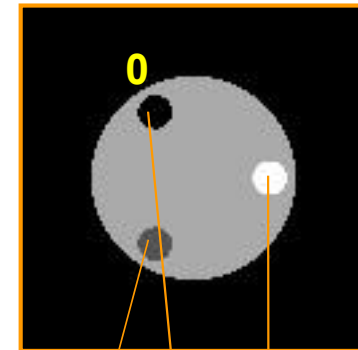
1 circle



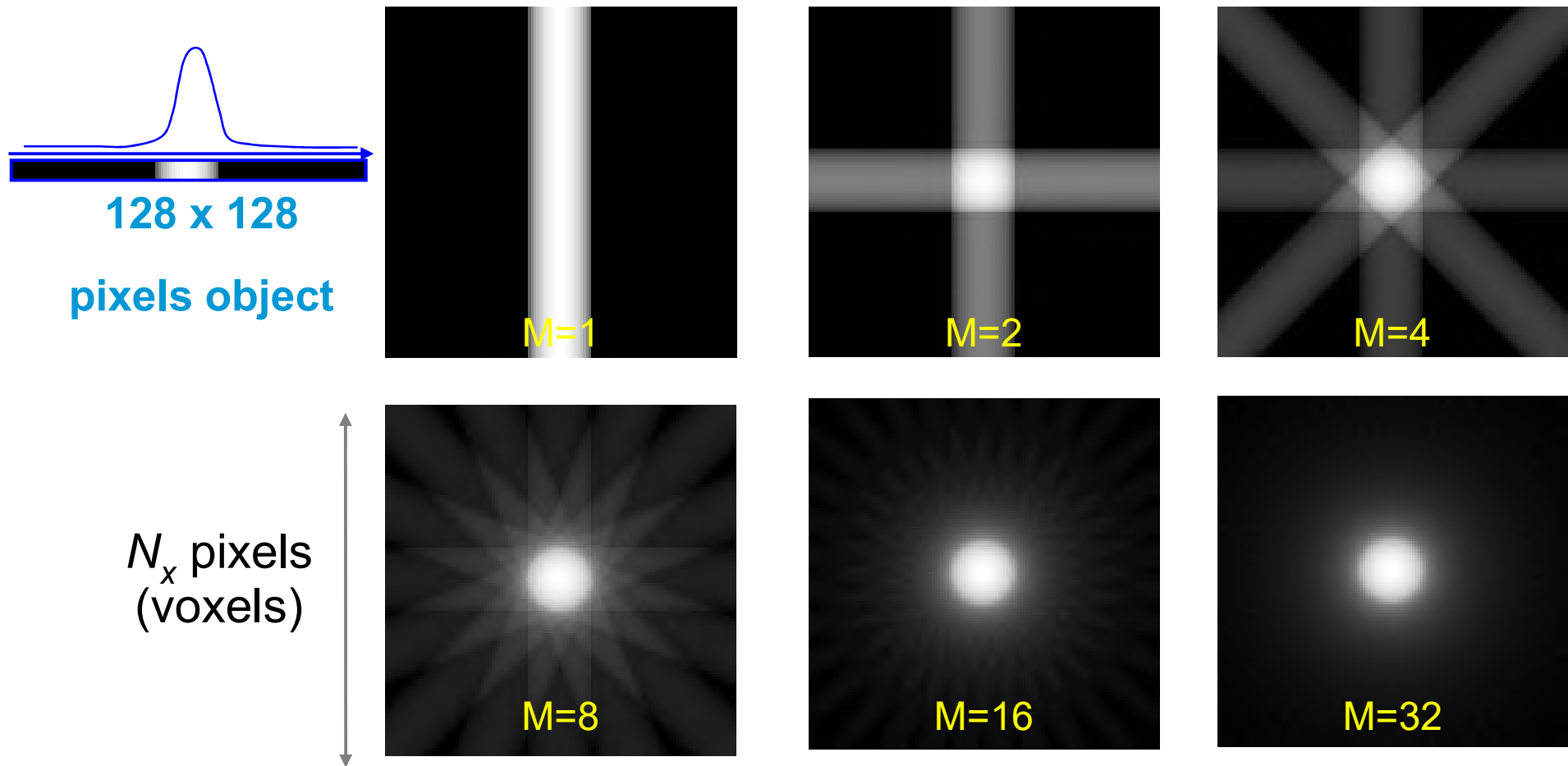
2 circles



3 circles



RECONSTRUCTION: (FILTERED) BACK-PROJECTION



Correct result using: **Filtered Back-projection**

Number of projections: Theory: $M = \frac{\pi}{2} N_x$ Practice: $M \approx N_x$

Absorption/Phase tomography: analytic method, FBP using e.g. Nabu, PyHST

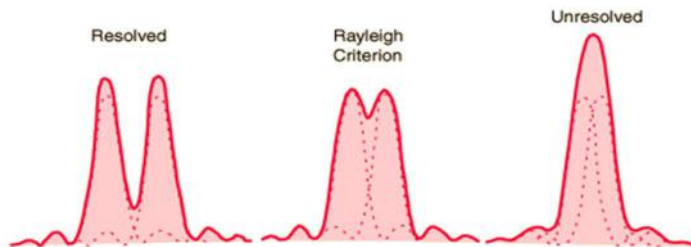
Diffraction/Fluorescence tomography: algebraic methods, MLEM, TV using e.g. ASTRA

Pixel / Voxel size

Often stated as 'resolution' in tomography!

Correct sampling requires: $f_{max} < f_{Nyquist} = \frac{1}{2 \text{pixelsize}}$

Rayleigh Criterion for resolving two adjacent objects



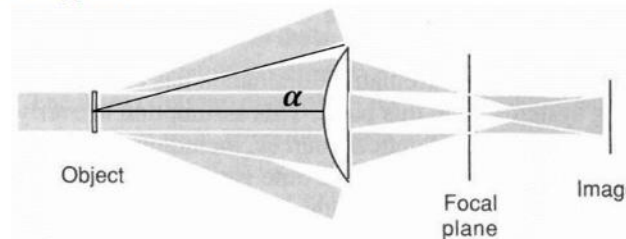
Point spread function - Transfer function

$$R_{\text{Rayleigh}} = c \lambda / \text{NA}$$

Noise-less world

Abbe criterion:

Resolution depends on **wavelength** and **numerical aperture**. There is an upper limit for a given wavelength.



$$\Delta x = \frac{\lambda}{2n \sin \alpha}$$

SPATIAL RESOLUTION (2)

Rose Criterion: influence of noise on spatial resolution

Photon statistics and/or dose limit the obtainable resolution
SNR > 5 for detection

e.g. tomography (Flannery 87) $N_{phot} \propto \frac{D^4}{R^4} \frac{\exp(\mu D)}{[\mu D (\sigma/\mu)]^2}$

With sample diameter D constant: if R ↓ then $N_{phot} \uparrow$ as $(1/R)^4$

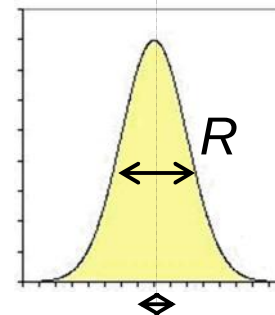
Detection Limit:

Smallest object that can be detected
will depend on contrast and noise
can be $\ll R_{Rayleigh}$ especially using phase contrast

Precision:

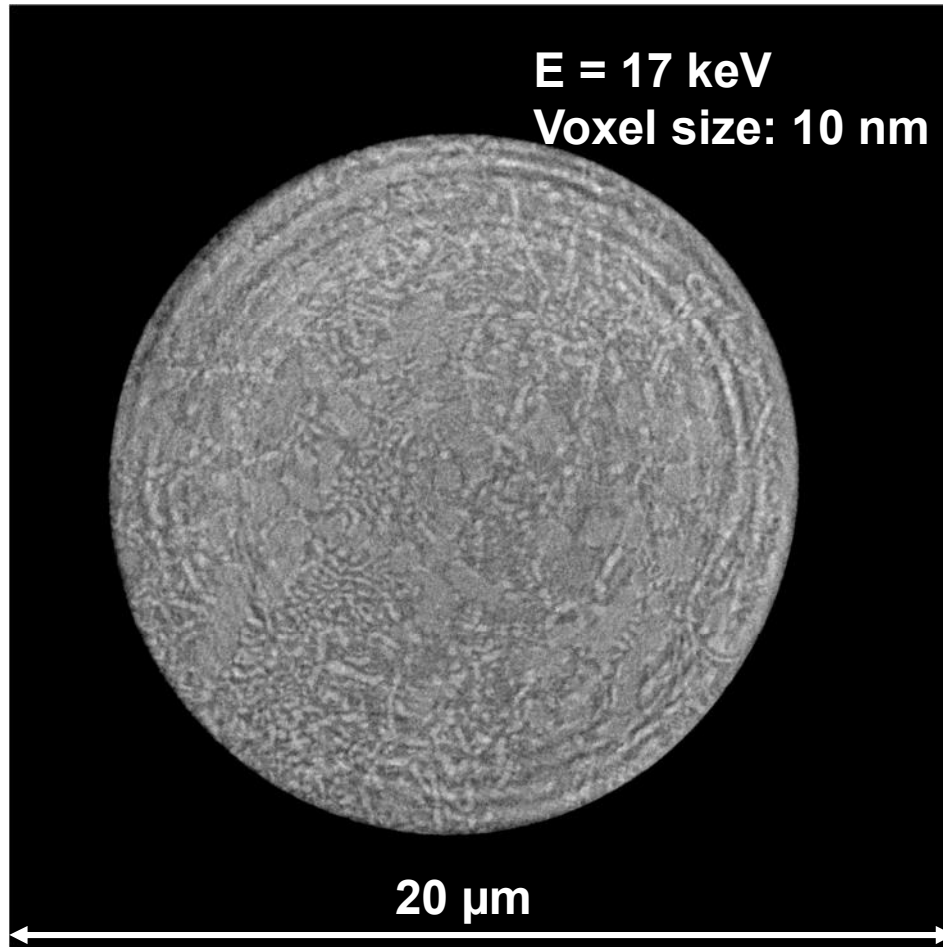
on the position of an object for example

$$Precision \propto \frac{R_{Rayleigh}}{\sqrt{N_{phot}}}$$

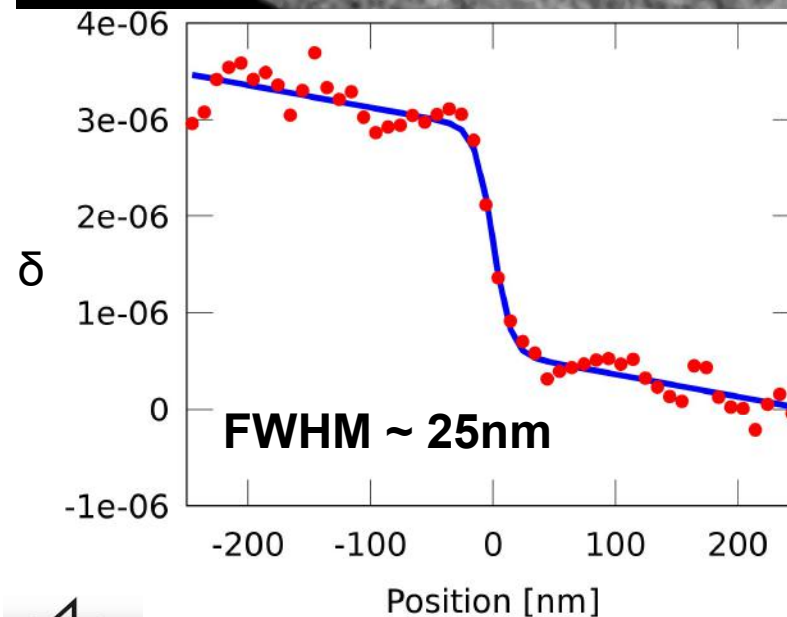
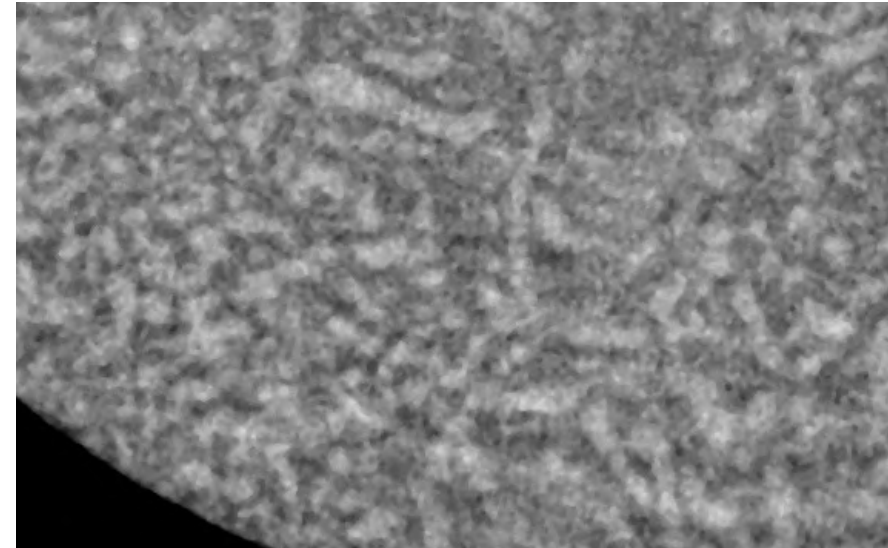


ESTIMATION OF THE 3D SPATIAL RESOLUTION

Edge Profile



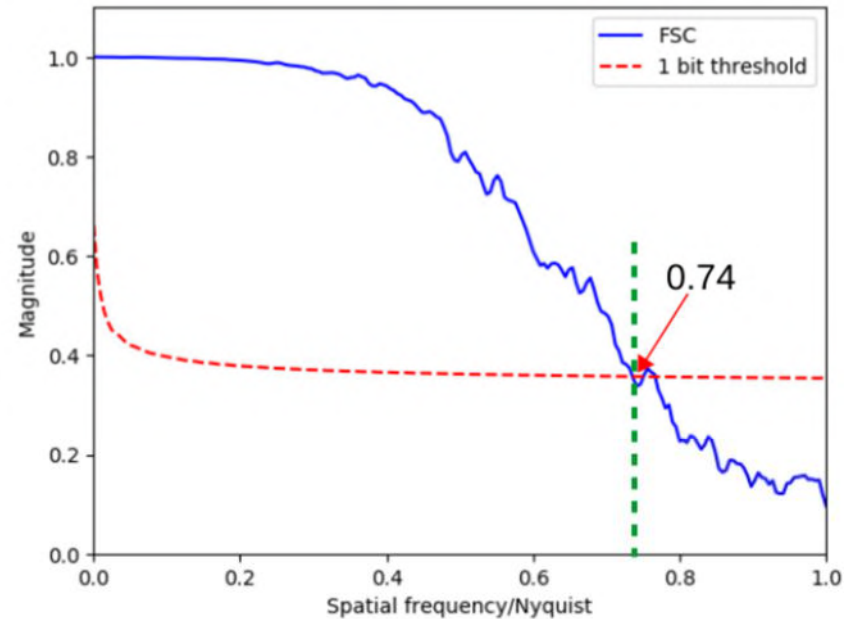
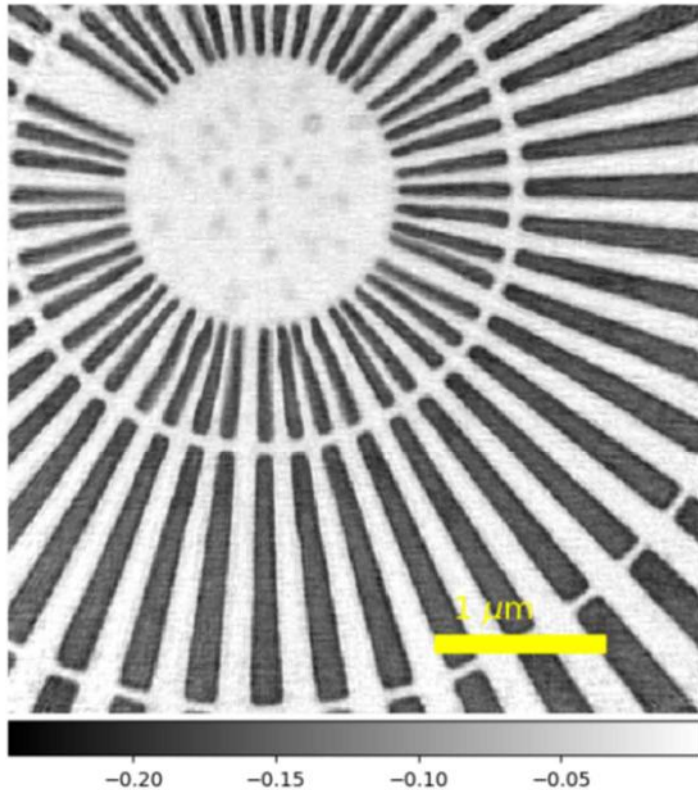
Ultrafine eutectic Ti/Fe alloy
by additive manufacturing



ESTIMATION OF THE 3D SPATIAL RESOLUTION

Fourier Shell Correlation

Actually measures the *consistency* of two measurements



Resolution estimate:

$$\frac{10 \text{ nm}}{0.74} = 13.51 \text{ nm}$$

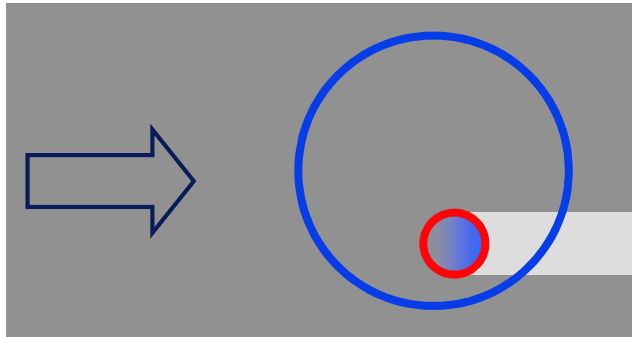
$$\text{FSC}_{12}(r_i) = \frac{\sum_{r \in r_i} F_1(r) \cdot F_2(r)^*}{\sqrt{\sum_{r \in r_i} F_1^2(r) \cdot \sum_{r \in r_i} F_2^2(r)}}$$

M. van Heel, M. Schatz,
Fourier Shell Correlation threshold criteria,
J. Struc. Biol. 151 (2005) 250.

ABSORPTION VS. PHASE

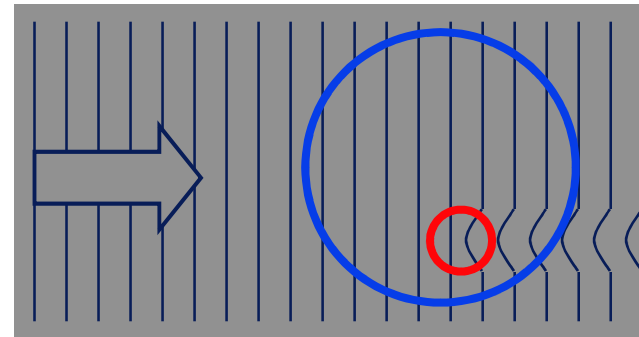
Simple transmission

Absorption



$$\beta \propto \mu$$

Phase



$$\delta \propto \text{electron density}$$

- Dream 1: **Low Dose**

Increase the energy

Absorption contrast ↓ replaced by phase contrast

- Dream 2: Improve the **Sensitivity**

Absorption contrast too low high spatial resolution

light materials

similar attenuation

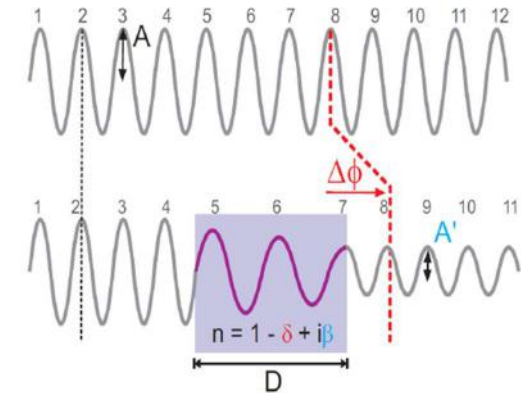
TRANSMISSION IMAGING

- Weak interaction with matter
- Refractive index n (X-rays):

$$n = 1 - \delta + i\beta$$

$$\delta \gg \beta$$

$$10^{-6} \quad 10^{-9}$$



from C. Grünzweig, PhD thesis

β – Absorption index

- photo-electric effect
 - Compton scattering
 - strong energy dependence
 - $\beta = (\lambda / 4\pi) \cdot \mu$
- linear attenuation coefficient μ

$$\beta = \frac{r_c \lambda^2}{2\pi V} \sum f_p''$$

δ – Refractive index decrement

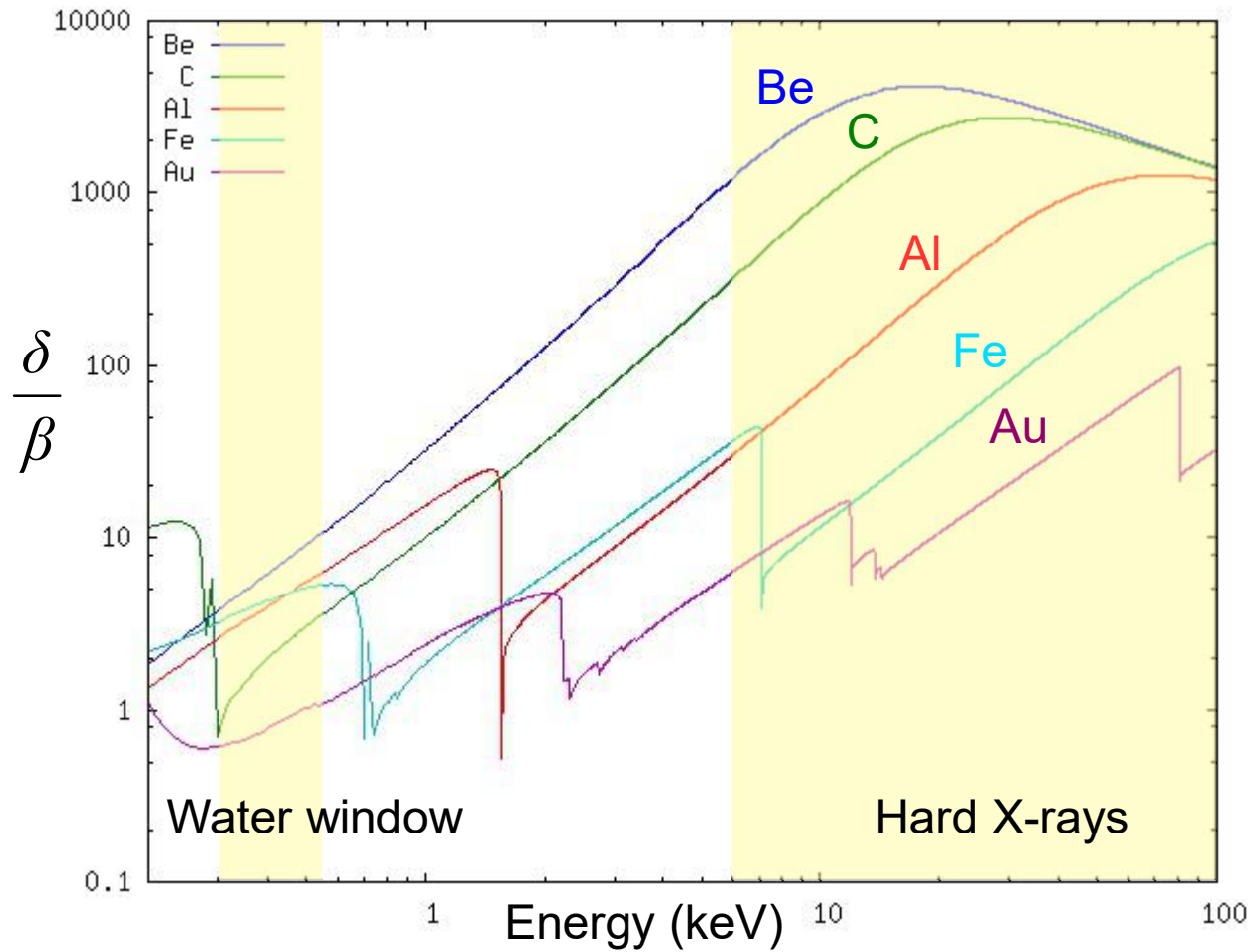
- proportional to electron density
- inversely proportional to energy²

$$\delta = \frac{r_c \lambda^2}{2\pi V} \sum (Z_p + f_p')$$

$$\approx 1.3 \cdot 10^{-6} \rho \lambda^2$$

ρ in g/cm³, λ in Å

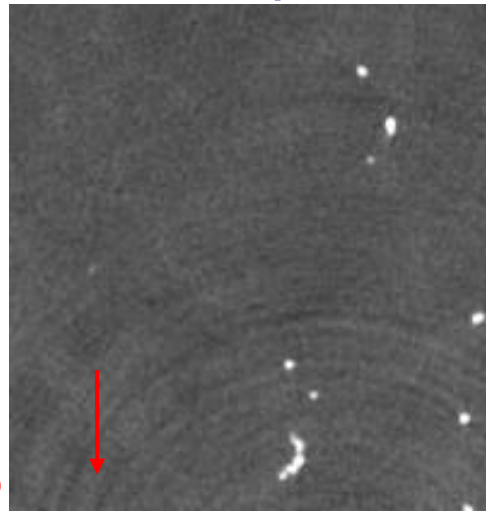
PHASE CONTRAST VS ABSORPTION



Gain of up to a few 1000 !

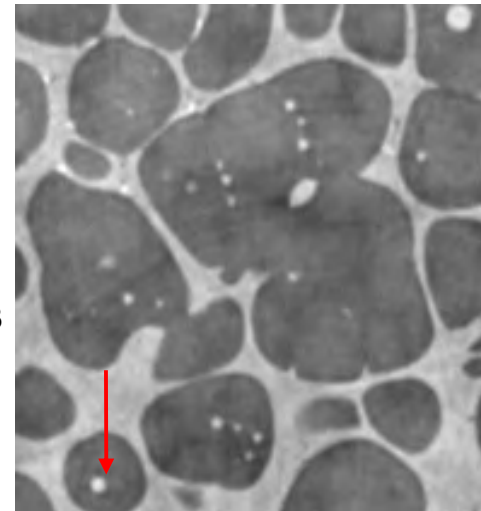
ABSORPTION VS. PHASE: AL/SI SEMI-SOLID ALLOY

Absorption



β -map

Phase contrast



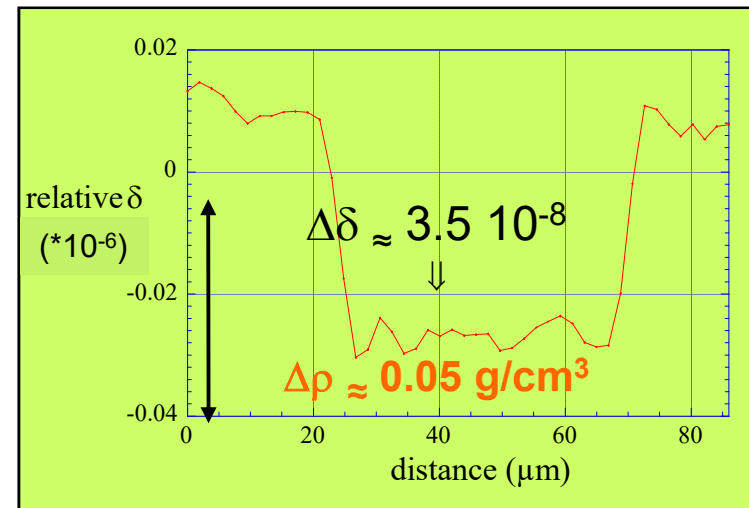
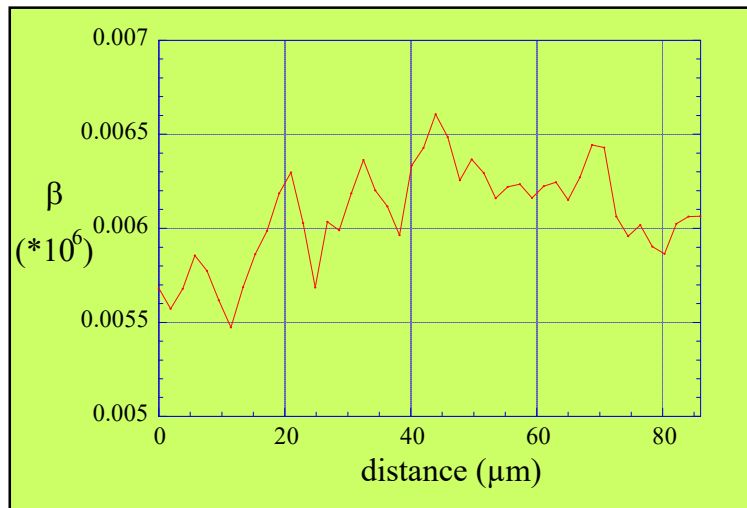
δ -map

$E = 18 \text{ keV}$

$50 \mu\text{m}$

$\rho_{\text{Al}} \sim 2.7 \text{ g/cm}^3$

$$\frac{\Delta\rho}{\rho} \approx 2\%$$



'Subtraction' of images around a K-edge:

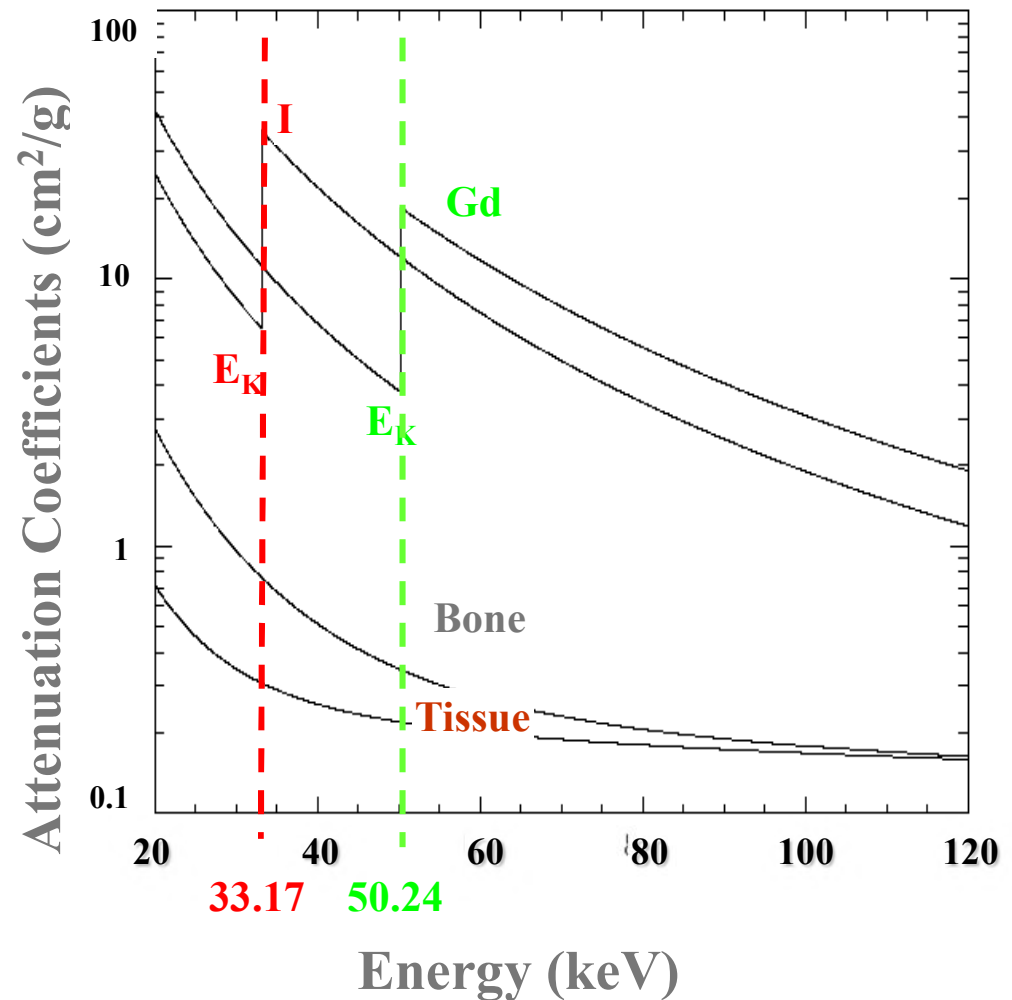
→ coronary angiography

→ brain permeability

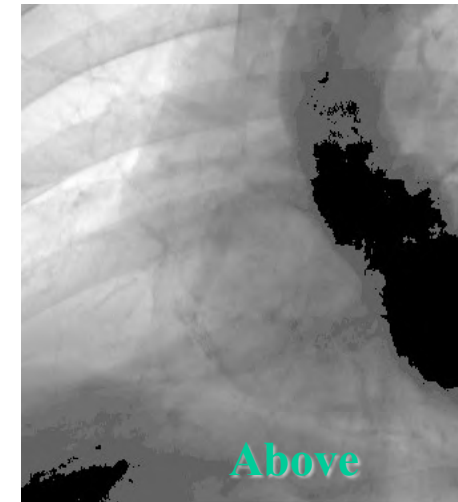
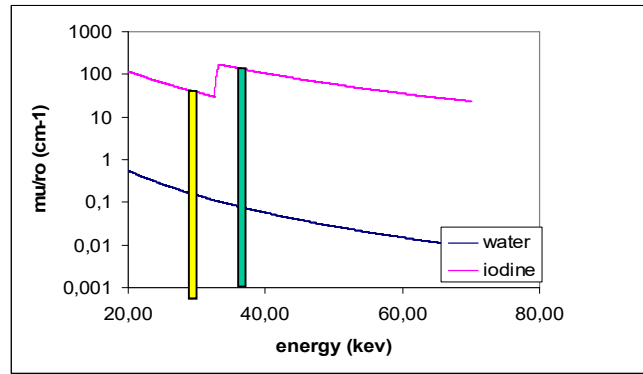
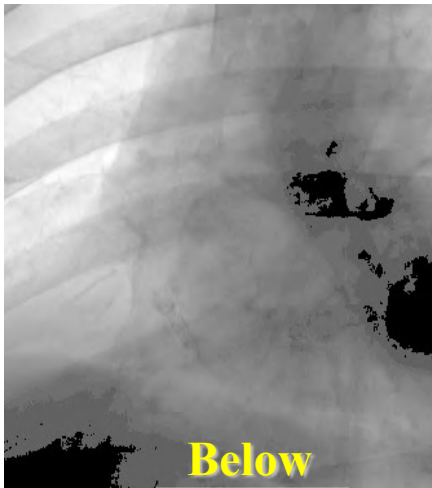
→ lung ventilation,

...

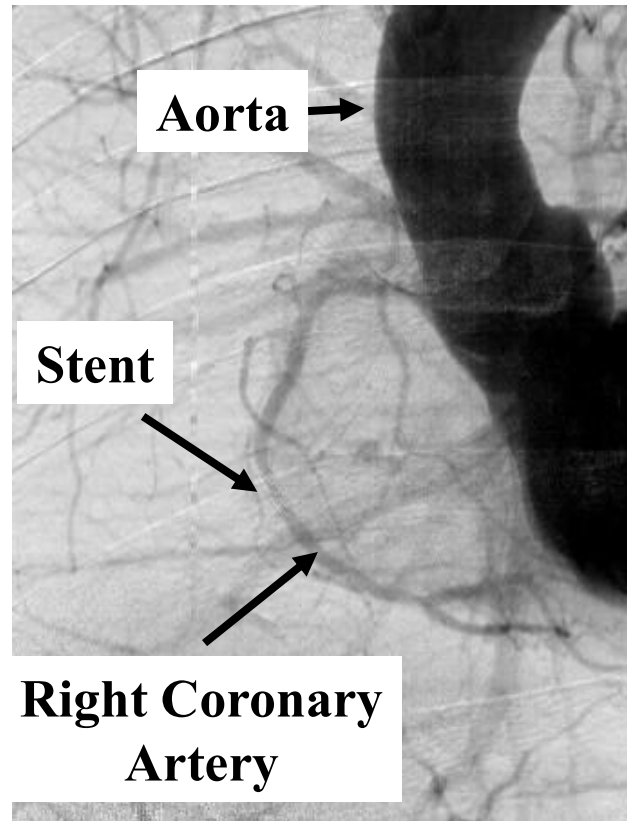
→ Concentration maps of I, Gd, ...



ABSORPTION: K-EDGE SUBTRACTION IMAGING



K-edge subtracted image:




Elleaume, Estève et al.

subtraction of images recorded above and below the “absorption edge” of iodine

Tissues display nearly the same absorption

→ Iodine is seen

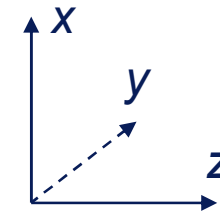
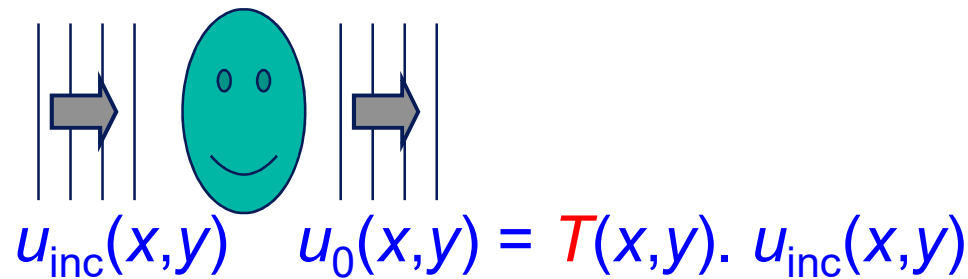
TRANSMISSION THROUGH A SAMPLE



refractive index $n = 1 - \delta + i\beta$

$$\Delta z \quad e^{ik_{medium}\Delta z} = e^{i\frac{2\pi}{\lambda}n\Delta z} = e^{i\frac{2\pi}{\lambda}\Delta z} \cdot e^{-i\frac{2\pi}{\lambda}\delta\Delta z} \cdot e^{-\frac{2\pi}{\lambda}\beta\Delta z}$$

Transmission function
 projection of the refractive index distribution

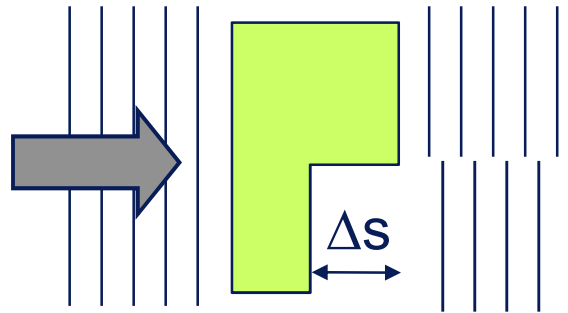


Amplitude $A(x,y) = e^{-B(x,y)} = e^{-\frac{2\pi}{\lambda} \int \beta(x,y,z) dz}$

$T(x,y) = A(x,y) \cdot e^{i\varphi(x,y)}$

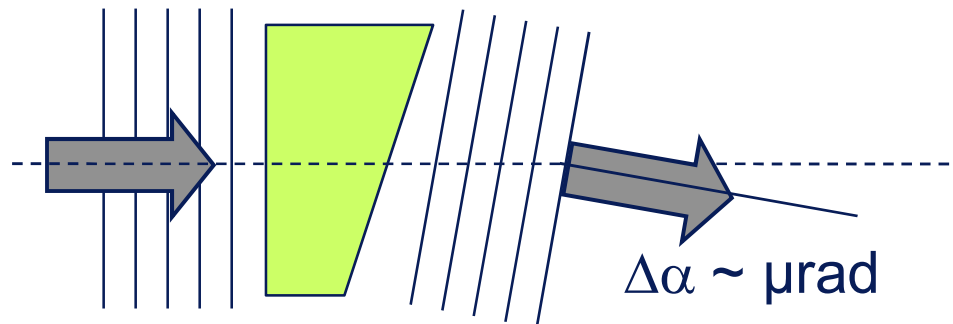
Phase $\varphi(x,y) = -\frac{2\pi}{\lambda} \int \delta(x,y,z) dz$

PHASE SENSITIVE TECHNIQUES



Phase Retardation

$$\Delta\varphi = -\frac{2\pi}{\lambda} \delta \cdot \Delta s$$



Deflection



Phase gradients

$$\Delta\alpha = -\frac{\lambda}{2\pi} \frac{\partial\varphi}{\partial x}$$

At zero distance:

Intensity $I_0 = |u_0|^2 = I_{inc} \cdot \exp\left(-\int \mu dz\right)$

⇒ all phase information is lost

SUMMARY PHASE CONTRAST IMAGING METHODS

Interferometry

$$\varphi$$

Differential Phase Contrast Imaging

Analyser-based PCI

$$\frac{\partial \varphi}{\partial x} + \text{Dark-field}$$

Grating-based PCI

$$\frac{\partial \varphi}{\partial x} + \text{Dark-field}$$

Speckle-based PCI

$$\frac{\partial \varphi}{\partial x, y} + \text{Dark-field}$$

Edge Illumination PCI

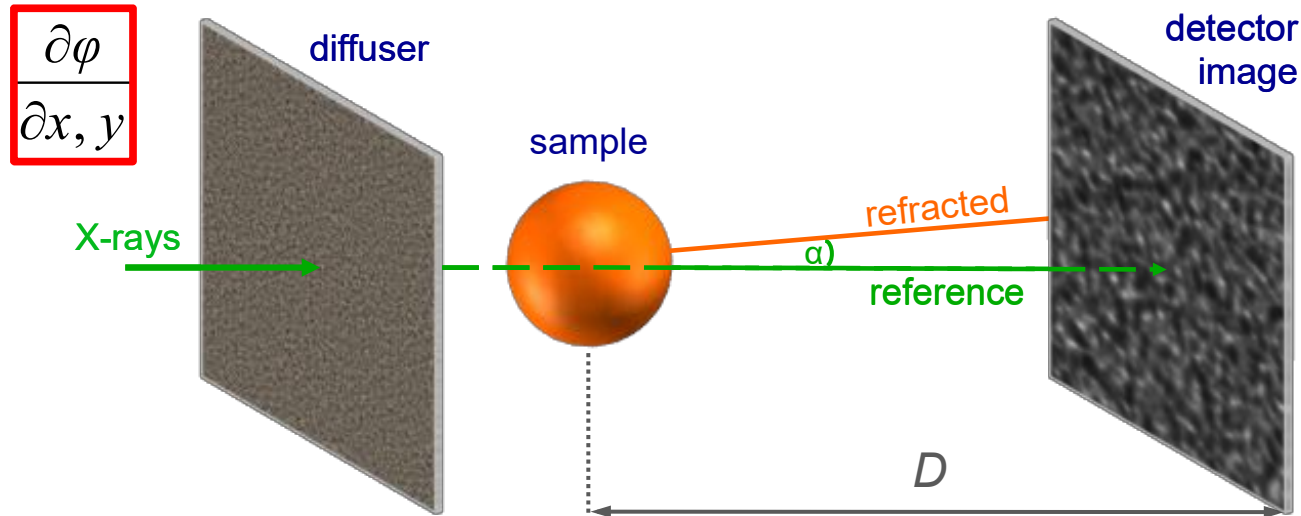
$$\frac{\partial \varphi}{\partial x}$$

Propagation-based

$$\frac{\partial^2 \varphi}{\partial^2 x} + \frac{\partial^2 \varphi}{\partial^2 y}$$

DIFFERENTIAL PHASE CONTRAST IMAGING

Speckle-based Phase Contrast Imaging



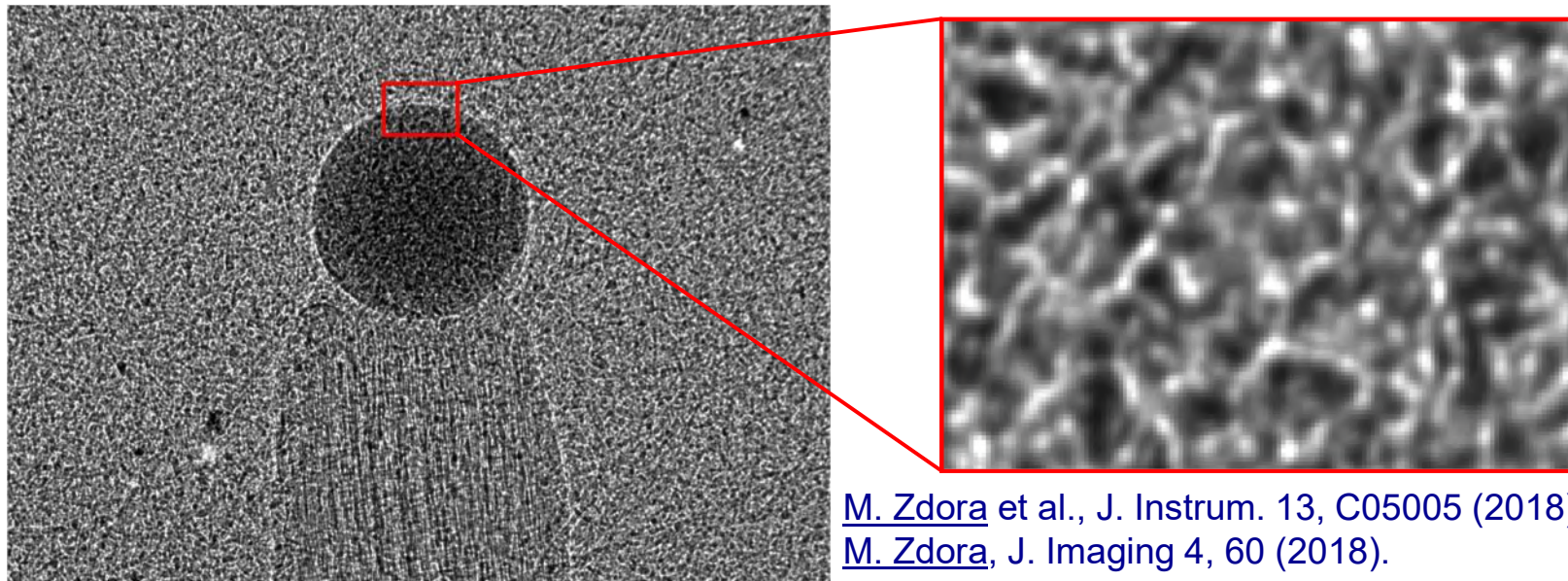
S. Berujon et al., Phys. Rev. A 86, 063813 (2012).

K. Morgan et al., Appl. Phys. Lett. 100, 124102 (2012).

$$\alpha_{x,y} = \frac{1}{k} \frac{\partial \phi}{\partial x, y} = \frac{v_{x,y}}{D}$$

refraction in sample

displacement of speckle pattern

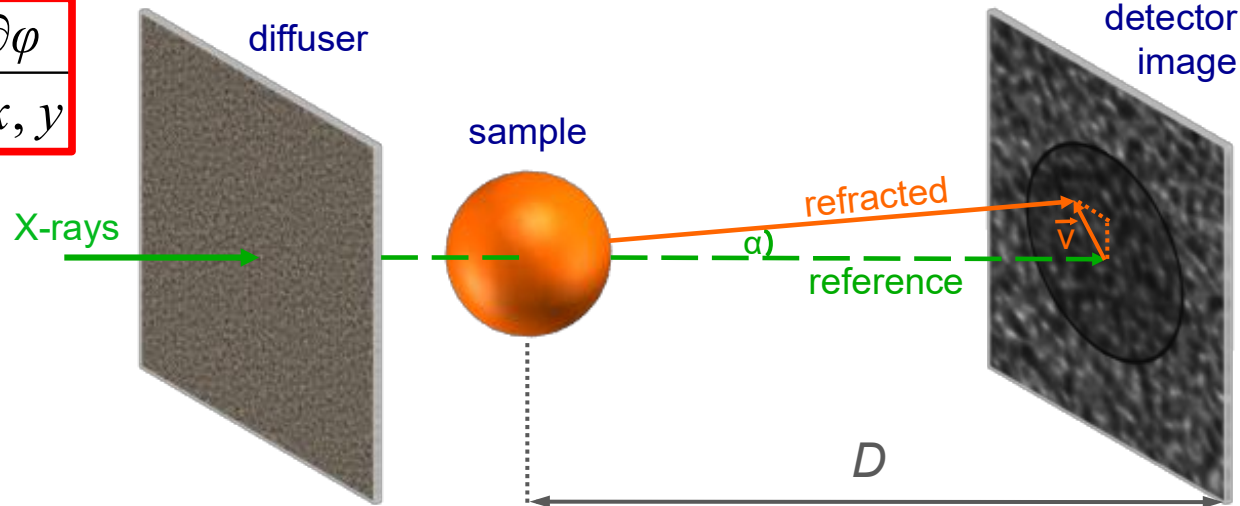


M. Zdora et al., J. Instrum. 13, C05005 (2018).
 M. Zdora, J. Imaging 4, 60 (2018).

DIFFERENTIAL PHASE CONTRAST IMAGING

Speckle-based Phase Contrast Imaging

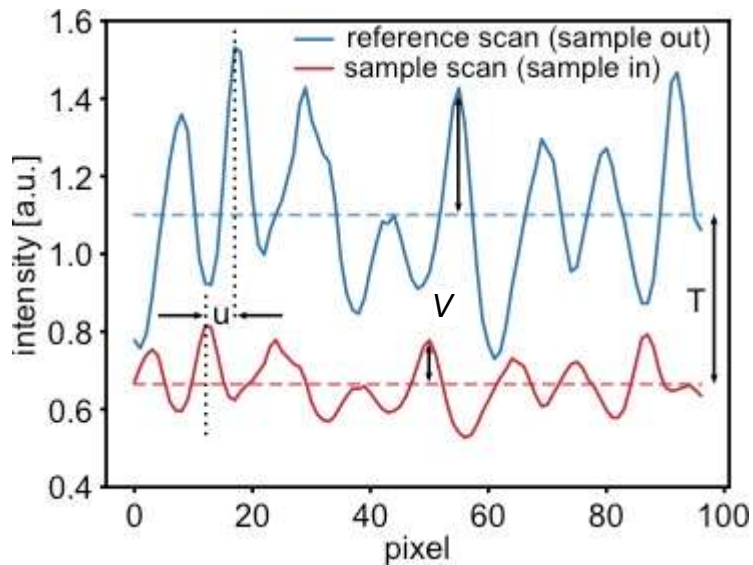
$$\frac{\partial \phi}{\partial x, y}$$



S. Berujon et al., Phys. Rev. A 86, 063813 (2012).

K. Morgan et al., Appl. Phys. Lett. 100, 124102 (2012).

$$\alpha_{x,y} = \frac{1}{k} \frac{\partial \phi}{\partial x, y} = \frac{v_{x,y}}{D}$$



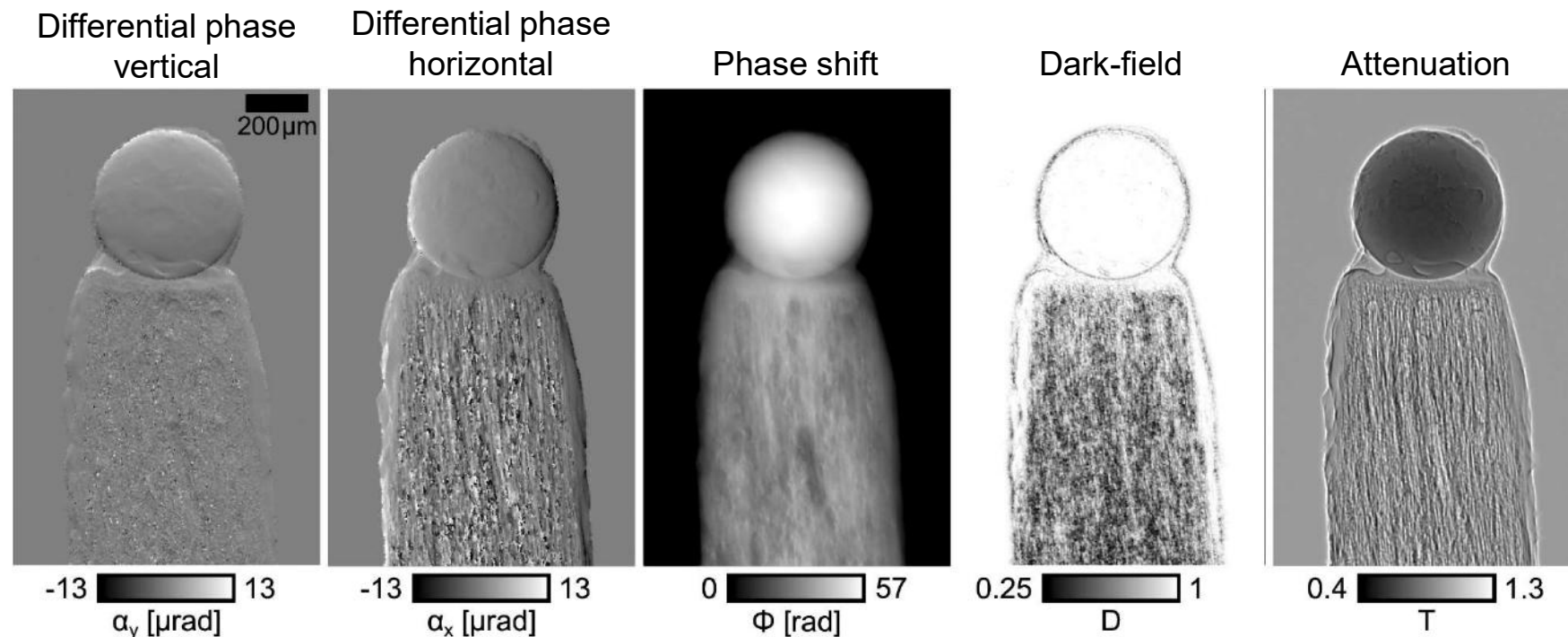
3 complementary image signals:

- Differential phase in horizontal **and** vertical direction
- Transmission
- Dark-field (scattering)

M. Zdora et al., J. Instrum. 13, C05005 (2018).

DIFFERENTIAL PHASE CONTRAST IMAGING

Speckle-based Phase Contrast Imaging



[M. Zdora et al., J. Instrum. 13, C05005 \(2018\).](#)

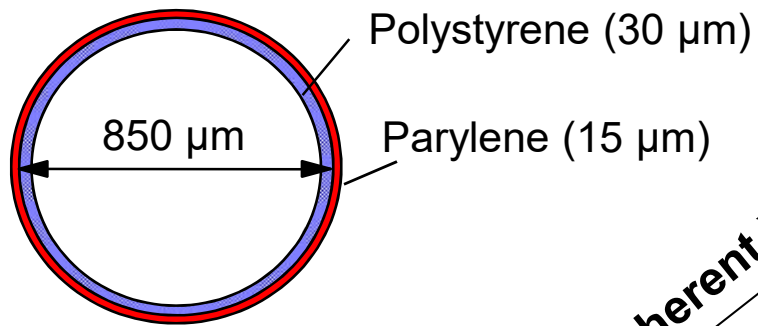
[M. Zdora, J. Imaging 4, 60 \(2018\).](#)

3 complementary image signals:

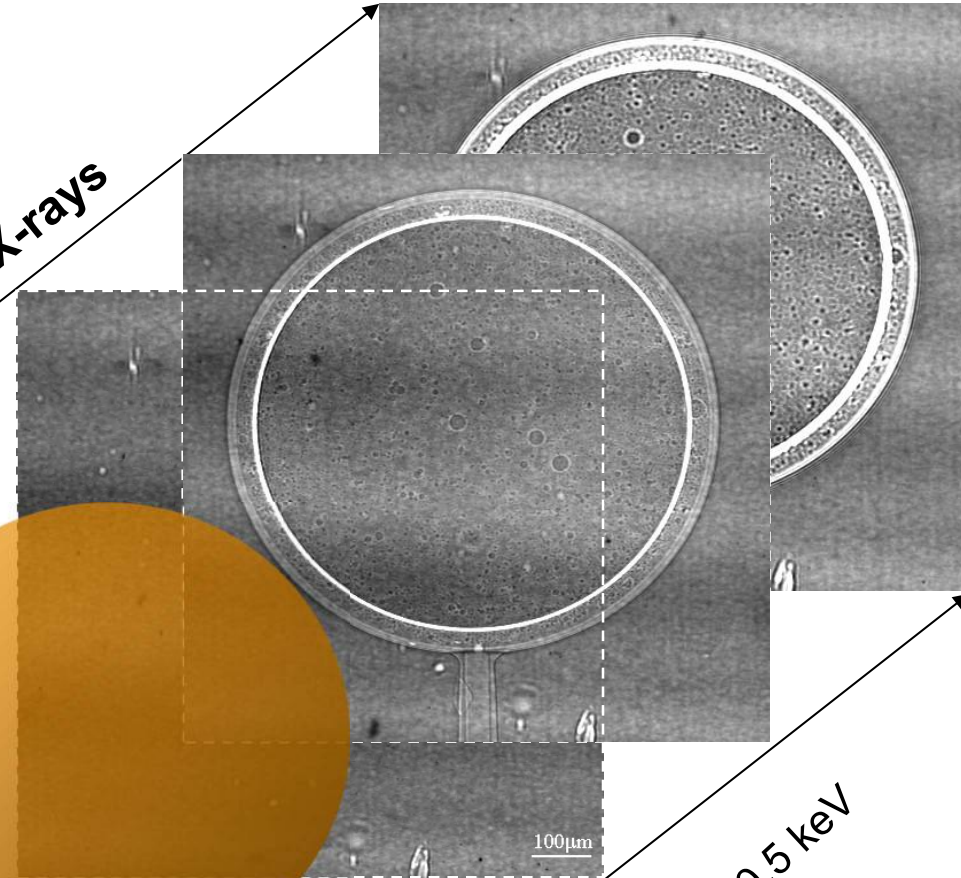
- Differential phase in horizontal **and** vertical direction
- Dark-field (scattering)
- 'Attenuation'

PROPAGATION BASED PHASE CONTRAST IMAGING

In-line Holography Fresnel diffraction



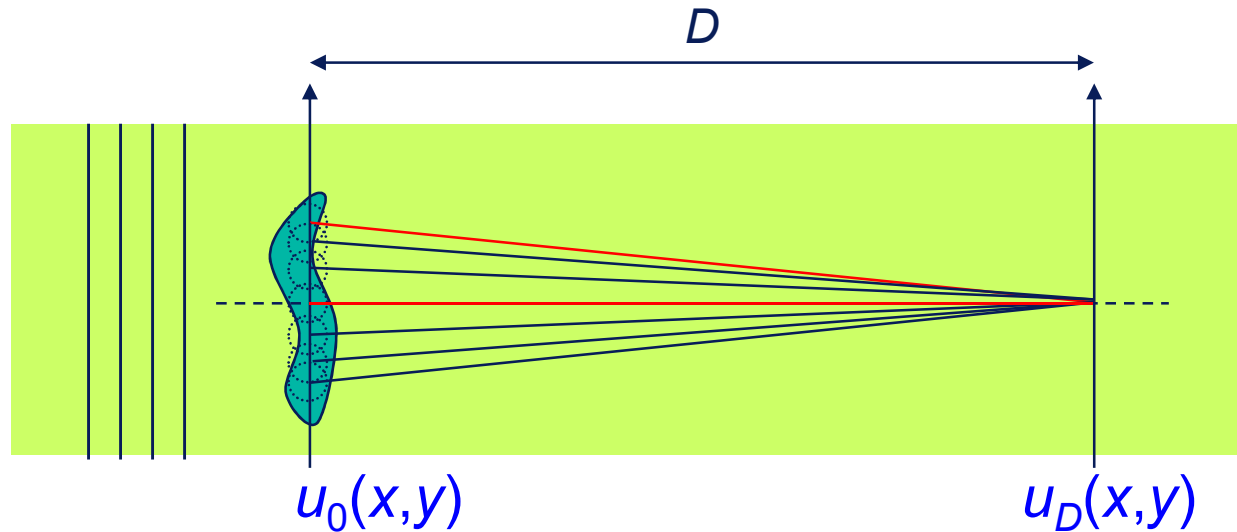
Coherent X-rays



Attenuation at zero distance
Phase contrast downstream

$E=20.5 \text{ keV}$

FRESNEL DIFFRACTION IN REAL SPACE

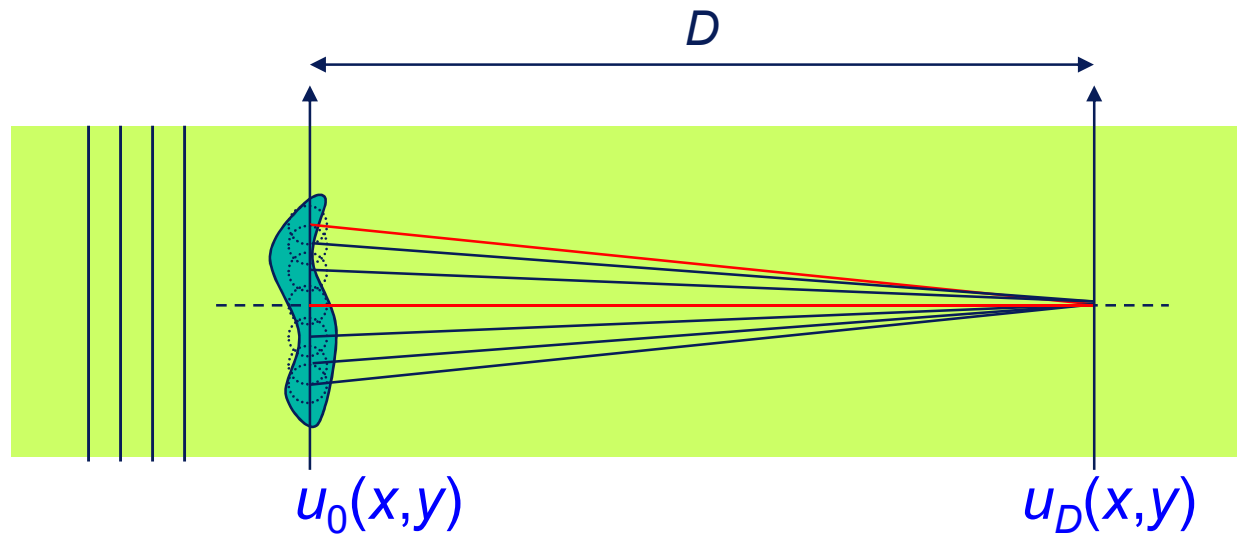


- Fresnel Integral (1D)

$$u_D(x) = \frac{1}{\sqrt{i\lambda D}} \int_{-\infty}^{+\infty} u_0(x_0) \exp\left[-i \frac{\pi}{\lambda D} (x - x_0)^2\right] dx_0$$

- Convolution in real space
Multiplication in reciprocal space
- Small angles paraxial approximation

FRESNEL DIFFRACTION IN REAL SPACE



- *In principle*: complete object contributes to a point of the image
In practice: only finite region: first Fresnel zone

$$\text{radius } r_F = \sqrt{\lambda D}$$

- First Fresnel zone determines the sensed lengthscale
Distance to be most sensitive to object with size a $D_{opt} = \frac{a^2}{2\lambda}$

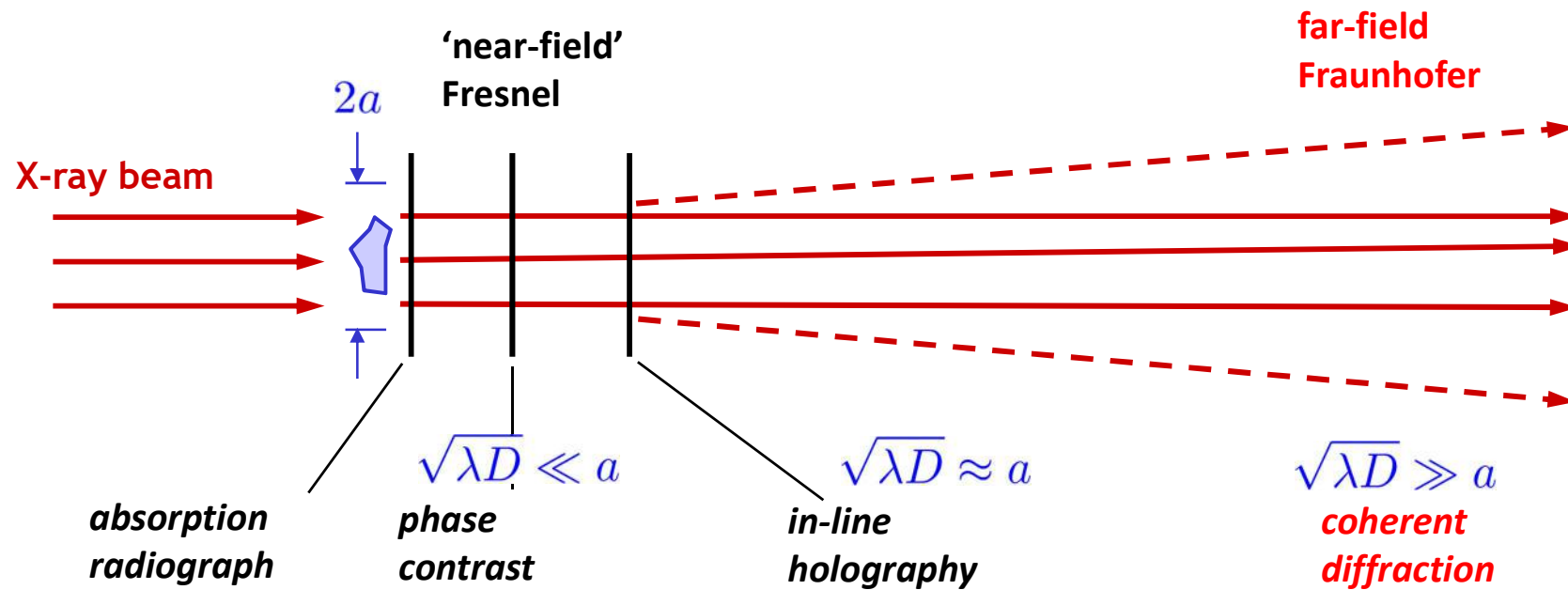
For example at $\lambda = 0.5\text{\AA}$ (25 keV)

$$a = 1 \mu\text{m} \quad \Rightarrow \quad D = 10 \text{ mm}$$

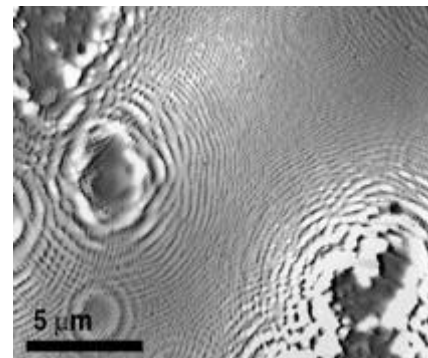
$$a = 40 \mu\text{m} \quad \Rightarrow \quad D = 16 \text{ m}$$

- Fresnel Integral becomes Fourier Transform if $\sqrt{\lambda D} \gg$ object size

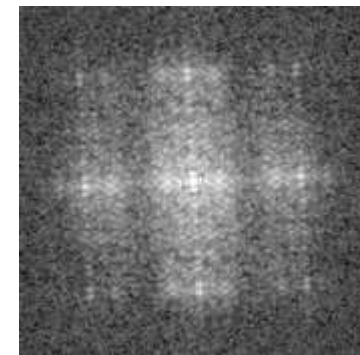
IMAGING REGIMES WITH COHERENT X-RAYS



Kagoshima (1999)



Jacobsen (1990)



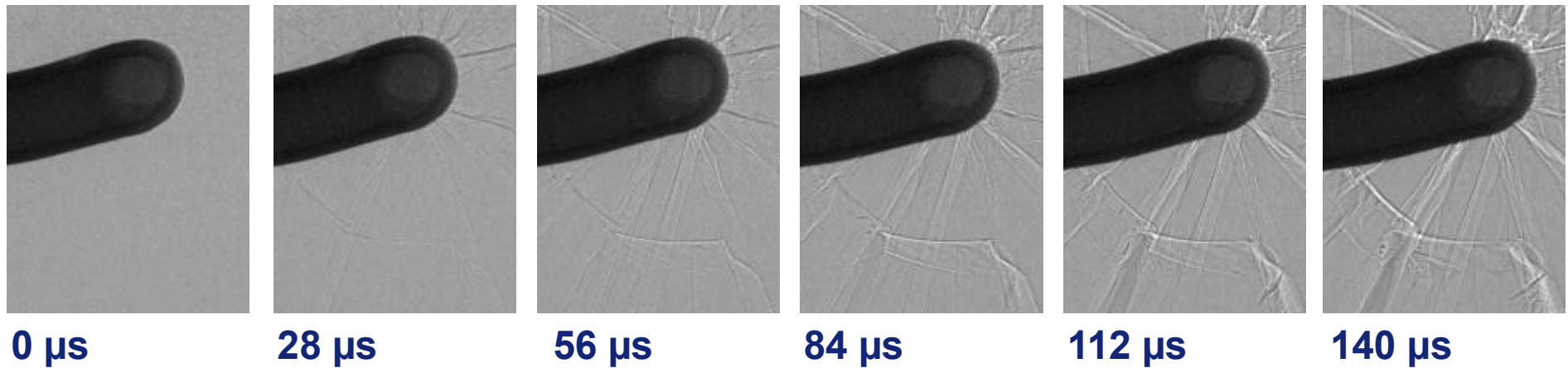
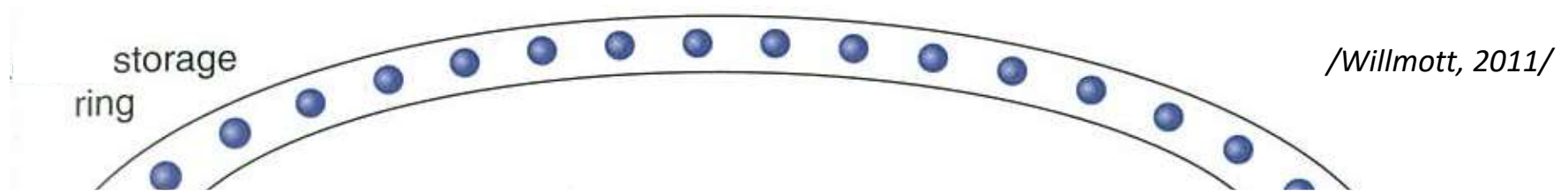
Miao (1999)

Courtesy Prof. Oleg Shpyrko (UCSD)

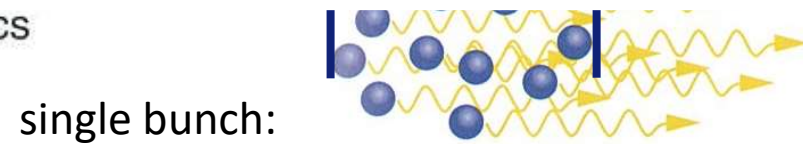
Fresnel number $F = \frac{a^2}{\lambda D}$

←

SINGLE-BUNCH PHASE CONTRAST IMAGING



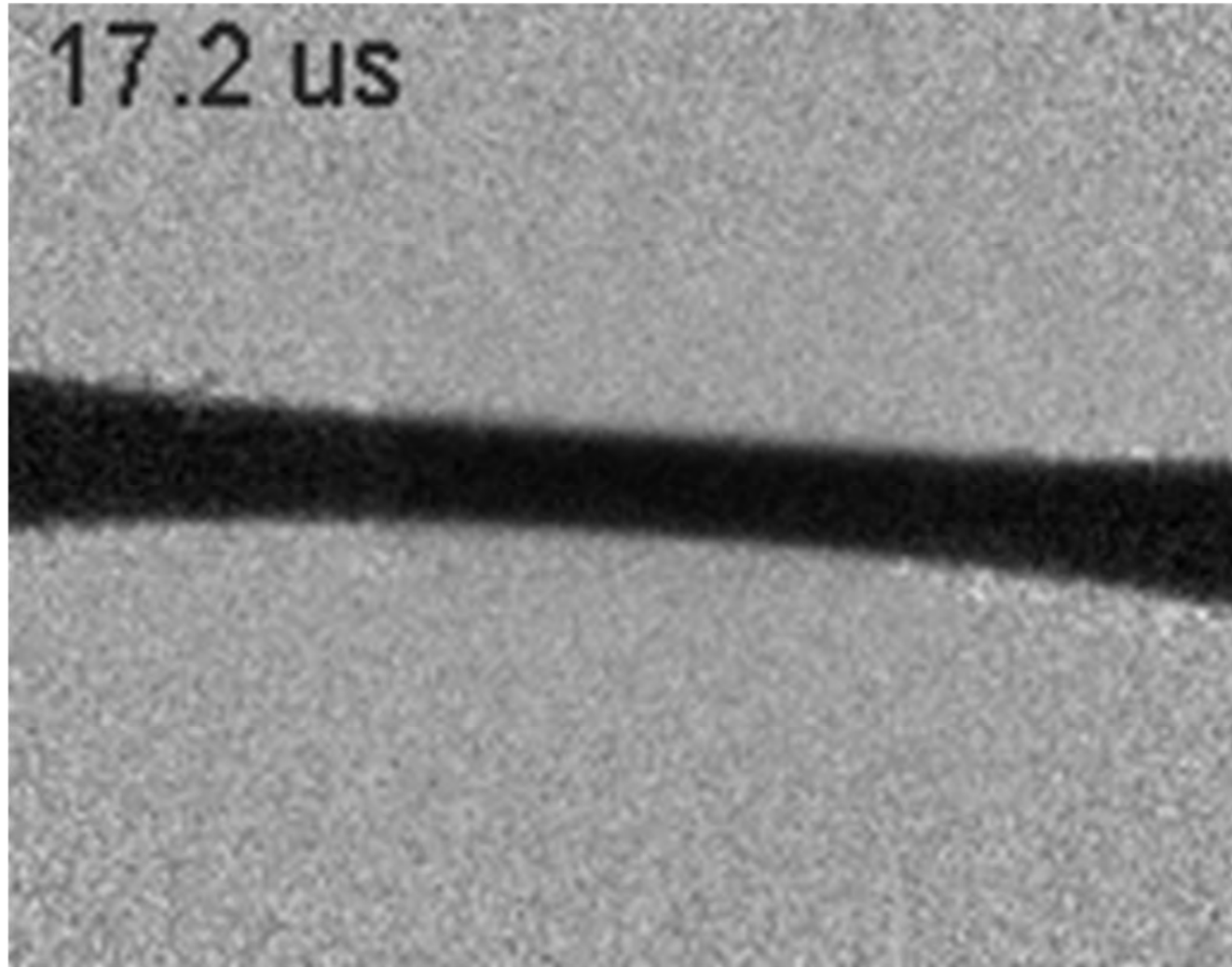
optics



- revolution 2.8 μs
- 4 bunches = 1.4 MHz frame rate
- Approx. 160 ps flash exposure

Rack, Scheel, Reichert et al.,
J Synch Rad 21, no. 4 (2014)

Electric arc ignition during fuse operation



lic connector
erite body
z sand
lic element

*M. P. Olbinado et al, MHz frame rate hard X-ray phase-contrast imaging using synchrotron radiation
Optics Express, vol. 25 (12), 13857-13871 (2017)*

M Olbinado, A Rack

The European Synchrotron



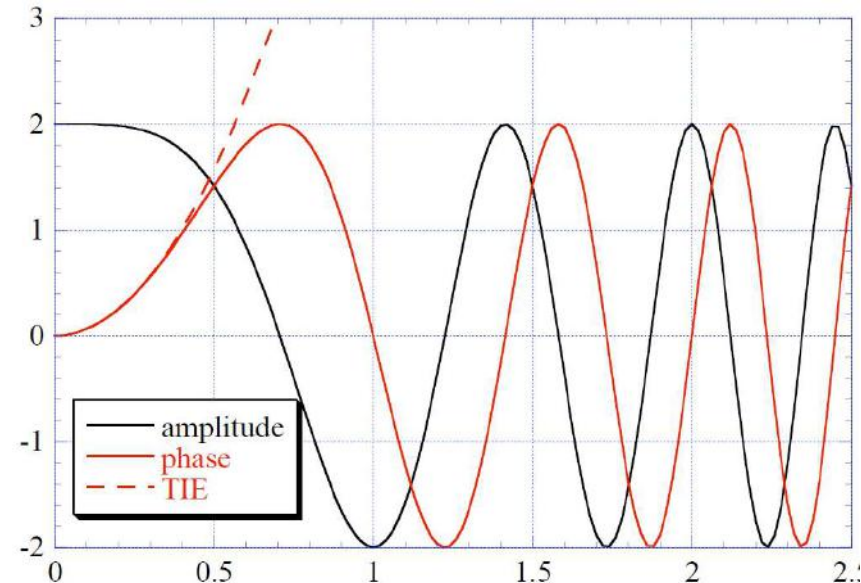
Linearization with respect to defocus D

Australian School: K. Nugent, T. Gureyev, D. Paganin

TIE (transport of intensity)

$$\frac{\partial I}{\partial z} = -\frac{\lambda}{2\pi} \nabla(I \nabla \phi)$$

CTF



Linearization with respect to object

Flemish School: D. Van Dyck, P. Cloetens, JP Guigay

Transfer Function Approach / Focus variation

Schiske, P. (1968). Zur Frage der Bildrekonstruktion durch Fokusreihen.

Combined in 'Mixed Approach'

JP Guigay, M Langer, R Boistel, P Cloetens, Opt. Lett., 32, 1617 (2007)

PAGANIN APPROACH (TIE VARIANT)

- Single distance reconstruction for **homogeneous objects**
Valid for **short distance, arbitrary absorption (!) and constant δ/β**
Paganin, D. et al, J. Microsc., 206, 33-40 (2002).
- Pro's:
 - Single distance
 - Most correct of simple approaches (arbitrary absorption)
 - Robust: homogeneity assumption has regularizing effect
- Contra's:
 - Weak distance, weak contrast \rightarrow contrast is not optimized
 - Not adapted to very inhomogeneous objects
 - Low-pass filter \rightarrow resolution is deteriorated

$$\text{resolution} \approx \sqrt{\lambda D}$$

$$\int \mu dz = -\ln \left(FT^{-1} \left\{ \frac{FT(I_D)}{\pi \lambda D f^2 \frac{\delta}{\beta} + 1} \right\} \right)$$

BM18: HIERARCHICAL PHASE-CONTRAST TOMOGRAPHY

Main techniques:

- Hierarchical tomography
- Propagation phase-contrast imaging

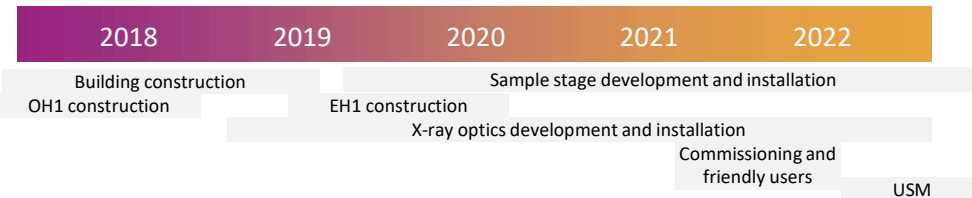
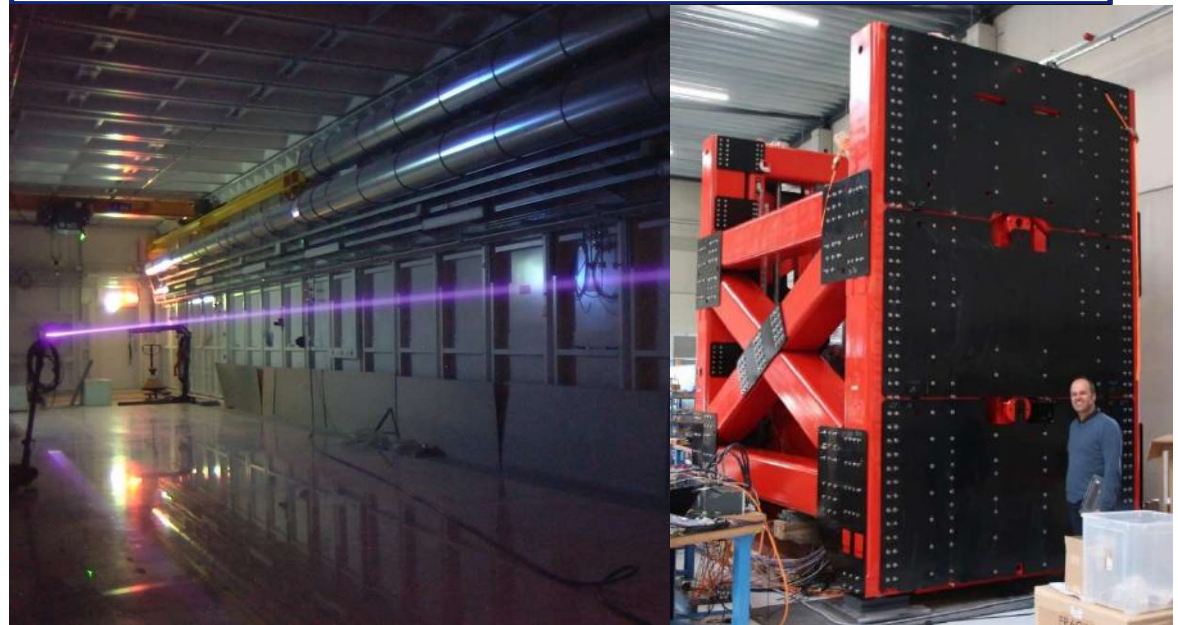
Main beamline specifications:

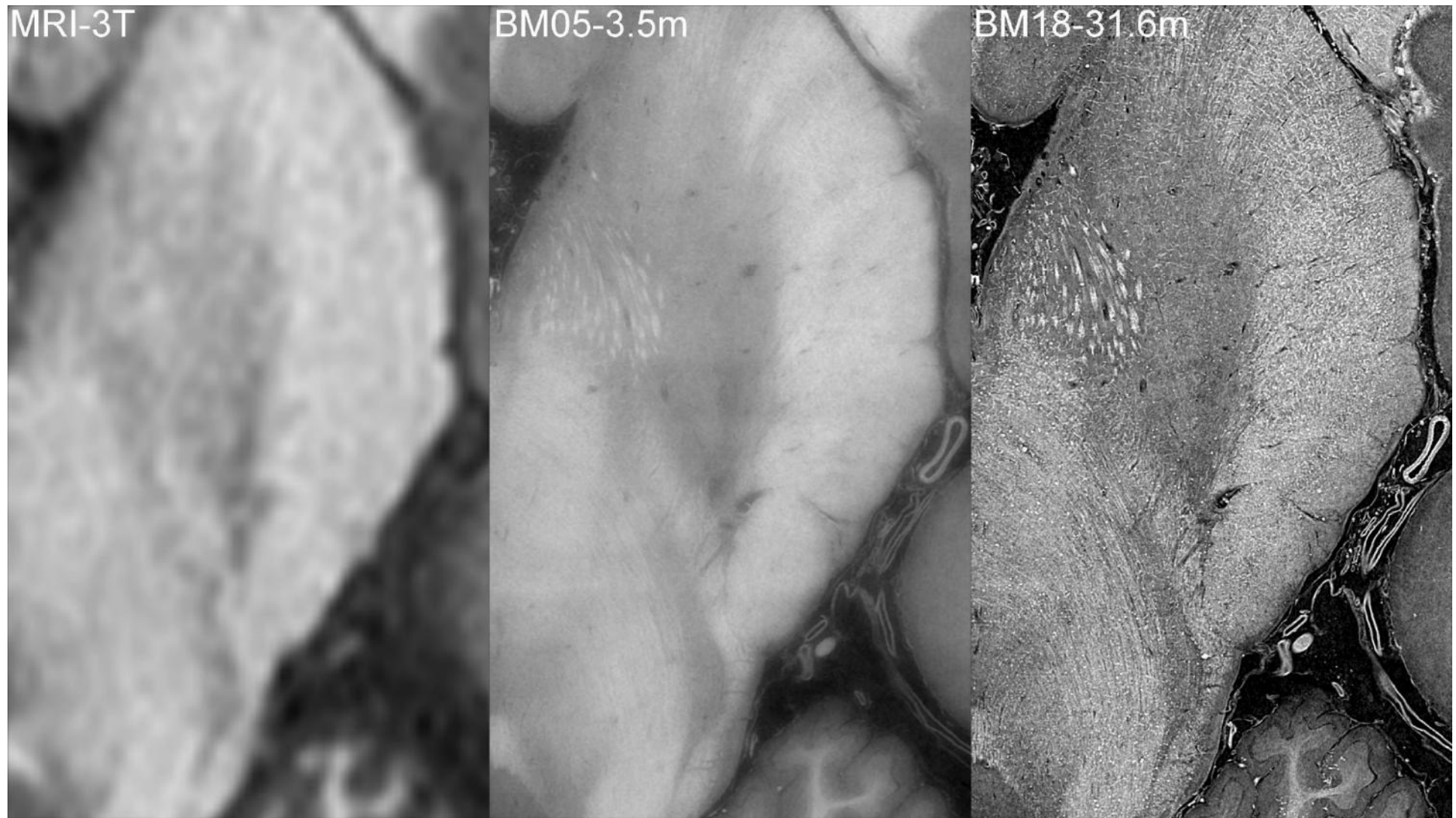
- Energy range: **80-240 keV** (*25-280 in the future*)
- 220m long beamline, up to 36m for propagation phase-contrast
- Current sample size **0.5m and 30 kg**
- Future sample size up to **2.5m and 300 kg**
- High automation level for high throughput (*regular improvements*)

EBS and refurbishment improvements:

- *Smallest possible X-ray source of the EBS*
- *Beam of 35cm with highest coherence worldwide for high-energy X-ray imaging*
- *Large pixel range (0.85 - 120 μm)*

45m long experimental hutch with large polychromatic beam at high energy



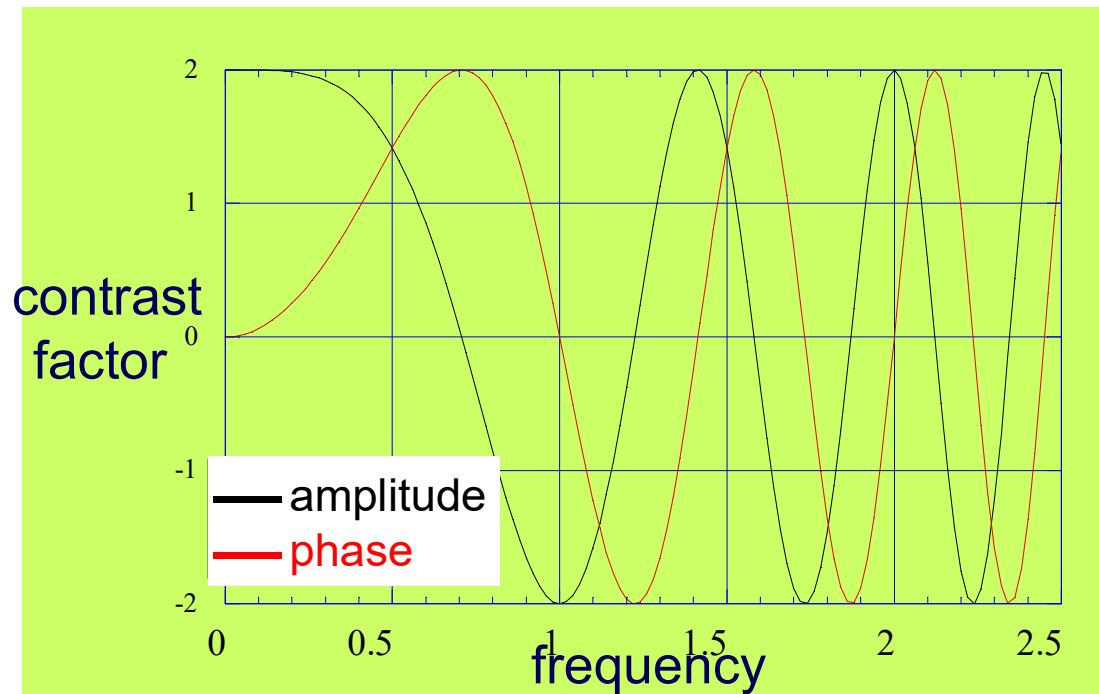


CONTRAST TRANSFER FUNCTIONS

- Fourier Transforms of the intensity and phase are linearly related

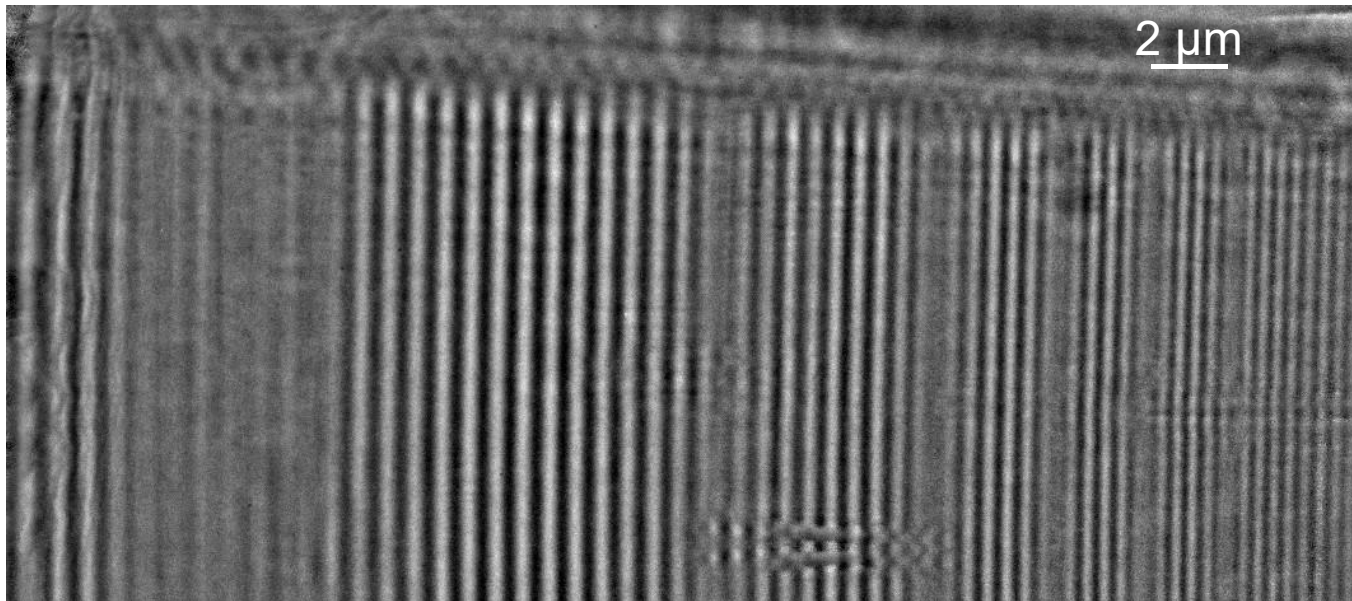
$$I_D(\mathbf{f}) = \delta_{\text{Dirac}}(\mathbf{f}) - 2 \underbrace{\cos(\pi\lambda D\mathbf{f}^2)}_{\text{amplitude contrast factor}} \cdot B(\mathbf{f}) + 2 \underbrace{\sin(\pi\lambda D\mathbf{f}^2)}_{\text{phase contrast factor}} \cdot \varphi(\mathbf{f})$$

valid in case of a slowly varying phase, weak absorption



CONTRAST TRANSFER FUNCTIONS

Decreasing linewidth
Increasing spatial frequency



period ≈ 720 nm

period ≈ 530 nm

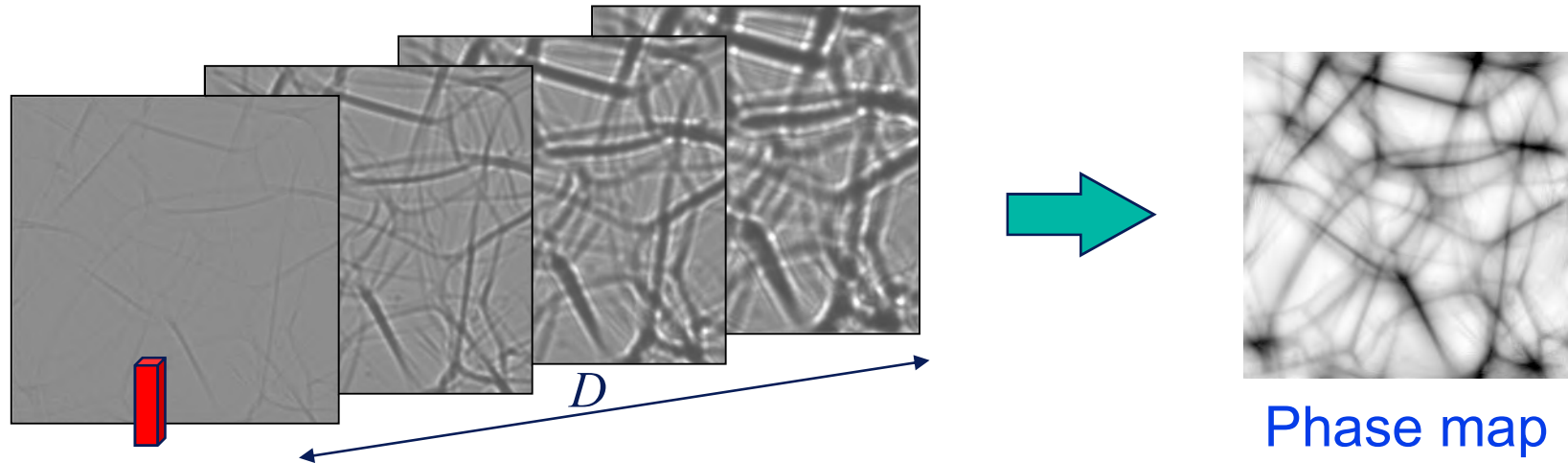
period ≈ 610 nm

Object invisible !

Contrast depends strongly on period or spatial frequency

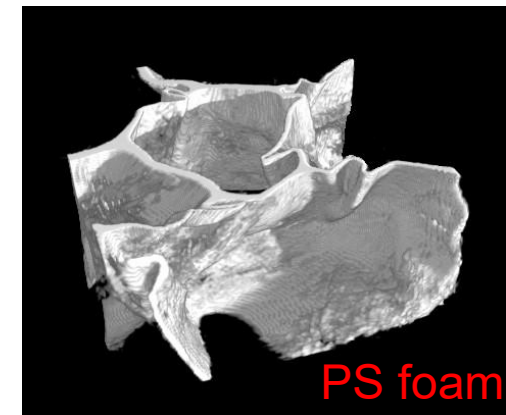
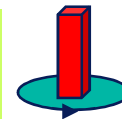
HOLO-TOMOGRAPHY

1) **phase retrieval** with images at different distances



2) **tomography**: repeated for ~ 1000 angular positions

3D distribution of δ or the electron-density
improved resolution
straightforward interpretation
processing



P.Cloetens et al., Appl. Phys. Lett. 75, 2912 (1999)

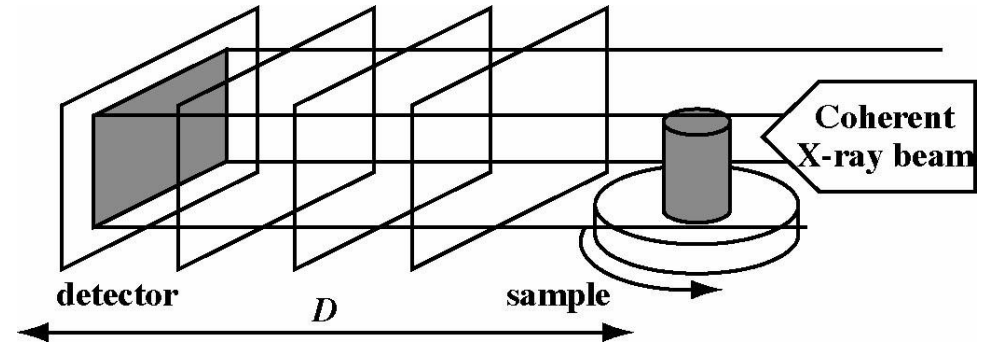
JP Guigay, M Langer, R Boistel, P Cloetens, Opt. Lett., 32, 1617 (2007)

M. Langer, P Cloetens, A Pacureanu, F Peyrin, Opt. Lett., 37, 2151 (2012)

X-RAY MICROSCOPY: FULL-FIELD SETUPS

Parallel beam imaging:

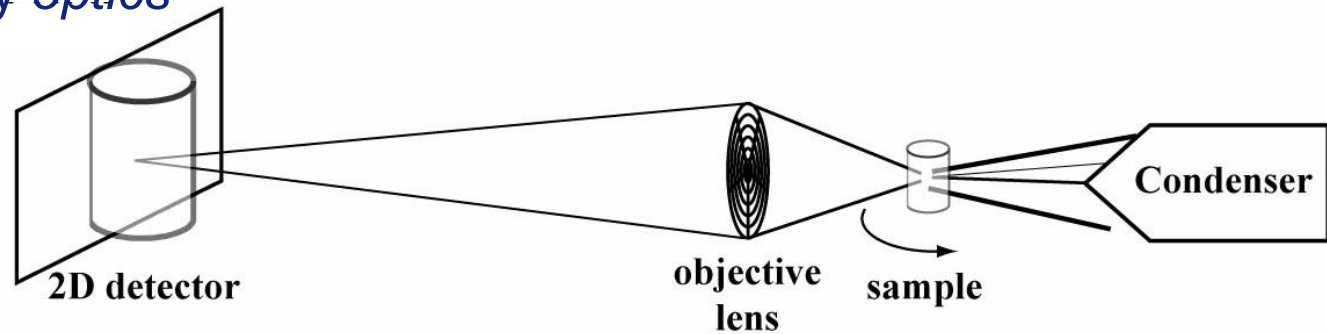
Absorption + Phase + Bragg
Dose (in)efficient, ultra fast
Resolution limited by *detector*



Full-field microscopy:

Absorption + Phase (+ Bragg)
Dose inefficient, fast
Resolution limited by *optics*

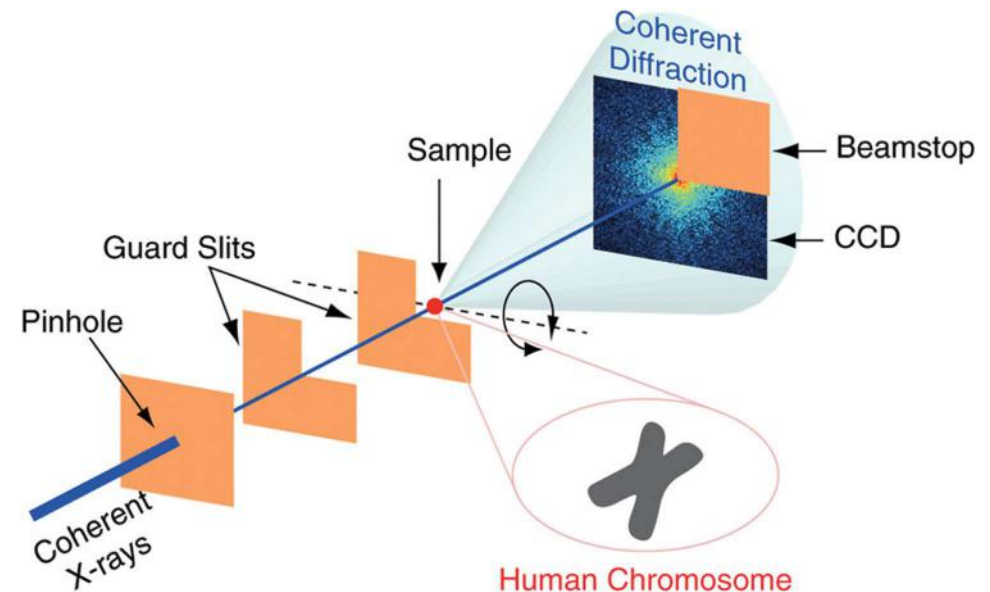
TXM (Transmission X-ray Microscopy)



X-RAY MICROSCOPY: COHERENT DIFFRACTION IMAGING SETUPS

CDI:

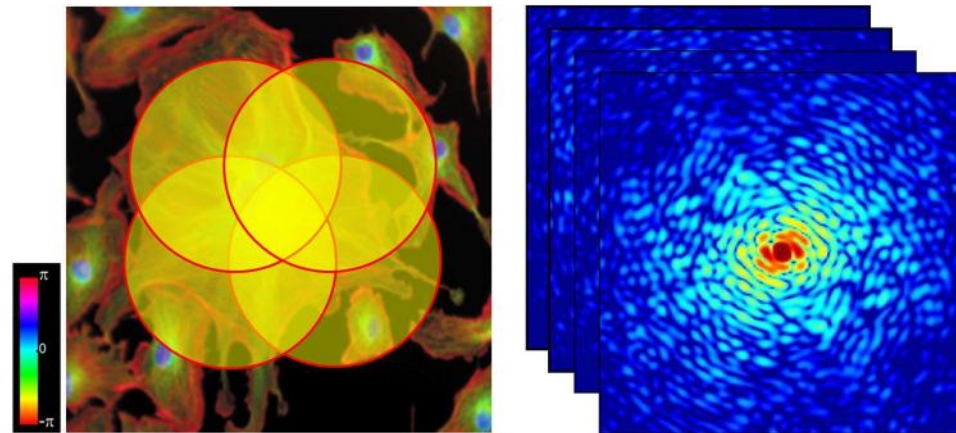
Phase contrast + Bragg
Dose efficient, ~fast, less robust
Small, isolated object
Resolution limited by *coherent flux*



Nishino et al, PRL 102, 018101 (2009)

Ptychography:

Phase contrast (+ Bragg)
Dose efficient, slow, more robust
'Extended' object
Resolution limited by *coherent flux*



M. Dierolf et al, Europhysics New 39, 22 (2008)

X-RAY MICROSCOPY: NANO-FOCUS SETUPS

S(T)XM Scanning (Transmission) X-ray Microscopy:

Micro-analysis

Slow

Rich, trace elements

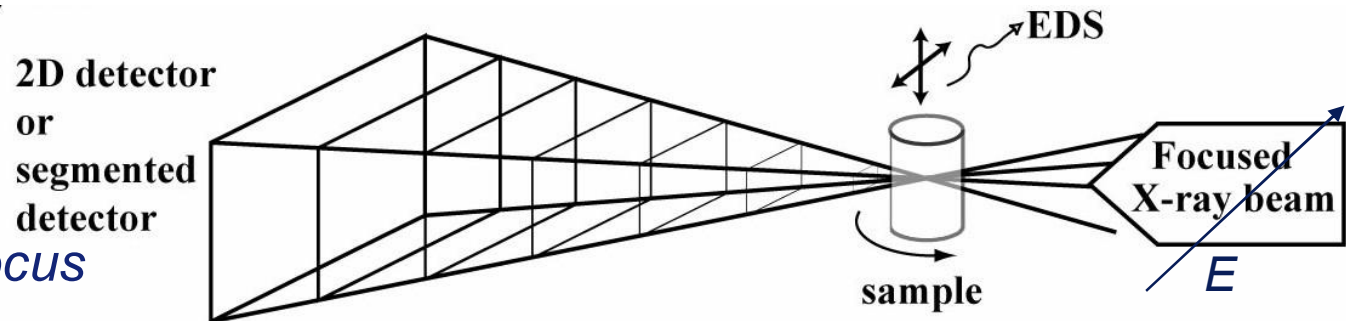
Phase contrast

Micro-fluorescence

Micro-diffraction

Micro-spectroscopy

Resolution limited by *focus*

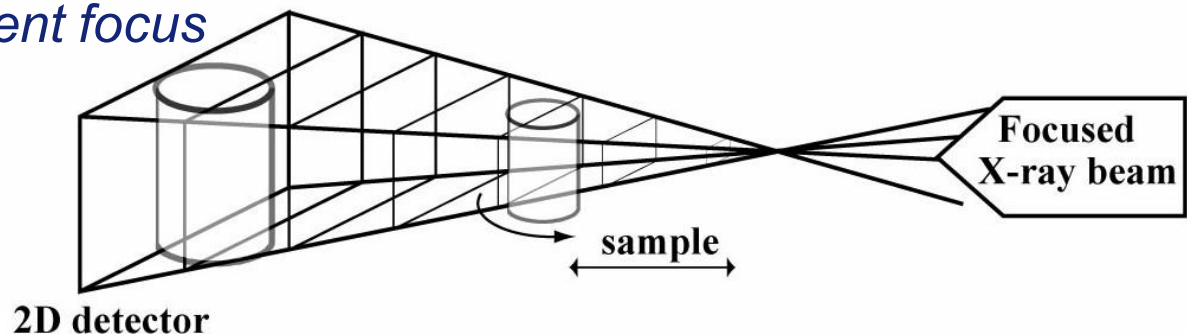


Projection X-ray Microscopy or Holography:

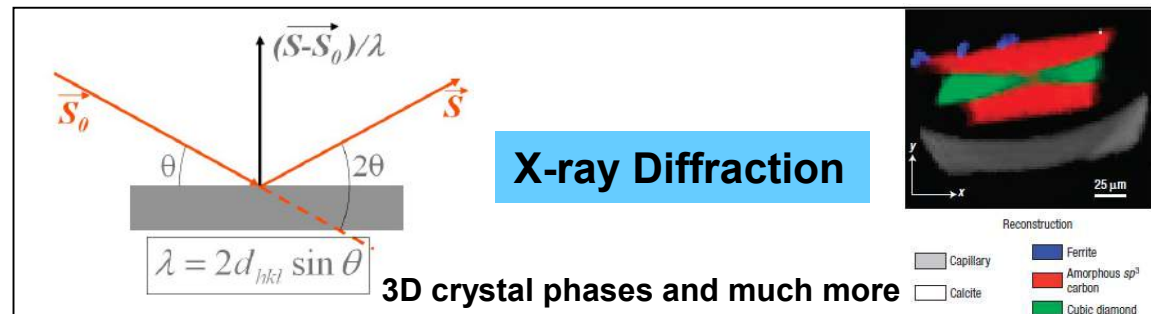
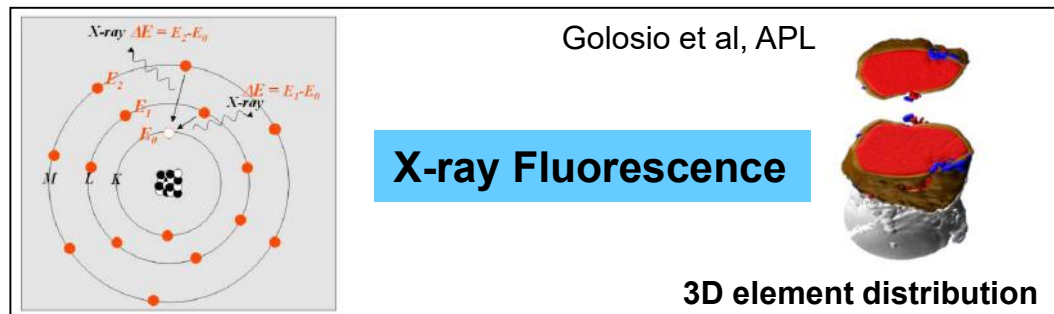
Phase contrast

Dose efficient, fast

Resolution limited by *incoherent focus*



GENERALIZED TOMOGRAPHY

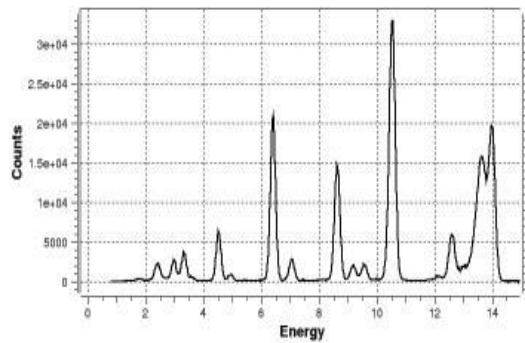


Courtesy of P. Bleuet

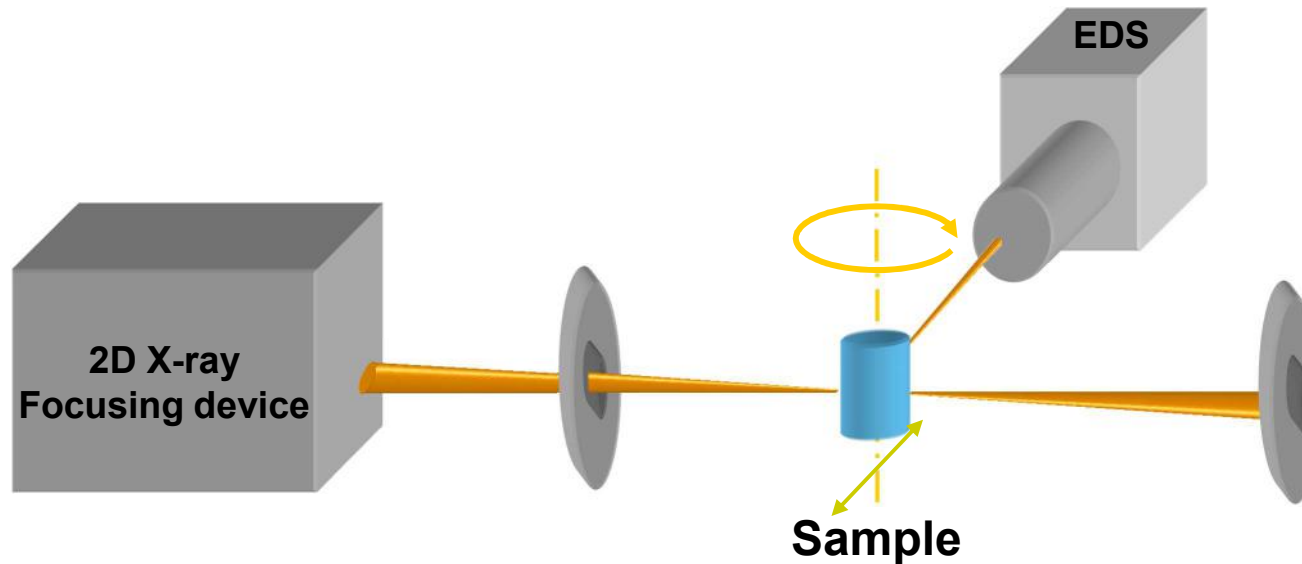
X-RAY FLUORESCENCE TOMOGRAPHY

Back to 1st generation scanner!

Pixel- by-pixel acquisition

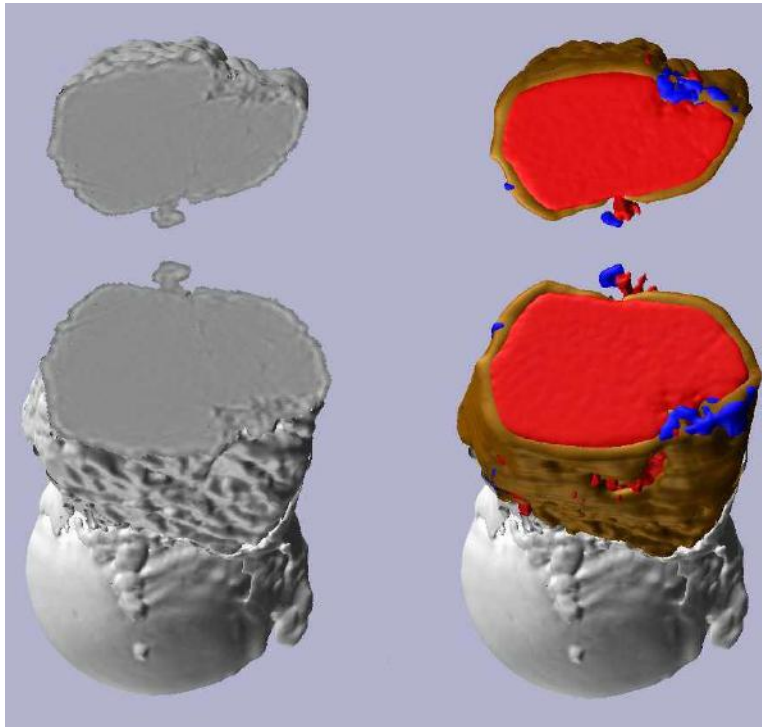


2D-Slice or 3D-Volume



Self-absorption of fluorescence is major problem

3D rendering of a fly ash



3D rendering, fly ash particle

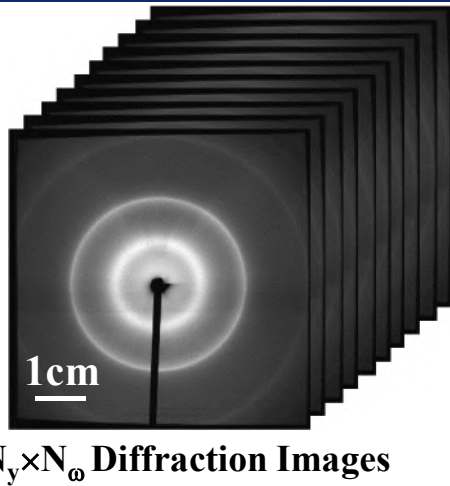
Left :
Transmission tomography

Right :
Distributions of
Rubidium
Manganese
Iron

Voxel size $3 \times 3 \times 3 \mu\text{m}^3$.

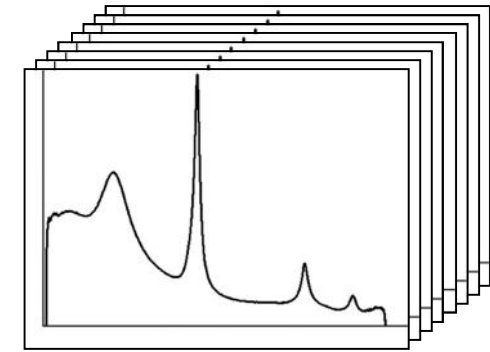
B. Golosio, et al., *J. Appl. Phys.* **94**, 145-156 (2003) and *Appl. Physics Letters*, (2004)

X-RAY (POWDER) DIFFRACTION TOMOGRAPHY

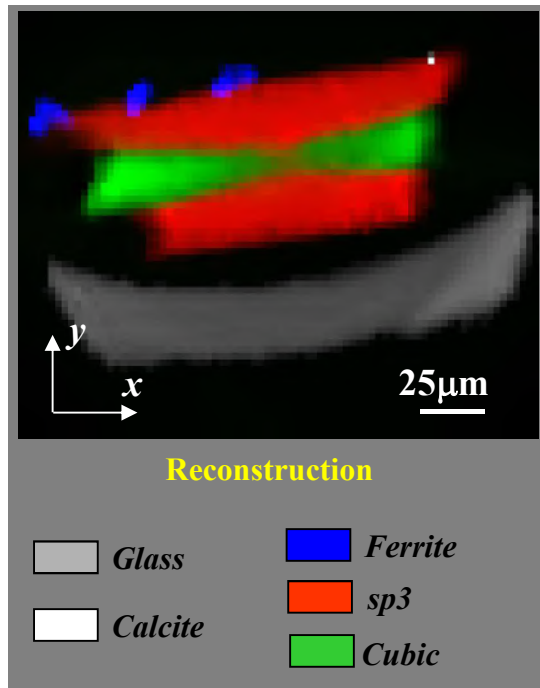


Azimuthal Integrations

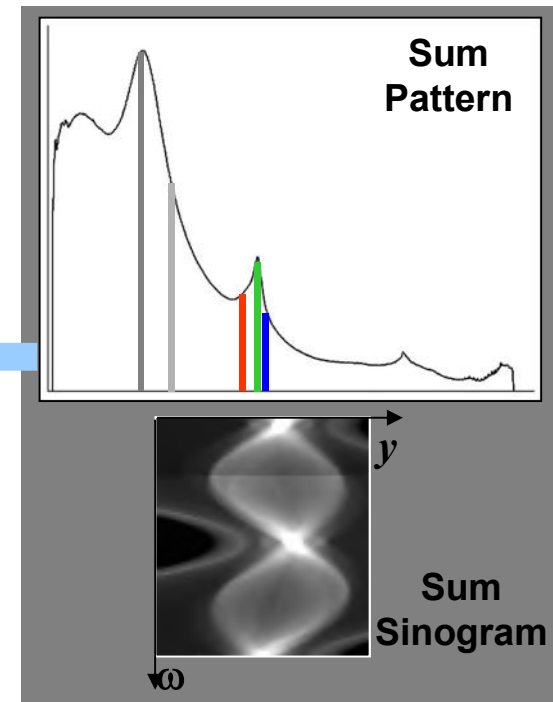
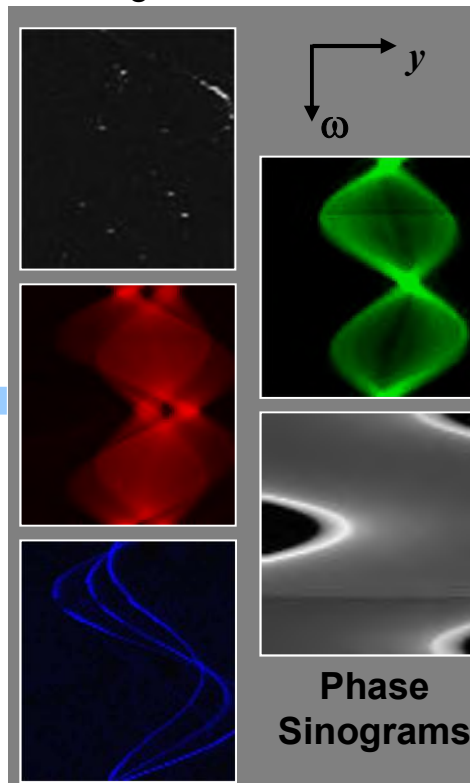
ID22
 Energy = 18keV
 Spot size = 1.6x2.3 μm (VxH)
 81 steps / angle
 90 angles / 360°



$N_y \times N_\omega$ Diffraction Patterns



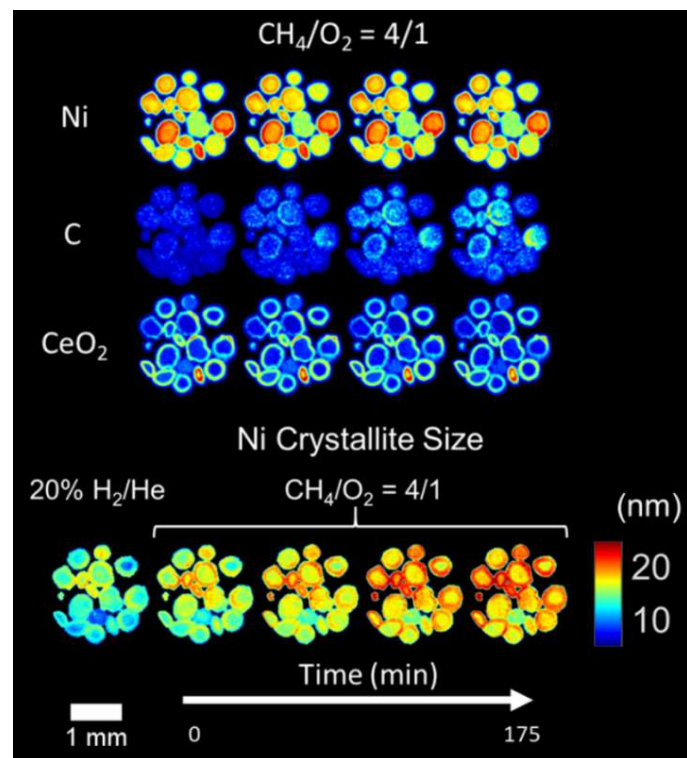
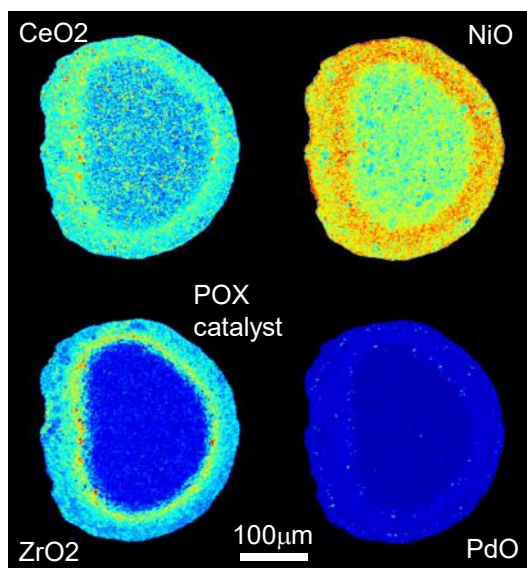
Algebraic
 Reconstruction
 Technique



OPERANDO CATALYSIS

Chemical evolution of a catalyst under working conditions

Phases distribution maps in a catalyst



SAXS TENSOR TOMOGRAPHY



Scanning SAXS in 3D: A 6D reconstruction

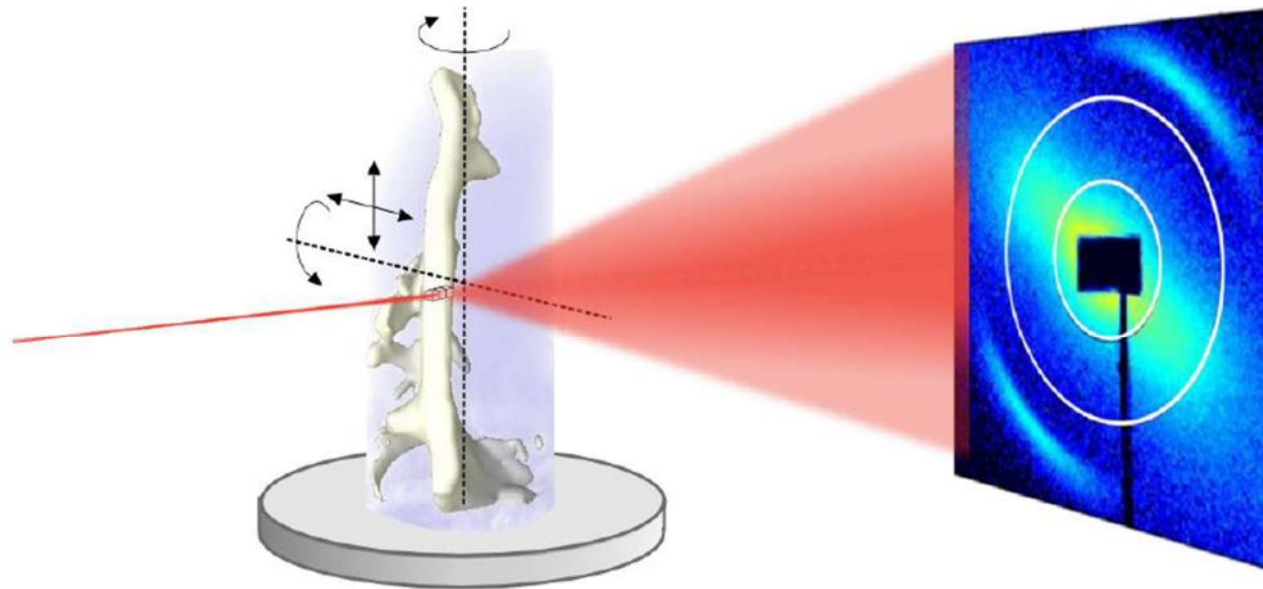
SAXS tensor tomography

Reconstructing 3D real space and 3D reciprocal space

measurements around two axis needed



Marianne Liebi
Now at MAX IV

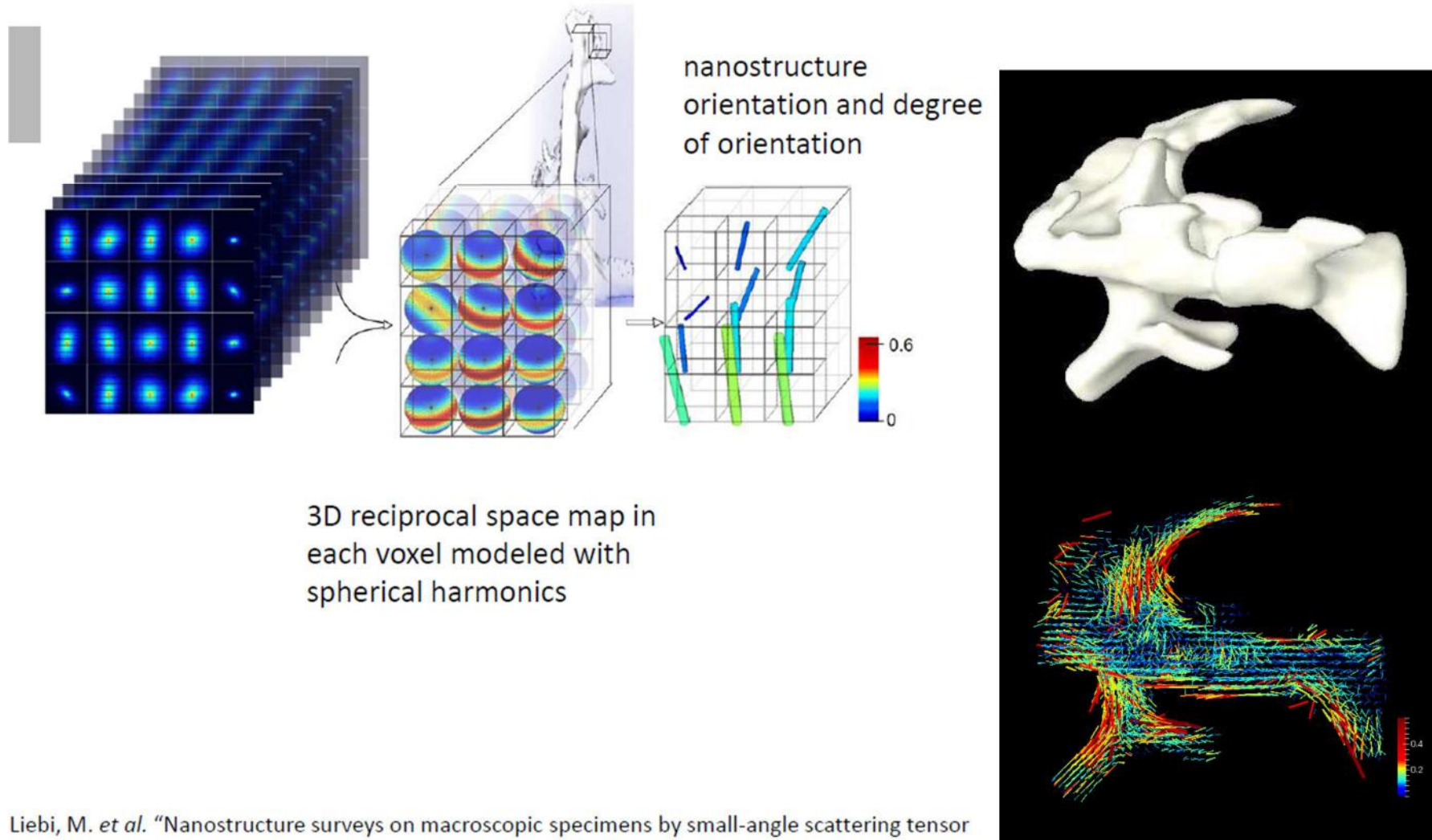


SAXS TENSOR TOMOGRAPHY

PAUL SCHERRER INSTITUT

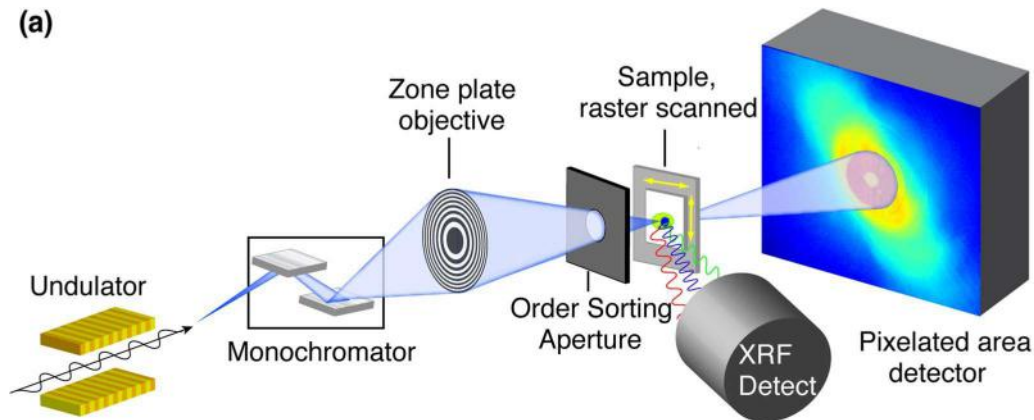


Scanning SAXS in 3D: A 6D reconstruction



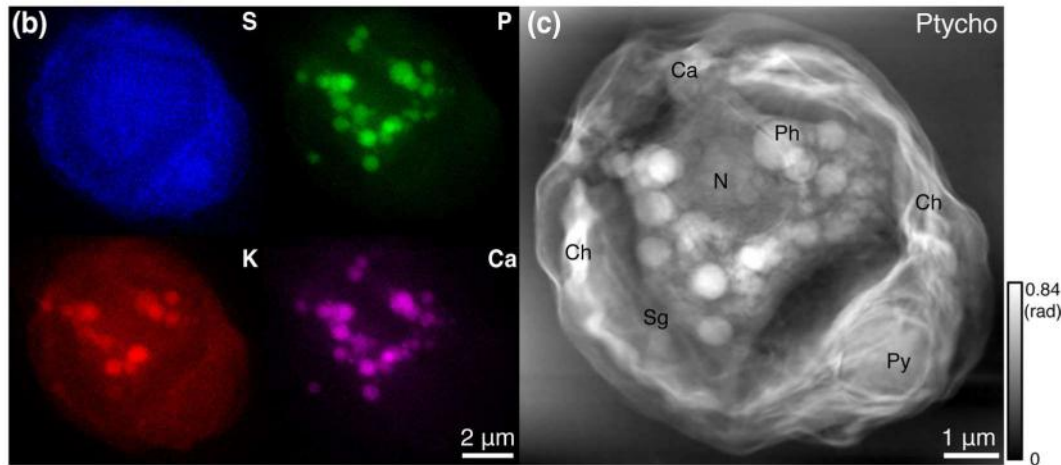
Liebi, M. *et al.* "Nanostructure surveys on macroscopic specimens by small-angle scattering tensor tomography," *Nature* **527**, 349 (2015)

COMBINED X-RAY FLUORESCENCE AND PTYCHOGRAPHY



J Deng, D Vine,... Ch Jacobsen
Scientific Reports | 7: 445 | 2017

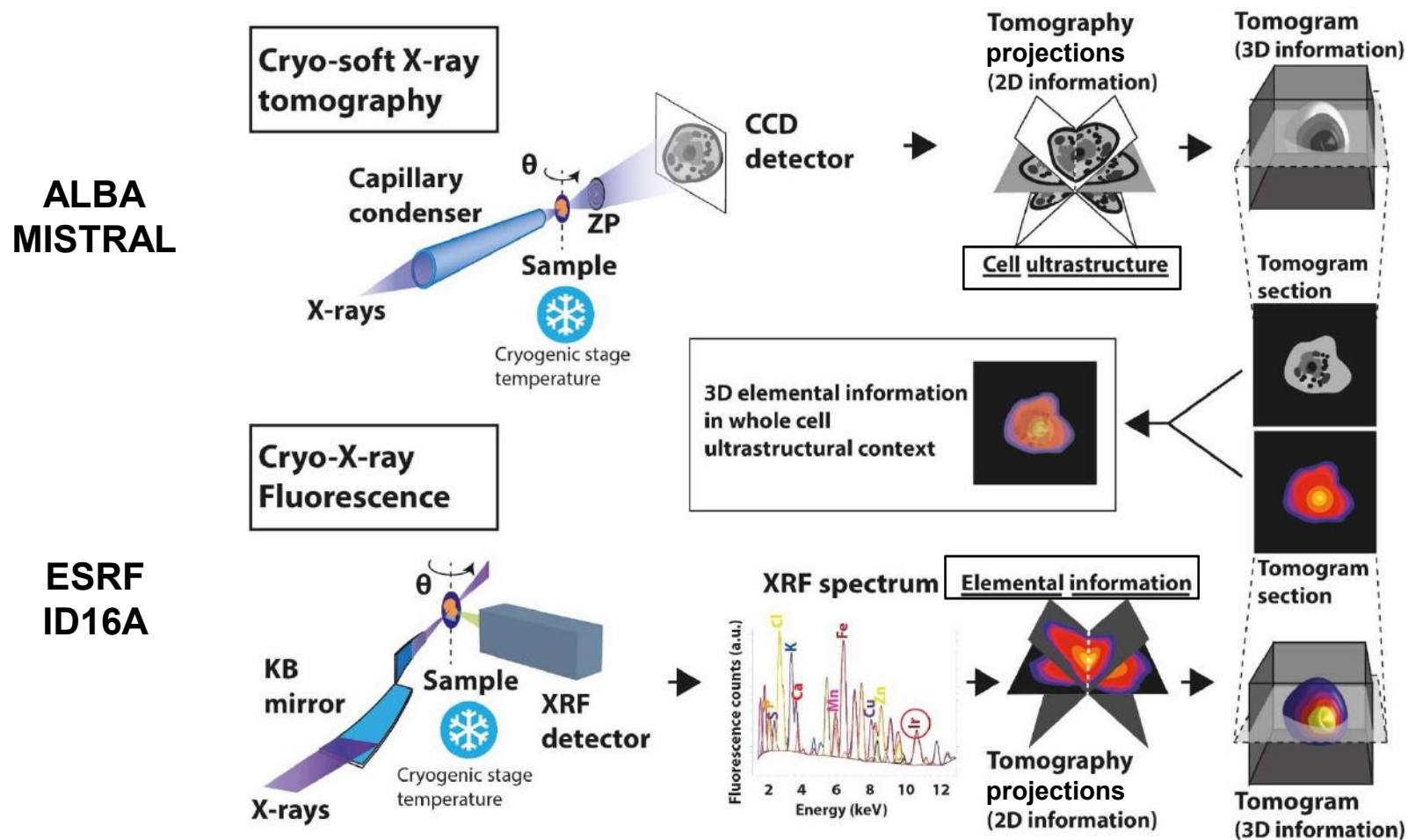
**Simultaneous,
using Continuous scanning
Energy: 5.2keV**



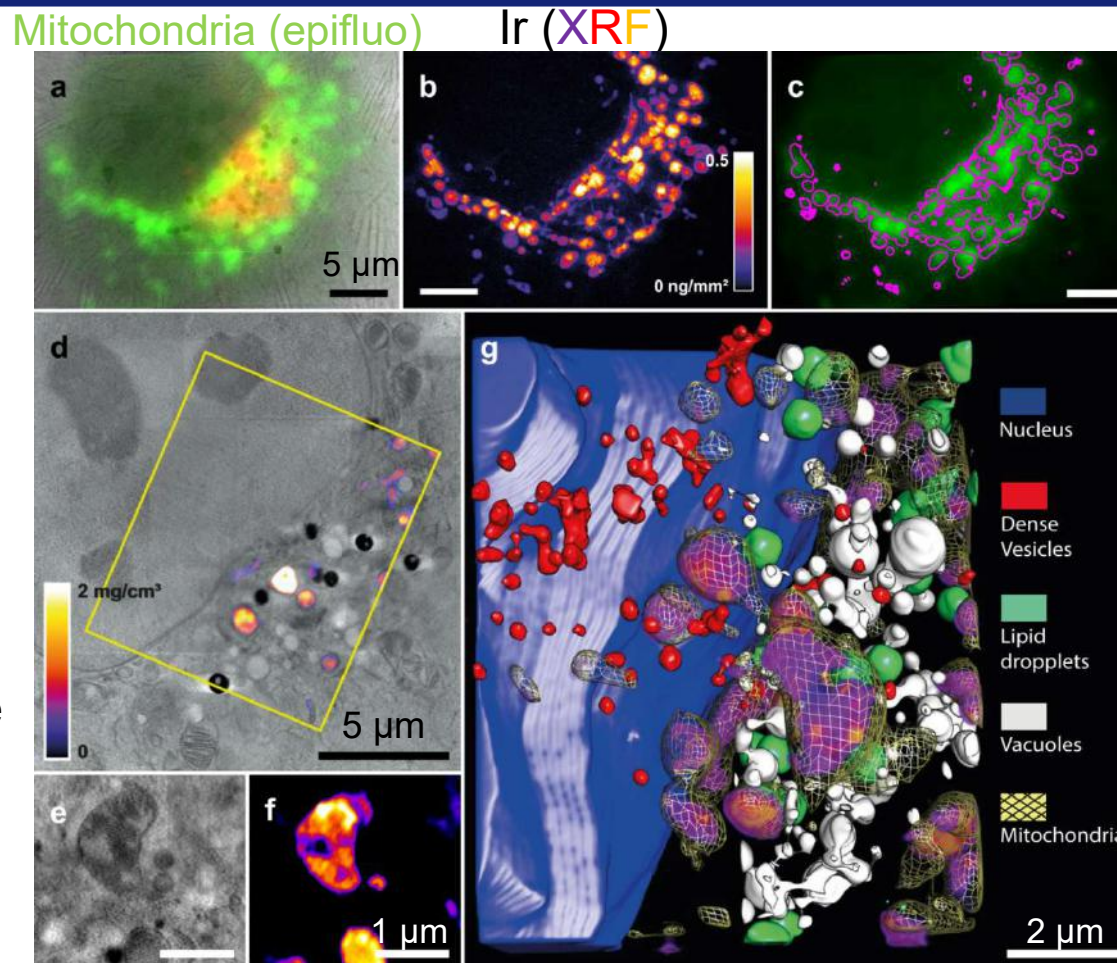
**Required coherence properties rather different (today)
Often preferred to do two successive experiments**

INTRACELLULAR LOCALIZATION OF POTENT ANTICANCER METALLODRUGS

Cryo 3D Correlative Microscopy (first time)



INTRACELLULAR LOCALIZATION OF POTENT ANTICANCER METALLODRUGS

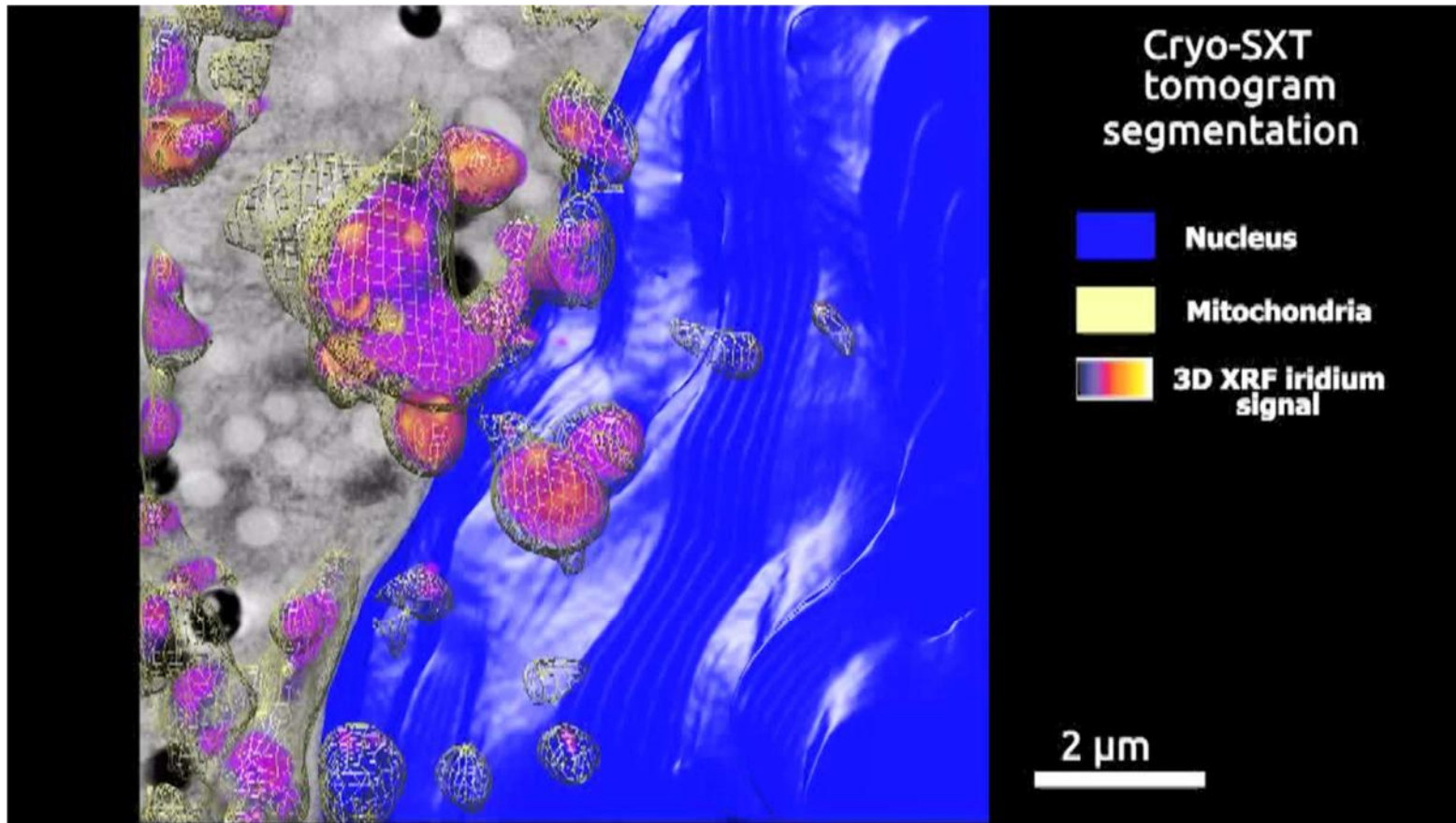


- Ir metallodrug is exclusively located in the *mitochondria*
- Triggers effects on cell death-related endogenous metals (Ca and K)

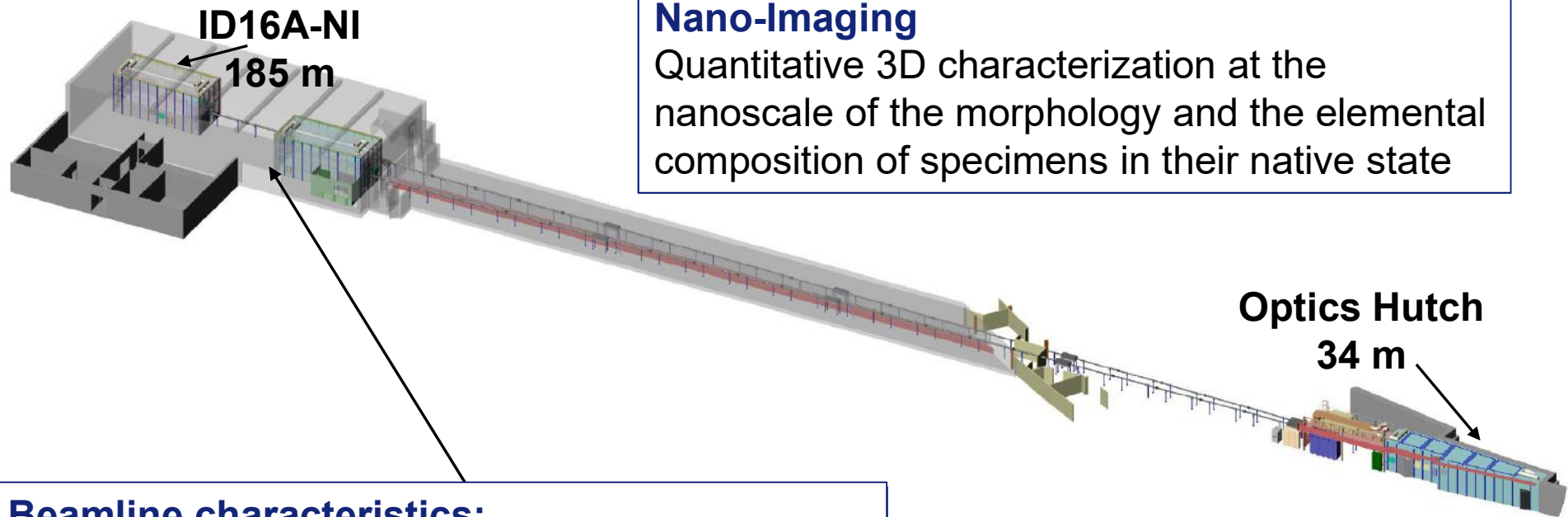
EBS outlook:

- *Time-dependent uptake* of drugs in the cellular environment (*pseudo 4D*)

Cryo 3D Correlative Microscopy



ID16A-NI: NANO-IMAGING BEAMLINE

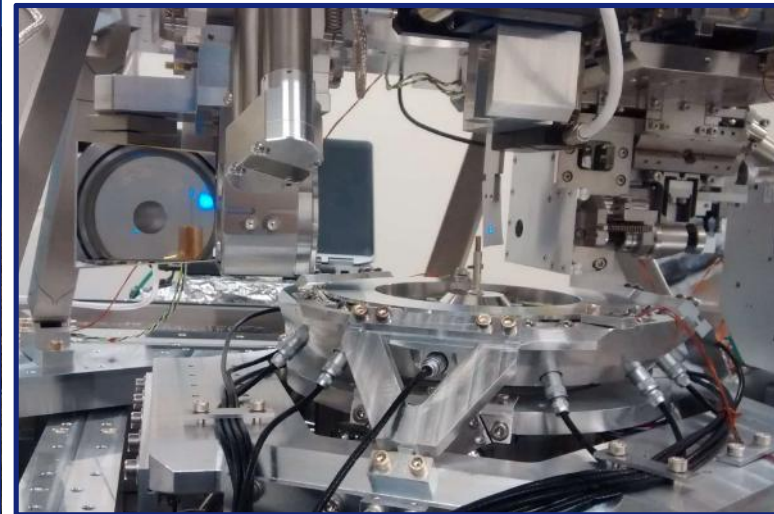


Nano-Imaging

Quantitative 3D characterization at the nanoscale of the morphology and the elemental composition of specimens in their native state

Beamline characteristics:

- Techniques: fluorescence analysis, in-line phase contrast, near-field ptychography
- **Nano-Focus:** (12) 25-35 nm
- Energy: **17 keV & 33.6 keV**
- Flux: $1.2 \cdot 10^{13}$ ph/s & $2.7 \cdot 10^{12}$ ph/s @ 0.7%
in principle...
- Operation: under vacuum, room temperature or **cryo** workflow

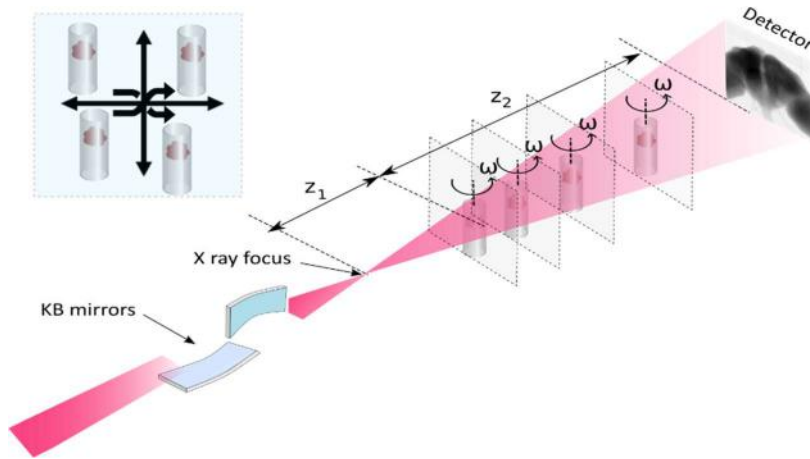


ID16A End-station

X-RAY PHASE CONTRAST

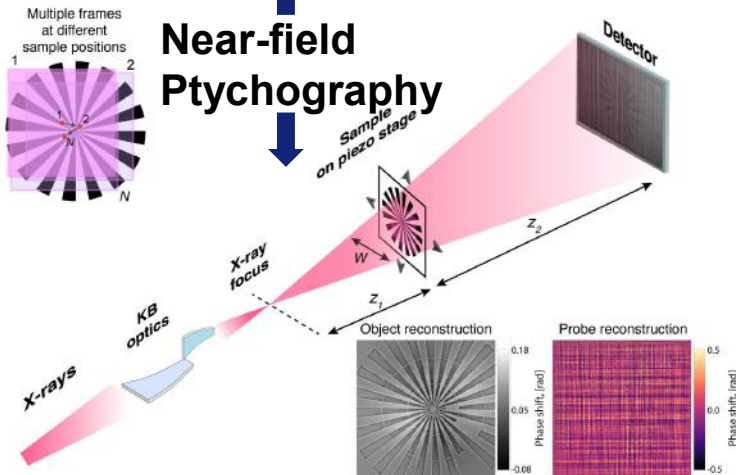
X-RAY FLUORESCENCE

Holography



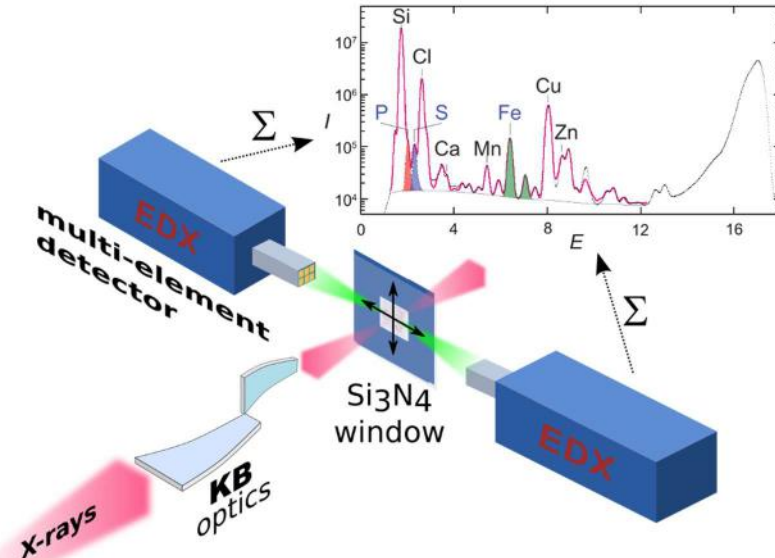
Morphology
Electron Density distribution

Near-field Ptychography



Nano
Cryo

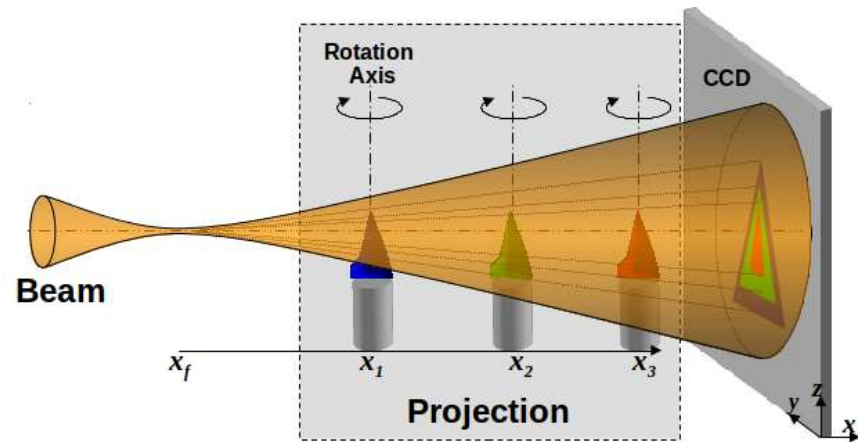
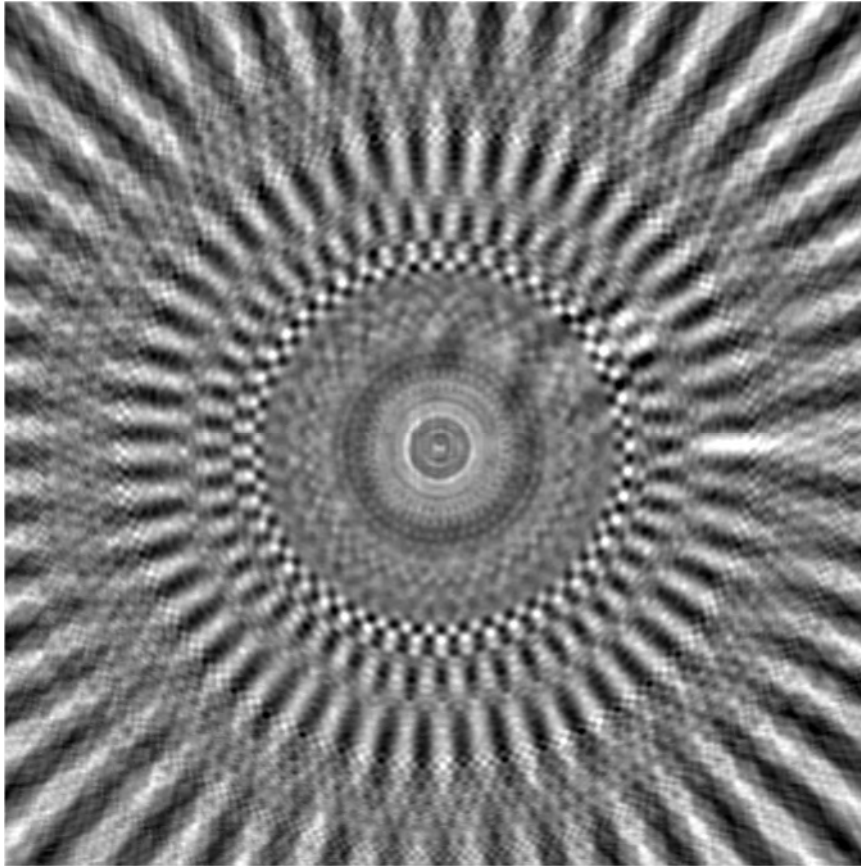
Label free chemical composition
(Trace) Element distributions



- **Quantitative** 3D reconstructions of $\rho(x,y,z)$, $c_{el}(x,y,z)$
- **Correlative** approaches (Soft X-rays, EM, STED)

50ms dwell-time	Phosphorus	Sulfur	Potassium	Zinc
Limit of detection	1.2 10^{-18} g (22,500 atoms)	3.9 10^{-19} g (7,300 atoms)	3.3 10^{-19} g (5,100 atoms)	1.9 10^{-20} g (180 atoms)
Limit of quantification	2.4 10^{-18} g (47,700 atoms)	8.7 10^{-19} g (16,300 atoms)	7.7 10^{-19} g (11,400 atoms)	3.6 10^{-20} g (330 atoms)

HOLOGRAPHY: PHASE RETRIEVAL



Least squares minimization of cost functional

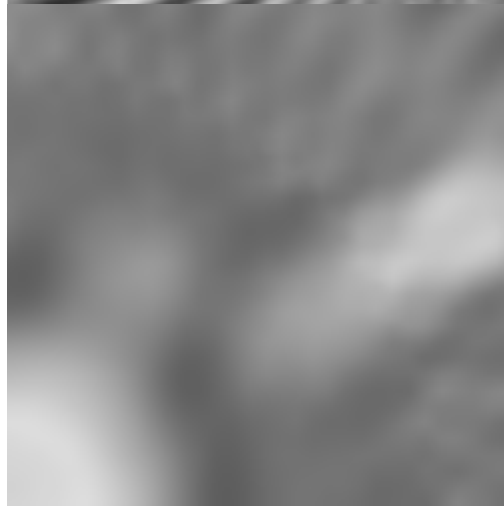
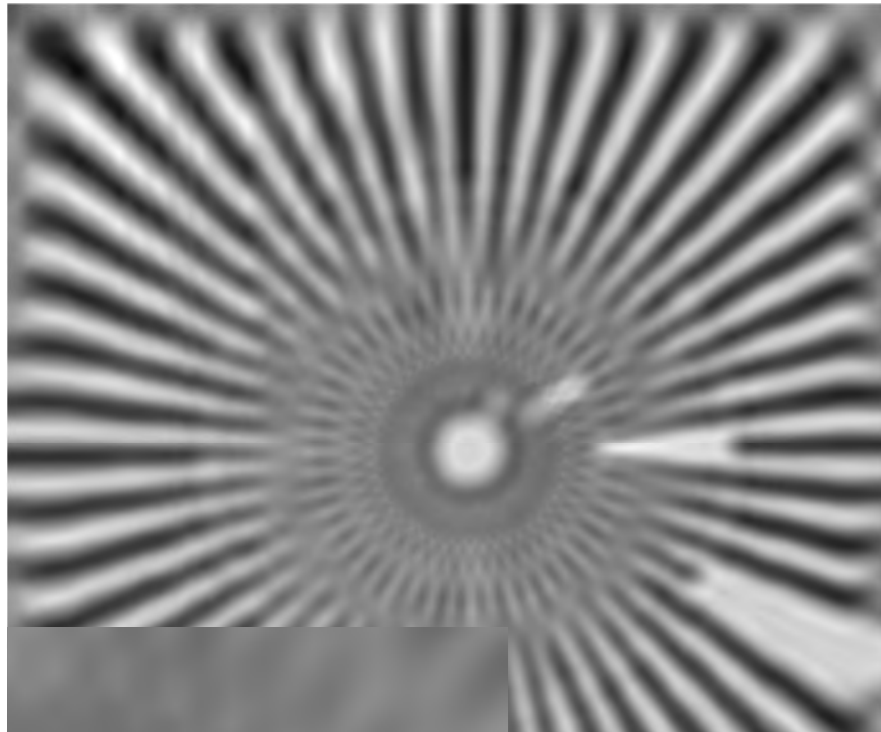
$$K = \sum_m |I_m^{\text{exp}}(f) - I_m^{\text{calc}}[\phi(f)]|^2$$

P.Cloetens et al., Appl. Phys. Lett. 75, 2912 (1999)

P.Schiske, (1968). Zur Frage der Bildrekonstruktion durch Fokusreihen

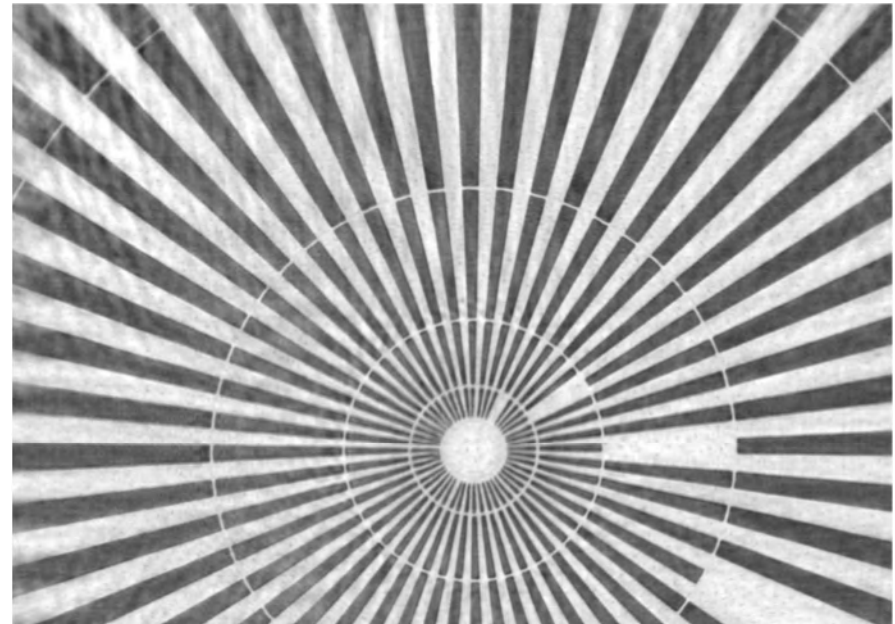
HOLOGRAPHY: PHASE RETRIEVAL

Single Distance Paganin

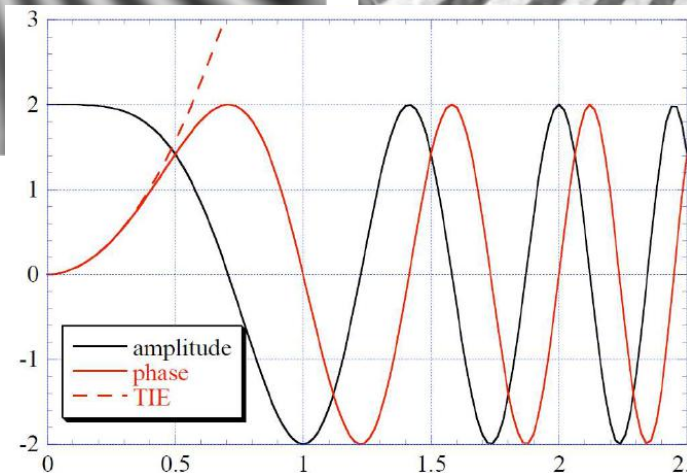


2.5 μm

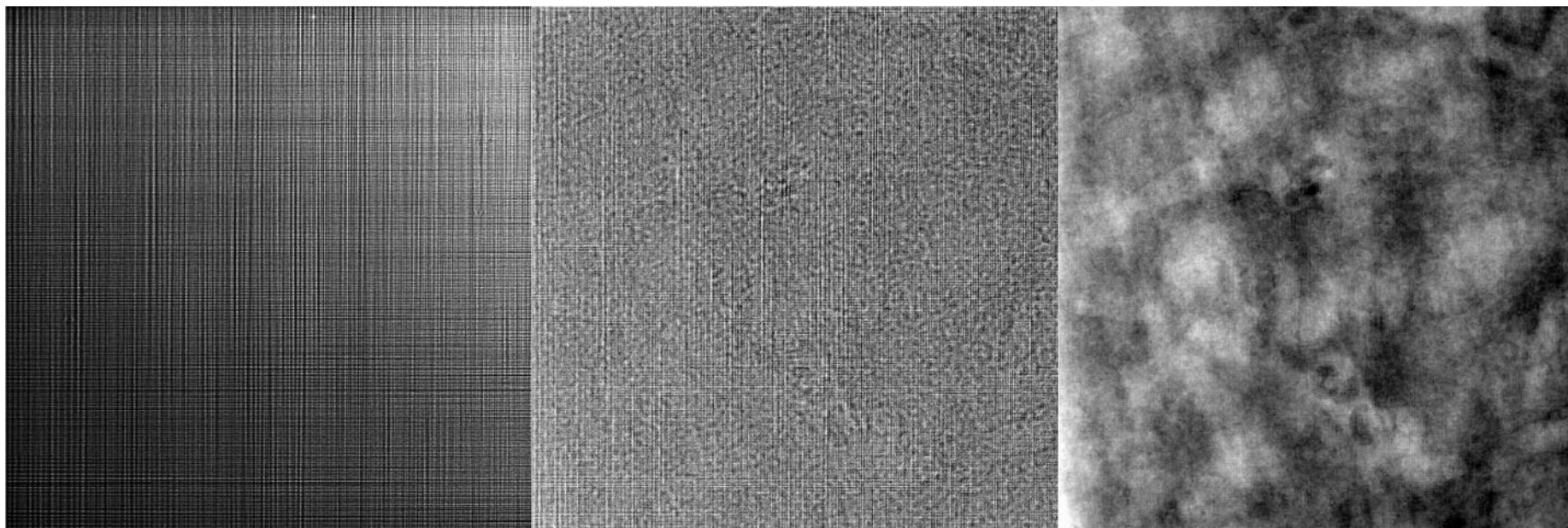
Multiple Distance Transfer Function



2.5 μm



THE POWER OF PHASE CONTRAST ILLUSTRATED ON MOUSE CORTEX TISSUE

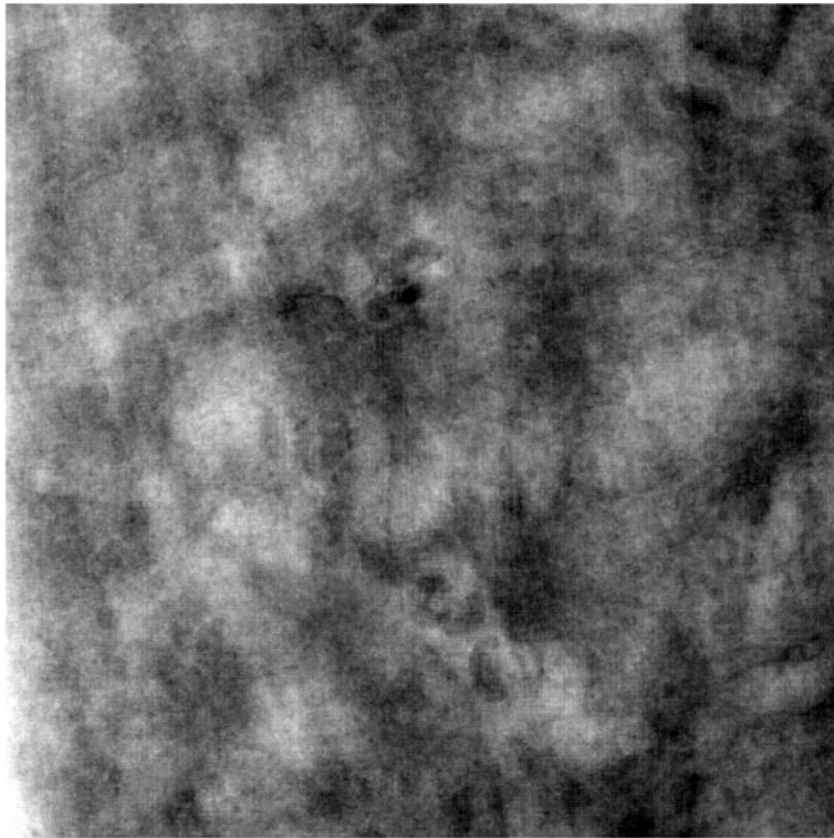


Raw data recorded by the detector → Empty beam corrected holograms → Phase maps

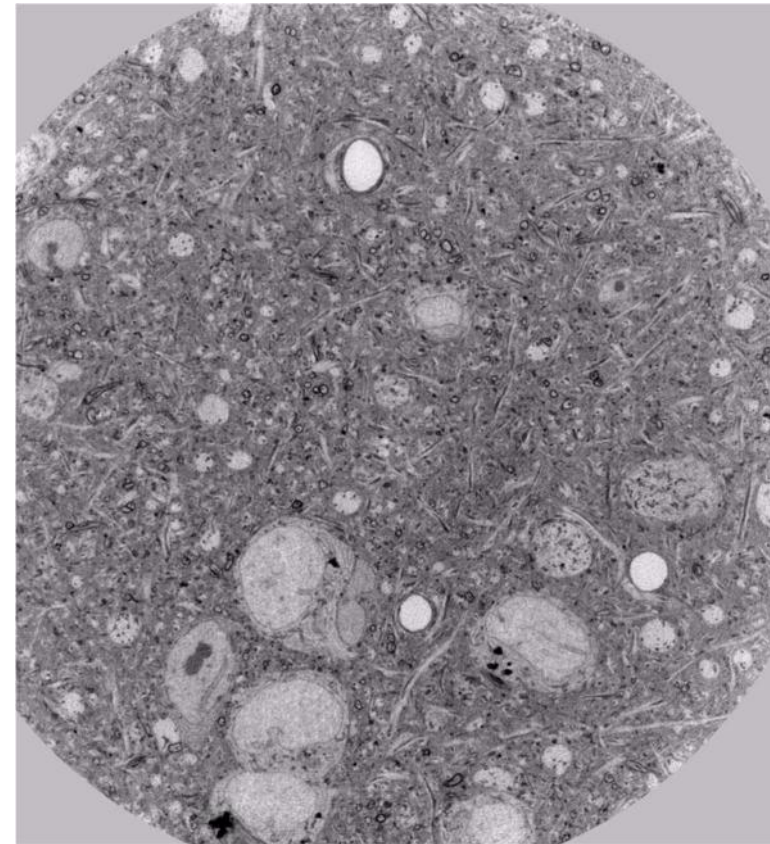


RESOLVING NEURAL NETWORKS (CONNECTOMICS)

Connectomics in mouse cortex – complementarity with FIB-SEM & TEM
Data collection ~4h



Phase maps



Pixel size 40 nm

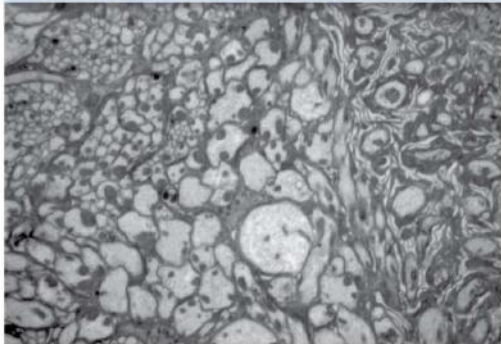
Tomographic
reconstruction



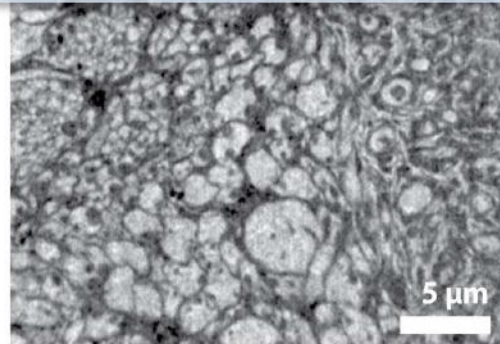
Kuan, Maniates, Lee, Pacureanu* et al. Nat. Neuro. 2020*

X-RAY HOLOTOMOGRAPHY ENABLES MULTISCALE IMAGING OF BRAIN VOLUMES WITH EM-LIKE QUALITY

Drosophila neuronal dendrites

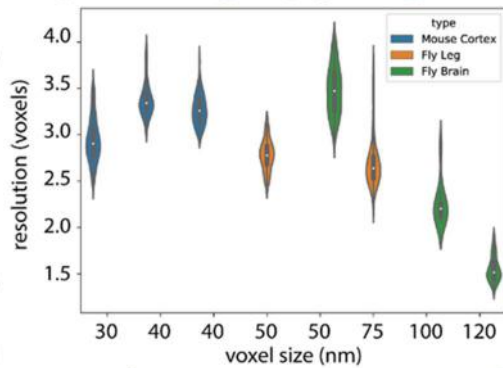
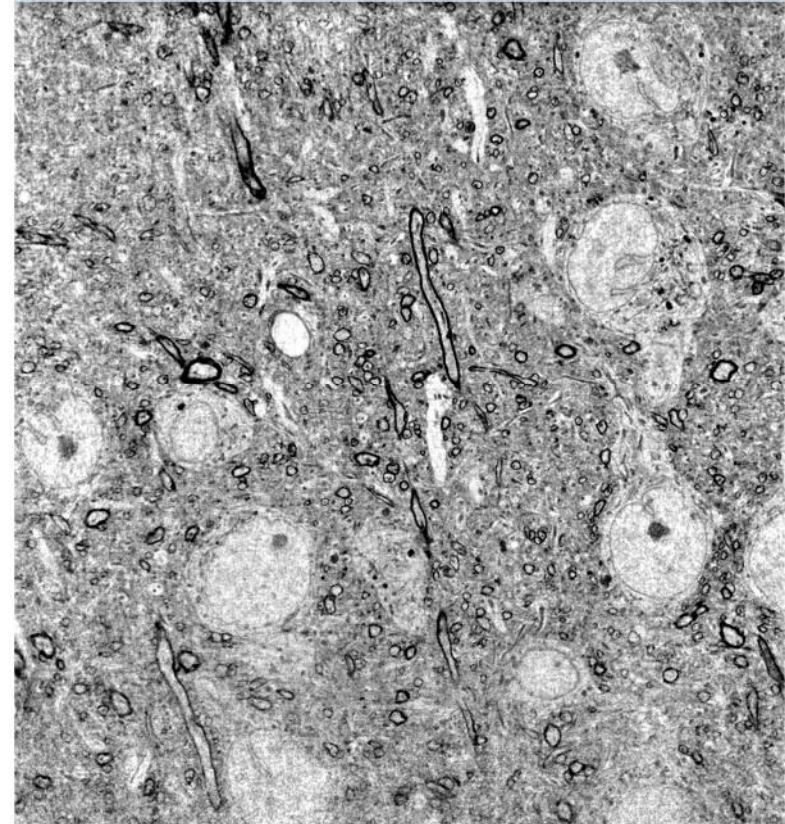


Transmission Electron
Microscopy
12 nm pixels



X-ray holographic
nano-tomography
50 nm pixels

Mouse cortex 30 nm voxel size

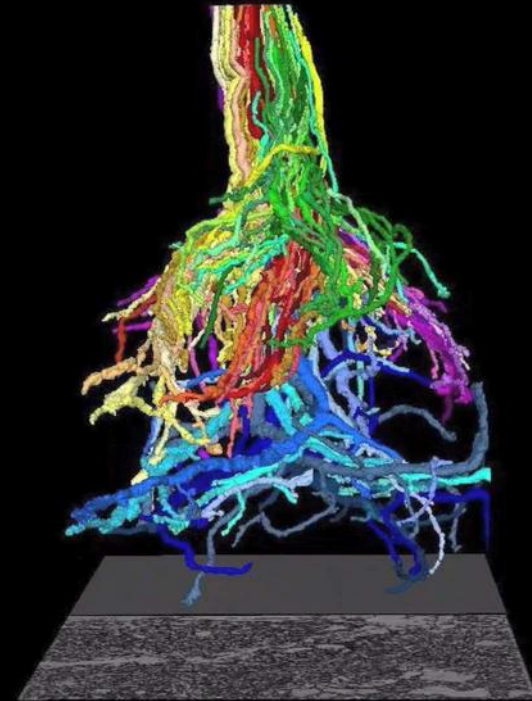
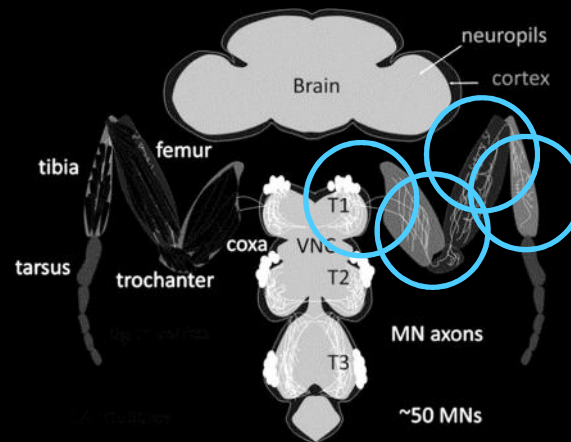
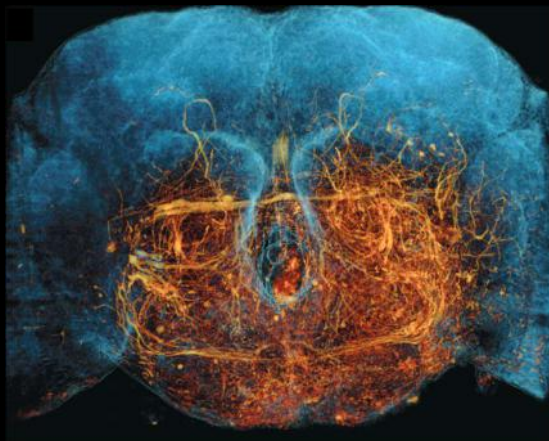
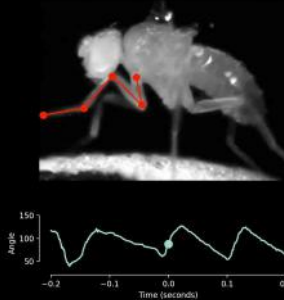


Thanks to EBS spatial resolution and throughput will be improved
 → Synaptic resolution, large volumes, multiple samples

DENSE NEURONAL RECONSTRUCTION IN MILLIMETER SIZED SAMPLES

HOW NEURONS CONTROL AND COORDINATE WALKING

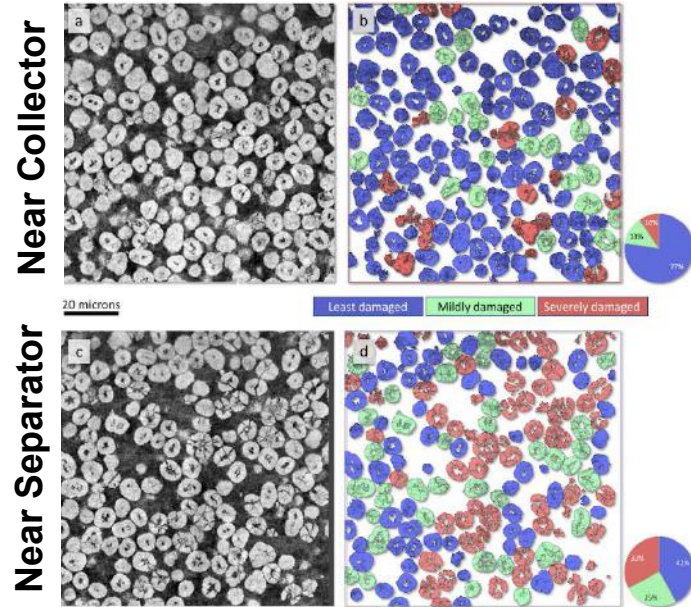
- *Drosophila* brain → first 3D image obtained with serial-section electron microscopy (Bock et al. *Cell* 2018). **Data collection took ~ 2 years.**
- X-ray holographic tomography → data collection for a brain takes **4 – 24 hours** f (voxel size).
- Legs are nearly impossible to section for EM → X-ray imaging was the only way to access the 3D information.



Automatic reconstruction of neurons connecting VNC (spinal cord) to muscles in the leg of *Drosophila*

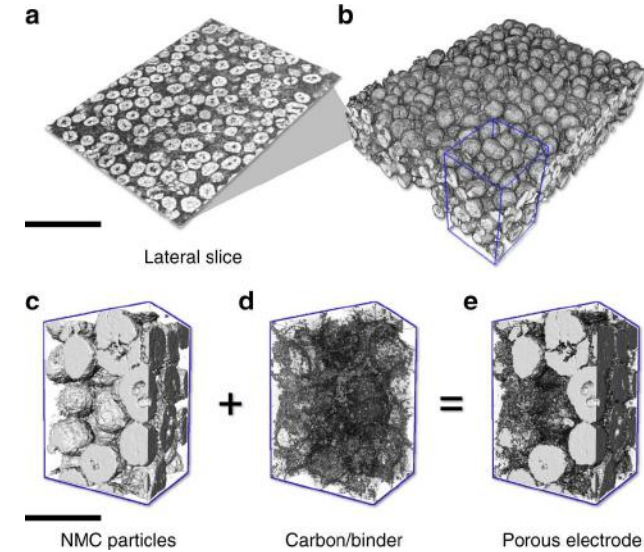
QUANTIFICATION OF DEGRADATION IN LI-ION BATTERIES

Fracturing of secondary particles

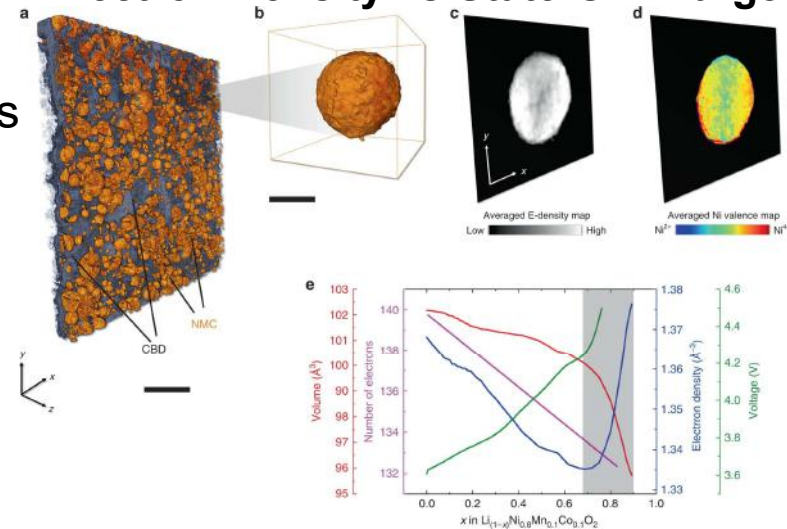


Li-NMC cathode

NMC-CBD detachment



Electron Density vs State-Of-Charge



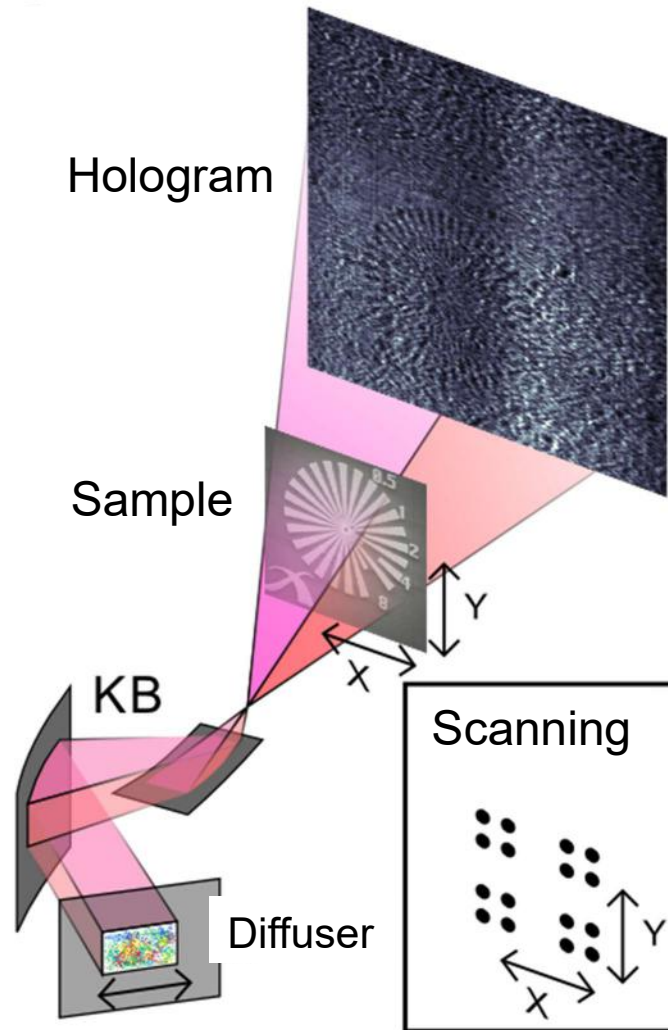
Heterogeneous fracturing of secondary particles in Ni-rich cathode materials

Quantification by Machine Learning of particle - carbon/binder detachment

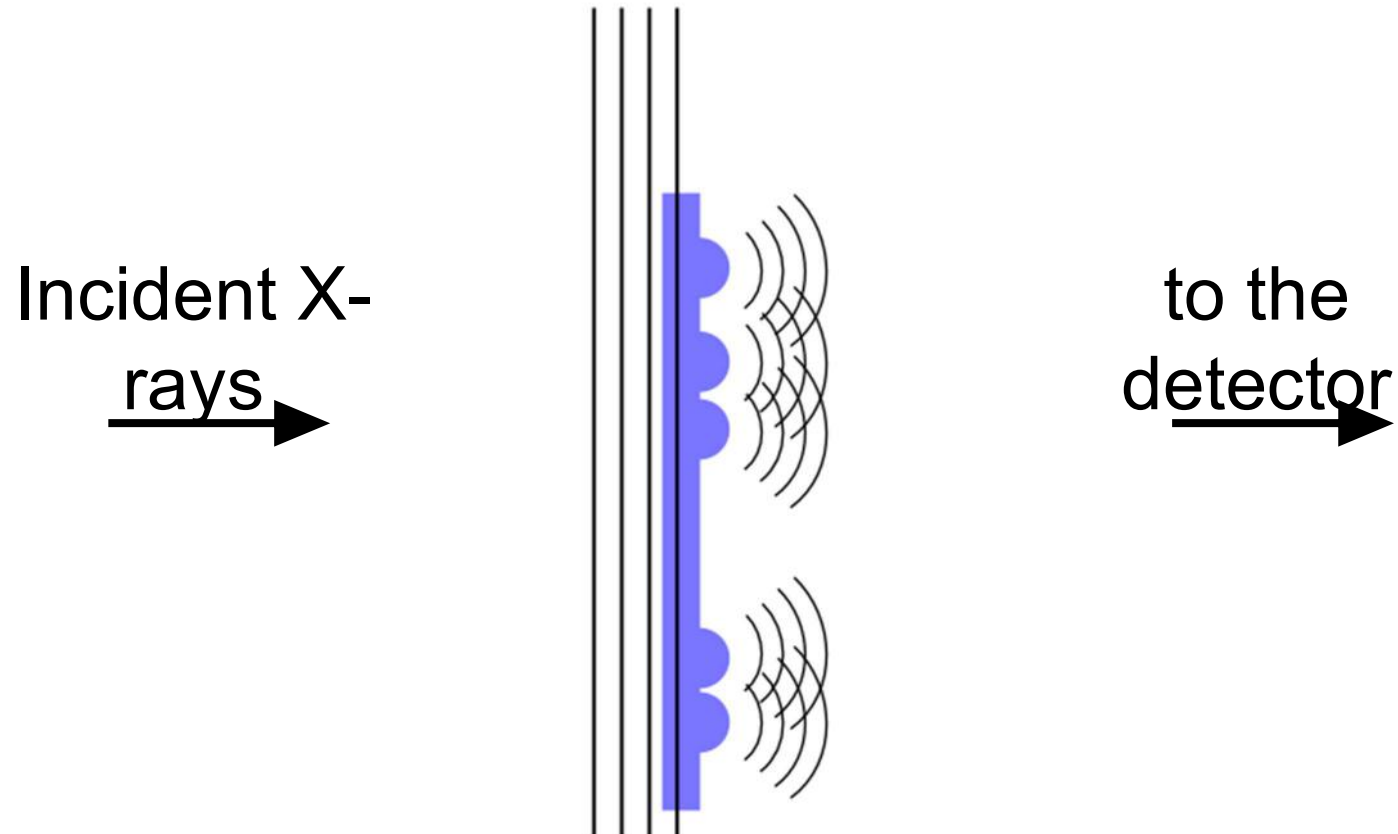
Electron density as a proxy for the State-Of-Charge (SOC)

Yang, Liu et al. *Adv. Energy Mater.* 2019
 Jiang, Liu et al. *Nat. Commun.* 2020

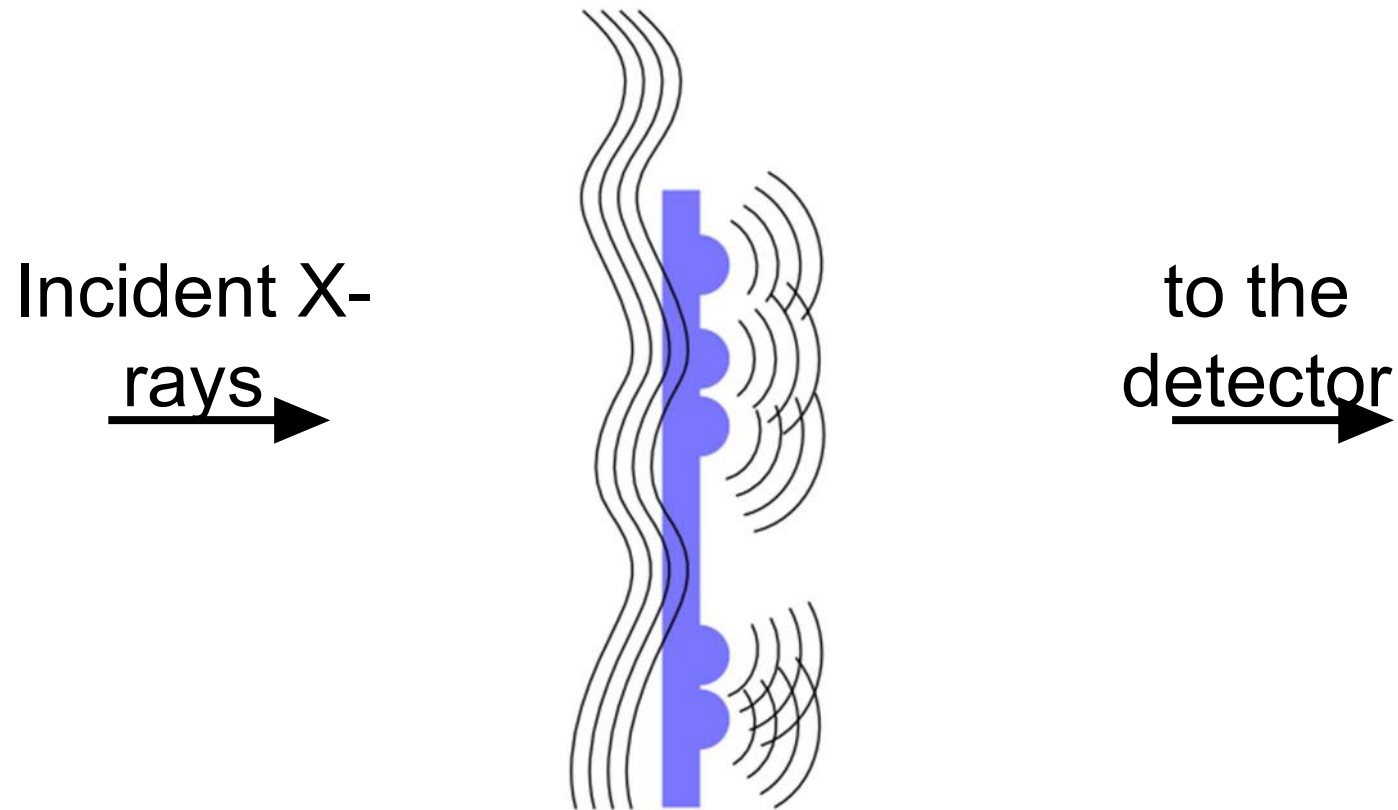
Near-field ptychography with a structured illumination



A different flavor of transverse diversity

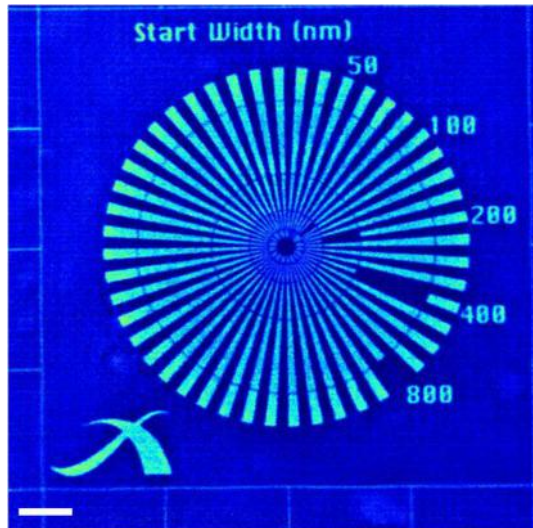


A different flavor of transverse diversity

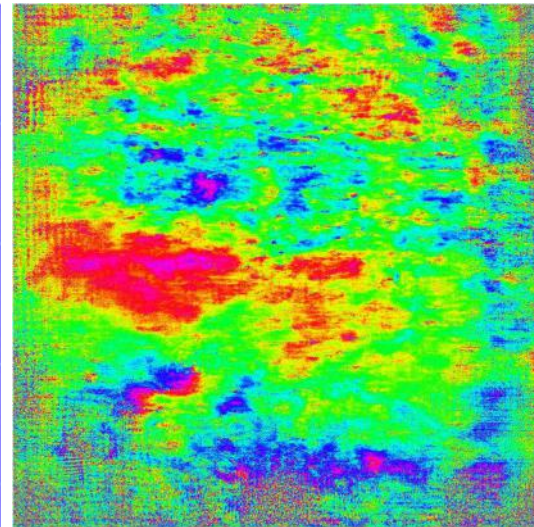


NEAR-FIELD PTYCHOGRAPHY

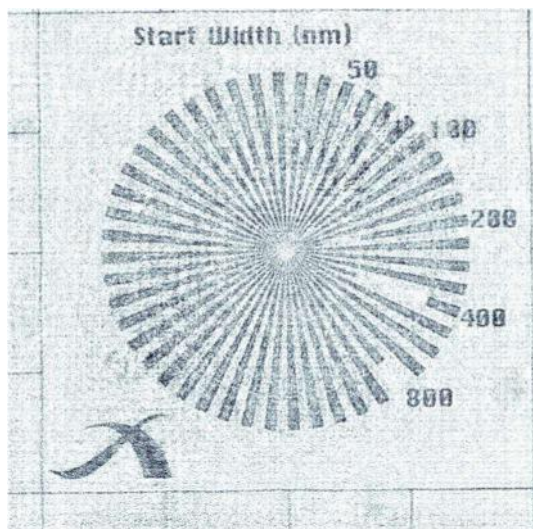
Object
phase



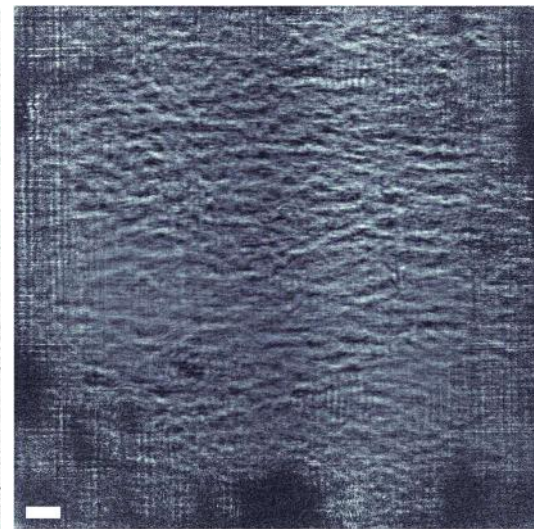
Probe
phase



Object
amplitude

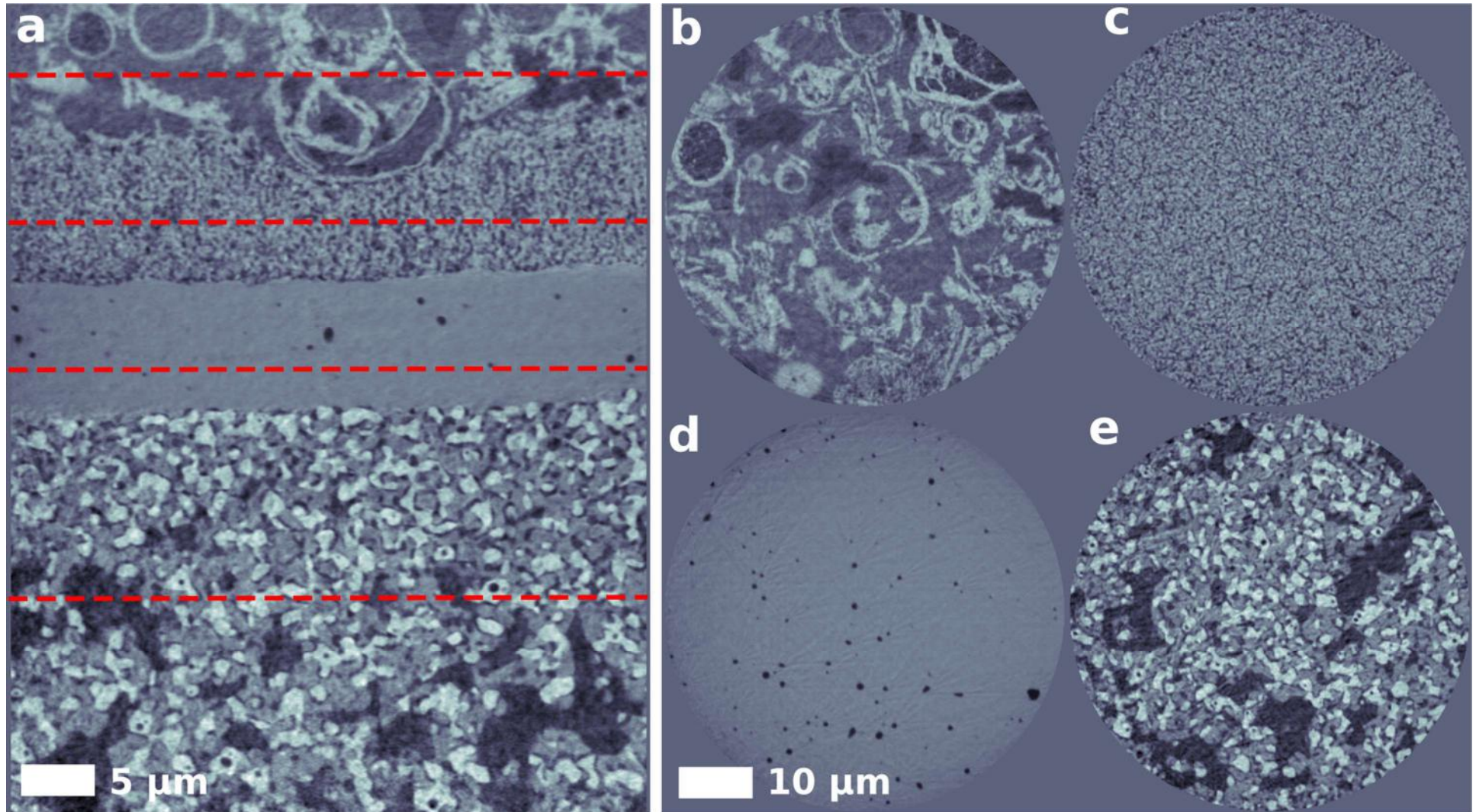


Probe
amplitude



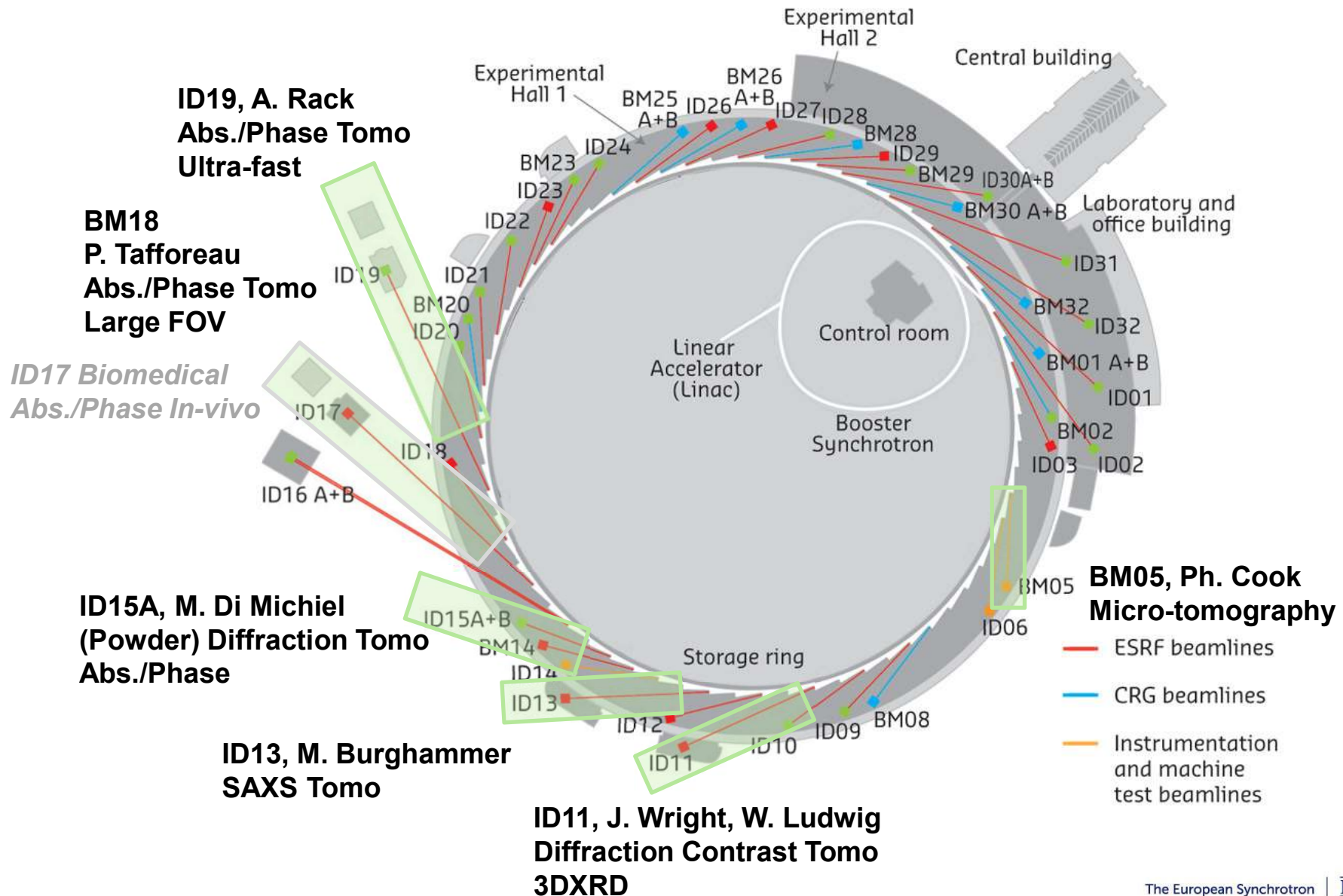
NEAR-FIELD PTYCHOGRAPHY

Successfully extended to (local) tomography!



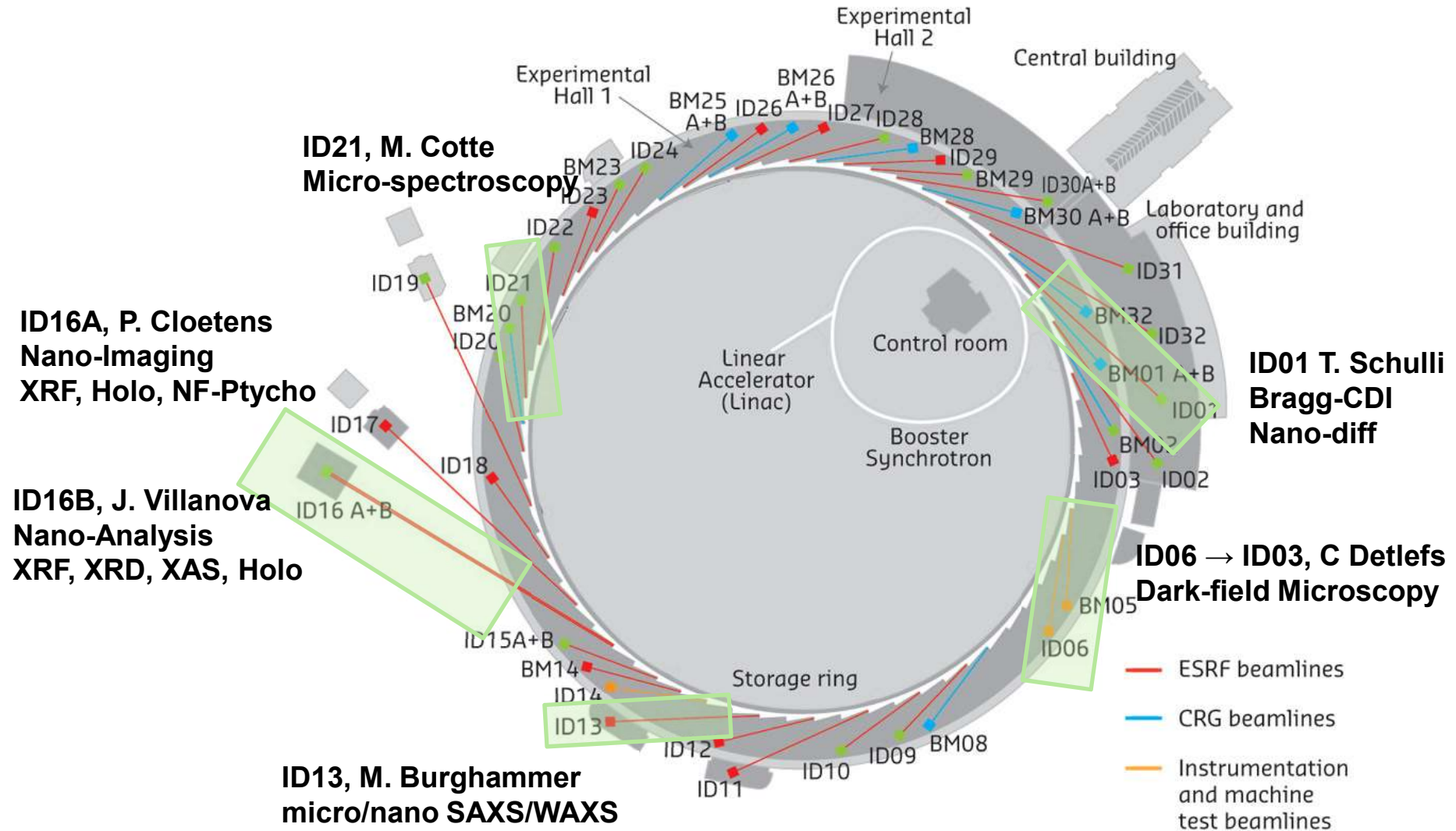
ESRF: THE EUROPEAN SYNCHROTRON

Main beamlines involved in X-ray Imaging



ESRF: THE EUROPEAN SYNCHROTRON

Main beamlines involved in X-ray Microscopy





Thank you for your attention