X-ray Imaging & Microscopy Applications and Future Opportunities

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⇒ Motivation: proposed energy recovery linac (ERL) x-ray source scientific needs in imaging & in synchrotron science
⇒ Overview & examples: x-ray imaging & microscopy applications
⇒ Discussion: why ERL ideal for advancing x-ray microscopy
⇒ New frontier of x-ray science: structure of nonperiodic specimen by imaging & microscopy
X-ray Science

Protein Data Bank: Deposits / year
~85% structures by x-ray crystallography

CHESS

2003 Nobel Prize in Chemistry:
Roderick MacKinnon
(Rockefeller Univ.)
1st K+ channel structure by x-ray crystallography based on CHESS data (1998)
X-ray Imaging & Microscopy

⇒ Century-old (since 1895), yet young scientific discipline
⇒ Highly dependent on x-ray source & optics
⇒ Rapid development after 3rd generation SR sources

William Röntgen
1st Physics Nobel (1901)

Hard X-ray Microscopy & Imaging
(search in INSPEC physics literature)
Energy Recovery Linac (ERL)

One option: ERL @ CESR

Georg Hoffstaetter
LEPP Journal Club
311 Newman Lab.
Friday, 3:45 pm
Oct. 24, 2003
ERL Properties

- ERL would be the world’s first high-intensity diffraction-limited hard x-ray source.
- ERL would produce a round point source of hard x-rays, ideal for imaging & microscopic applications.
- ERL would bring high coherence to hard x-ray regime, enabling new opportunities for imaging science.

\[ \varepsilon = \frac{\lambda}{4\pi} \]

**Diffraction limited source:**
\[ 2\pi\sigma'\sigma = \frac{\lambda}{2} \]

**ERL emittance (0.15Å)**

**ESRF emittance (4nm x 0.01nm)**

**Diffraction limited @ 8keV (0.123Å)**
Nature's Dimensions

Size (m)

- Plants & Animals
- Micro-organisms
- Cells
- Naked eye
- Optical microscopy
- X-ray radiology
- Ultrasound, MRI
- Electron microscopy
- TEM
- X-ray crystallography
- NMR
- AFM
- STM
Three Imaging Categories

Medical Imaging
- low contrast tissues
- real time imaging
- high resolution

Cellular Imaging
- subcellular organelles
- protein locations
- natural state

Molecular Imaging
- without need of crystals
- atomic resolution
- less damage

Plants & Animals
- onion cell
- cheek cell
- ant

Organisms
- human hair
- Helisoma

Cells
- microchip
- Ge/Si dots
- Si pillars

Organelles
- Reovirus
- Ribosome
- Hemoglobin

Small molecules
- Cystine
- H₂O

Atoms
- Si (111)7x7

Size (m)

10⁻¹ 10⁻² 10⁻³ 10⁻⁴ 10⁻⁵ 10⁻⁶ 10⁻⁷ 10⁻⁸ 10⁻⁹ 10⁻¹⁰
X-ray Microscopy Basics

- Direct imaging (radiography)
- Transmission microscope (TXM)
- Scanning microscope (SXM)
- Far-field diffraction
- Holography
X-ray Contrast

Refraction index: \( n = 1 - \delta - i\beta \)

\[
E(z) \sim E_0 e^{-i2\pi(-\delta-i\beta)z/\lambda} \sim E_0 e^{i2\pi\delta z/\lambda - 2\pi\beta z/\lambda}
\]

\[
I(z) \sim |E(z)|^2 \sim I_0 e^{-4\pi\beta z/\lambda}
\]

⇒ Absorption contrast: \( \mu z = 4\pi\beta z/\lambda \sim \lambda^3 \)

⇒ Phase contrast: \( \phi(z) = 2\pi\delta z/\lambda \sim \lambda \)

Mori et al. (2002): broken rib with surrounding soft tissue
Phase vs. Absorption Contrast

- Phase contrast is $10^4$ higher than absorption contrast for protein in water @ 8keV
- Required dose reduced due to phase contrast

Kagoshima et al. (2001): protein $C_{94}H_{139}N_{24}O_{31}S$
$\rho=1.35\text{g/cm}^3$, $t=0.1\mu\text{m}$ in $10\mu\text{m}$ water

Kirz (1995): 0.05$\mu\text{m}$ protein in 10$\mu\text{m}$ thick ice

$C_{94}H_{139}N_{24}O_{31}S$
Examples of X-ray Imaging - I

- **Plants & Animals**
- **Humans**
- **Organisms**
- **Cells**
- **Organelles**
- **Macromolecules**
- **Medical Imaging** → low contrast tissues → real time imaging → high resolution
- **Cellular Imaging** → subcellular organelles → protein locations → natural state
- **Molecular Imaging** → without need of crystals → atomic resolution → less damage
Phase Enhanced X-ray Imaging

Three Ways to See Phases ....

- Interferometric imaging
- Diffraction enhanced imaging
- Phase radiography
Direct Phase Imaging

Image interpretation: $I(u, v) \Rightarrow \rho(x, y) = ?$

**Propagation based method:**
(near-field)
Nugent et al., PRL 77, 2961 (1996)
Paganin & Nugent, PRL 80, 2586 (1998)

**Fresnel diffraction method:**
(intermediate-field)
Wilkins et al, Nature 384, 335 (1996)
Phase Imaging with Interferometer


Photon Factory 17.7 keV

Blood vessels in mouse liver w/o contrast agent!

Phase-contrast x-ray CT of a rabbit liver tissue (5mm in diameter)

Cancerous lesion

rat cerebellum 12.4 keV same dose
Radiography of soft tissue of the foot and ankle with diffraction enhanced imaging

Jun Li, Zhong Zhong, Roy Lidtke, Klaus E. Kuettner, Charles Peterfy, Elmi A. Aliyev and Carol Muehlenman

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National Synchrotron Light Source, Bldg. 725D, Brookhaven National Laboratory, Upton, NY 11973, USA
W.M. Scholl College of Podiatric Medicine, Finch University, The Chicago Medical School, North Chicago, IL, 60064, USA
SYNAR, Inc, San Francisco, CA 94105, USA

The major soft tissue structures that can be identified in DEI (not visible in absorption radiograph) include the two major tendons of the toe, the fat pad under the ball of the foot and the skin.

Conventional absorption image of the great toe

Diffraction enhanced image
Tracheal Respiration in Insects Visualized with Synchrotron X-ray Imaging

Mark W. Westneat,1 Oliver Betz,1,2 Richard W. Blob,1,3 Kamel Fezzaa,4 W. James Cooper,1,5 Wah-Keat Lee4
Field museum of Chicago & APS, Argonne National Lab.

- Animal functions
- Biomechanics
- Internal movements
- New findings not known before

ERL would extend these studies to much higher lateral resolution and faster time scales


wood beetle

bug_s1.avi
Point-Source Projection Microscopy with ERL

ERL: 3cm-long '2 + 2' ID 0.3 mrad

Focusing optic: 1m-long ML KB mirrors @ 1°
double triangle Si (111) @ 10°

S = 10µm

50m 0.5m 4mm

s = 0.1µm

30 mrad

Resolution d = 0.1µm
Required pixel size: <0.2mm

M = 8m/4mm = 2000x
Image size: 24cm
Field view: 0.12mm

Ultimate resolution d = 6µm/2000 = 3nm
if focal spot size of 3nm can be achieved

ERL has the potential to improve lateral resolution in direct phase imaging by two orders of magnitude
Summary on Medical & Small Animal Imaging

- Phase-enhanced x-ray imaging allows observation of weak-absorbing features that are otherwise not observable with conventional radiography, with less radiation dosage.

- Many experiments can be done at existing synchrotron sources, but real-time (or faster) imaging at high spatial resolution of ~1\( \mu \)m or less appears to be difficult.

Projection Microscopy with ERL:

- Would improve lateral resolution by 2-3 orders of magnitude, down to sub-\( \mu \)m scales.

- With fast detectors such as pixel-arrays, would offer ultra-fast high-resolution x-ray radiography at video frequency of MHz.

- With short x-ray pulses, ERL offers the potential for flash imaging at time scales of < 1 ps.
Examples of X-ray Imaging - II

### Size (m)

- $10^{-1}$
- $10^{-2}$
- $10^{-3}$
- $10^{-4}$
- $10^{-5}$
- $10^{-6}$
- $10^{-7}$
- $10^{-8}$
- $10^{-9}$
- $10^{-10}$

#### Plants & Animals
- Plants
- Animals

#### Organisms
- Cells
- Organelles
- Macromolecules

### Medical Imaging
- low contrast tissues
- real time imaging
- high resolution

### Cellular Imaging
- subcellular organelles
- protein locations
- natural state

### Molecular Imaging
- without need of crystals
- atomic resolution
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Shen – October 20, 2003
Challenges in Cell Biology

- The **basic information** about the organization of cells and subcellular structures is critical for our understanding of cellular functions – central theme in cell biology.

- The **challenge** in cell biology has been to obtain the best resolution **3D morphological information** about cells that are examined in a state most closely resembling their natural environment.

- **X-ray microscopy** is proving to be a powerful method in that (a) it provides far better resolution than confocal laser microscopy, and (b) one can examine whole, fully-hydrated cells, avoiding potential artifacts introduced by the dehydration, embedding and sectioning that is required for electron microscopy.

- **Cellular imaging** is of critical importance in the post-genomic era as we face the daunting task of determining the **function** of the vast number of genes and gene products identified as a result of modern molecular biology techniques.

*Transmission x-ray microscope image of mouse 3T3 fibroblasts, a type of connective-tissue cell, with spatial resolution of 36 nm, clearly shows features -- such as nucleoli and the sharp nuclear membrane -- not resolvable with optical confocal microscopy.*
Subcellular Imaging with Labels

- Nuclear pore complex (NPC): a large (50-100 MD) collection of proteins which organize the ~9 nm openings in nuclear membranes of eukaryotic cells.

C. Larabell (LBNL): cell biologist, using confocal & electron microscopy

- Immunocytochemistry: a method for identifying structure-function relationships of cells and proteins in cells by looking at the subcellular location of these proteins

- Critical proteins inside cells are labeled so that X-rays could be used to identify them

- X-ray microscopy gives cell biologists a whole new way of looking at their samples

**FIGURE 3.** Distribution of nuclear pore complexes (NPC) in tumor mammary epithelial cells. The left image is the control, which was not exposed to primary anti-NPC antibodies but did receive secondary gold-tagged antibodies and silver enhancement, and is free of label. Blue dots in the right image are antibody labeled, silver enhanced NPC molecules. Scale bar = 1 μm.
Cryo-Tomography of Whole Hydrated Cells

- Flash-frozen, whole, fully hydrated cells
- Soft x-ray microscope, depth of field ~10µm

=> Unique 3D information about cells and interactions of intracellular organelles

Drosophila embryonic cell
(G. Schneider, LBNL)

Green = nucleolus
Gold = sex-determining protein
(labeled with 1nm Au & Ag-enhanced)
Summary on Cellular Microscopy

• X-ray microscopy is beginning to show its potential as a powerful tool in cell biology
• X-ray microscopy could have high impact on cell biology in a way that is similar to what synchrotron x-ray crystallography is doing on molecular biology
• Substantial increase in demand for high-brightness x-ray microscopes is expected for the next decade

Cellular Microscopy with ERL:

ERL would be an ideal x-ray source to satisfy the need for high-quality cellular and subcellular imaging. It would:

• improve soft x-ray microscope brightness by 2-3 orders of magnitude
• deliver an ultra-small, round, isotropic resolution function
• bring high brilliance x-ray microscopy to hard x-ray regime, allowing (a) possibility with phasing contrast and less dose requirement, (b) substantial increase in depth of view for 3D tomography, and (c) extended range of elemental labels in the higher energy region.
Examples of X-ray Imaging - III

- Medical Imaging:
  - low contrast tissues
  - real time imaging
  - high resolution

- Cellular Imaging:
  - subcellular organelles
  - protein locations
  - natural state

- Molecular Imaging:
  - without need of crystals
  - atomic resolution
  - less damage
Molecular Imaging & Microscopy

- Molecular imaging requires lateral resolution \(< 10\text{nm}\) \(\Rightarrow\) current limit on optics
- To move beyond the limit, lensless imaging or diffraction imaging using coherent beam is an attractive alternative

- Diffraction imaging is analogous to crystallography, but for noncrystalline materials
- Coherent diffraction from noncrystalline specimen \(\Rightarrow\) continuous diffraction pattern
- Spatial resolution: essentially no limit. (only limited by \(\Delta\lambda/\lambda\) and weak signals at large angles)

- Present limitations: Lack of intense, coherent microbeams. ERL would change this dramatically.

soft x-ray diffraction reconstruction to 75 nm
Imaging Whole *Escherichia Coli* Bacteria Using Single Particle X-ray Diffraction

Jianwei Miao*,†, Keith O. Hodgson*,‡, Tetsuya Ishikawa§, Carolyn A. Larabell¶?, Mark A. LeGros**, and Yoshinori Nishino§


*E. Coli* bacteria ~ 0.5 µm by 2 µm
Labeled with maganese oxide
SPring-8, \( \lambda = 2 \) Å, pinhole 20 µm
Total dose to specimen ~ 8x10^6 Gray
Diffraction image to ~30nm resolution
Simulations Using ERL Beam

- Simulation of diffraction pattern using coherent beam from ERL, with statistical noise & missing beam-stop region.

- Assembly of 2900 gold atoms in 10nm box

- Image retrieval in collaboration with Elser (Physics, Cornell)

Coherent x-ray diffraction imaging offers the potential to go beyond the spatial resolution limit due to fabrication difficulties on x-ray optics. It could become the fundamental technique in molecular imaging.

It would, in principle, allow atomic resolution imaging on noncrystalline materials and do "crystallography" without the need for crystals. Resolution is only limited by radiation damage.

This type of experiments is completely limited by coherent flux available at existing sources, and will not become "bread & butter" measurements until the next generation of sources such as ERL comes along.

**Molecular Imaging with ERL:**

- ERL would offer 2 orders of magnitude increase in coherent flux compared to present-day synchrotron sources.
- It would be an ideal and essential hard x-ray source for molecular imaging using coherent diffraction.
Lateral resolution of most existing scanning x-ray microscopes (micro-probes) is limited by horizontal source size of synchrotron radiation.

ERL would completely remove this limitation, allowing diffraction-limited focal area 100-1000x smaller than currently available.

ESRF ID21: SXM 2-10 keV

⇒ transmission
⇒ fluorescence
⇒ photoemission electron
⇒ x-ray diffraction
Fluorescence Microtomography

C.G. Schroer (Aachen)
A. Snigirev (ESRF)

**ID22 ESRF:**
parabolic Al refractive lens
focal spot: 3 μm x 0.9 μm
scan step: 1 μm
132 projections in 360°

**Specimen:**
mycorrhizal root of tomato plant, grown on heavy metal polluted soil
Differential-Aperture X-ray Microscopy (DAXM)

Cargill (intro to Larson), Nature, 2002

"The availability of ... DAXM... provides a direct, - and previously missing - link between the actual microstructure and evolution in materials and the results of numerical simulations ...on mesoscopic length scales"

These experiments are microbeam flux limited. ERL would extend realm.
Conclusions

Synchrotron x-ray imaging & microscopy has made substantial progresses in all three areas of imaging science. With future sources such as ERL:

- it would open up molecular structural science from crystal-based today to noncrystalline & nanocrystalline materials
- It would make XRM a powerful tool in cellular biology, just like what x-ray crystallography is today to molecular biology
- It would allow real-time, phase-contrast x-ray imaging at <0.1µm resolution in medical & small animal imaging as well as in other biological specimens

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<tr>
<th>Plants &amp; Animals</th>
<th>Atoms</th>
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<tbody>
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<td>Organisms</td>
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X-ray microscopy: Chris Jacobsen (Stony Brook) Janos Kirz (Stony Brook) Ian McNulty (APS)

Thank You!