STATUS OF THE NANO-IMAGING BEAMLINE ID16A

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ID16A

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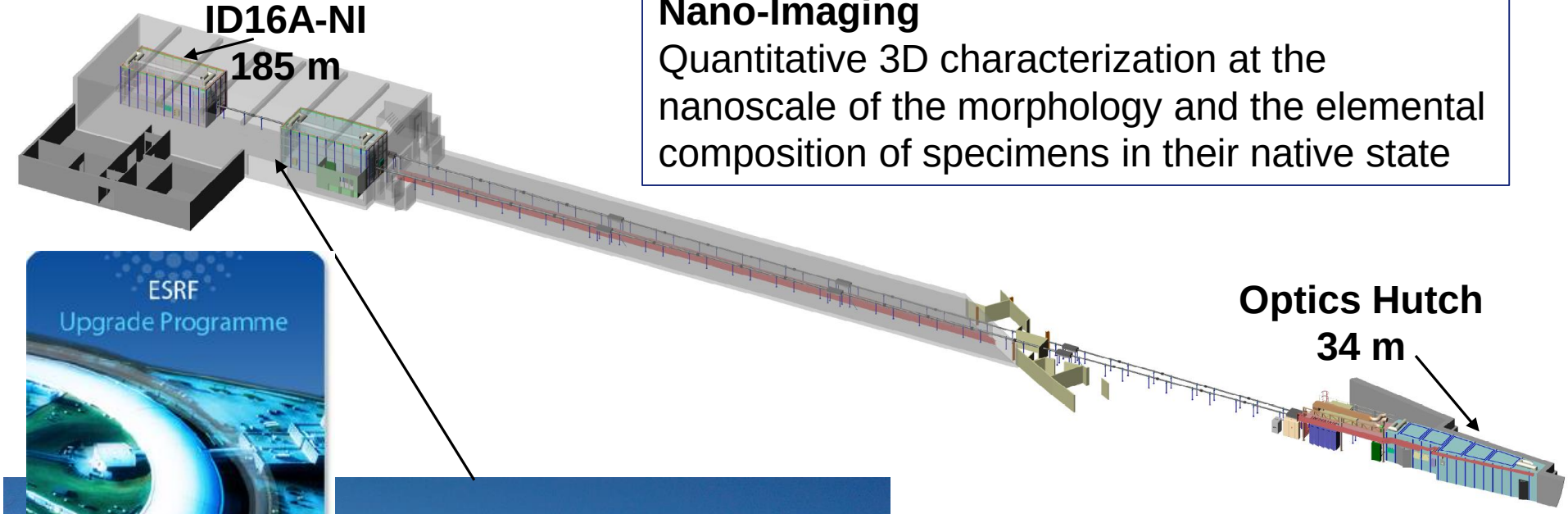
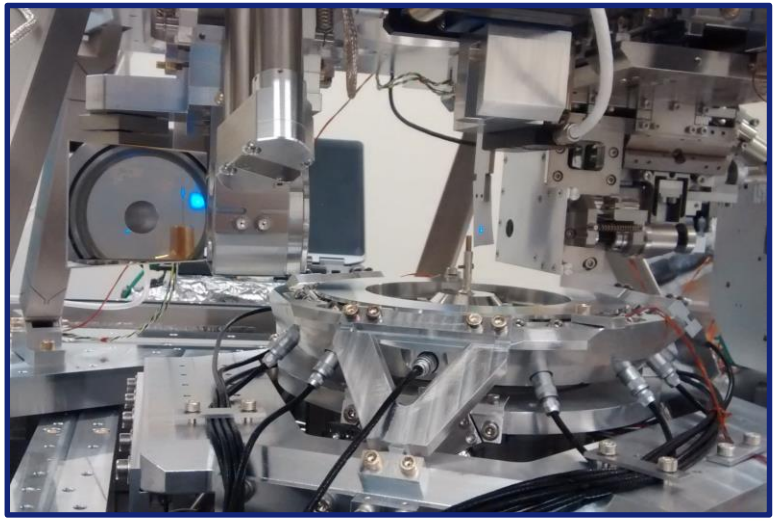
The European Synchrotron | ESRF

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



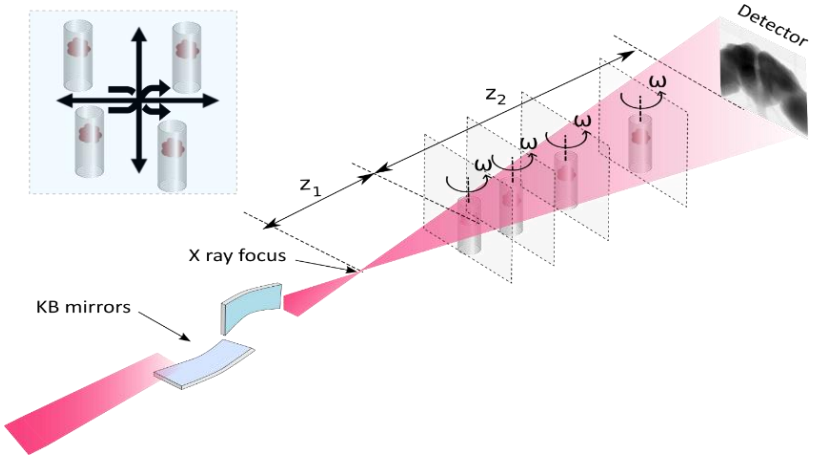

ID16 Satellite Building

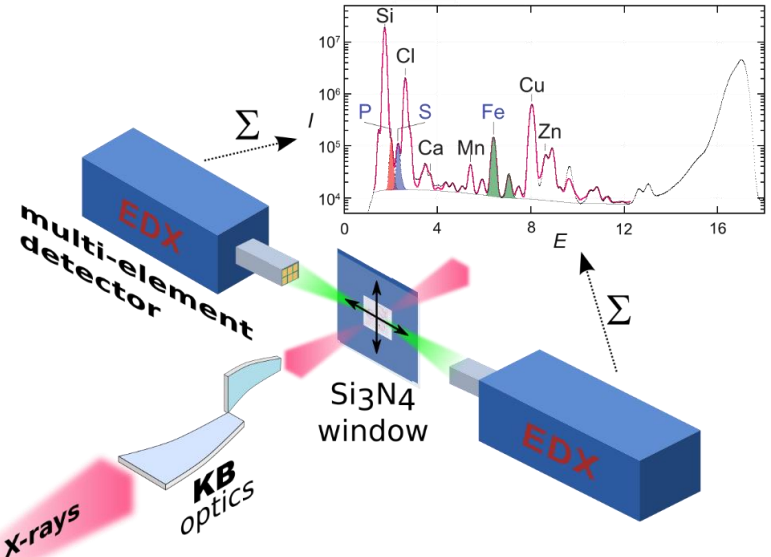

ID16A End-station

X-RAY PHASE CONTRAST

X-RAY FLUORESCENCE

Holography



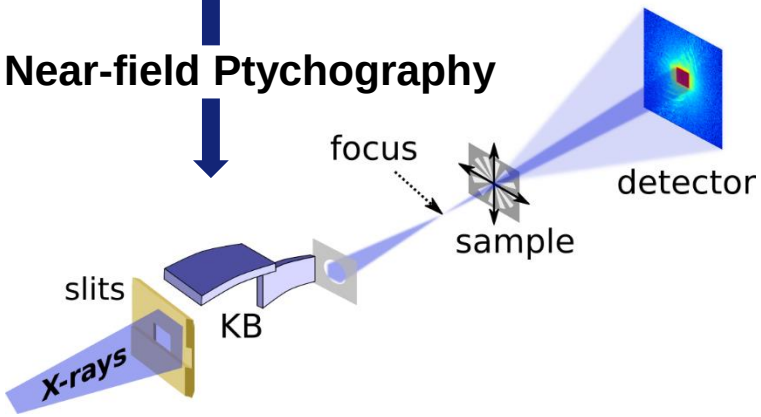


Morphology
Electron Density distribution

Nano
Cryo

Label free chemical composition
(Trace) Element distributions

Near-field Ptychography

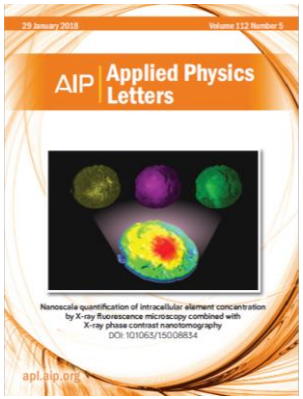


Ptychography

- Output:
  3D reconstructions of $\rho(x,y,z)$, $c_{el}(x,y,z)$

COMBINED PHASE AND X-RAY FLUORESCENCE

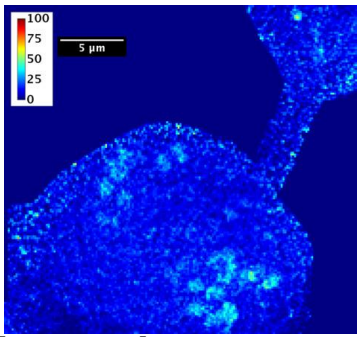
Quantitative correlative approach



C. Gramaccioni et al., Appl. Phys. Lett. 112, 053701 (2018)

2D molarity/concentration

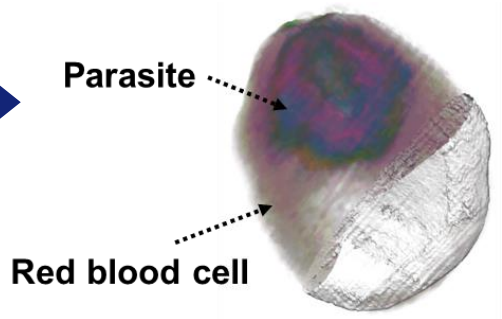
2D mass fraction



Kosior et al., J. Struct. Biol. 177, 239 (2012)

2D XRF	3D phase contrast
2D phase contrast	3D XRF

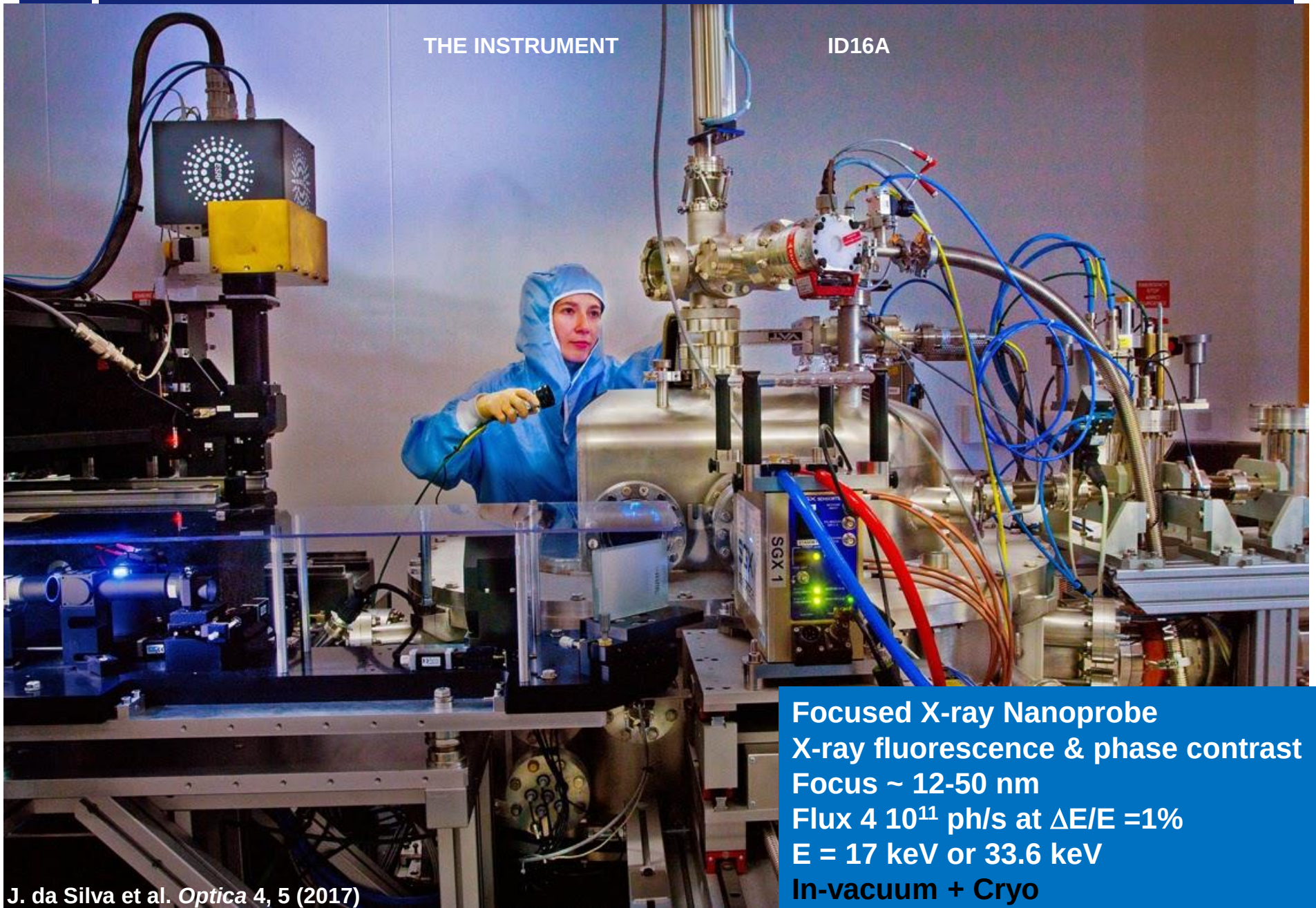
3D mass fraction



3D mass fractions
Fe / S / P

Yang et al., Anal. Chem. 91, 6549 (2019)

ID16A-NI: NANO-IMAGING BEAMLINE



Focused X-ray Nanoprobe
X-ray fluorescence & phase contrast
Focus ~ 12-50 nm
Flux 4 10^{11} ph/s at $\Delta E/E$ =1%
E = 17 keV or 33.6 keV
In-vacuum + Cryo

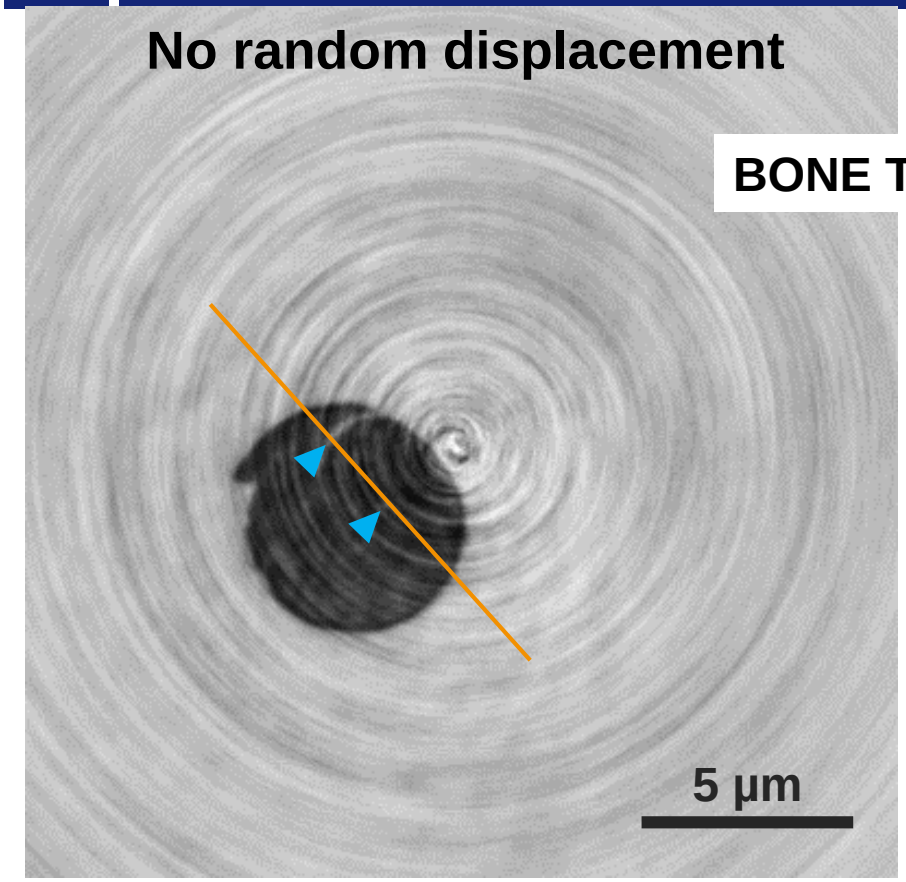
J. da Silva et al. *Optica* 4, 5 (2017)

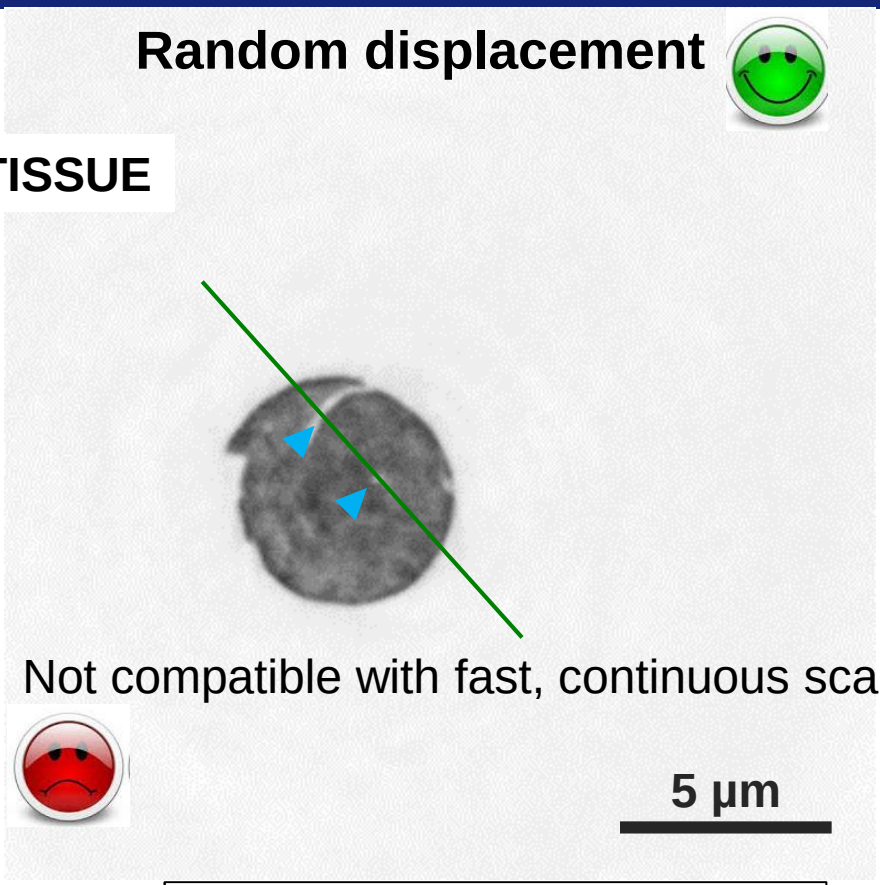
RANDOM SAMPLE DISPLACEMENT

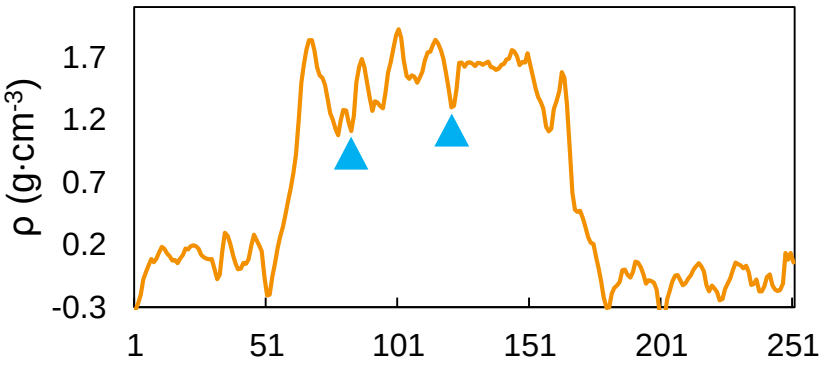
No random displacement

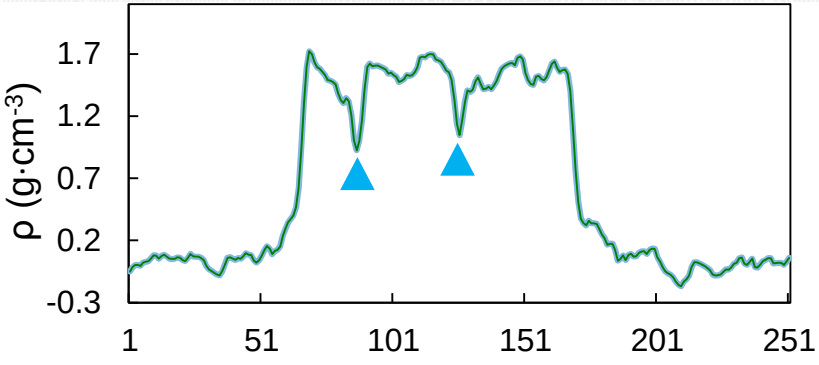
Random displacement

BONE TISSUE









M. Hubert, A. Pacureanu, et al. Appl. Phys. Lett., 112, 203704 (2018)

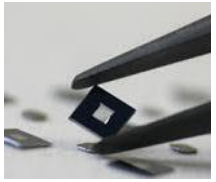
CRYOGENIC WORKFLOW FOR BIOLOGICAL SAMPLES

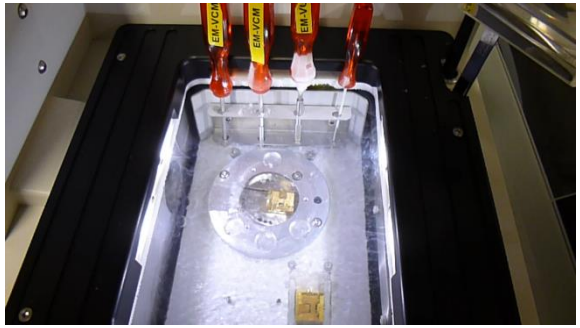
Preserve biological samples close to their native state (**frozen hydrated**)

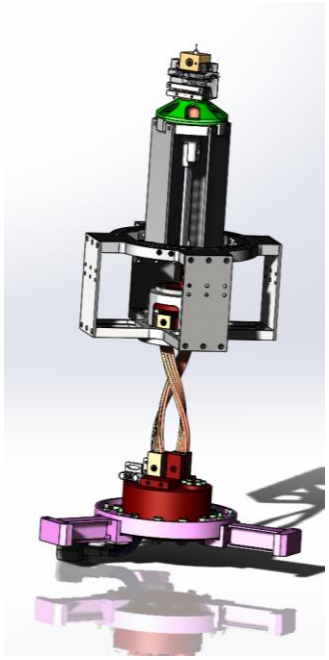
- Avoid redistribution of elements, morphological changes
- Avoid (limit) radiation damage

Cryogenic sample preparation → **Cryo loading chamber and transfer system** → **Cryogenic cooling of the sample stage**

Plunge freezing

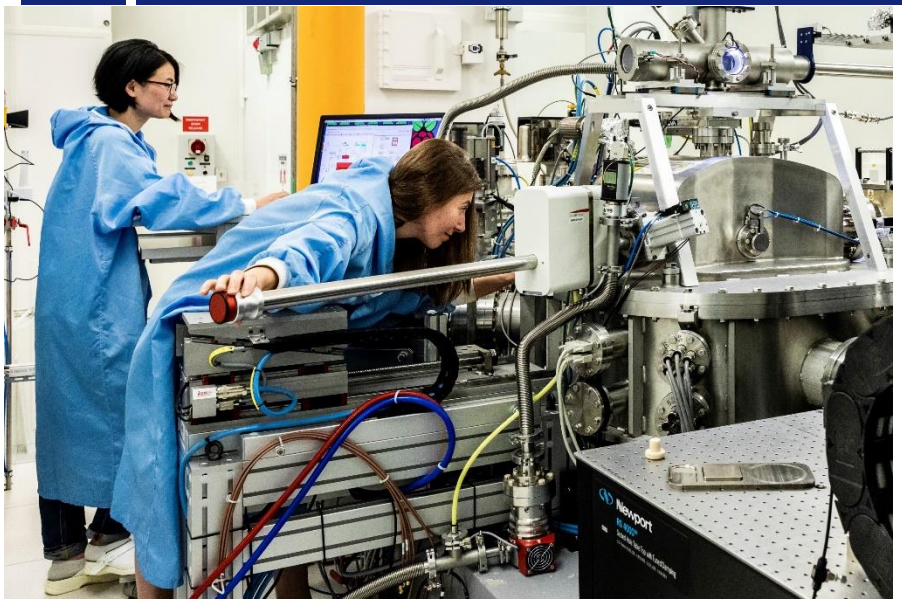


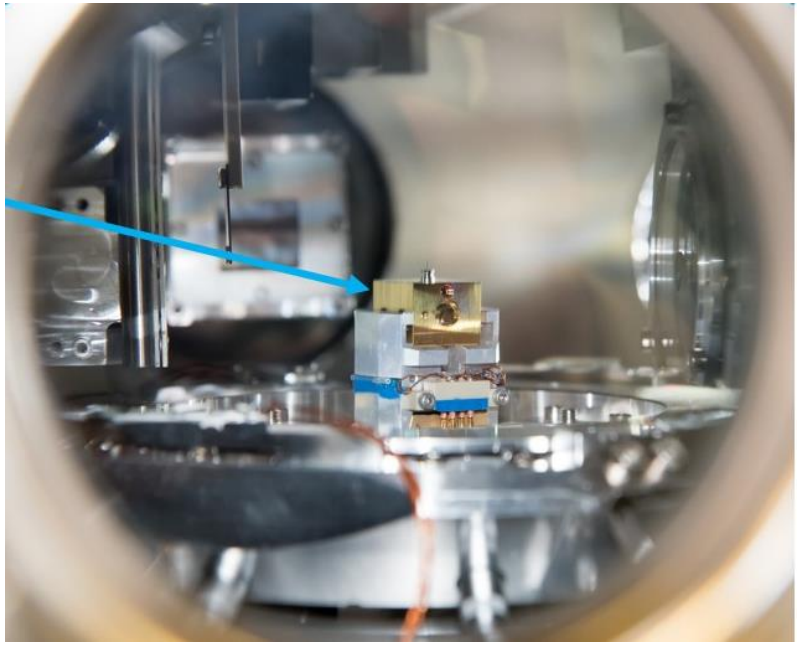




P. van der Linden , S. Bohic, F. Villar, L. Andre

CRYOGENIC WORKFLOW FOR BIOLOGICAL SAMPLES





Leica EM-VCT
Cryo-transfer system

Integrated into the “Hexapiezo”
Highly precise and stable
rotation (<20nm) and scanning (<5nm)

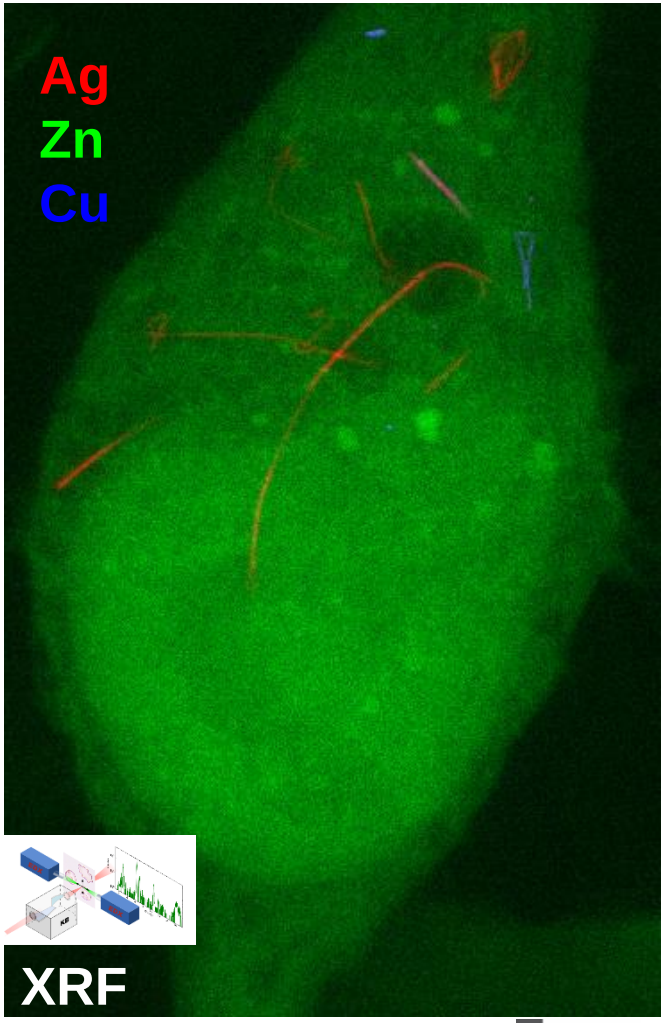
CRUMPLING OF SILVER NANOWIRES BY ENDOLYSOSOMES STRONGLY REDUCES TOXICITY

Fibroblast cell

Cryo

Phase map

15 nm pixel size



XRF

50 nm pixel size

33 nm Ag nanowires

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

UGA Univ. Grenoble Alpes

BERKELEY LAB
LAWRENCE BERKELEY NATIONAL LABORATORY

ESRF

CRYO CORRELATIVE LIGHT X-RAY MICROSCOPY

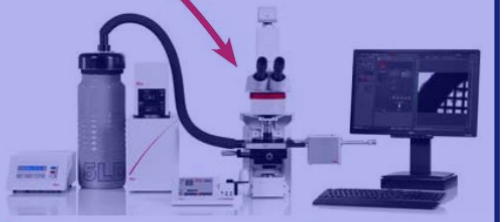
High Pressure Freezing
Leica EM ICE

IBS / GIN / Users

Cryo-Ultramicrotomy
Leica EM UC7
Leica EM FC7

New

Cryo-Transfer-System

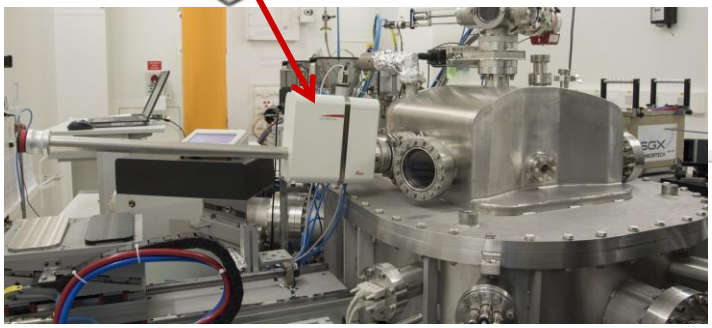


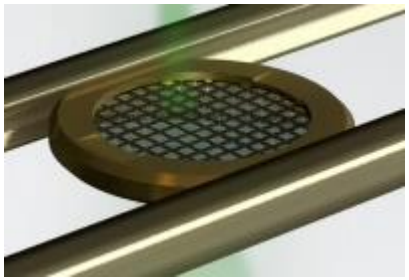
Cryo Light Microscopy
Leica EM Cryo CLEM
on Leica DM6 FS

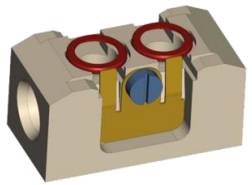
Grid Plunging
Leica EM GP

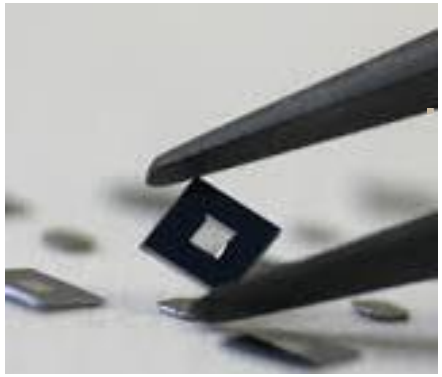
ID16A Users

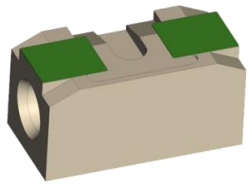
M Software
d correlation
ctures in EM









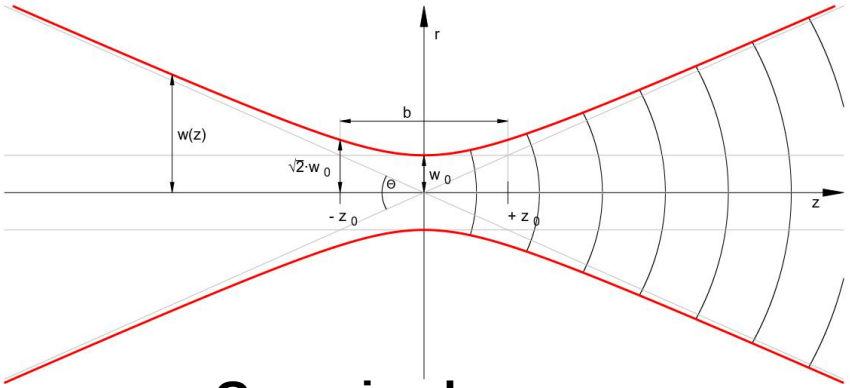


Si_3N_4 window and
Autoloader compatible

<u>Benefits</u>

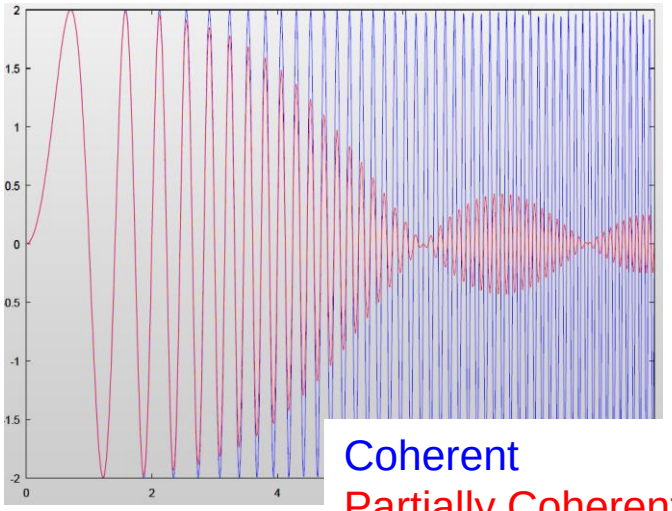
Inspect samples under cryo conditions before X-rays
Preselect regions of interest (coordinate transfer)
Label proteins and/or intracellular components

FRESNEL PROJECTION MICROSCOPY: ULTIMATE RESOLUTION



Gaussian beam

CTF



Coherent

Partially Coherent

Factors limiting the spatial resolution:

Fresnel diffraction : $1/2\sqrt{\lambda D}$

Phase retrieval through *phase diversity*

Different distances

Near-field ptychography (M Stockmar et al, Scientific Reports, 2013)

Incoherent contributions to the nanofocus

Mechanical vibrations

Electron source size

$\mu_{12}(\lambda D f)$

Not just brightness, coherence limited

1 nm contribution, demagn. 1000 → 1 µm source size

DEPTH OF FIELD AT HIGH RESOLUTION

Number of pixels *N*

$$N = \frac{\text{max 3D field of view}}{\text{resolution element}}$$

Absorption
Depth of field
Multiple scattering

Optics
Interaction strength
Photon statistics

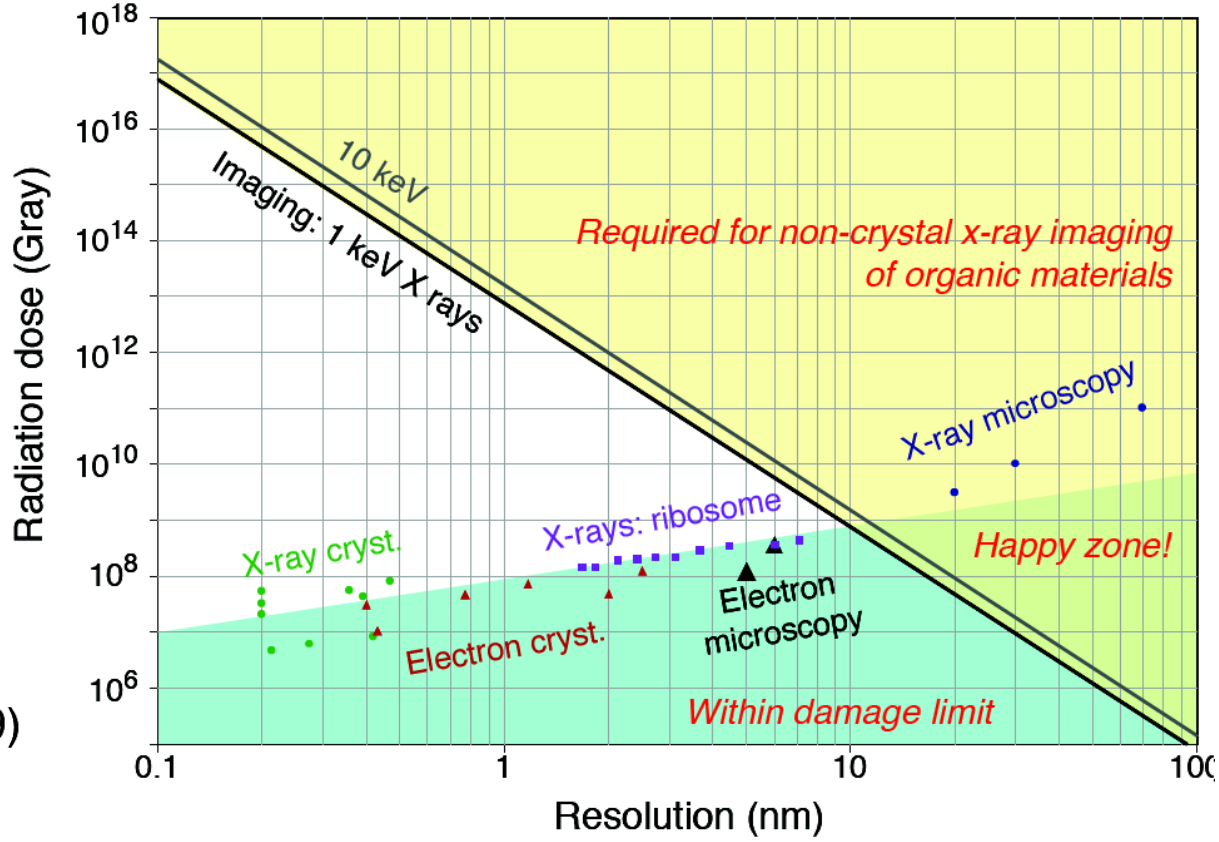
3D Imaging from *projections*

$$N = \frac{\text{depth of field}}{\text{Rayleigh resolution}} = \frac{2\frac{\lambda}{\text{NA}^2}}{0.61\frac{\lambda}{\text{NA}}} = \frac{2}{0.61\text{NA}} = \frac{2}{0.61^2}\frac{\text{resolution}}{\lambda}$$

NA << 1
λ << resolution

'Hard' X-rays are favorable

RADIATION DAMAGE AND DOSE AT HIGH RESOLUTION

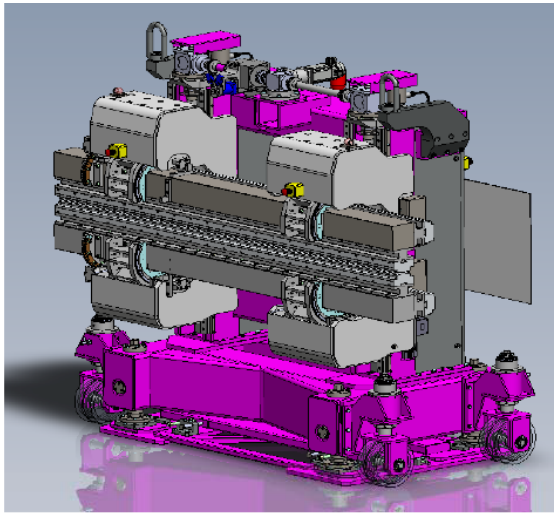


Howells *et al.*, *J. Electron. Spec. Rel. Phen.* **170**, 4 (2009)

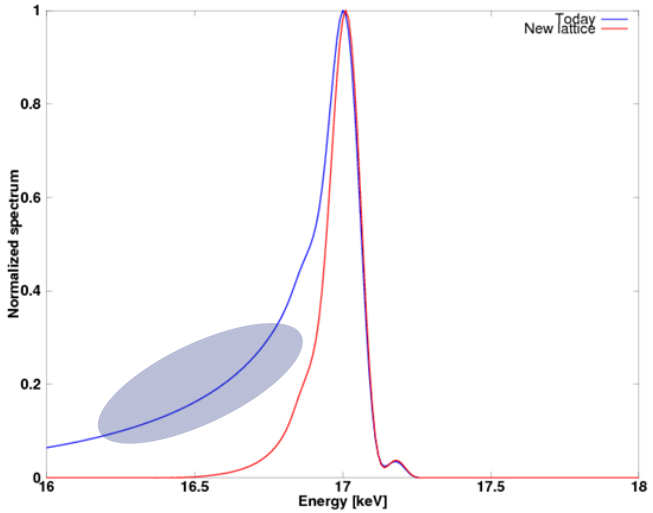
- Fast freezing and cryo-microscopy partially solves the issue
- XRF (50ms dwell-time):
  - 2D: ~10^{10} Gy @ 20nm pixel
  - 3D: 2 10^{9} Gy @ 120nm voxel
- Holographic nanotomography (200ms exp.):
  - 3D: 10^{8} Gy @ 10nm voxel

ESRF-EBS: ID16A SOURCE FEATURES

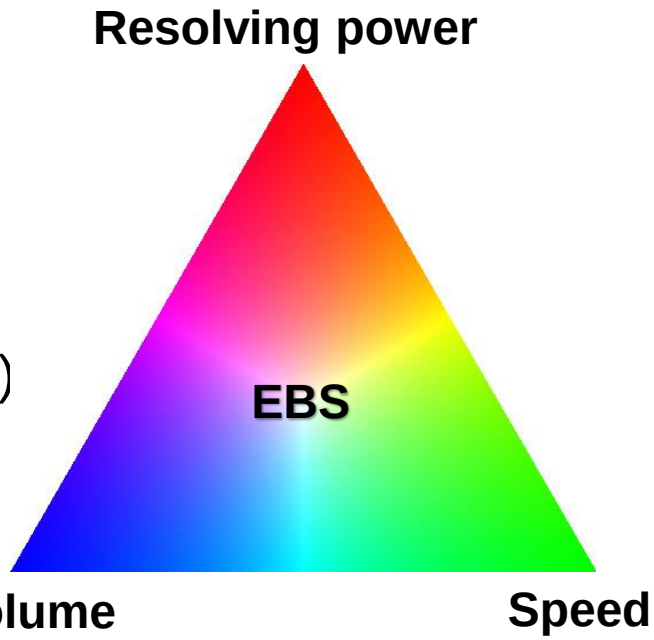
- Ideally suited for the production of **round, pink nano-beams**
- **Increase** by about **30** of **flux density, 25 of flux**, **>10^{13} ph/s!!!**
- **Spectral bandwidth from 1% → 0.7%**
- *Further decrease of focal spot size is mostly optics limited*
- **Improved spatial resolution and sensitivity** in coherent imaging (better coherence and higher flux)



2.3 m Revolver support



ESRF-EBS: ID16A ORIENTATIONS



X-ray Fluorescence

- Towards **single atom limit of detection** (Zn, Fe)
- **Larger number of replicates**
- **Routine 3D cryo XRF** with full resolution

Coherent Imaging

- Focus on **near-field configuration** (through-put, sample size, sensitivity)
- **Huge 3D problems**

  e.g. Connectomics (1mm^3 brain with ‘synaptic resolution’)

Correlative (X-ray) microscopy

- Online: Phase & Fluo
- Offline: Cryo CLXM & (cryo-)EM

ESRF-EBS: ID16A ENABLING TECHNOLOGY

- End-station currently dismantled!
- Faster nano-positioning
  New faster in-vacuum rotation and improved cryo-environment
- New KB Vertical Focusing Mirror @ 17 keV
- Imaging detector:
  faster (sCMOS) and larger (2K x 2K → 4K x 4K or 6K x 6K)

~2GB/s

- Homogeneity of software
  in the frame of ESRF Tomography Strategy

- Sample preparation
- Dose management
- …