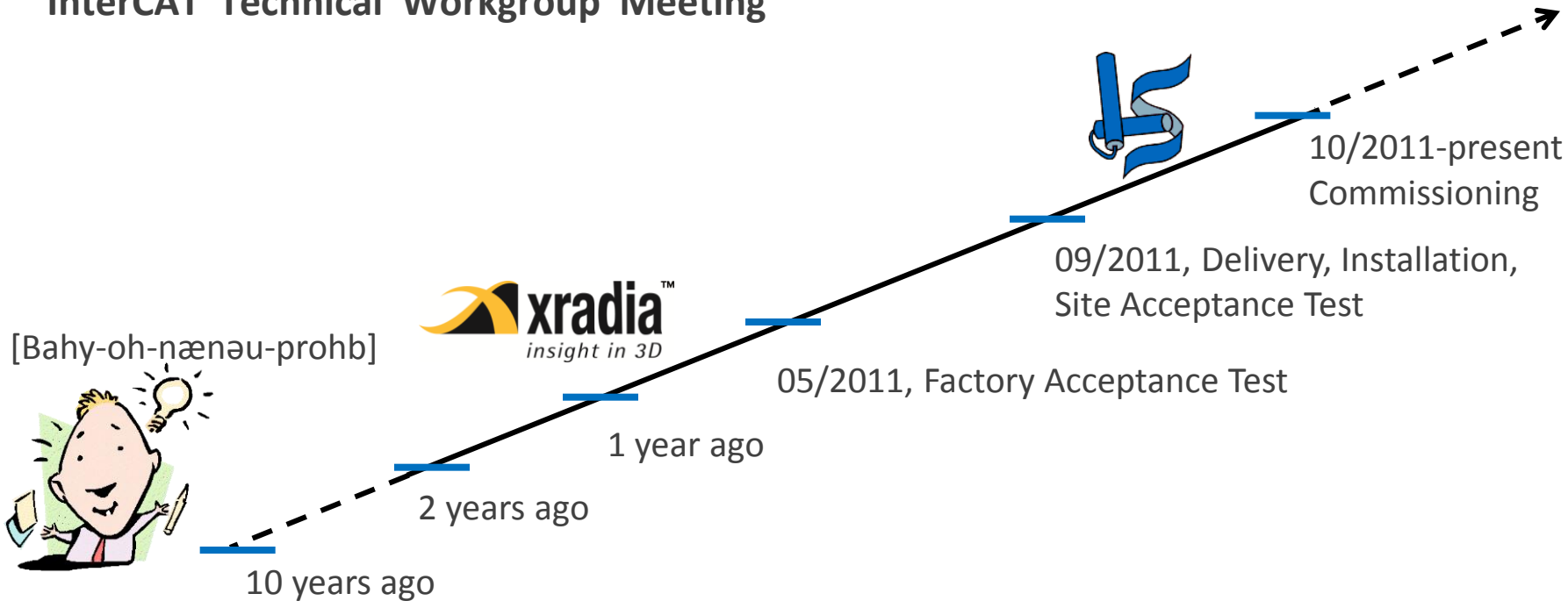
An Early View of the Bionanoprobe

Si Chen

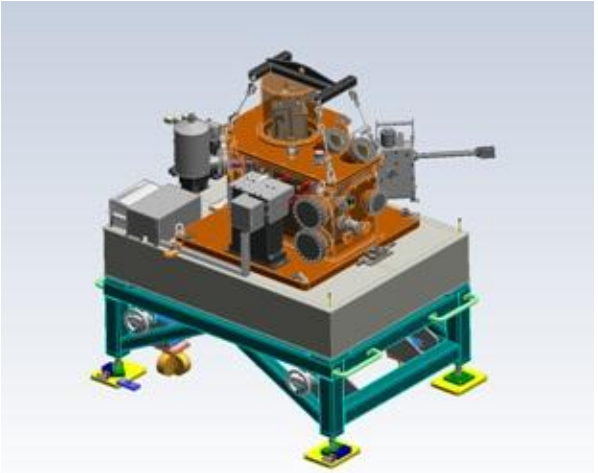
March 15, 2012

InterCAT Technical Workgroup Meeting



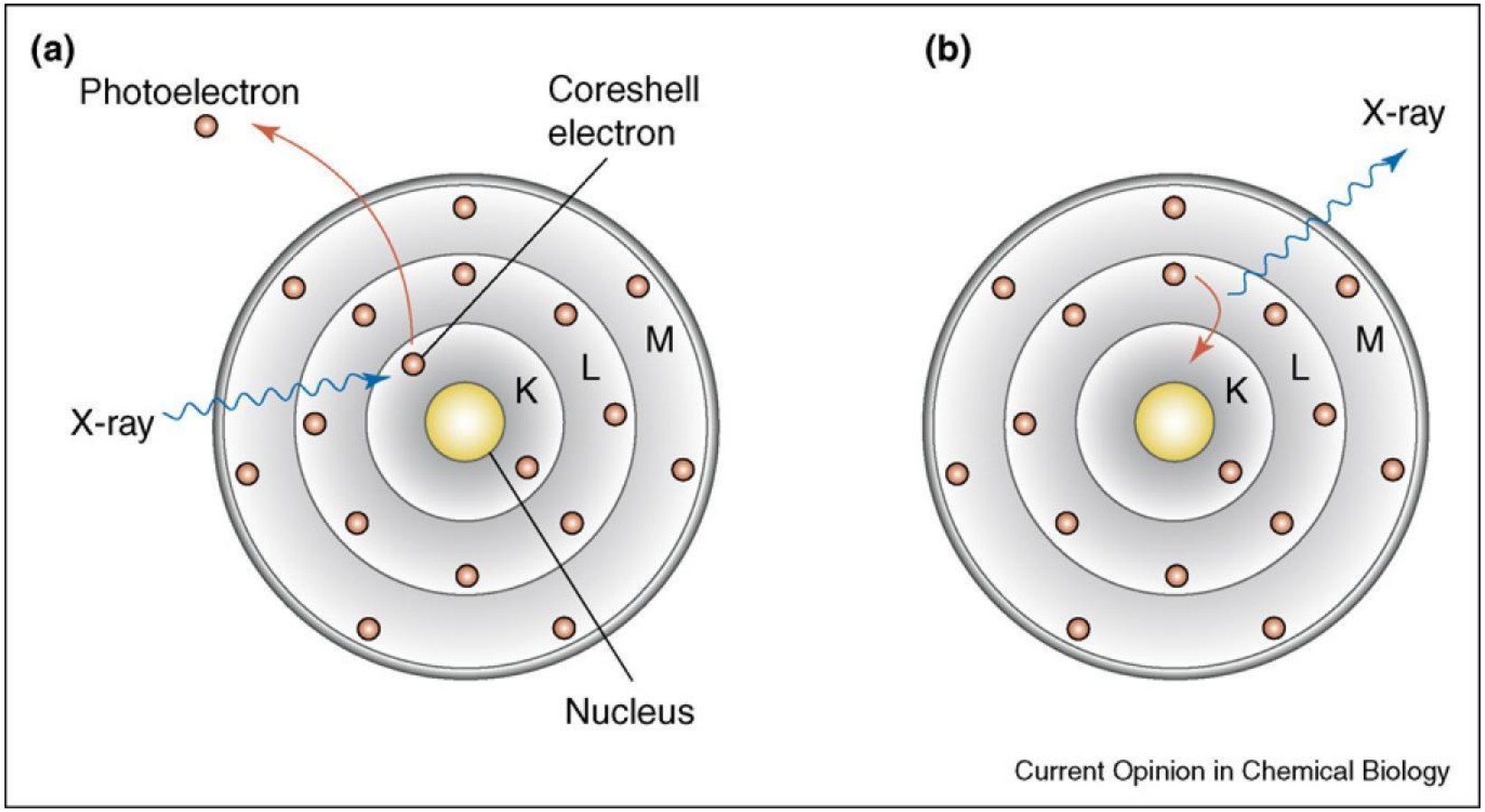
Outlines

- Concept of a hard X-ray fluorescence microscope
- Motivation for developing the Bionanoprobe
- System design of the Bionanoprobe
- Preliminary results
- Summary



Bionanoprobe

Basic Principle of X-ray Fluorescence

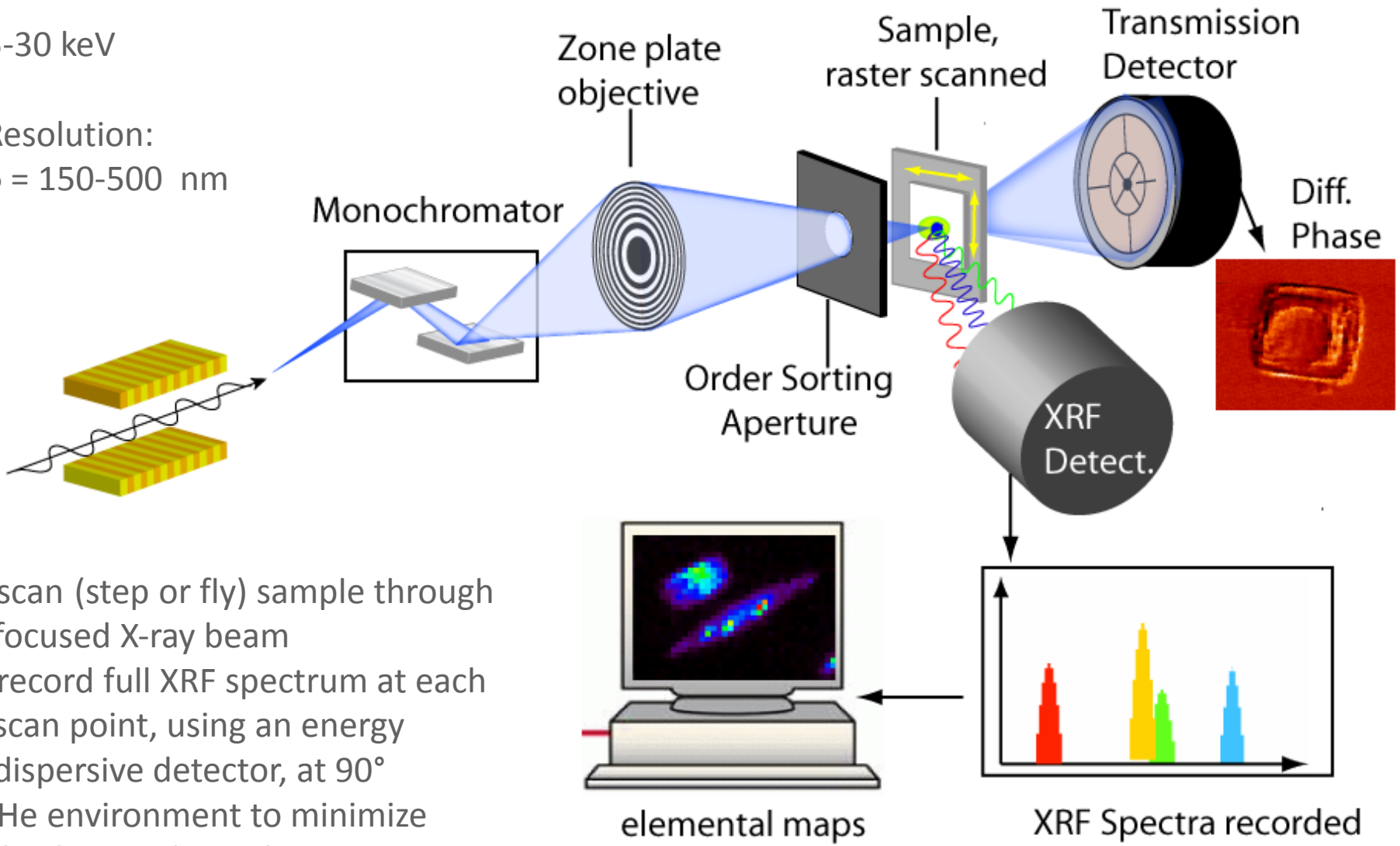


C. Fahrni, Current Opinion in Chemical Biology (11), 2007

Schematic of a Hard X-ray Fluorescence Microscope

5-30 keV

Resolution:
δ = 150-500 nm



- scan (step or fly) sample through focused X-ray beam
- record full XRF spectrum at each scan point, using an energy dispersive detector, at 90°
- He environment to minimize background, air absorption
- data acquisition: Epics; visualization: IDL / MAPS

B. Hornberger et al, J Synchrotron Radiat 15(Pt 4), 2008
de Jonge et al, Phys Rev Lett 100(16), 2008

Schematic NOT to scale

What has been available?

X-ray fluorescence microprobes at Sector 2 optimized for life science applications:

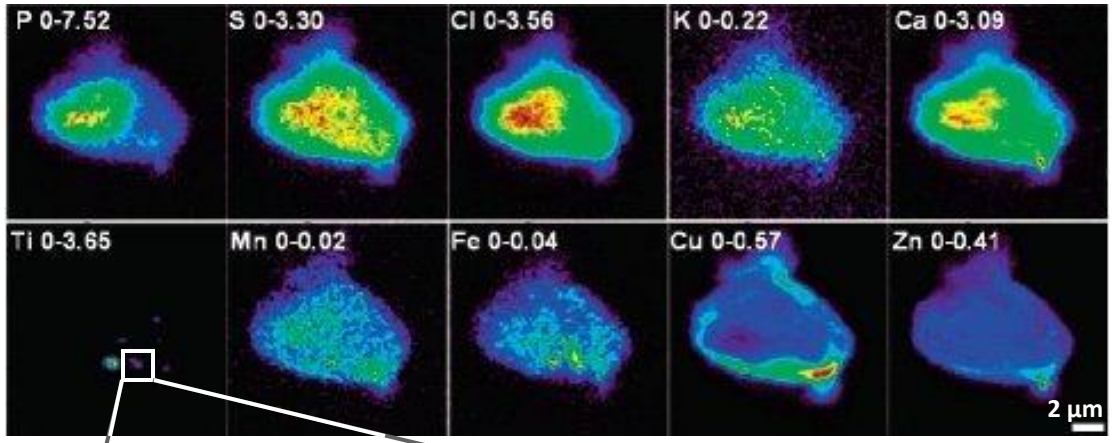
2-ID-D:

- XRF mapping and micro-spectroscopy (determine local oxidation state via micro-XANES)
- spatial resolution: 150 nm (high resolution), 400 nm (high flux and micro-spectroscopy)
- cryogenic capabilities (under commissioning)

2-ID-E:

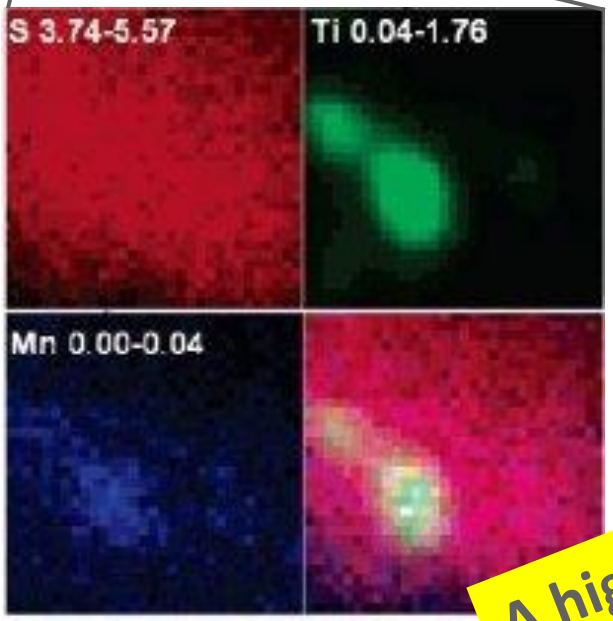
- XRF mapping
- spatial resolution: 250 nm (high resolution), 400 nm (high flux)
- fly-scan overview scanning, tomography

Example results from 2-ID-D



Elemental concentrations ($\mu g \cdot cm^{-2}$) in a whole rat pheochromocytoma cell transfected with mitochondrion-specific TiO_2 nanoconjugates.

13 µm x 12.8 µm, step: 0.2 µm



scan area:
1.5 µm x 1.5 µm

step size:

A higher spatial resolution is needed!

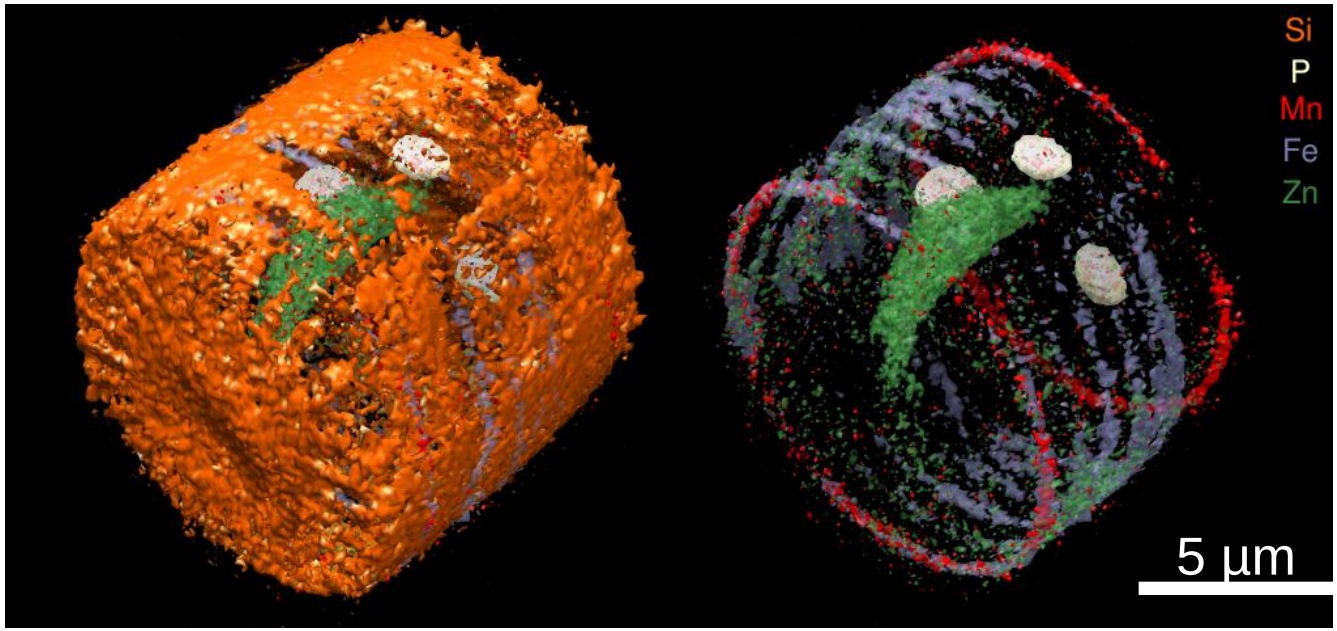
[Paunesku *et al*. Nano Letters 2

http://micro.magnet.fsu.edu/cells/mitochondria/images/mitochondriafigure1.jpg

Example results from 2-ID-E

Cyclotela

[de Jonge *et al*. PNAS 2010]



(a) with the siliceous cell wall **(b) without the cell wall**

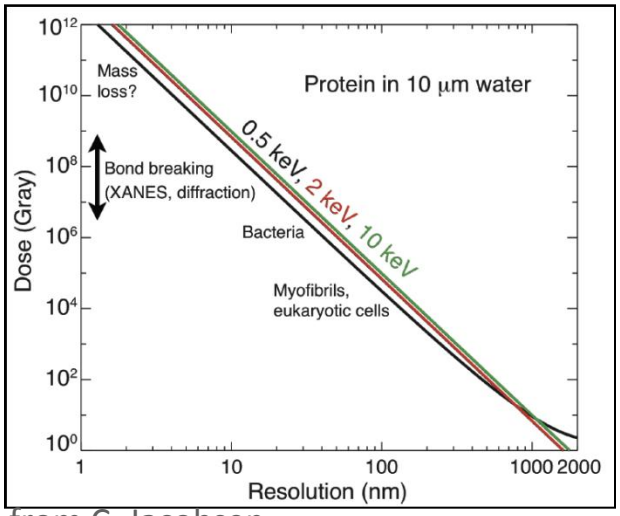
3-D renderings of trace elemental dist… in a plunge-frozen and freeze-dri… …ater diatom; reconstruction resolu… …00 nm

A higher spatial resolution is needed!

Why do we need the Bionanopobe?

- High spatial resolution: 30 nm

  Advanced optics; Precise motion control
- Protection from radiation damage



from C. Jacobsen

~~wet~~

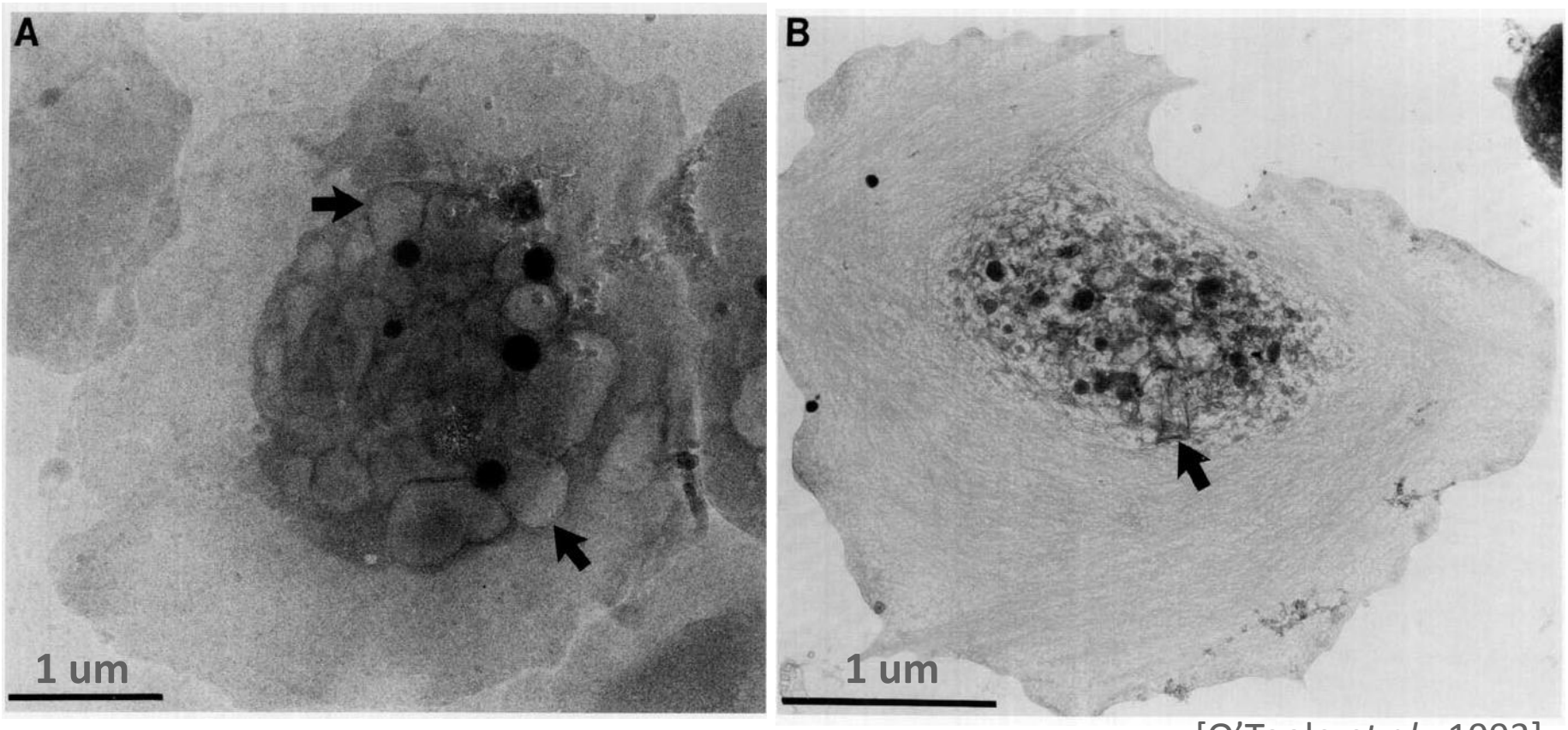
frozen-hydrated

- Automated sample change
- High speed

- 7 zone plates (5-35 keV)
- Piezo stages (<5 nm step)
- interferometer system
- cryogenic environment
- high vacuum
- sample handling robot
- fly-scan mode
- kinematic sample stage for tomography

Bionanoprobe:
a scanning X-ray nanoprobe with cryo-capabilities
***first* microscope of its kind**

Cryogenic capability enables biosamples to be studied in their froze-hydrated state

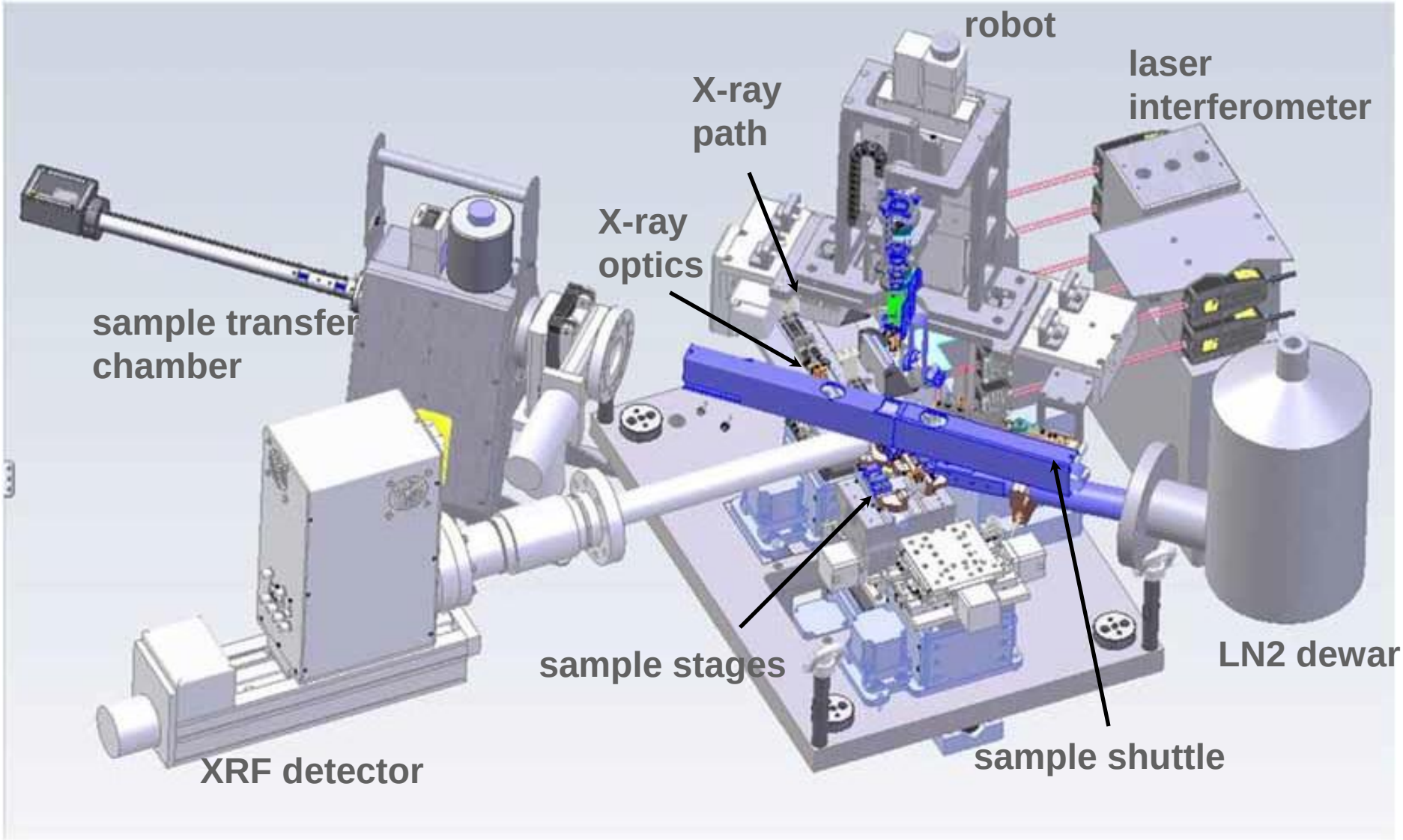


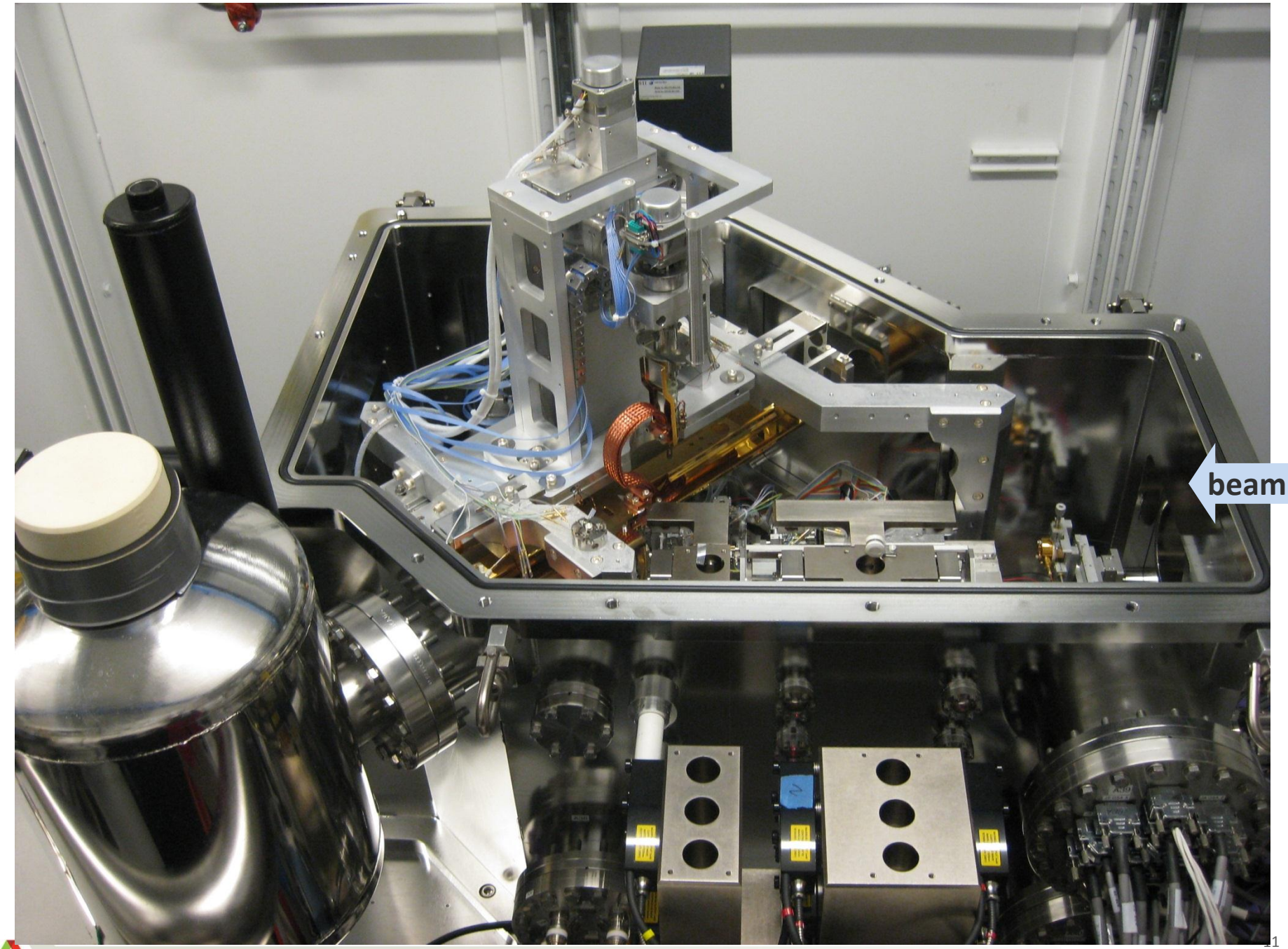
[O'Toole *et al.*, 1993]

frozen-hydrated platelet, imaged at -160˚C

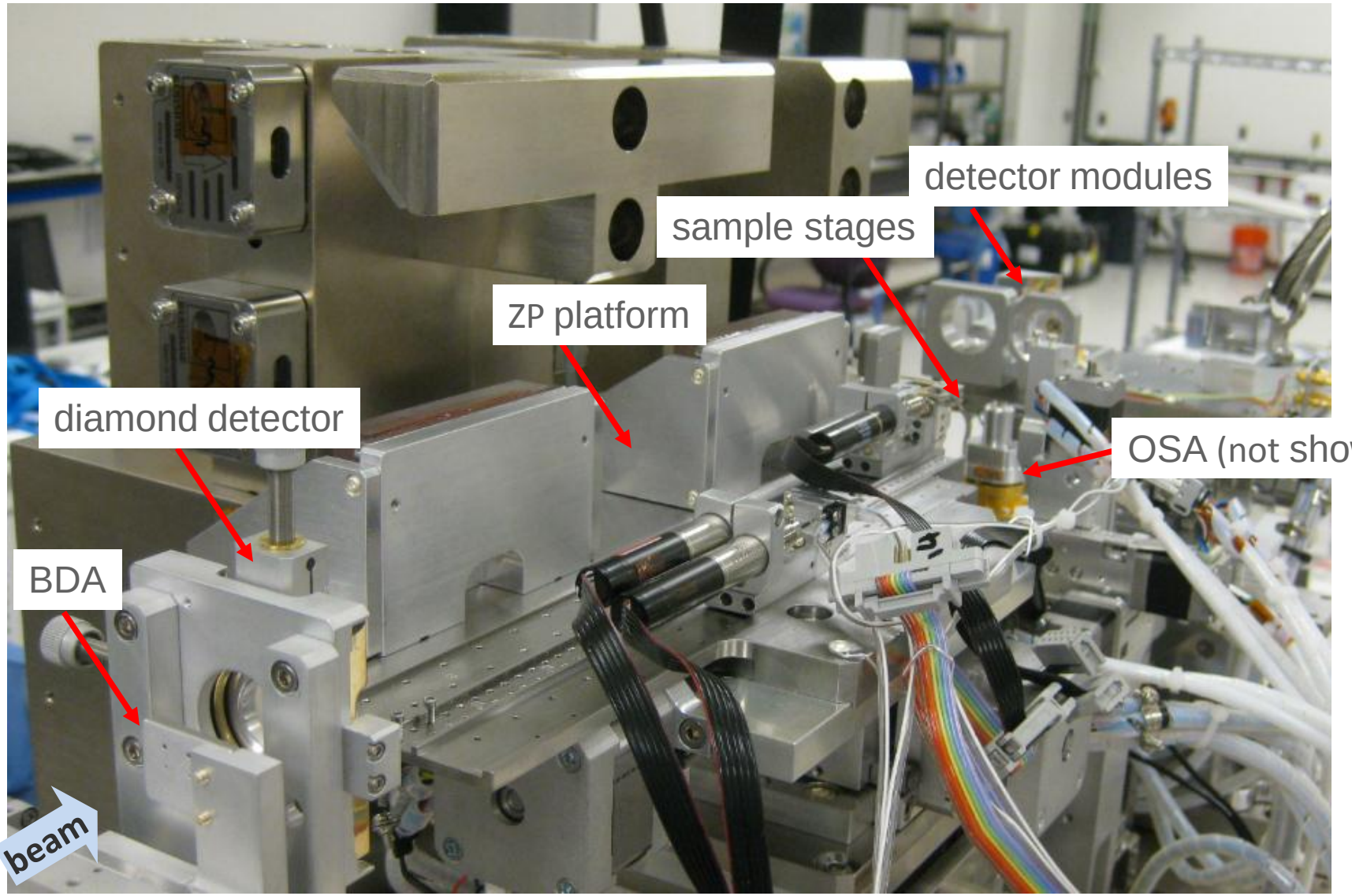
chemically-fixed, dehydrated platelet, imaged at room temperature

Schematic of the Bionanoprobe




beam

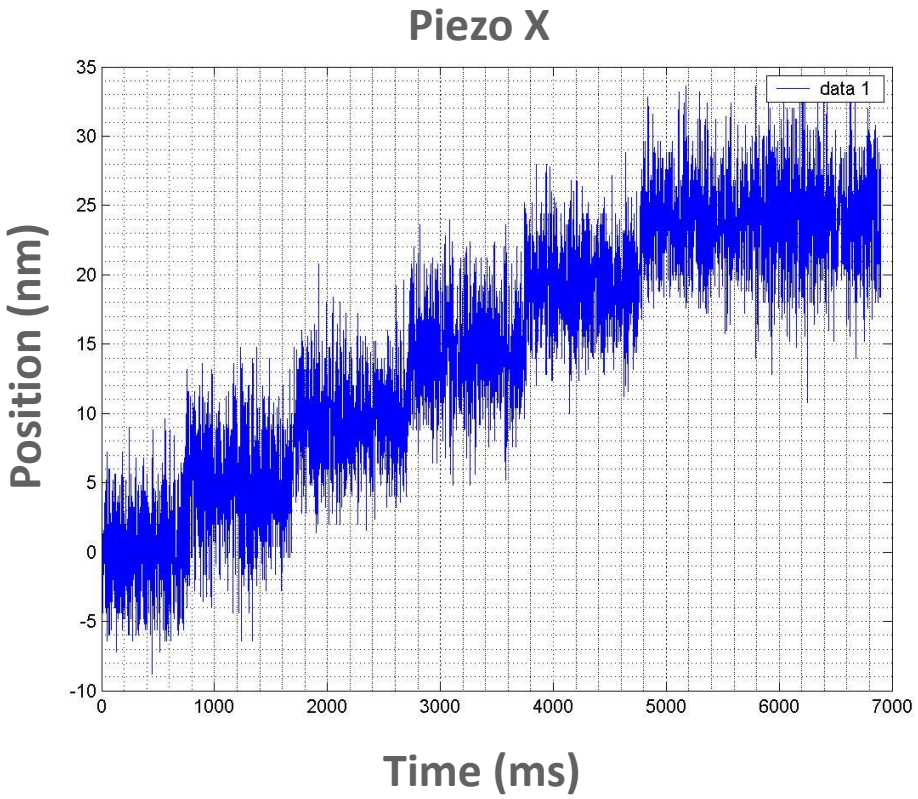
Optical Assembly

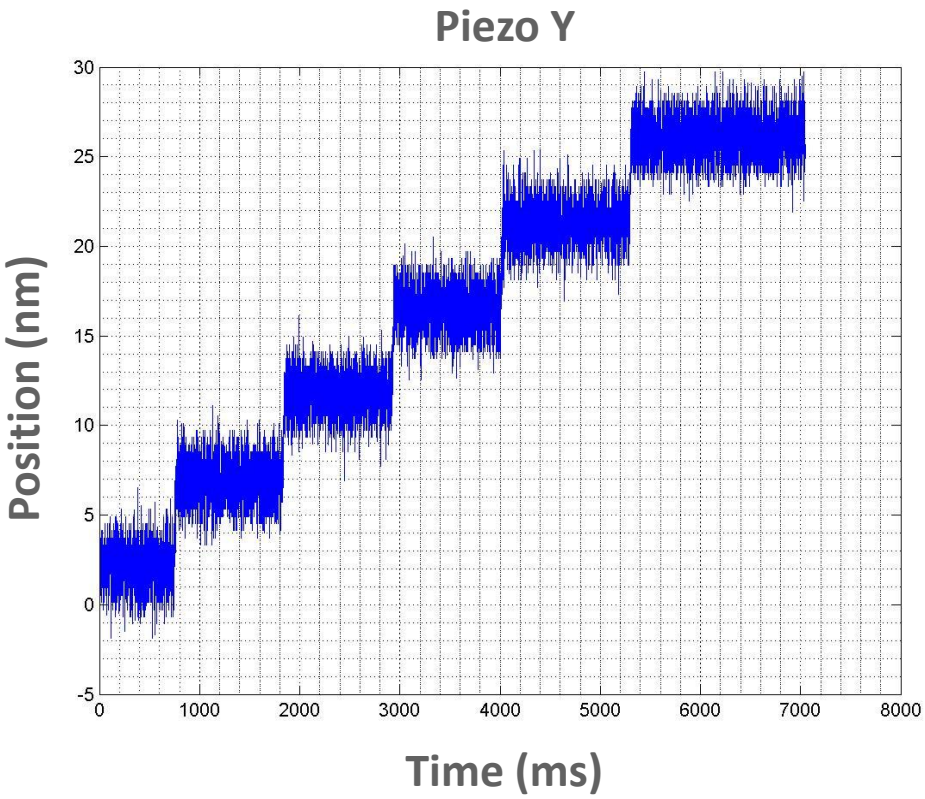


Positioning Stability of Piezo Stages (closed-loop mode)

(results from the Factory Acceptance Test)

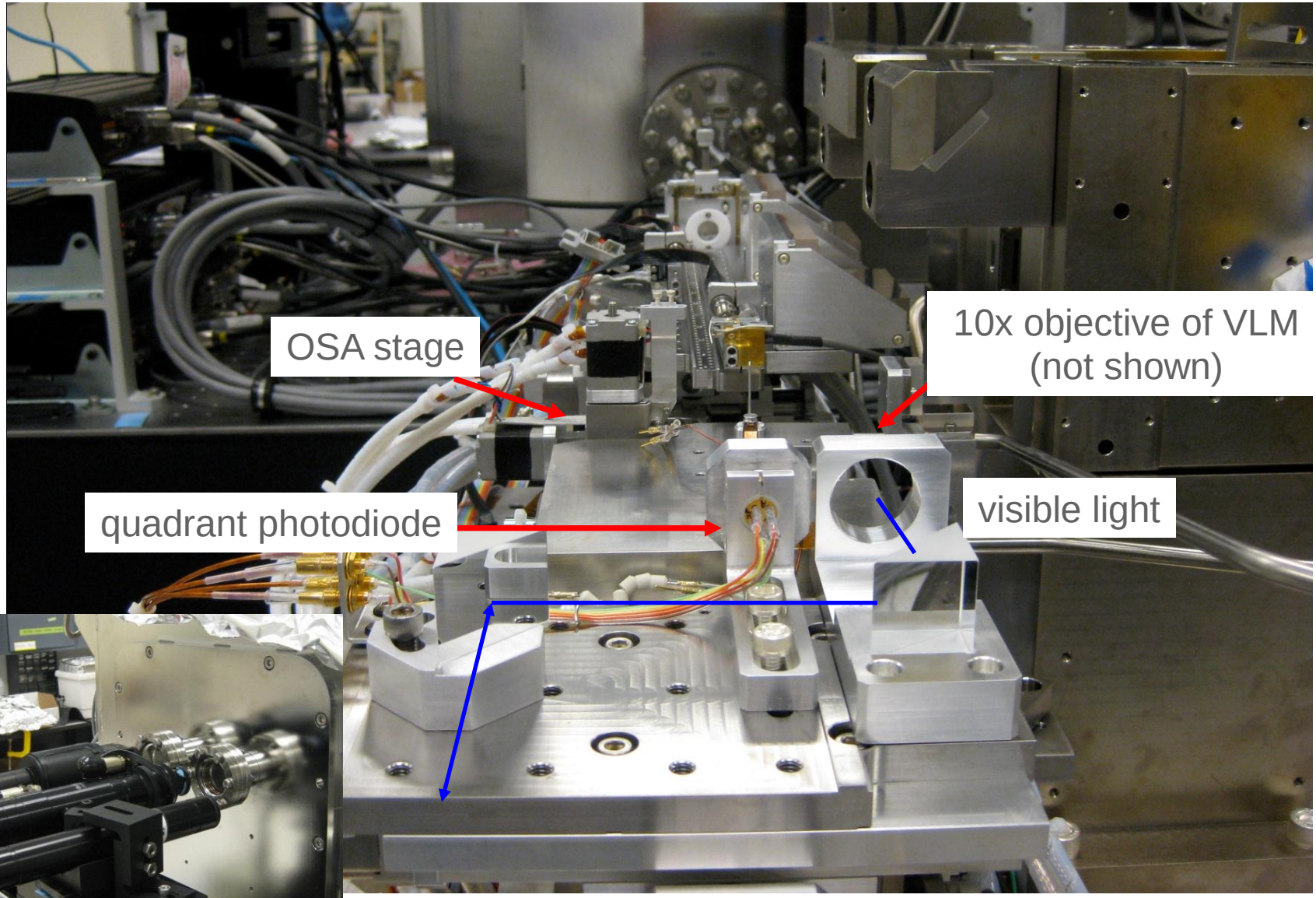
- 5-nm incremental steps achieved
- RMS noise (X) < 3.5 nm, RMS noise (Y) < 1.5 nm



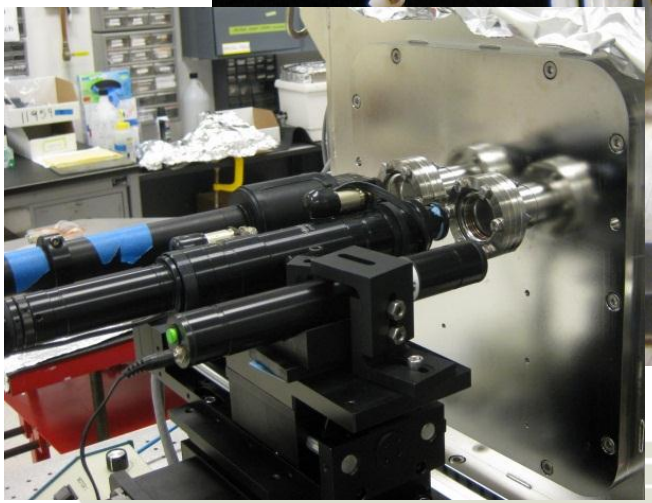


200 Hz sampling rate

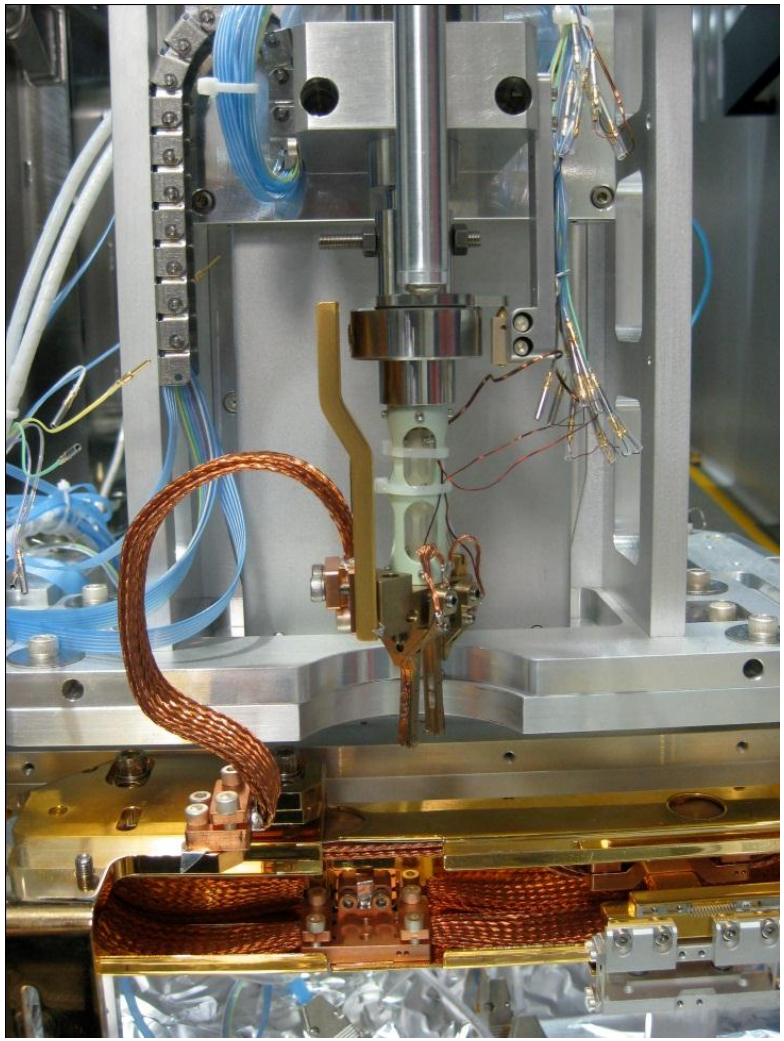
Detector Modules

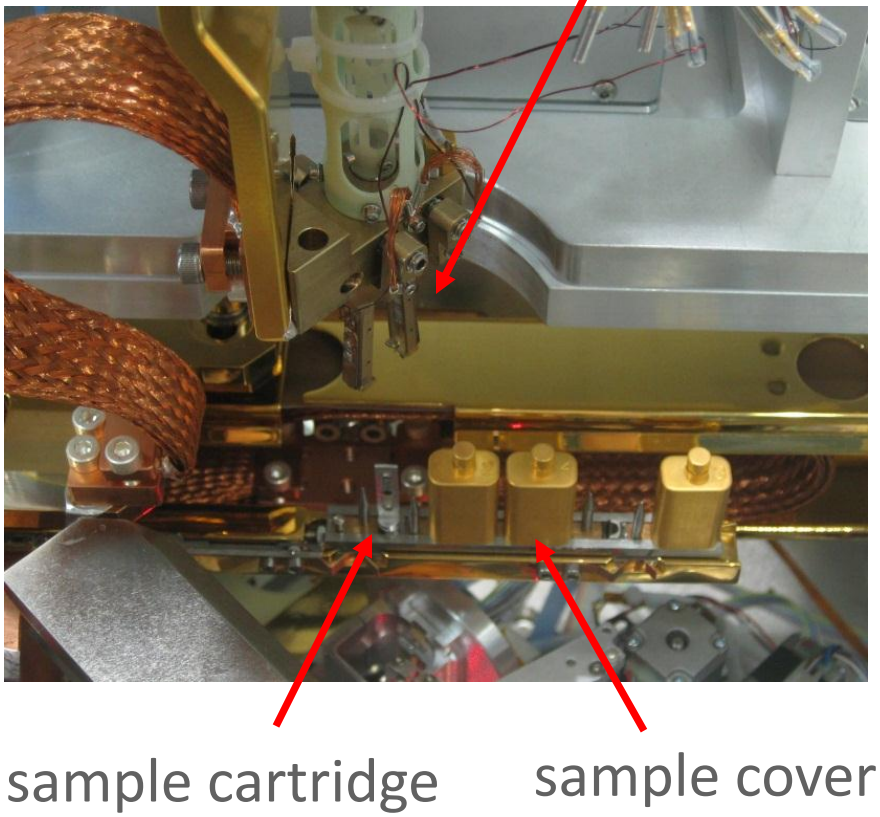


camera modules

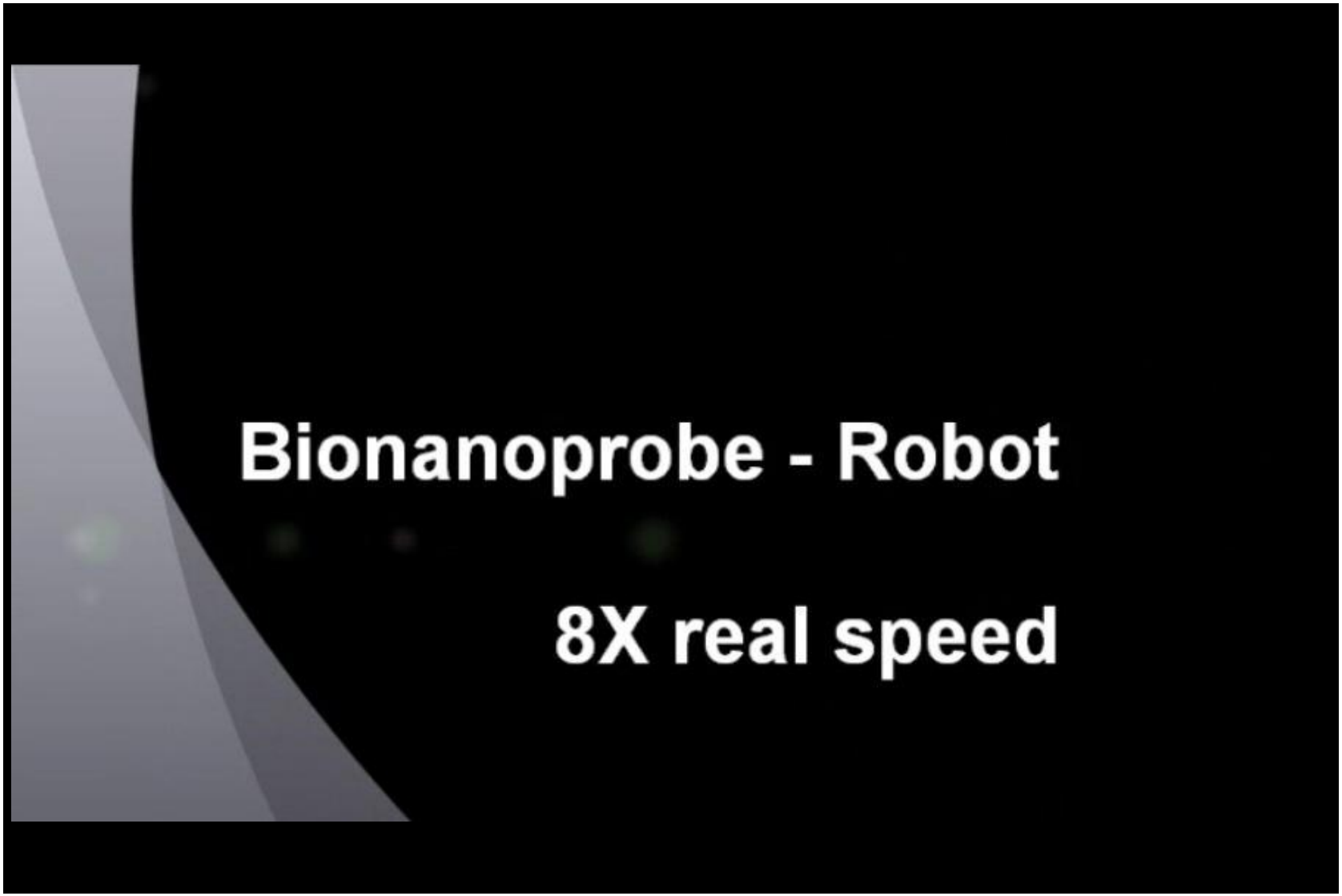


Sample Handling Robot





How does the robot work?



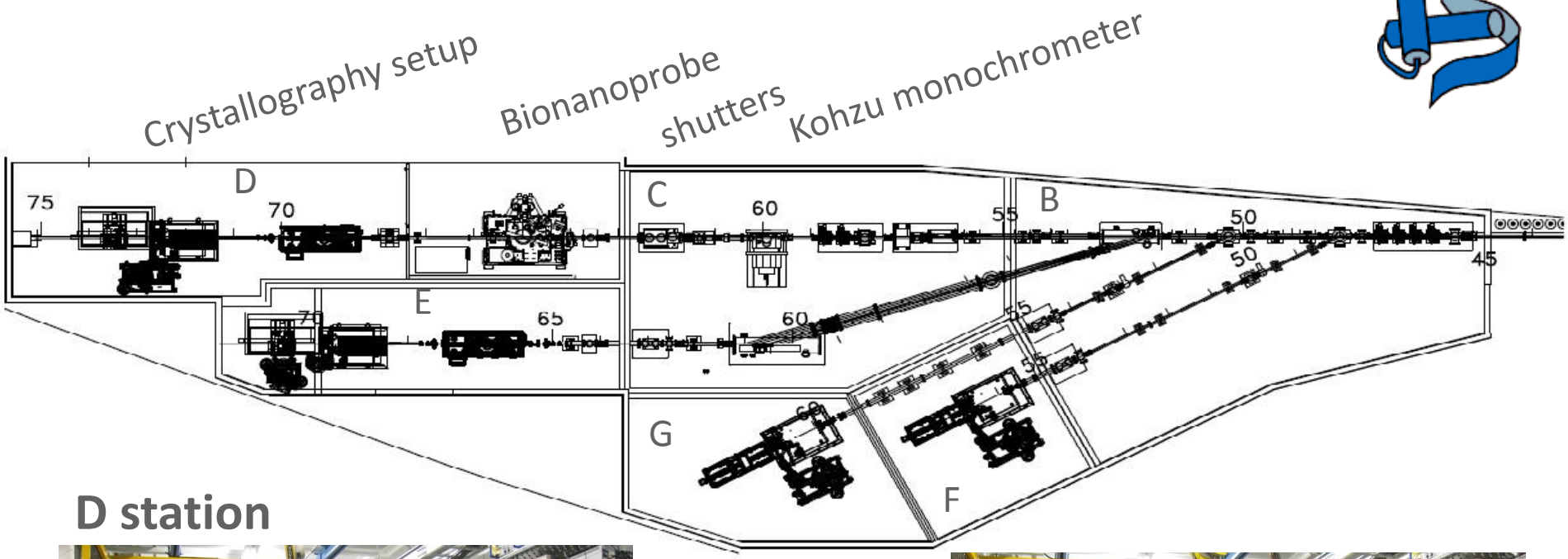
May, 2011

Xradia, Inc, Ca

September, 2011

LS-CAT, IL

Outline of the beamline at LS-CAT



D station

Bionanoprobe

Commissioning at room temperature (300 K)

- Beam energy: 10 keV
- White beam slits closed down to 50 µm in horizontal direction
- Zone plate: single zone plate 70-160-7 (N_2 encapsulated)

  -- outermost zone width (Δr): 70 nm

  -- focal length: 90 mm

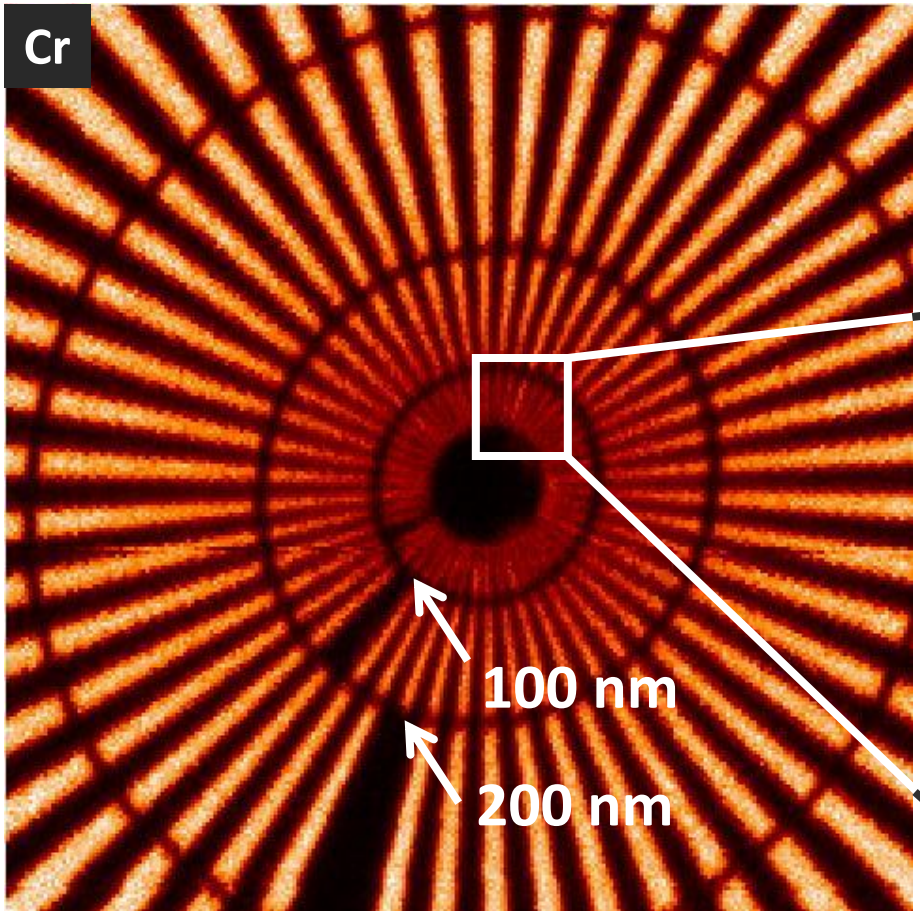
  -- depth of focus: $\pm\frac{1}{2}\frac{\lambda}{(NA)^2} = \pm 80\ \mu m$

  -- theoretical **Rayleigh resolution** (1.22 Δr): 85.4 nm

  -- theoretical **Modulation Transfer Function** limit: 35 nm

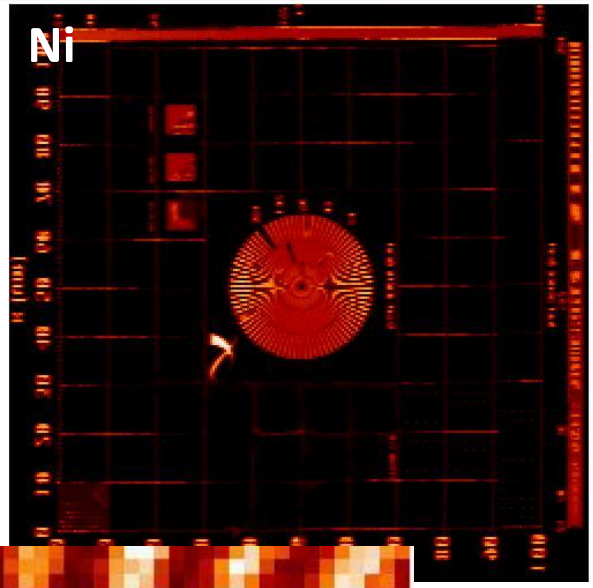
The exciting moments

XRF imaging of Ni/Cr test patterns at room temperature (300 K)

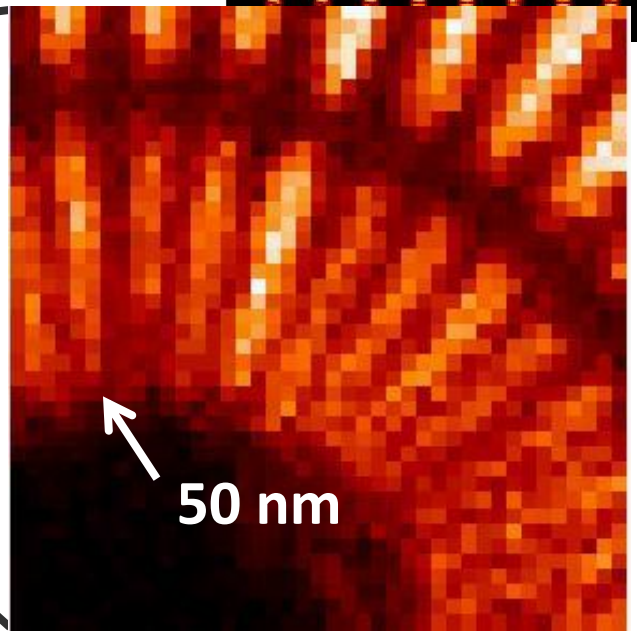


2 µm

scan area: 12 µm x 12 µm
step size: 50 nm



scan area:
120^2 µm^2
step size:
480 nm



200 nm

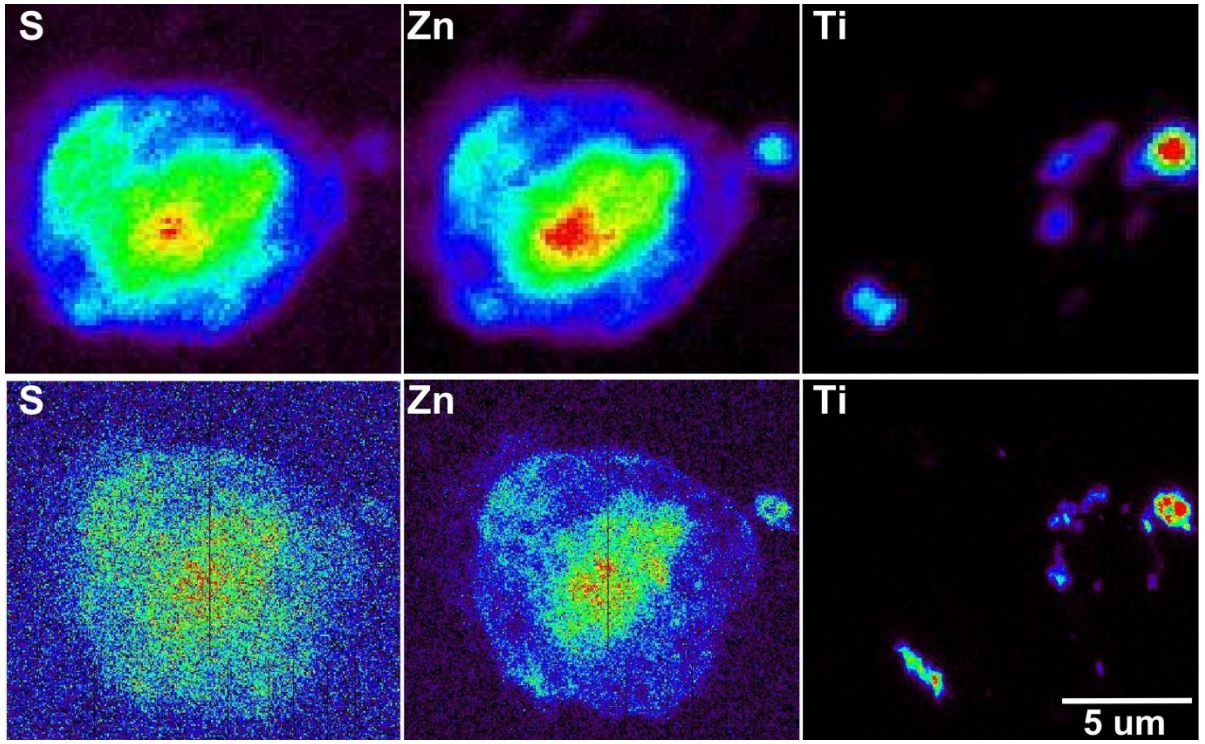
scan area: 1.4 µm x 1.4 µm
step size: 35 nm

Comparison of 2-ID-E and the Bionanoprobe

paraffin embedded Hela cell transfected with TiO_2-nanocomposites and maintained at room temperature

2-ID-E microprobe
step-scan
step size: 200 nm
dwell time: 2 s/pixel

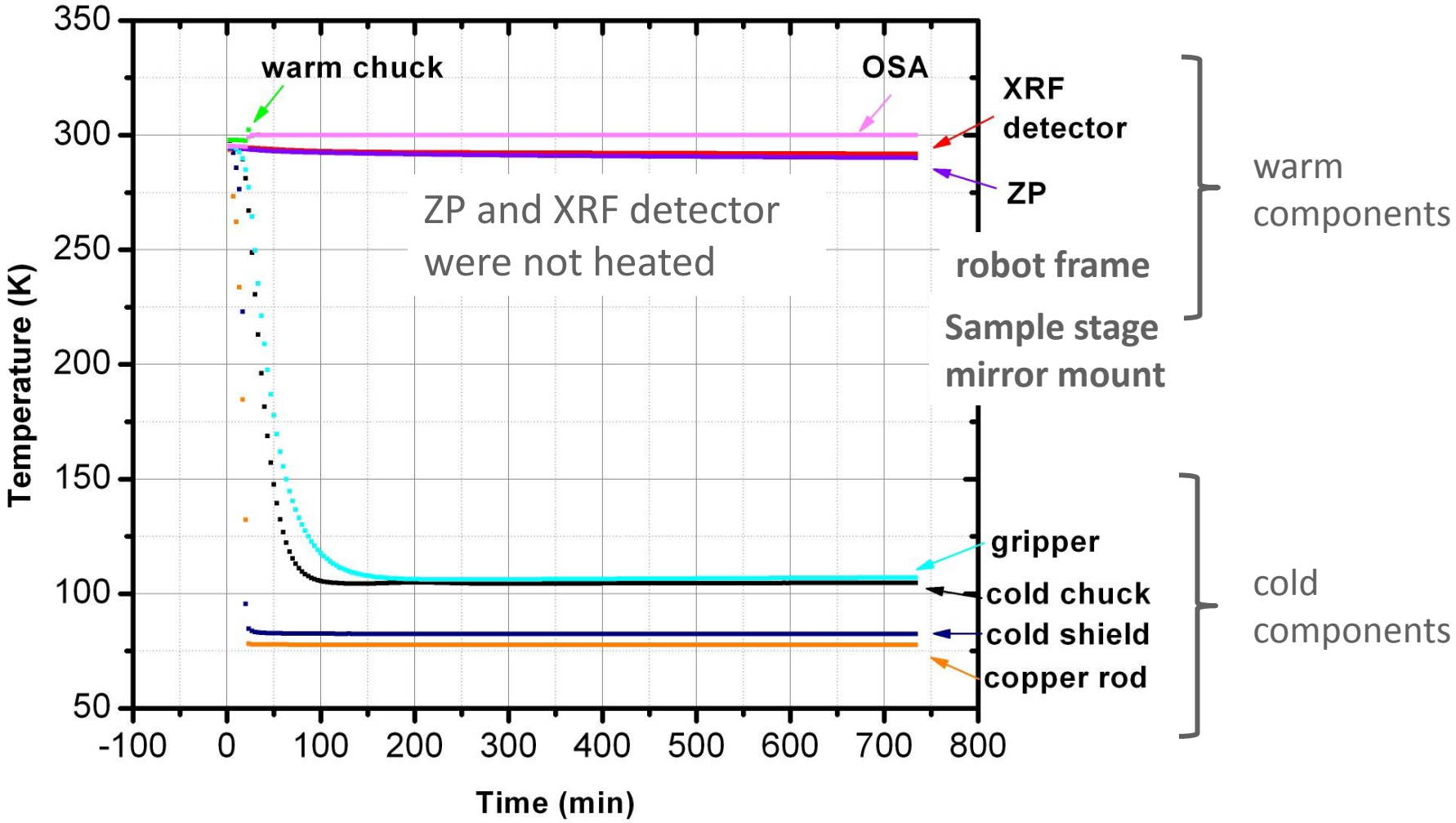
Bionanoprobe
step-scan
step size: 50 nm
dwell time:
250 ms/pixel



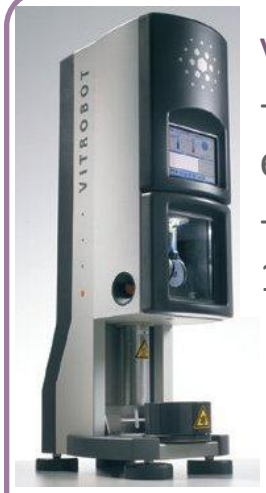
Commissioning at cryogenic temperature

- Beam energy: 10 keV
- White beam slits closed down to 50 µm in horizontal direction
- Zone plate: single zone plate 70-160-7
- Temperature control in the system
- Sample handling in cryogenic conditions

Thermal control for cryogenic conditions

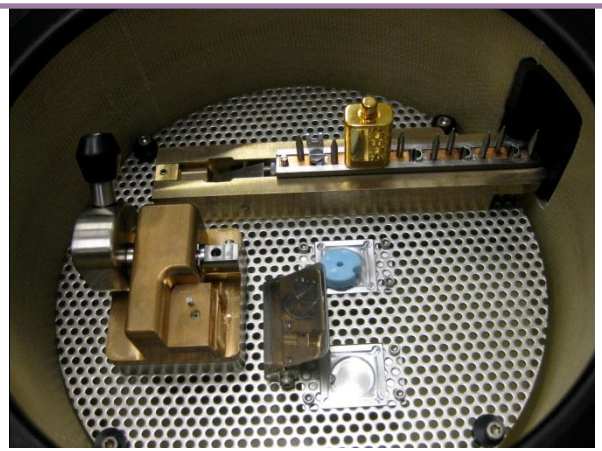


Cryo-sample preparation and transfer



vitrification
- cryogen: liquid ethane at 90 K
- cooling rate: 10^5 ˚C·s^{-1}

FEI Vitrobot Mark IV



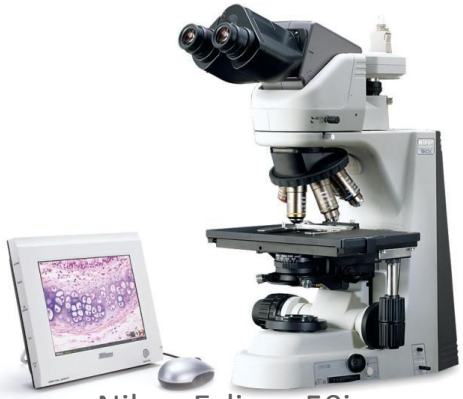
BNP workstation
sample transfer in liquid nitrogen

BNP transfer chamber
< 110K

BNP:Cryo-XRF

visualization
at -180˚C

Instec cold stage



Nikon Eclipse 50i

XRF at room T.

~~dehydration~~
~~chemical fixation~~
~~wash ...~~

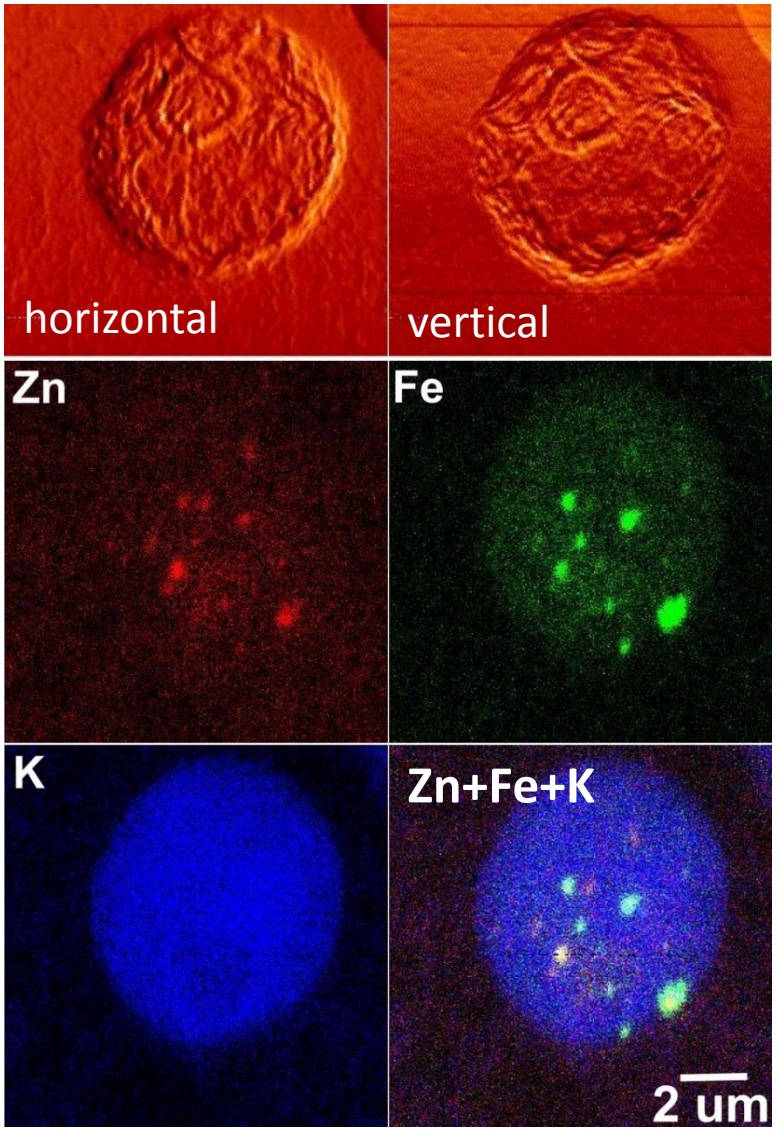
Frozen-hydrated algae cell (<110 K)

- *Chlamydomonas reinhardtii* (single cell green algae) sample in its culture medium
- plunge frozen in liquid ethane; transferred below 110 K
- fly-scan mode

  area: 10.5 µm x 10.5 µm

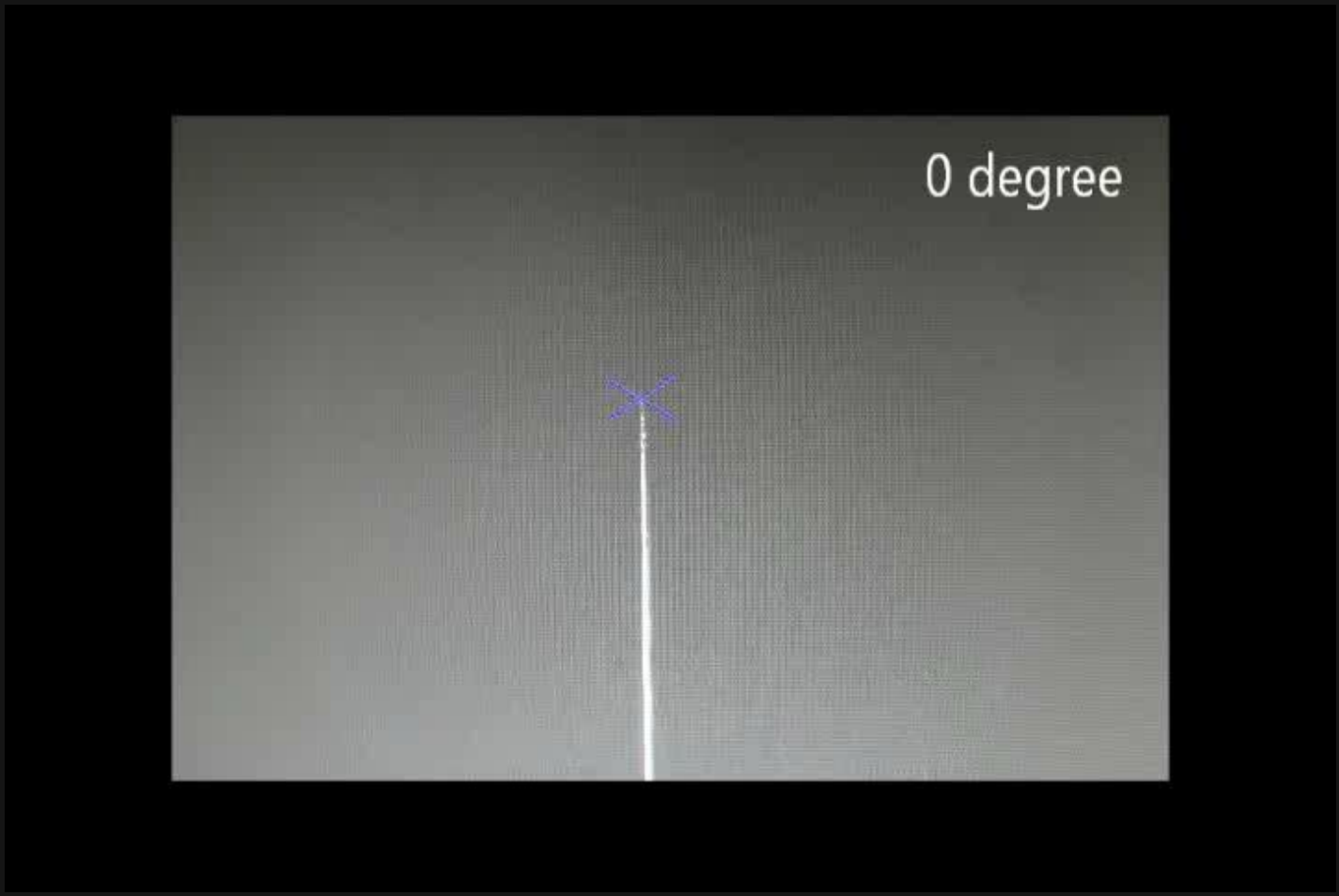
  step size: 35 nm

  dwell time: 250 ms/pixel



XRF tomographic dataset collection

W-tip



rotation
-20˚ -- +20˚
with a 10˚ step

2D projection
X: 20 µm
Y: 30 µm

rotation, center, focus -- 2D scan -- rotation, center, focus -- 2D scan …

Hela cells: transfected with Fe_3O_4/TiO_2 nanocomposites, chemically fixed, frozen-hydrated

Optical microscope
for visualization

↓

Bionanoprobe
overview scan (ZP: 70-160-14s)

↓

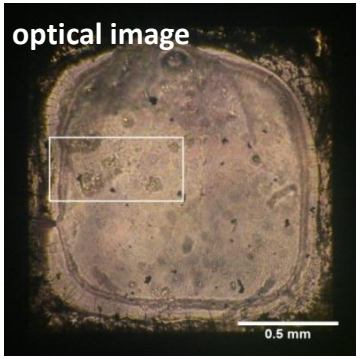
2D image -- fly-scan mode
area: 30 µm x 24 µm
step size: 100 nm
dwell time: 200 ms/pixel
30˚ rotation

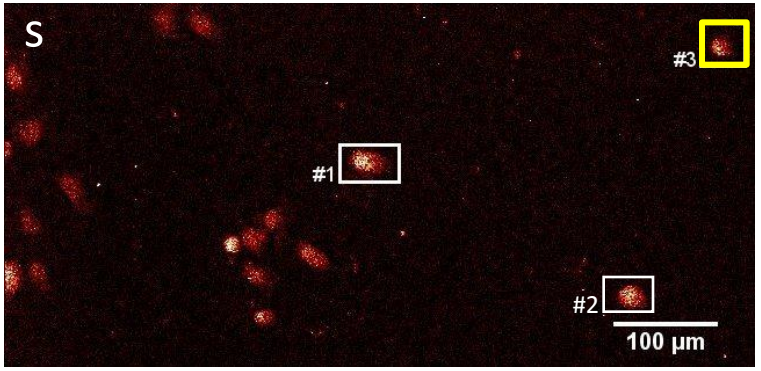
↓

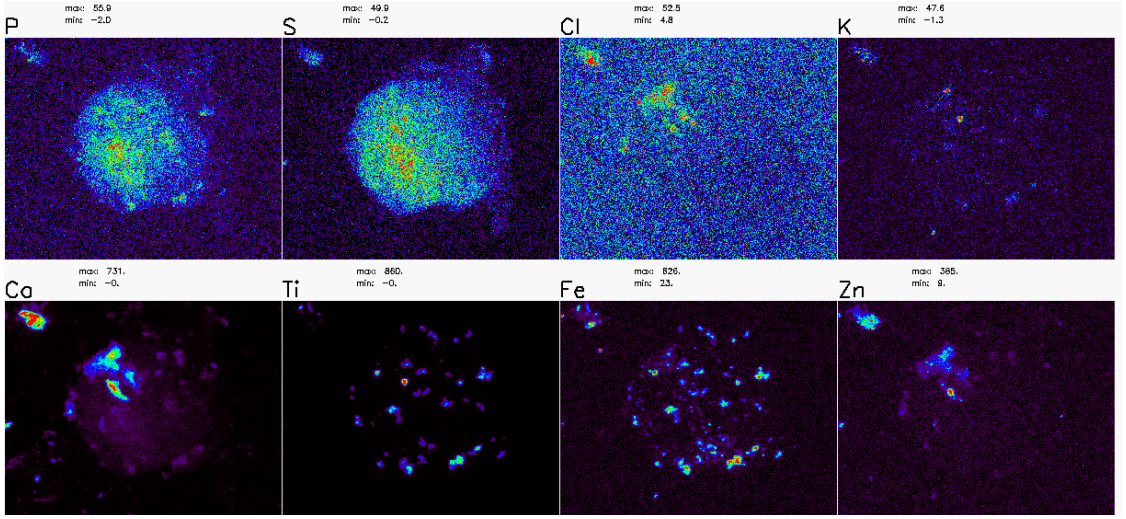
Tomographic dataset collection:
-66˚ -- + 66˚, 3˚ step
each projection:
step: 100 nm step,
dwell time: 30, 50 ms/pixel

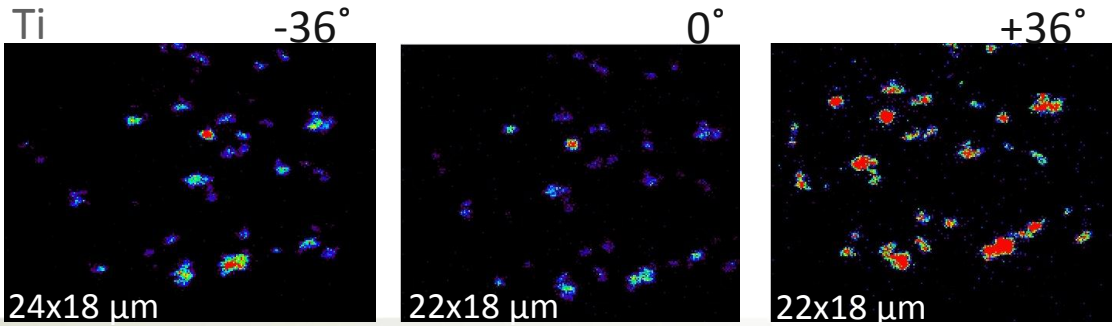
↓

Data reconstruction



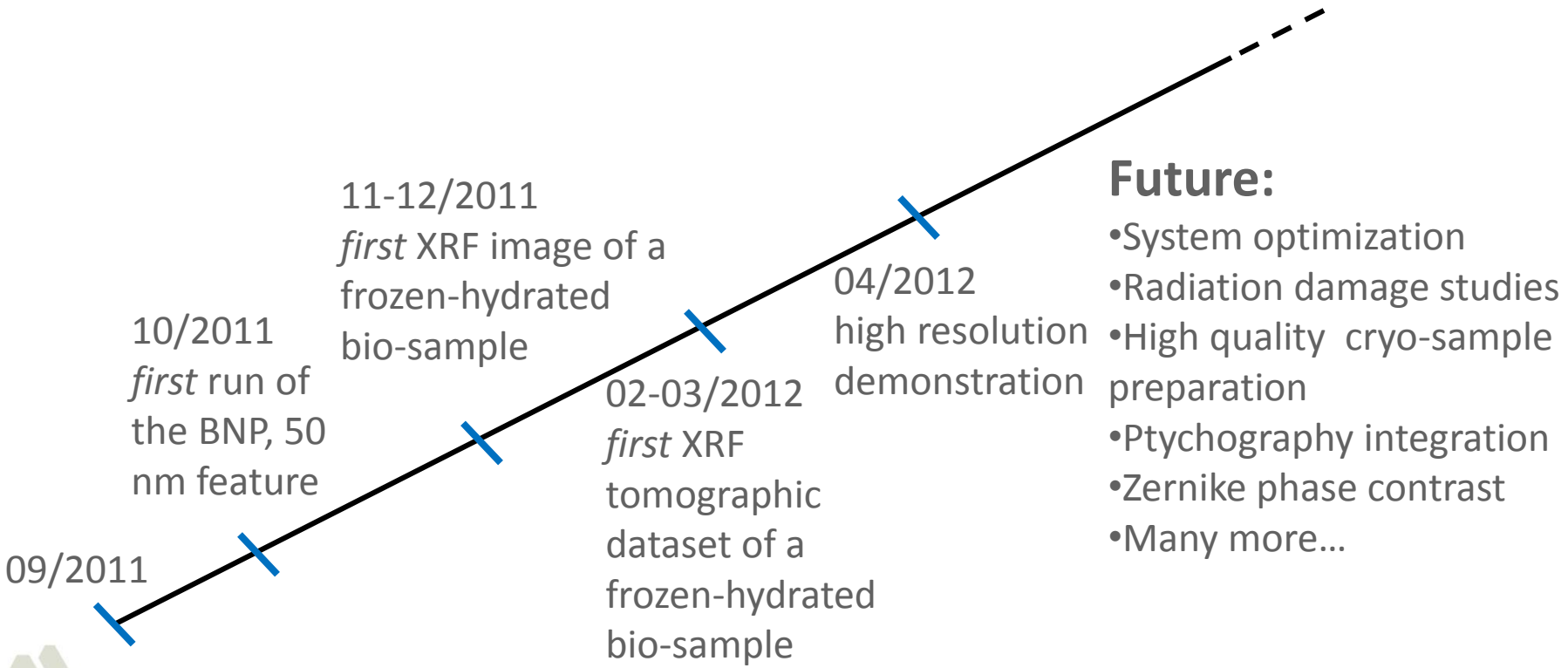






Summary

- The Bionanoprobe has provided us with new capabilities for high resolution studies of cryogenic specimens.
- Cryo-XRF microscopy makes it possible to visualize subcellular structures and to quantify trace elements in a manner close to the nature states of bio-samples without any fixation or dehydration.
- The Bionanoprobe is already reaching its initial performance target.



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