An Early View of the Bionanoprobe

Si Chen
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InterCAT Technical Workgroup Meeting

10 years ago
2 years ago
1 year ago
05/2011, Factory Acceptance Test
10/2011-present Commissioning
09/2011, Delivery, Installation, Site Acceptance Test

[Bahy-oh-nænæu-prohb]
Outlines

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

C. Fahrni, Current Opinion in Chemical Biology (11), 2007
Schematic of a Hard X-ray Fluorescence Microscope

- 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

de Jonge et al, Phys Rev Lett 100(16), 2008
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
Elemental concentrations ($\mu g \cdot cm^{-2}$) in a whole rat pheochromocytoma cell transfected with mitochondrion-specific TiO$_2$ nanoconjugates.

13 $\mu$m x 12.8 $\mu$m, step: 0.2 $\mu$m

A higher spatial resolution is needed!
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 distributions in a plunge-frozen and freeze-dried freshwater diatom; reconstruction resolution: ~400 nm

*A higher spatial resolution is needed!*
Why do we need the Bionanoprobe?

- High spatial resolution: 30 nm
  Advanced optics; Precise motion control
- Protection from radiation damage

Bionanoprobe: a scanning X-ray nanoprobe with cryo-capabilities

*first* microscope of its kind

- 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

from C. Jacobsen
Cryogenic capability enables biosamples to be studied in their froze-hydrated state.

Frozen-hydrated platelet, imaged at -160°C

Chemically-fixed, dehydrated platelet, imaged at room temperature

[O’Toole et al., 1993]
Schematic of the Bionanoprobe

- LN2 dewar
- Sample stages
- XRF detector
- Sample transfer chamber
- X-ray path
- X-ray optics
- Sample shuttle
- Laser interferometer
- Robot
Optical Assembly

- BDA
- ZP platform
- Sample stages
- Detector modules
- OSA (not shown)
- Diamond detector
- Beam

[Image of the optical assembly with labeled parts]
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
Sample Handling Robot

- Gripper
- Sample cartridge
- Sample cover
How does the robot work?

Bionanoprobe - Robot

8X real speed
Outline of the beamline at LS-CAT

Crystallography setup
Bionanoprobe
shutters
Kohzu monochromometer

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 \((N_2\text{ encapsulated})\)
  - outermost zone width \((\Delta r)\): 70 nm
  - focal length: 90 mm
  - depth of focus: \(\frac{1}{2} \frac{\lambda}{(NA)^2} = \pm80 \, \mu m\)
  - theoretical **Rayleigh resolution** \((1.22 \Delta r)\): 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)

- **Cr**
  - Scan area: 120² µm²
  - Step size: 480 nm

- **Ni**
  - Scan area: 1.4 µm x 1.4 µm
  - Step size: 35 nm

- Scan area: 12 µm x 12 µm
- Step size: 50 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

ZP and XRF detector were not heated

warm components:
- warm chuck
- OSA
- XRF detector

cold components:
- robot frame
- Sample stage
- mirror mount
- gripper
- cold chuck
- cold shield
- copper rod
Cryo-sample preparation and transfer

**Vitrification**
- Cryogen: liquid ethane at 90 K
- Cooling rate: $10^5 \, ^\circ C \cdot s^{-1}$

**Visualization**
- At -180°C

**BNP: Cryo-XRF**

**BNP Transfer Chamber**
- < 110K

**Sample Preparation and Transfer**
- BNP workstation
- Sample transfer in liquid nitrogen
- BNP transfer chamber

**Instec Cold Stage**

**Nikon Eclipse 50i**

**FEI Vitrobot Mark IV**

**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

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

W-tip

rotation
-20° -- +20°
with a 10° step

2D projection
X: 20 µm
Y: 30 µm
Hela cells: transfected with Fe$_3$O$_4$/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.

Future:
- System optimization
- Radiation damage studies
- High quality cryo-sample preparation
- Ptychography integration
- Zernike phase contrast
- Many more...

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
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<tbody>
<tr>
<td>09/2011</td>
<td>First run of the BNP, 50 nm feature</td>
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<tr>
<td>10/2011</td>
<td>First XRF image of a frozen-hydrated bio-sample</td>
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<tr>
<td>11-12/2011</td>
<td>First XRF image of a frozen-hydrated bio-sample</td>
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<tr>
<td>02-03/2012</td>
<td>First XRF tomographic dataset of a frozen-hydrated bio-sample</td>
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<tr>
<td>04/2012</td>
<td>High resolution demonstration</td>
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