Imaging Cellular Organelles

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Laboratory of Coherent X-ray Optics

- **Laboratory of Coherent X-ray Optics,**
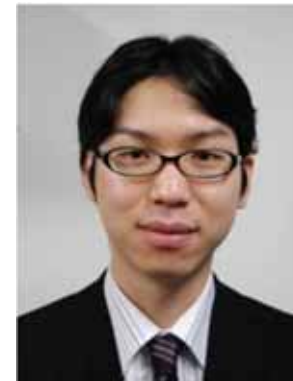 Research Institute for Electronic Science,
  Hokkaido University

Professor
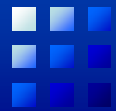**Yoshinori Nishino**

Assistant Professor
Marcus Newton

Assistant Professor
Takashi Kimura

Undergraduate
Students

Kei Soeta
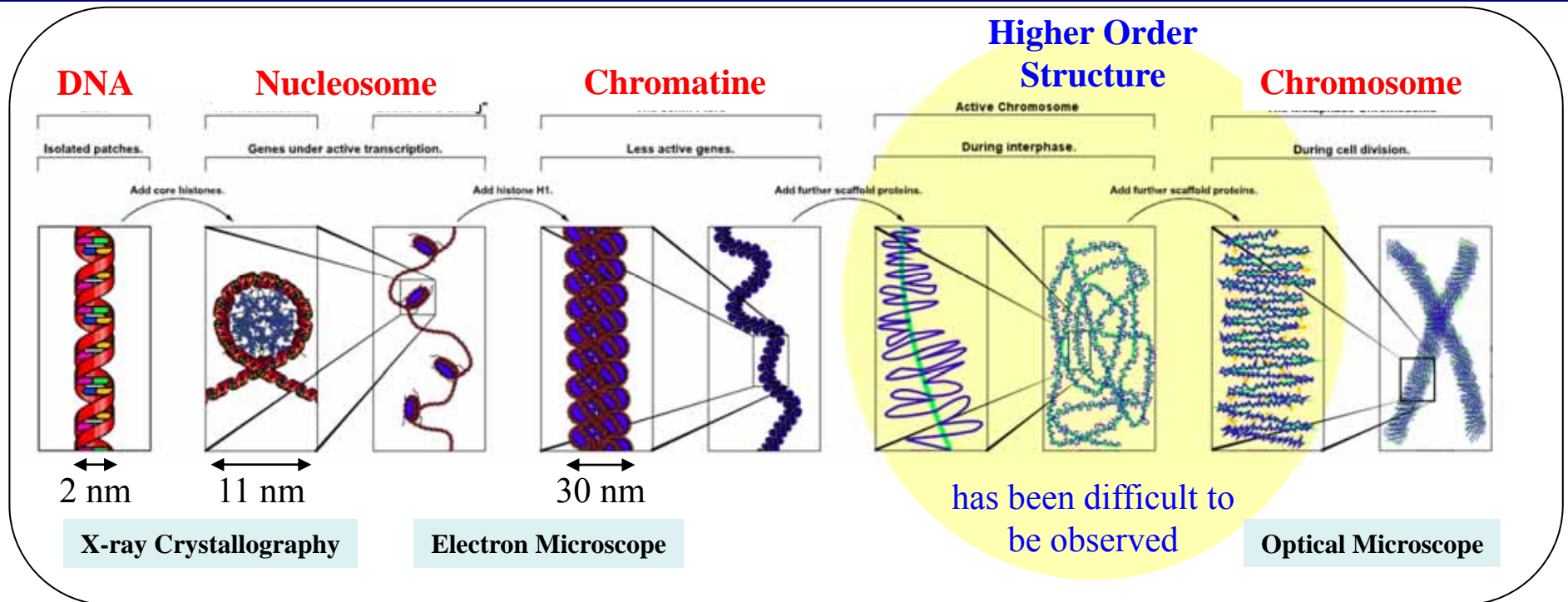**Chie Nagase**
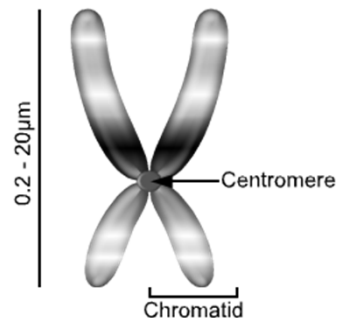**Arata Mori**

Secretary

Kiyo Ssaki

Outline
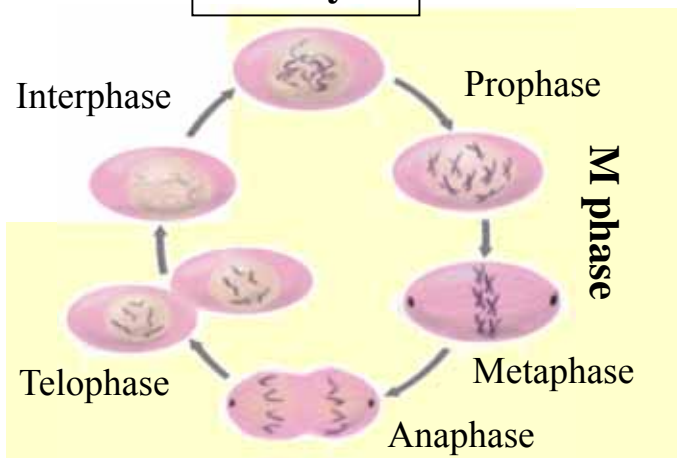
- Imaging Chromosome
- Discussions
  - Average Structure / Individual Structure
  - Focusing X-rays

Chromosomes

DNA   **Nucleosome**   **Chromatine**   **Higher Order Structure**   **Chromosome**

Active Chromosome

| Isolated patches. | Genes under active transcription. | Less active genes. | During interphase. | During cell division. |

Add core histones.   Add histone H1.   Add further scaffold proteins.   Add further scaffold proteins.

2 nm   11 nm   30 nm

X-ray Crystallography   **Electron Microscope**   has been difficult to be observed   **Optical Microscope**

Cell Cycle

Interphase   Prophase

M phase

Telophase   Metaphase

Anaphase

0.2 - 20μm

Centromere

Chromatid

Four Arm Structure:
a pair of sister chromatids attached to each other at the centromere

Coherent X-ray Diffraction

Synchrotron Radiation

iteration: 0

Coherent Diffraction Pattern

SPring-8 (Harima, Japan)
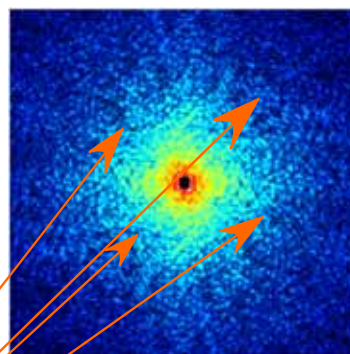
Phase Retrieval

Sample Image
Reconstruction
without the aid of Lenses

Fourier Transform

Sample

Coherent
X-rays
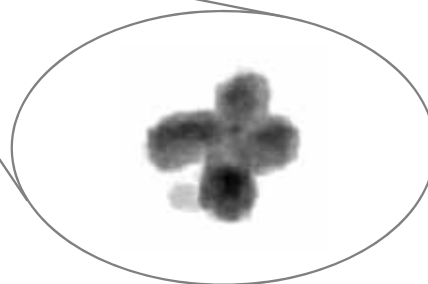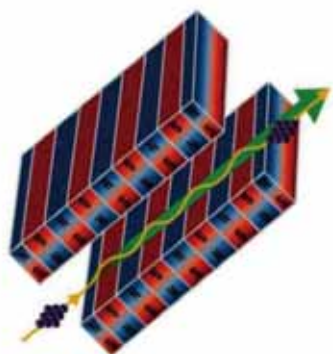
BL29XUL, SPring-8

Unstained Human Chromosome

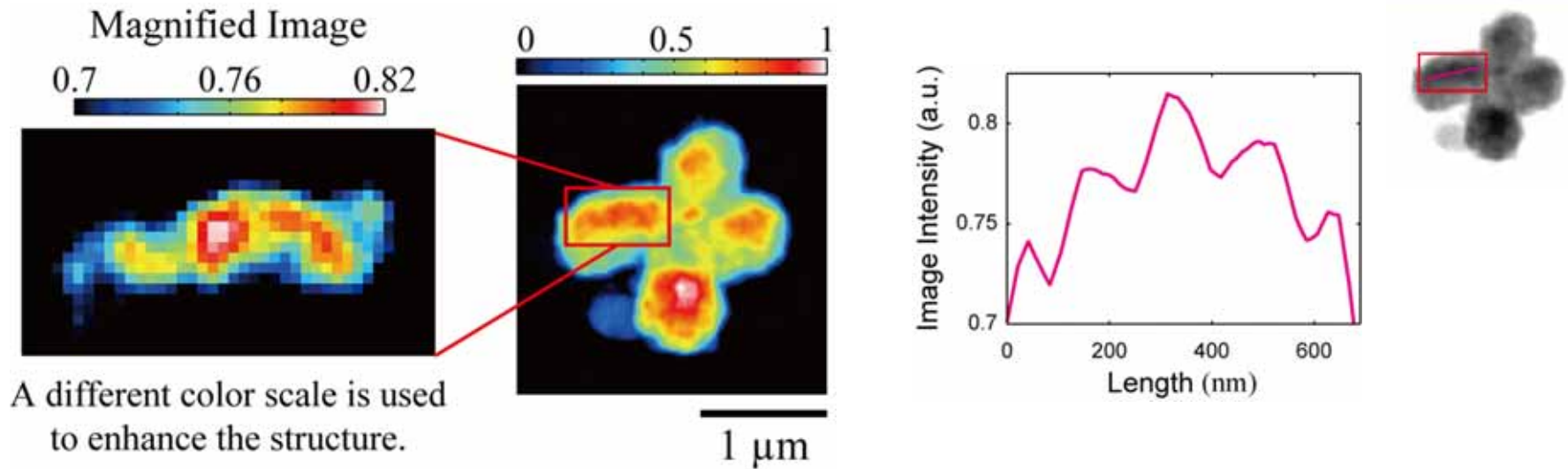50PRL .. Nishino, Y. Takahashi, N. Imamoto, T. Ishikawa, and K. Maeshima, Phys. Rev. Lett. **102**, 018101 (2009).

News Articles: nature Nature **457**, 238 (2009). physicstoday Physics Today, **62** (2), 20 (2009).
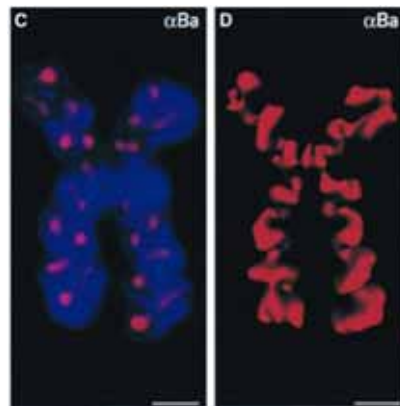
2D Observation of unstained human chromosome

Magnified Image

0.7   0.76   0.82

0   0.5   1

A different color scale is used to enhance the structure.

1 μm

Image Intensity (a.u.)

Length (nm)

spatial resolution = 38 nm

First observation of axial structure in unstained chromosome

Y. Nishino *et al.*, Phys. Rev. Lett. **102**, 018101 (2009).

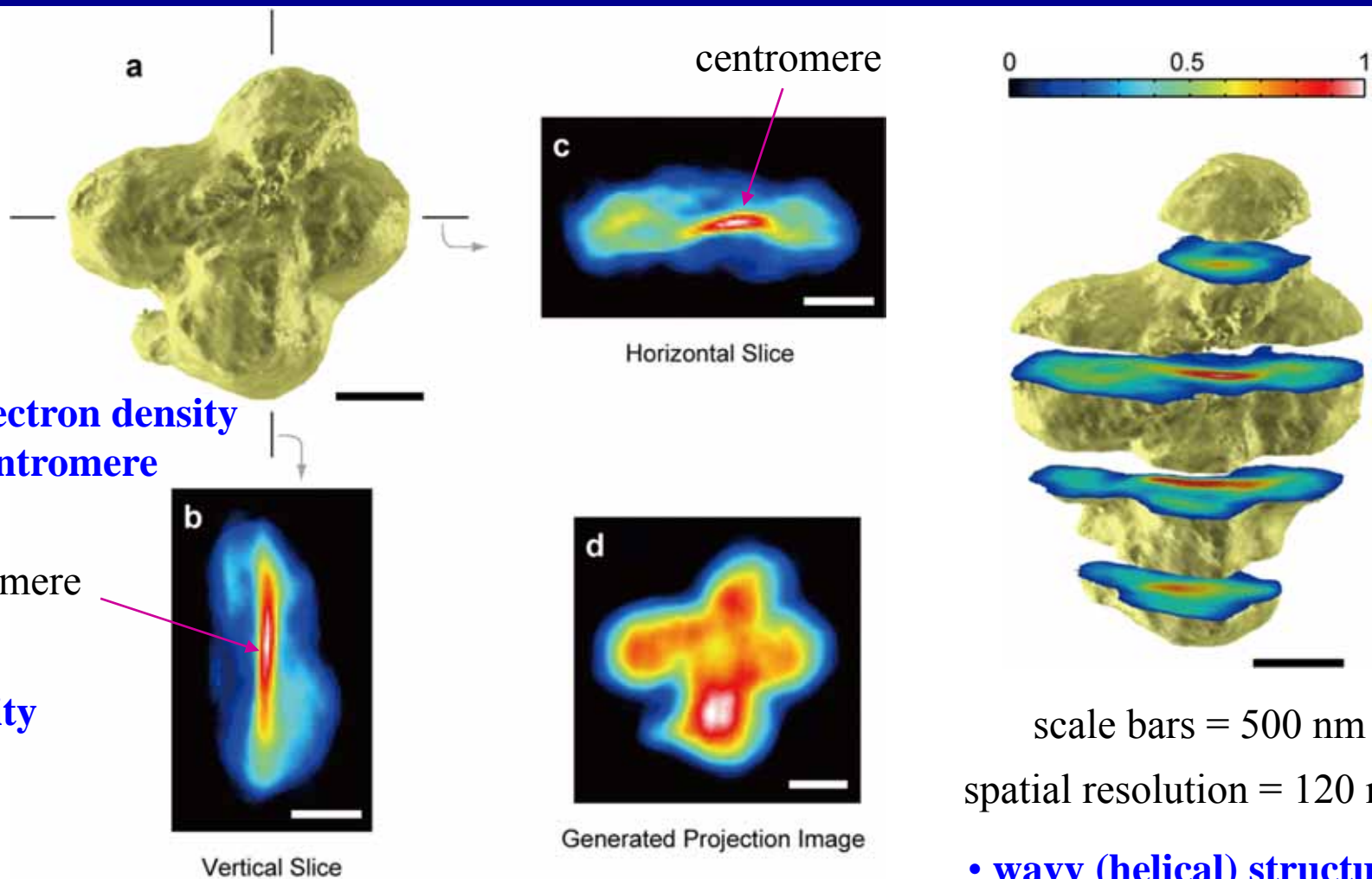Immunofluorescence Microscope Image

condensin antibody (red)

helically folded axial structure

E. Boy de la Tour & U.K. Laemmli, Cell 55, 937 (1988)

bar: 1 μm

K. Maeshima & U. K. Laemmli, Developmental Cell 4, 467 (2003)

3D observation of unstained human chromosome

centromere

Horizontal Slice

• **highest electron density around centromere**

centromere

• **high density near axis**

Vertical Slice

Generated Projection Image

scale bars = 500 nm

spatial resolution = 120 nm

• **consistent with 2D reconstruction**

• **wavy (helical) structure was not observed**

First observation of a cellular organelle in 3D by using hard X-rays

Y. Nishino *et al*., Phys. Rev. Lett. **102**, 018101 (2009).

Spatial Resolution

Estimated Dose

- Single diffraction: 4×10^8 Gy
- 3D diffraction: 2×10^{10} Gy

our 3D reconstruction
(close to feature-destroying dose line)

M. R. Howells et al., J. Electron Spectrosc. Relat. Phenom. **170**, 4 (2009) .

S. Marchesini et al., Optics Express 11, 2344 (2003).
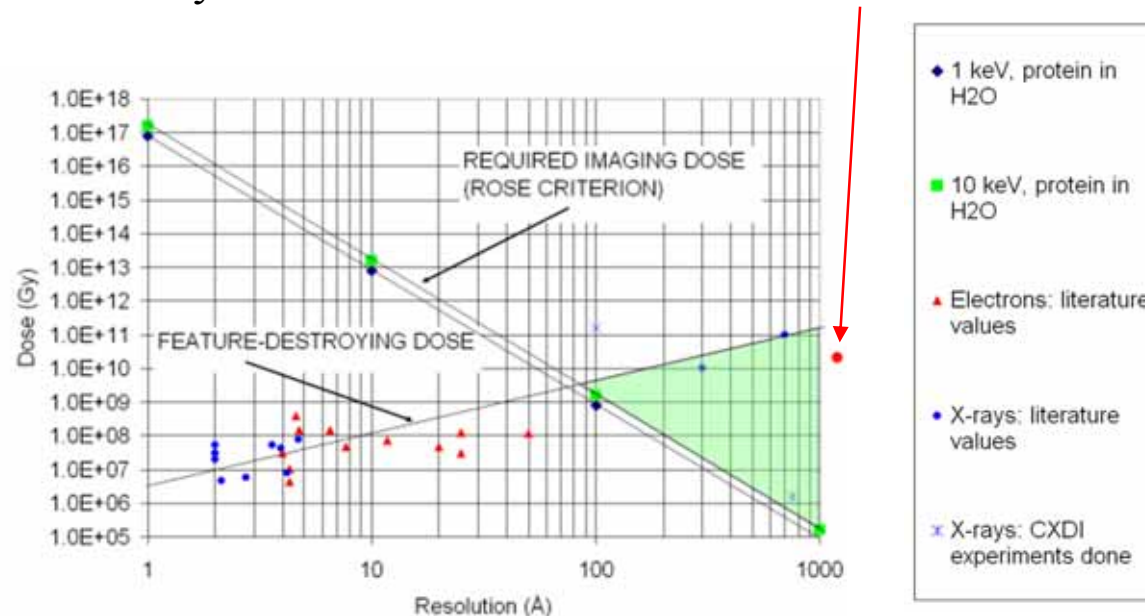
For higher spatial resolution

- cooling the sample cryogenically
- optimizing the dose
- improving the phase retrieval method

Average Structure / Individual Structure

Classical X-ray Methods

- X-ray Crystallography
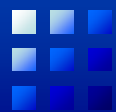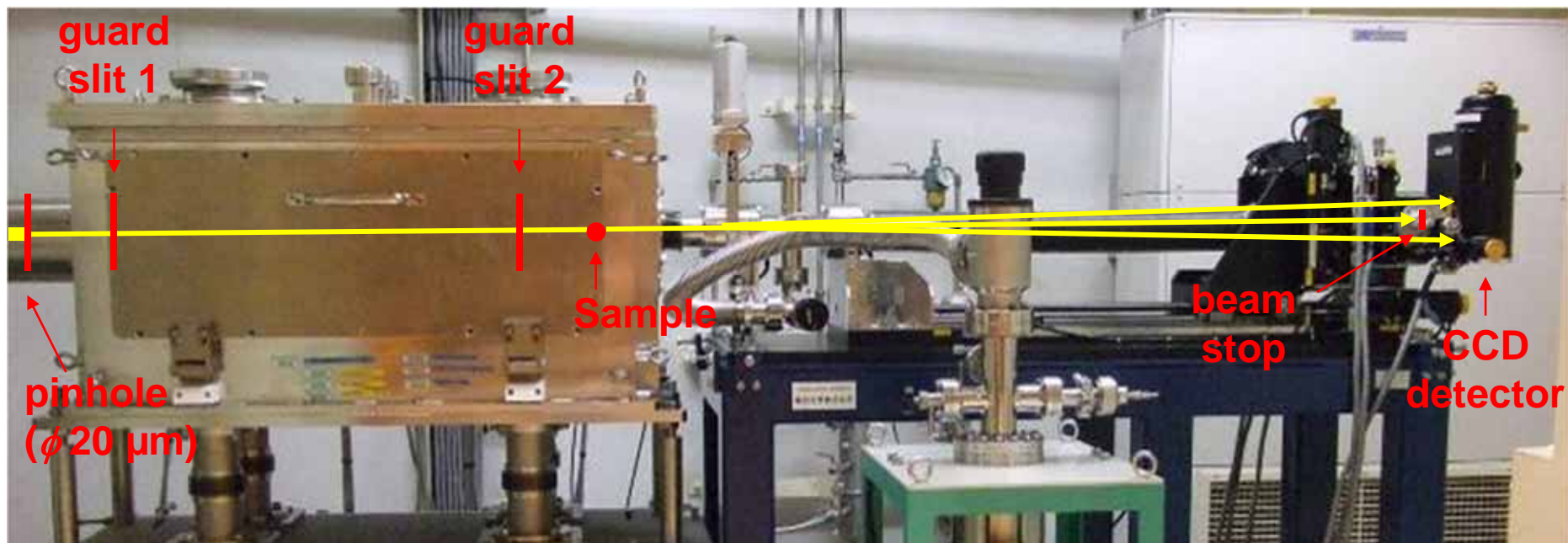- Small-Angle X-ray Scattering (SAXS)

Average Structure

Less Radiation Dose to Each Individual Biological Object
→ Higher Resolution

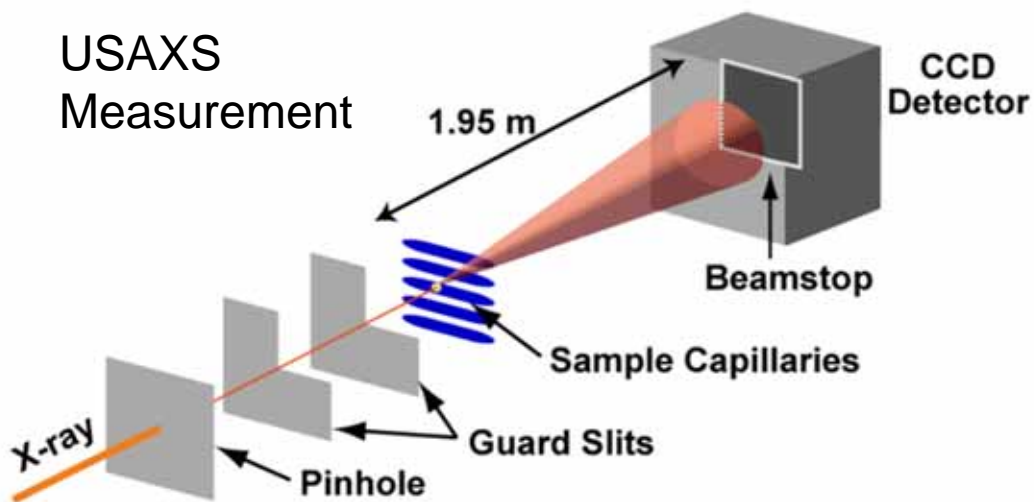Solution Scattering of Biomolecules

- for bio-molecules difficult crystallize
- nearly physiological conditions
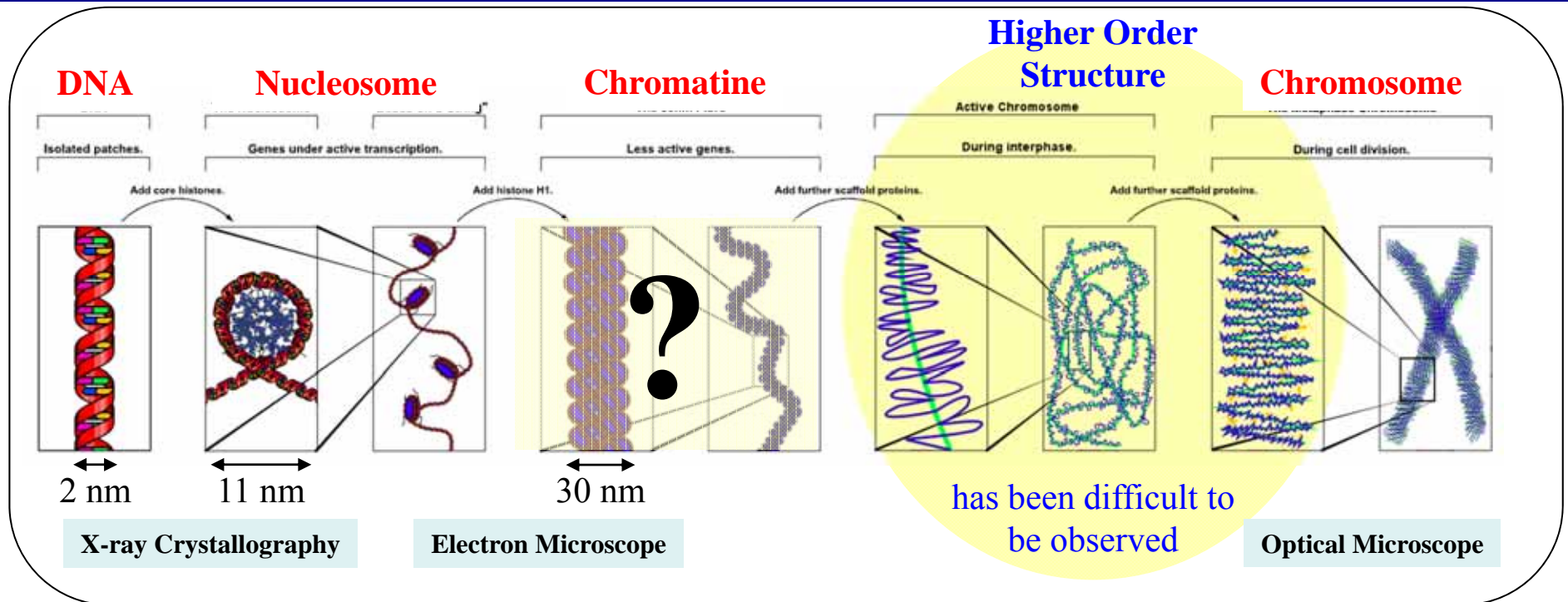
Diffraction Microscopy Instrument at SPring-8

guard slit 1

guard slit 2

Sample

beam stop

CCD detector

pinhole (ϕ 20 μm)

USAXS Measurement

1.95 m

CCD Detector

Beamstop

Sample Capillaries

Guard Slits
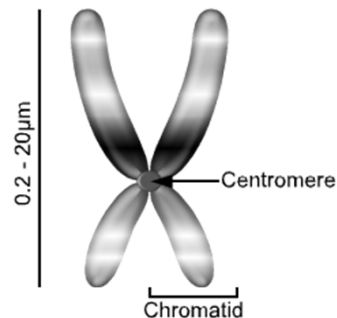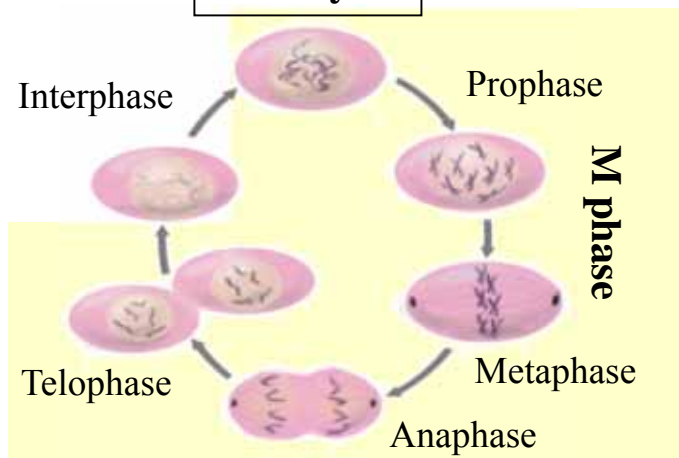
X-ray

Pinhole

Hard X-ray Beamline BL29XUL

• **CCD Detector:**
Princeton Instruments PI-LCX 1300
1300 × 1340 Pixels
Pixel Size: 20 μm × 20 μm
Direct Illumination
Deep Depletion

Chromosomes

DNA — **Nucleosome** — **Chromatine** — **Higher Order Structure** — **Chromosome**

Isolated patches. | Genes under active transcription. | Less active genes. | Active Chromosome During interphase. | During cell division.

Add core histones. | Add histone H1. | Add further scaffold proteins. | Add further scaffold proteins.

2 nm | 11 nm | 30 nm | ? | has been difficult to be observed

X-ray Crystallography | **Electron Microscope** | **Optical Microscope**

Cell Cycle

Interphase — Prophase — M phase — Metaphase — Anaphase — Telophase
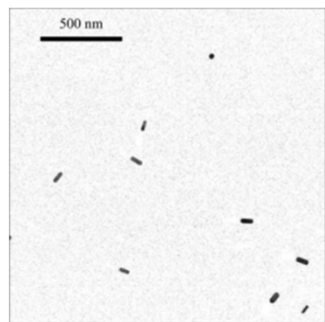

0.2 - 20μm — Centromere — Chromatid

Four Arm Structure:
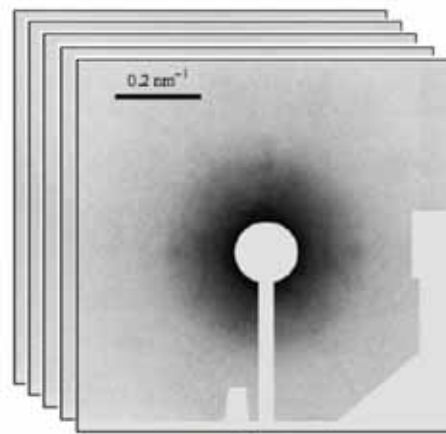a pair of sister chromatids attached to each other at the centromere
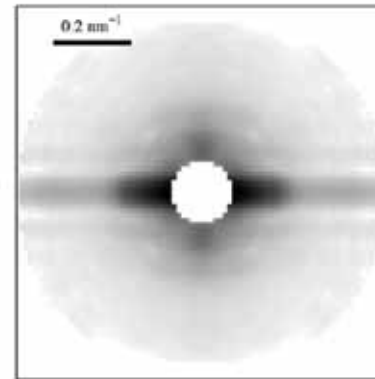
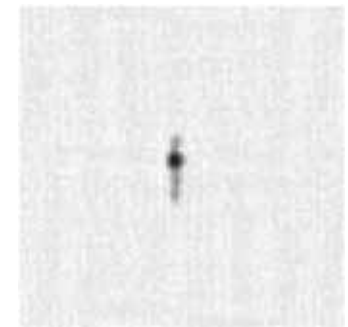# Fluctuation Microscopy

Z. Kam, Macromolecules, 10, 927 (1977)

TEM image of randomly oriented 90× 25 nm gold nanorods

many snapshot patterns, each with many disordered nanorods

Reconstruction of the *single nanorod* diffraction pattern
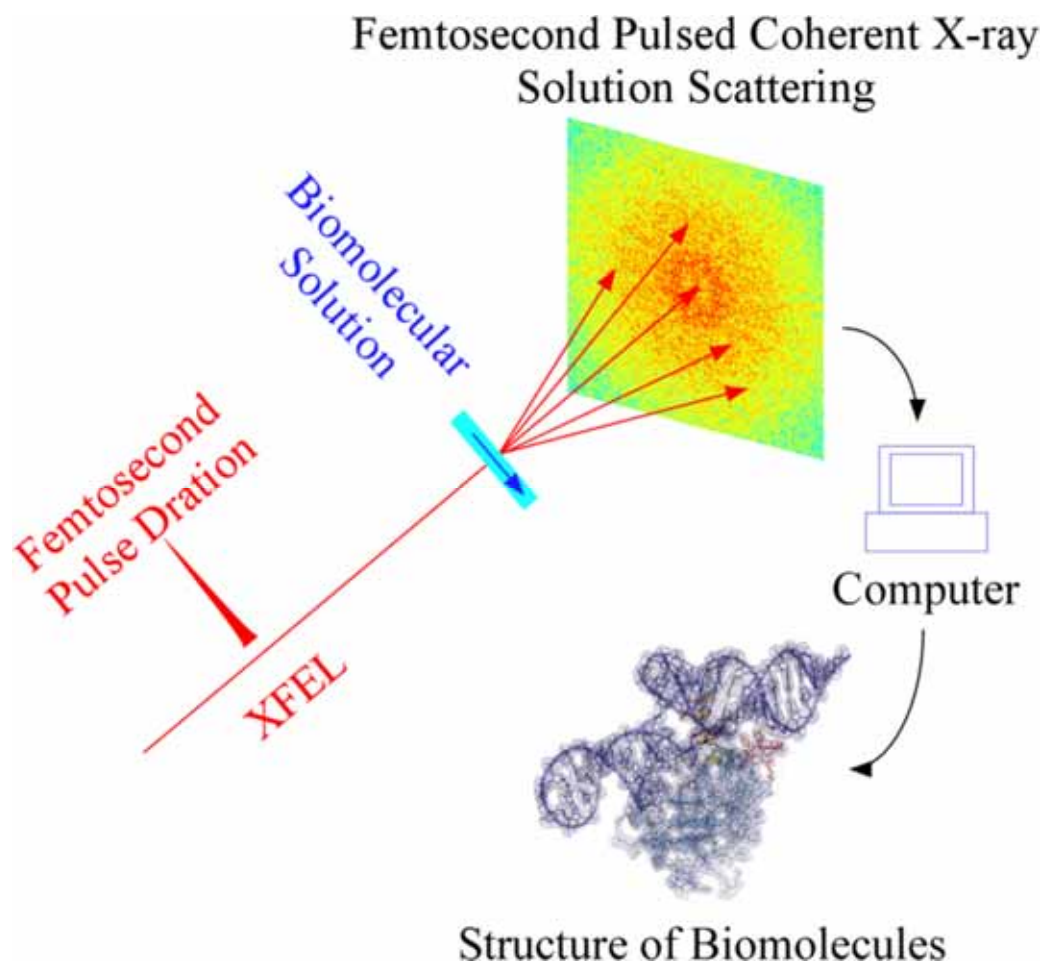
Reconstructed Image of gold nanorods

D. K. Saldin *et al*., PRL 106, 115501 (2011)

"The cross-correlation scheme has a disadvantage relative to the single-shot scheme both because of restrictions in the processing and the smaller number of photons scattered per particle."

V. Elser: arXiv:1007.3777v1

Pulsed Coherent X-ray Solution Scattering

Femtosecond Pulsed Coherent X-ray Solution Scattering

Biomolecular Solution

Femtosecond Pulse Dration

XFEL

Computer

Structure of Biomolecules

Conventional SAXS

Averaging

- Ensemble Average
- Time Average

Concentric Scattering Pattern
(1 Dimensional Data)

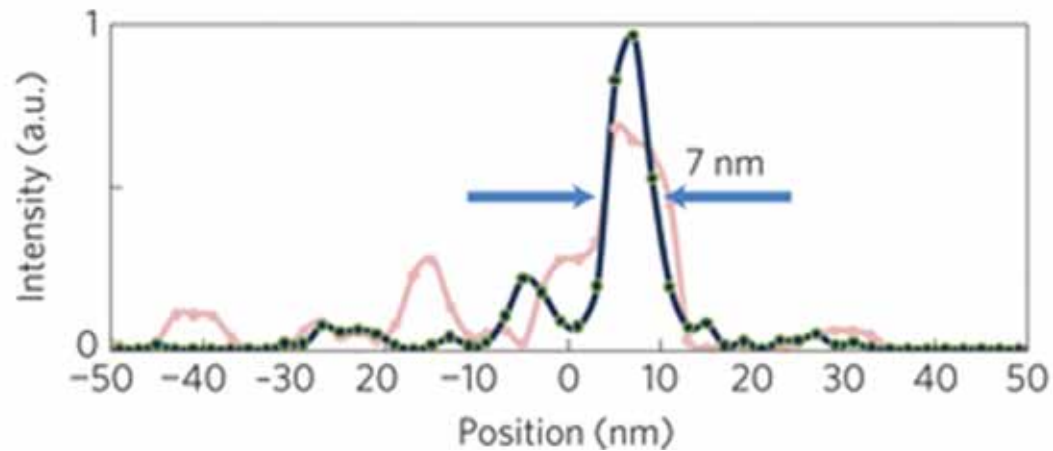## Solution Scattering with XFEL

Unaveraged Data Acquisition

- Illuminating a Small Number of Moleculses
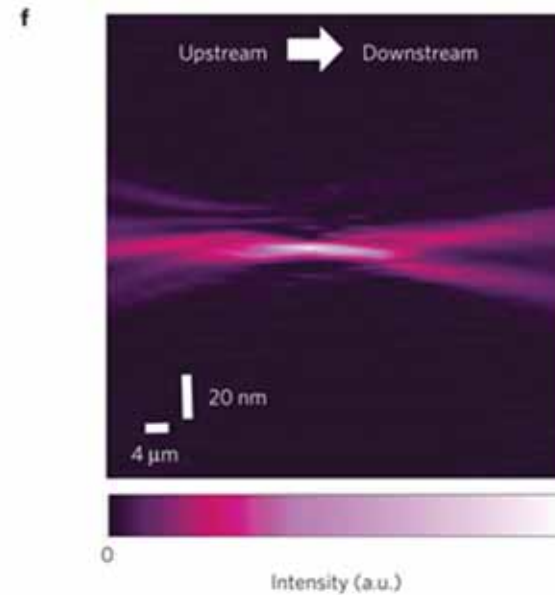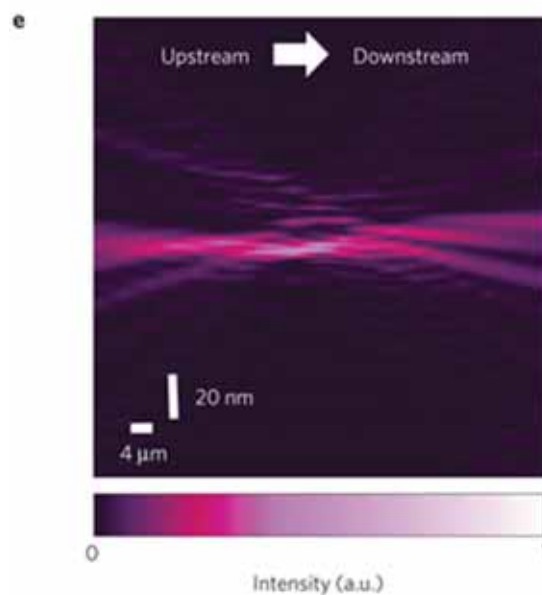- Ultrashort Pulse Duration

Speckled Coherent Scattering Pattern
(2-Dimensinal Data)

20 keV X-rays

Beamline Requirements

Upstream Mirror

Focal Point

Downstream Mirror

Light Source
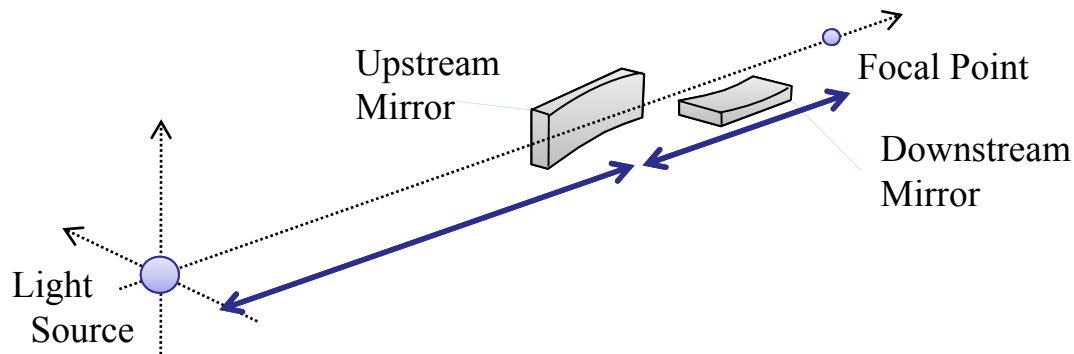
Geometrical Demagnification
= (Lens-to-Focus)/(Source-to-Lens)

Example:
Source Size: ~10 μm
Focal Size: ~10 nm
Working Distance ~100 mm
↓
Beamline Length ~100 m

1 km-long beam line

wave optics

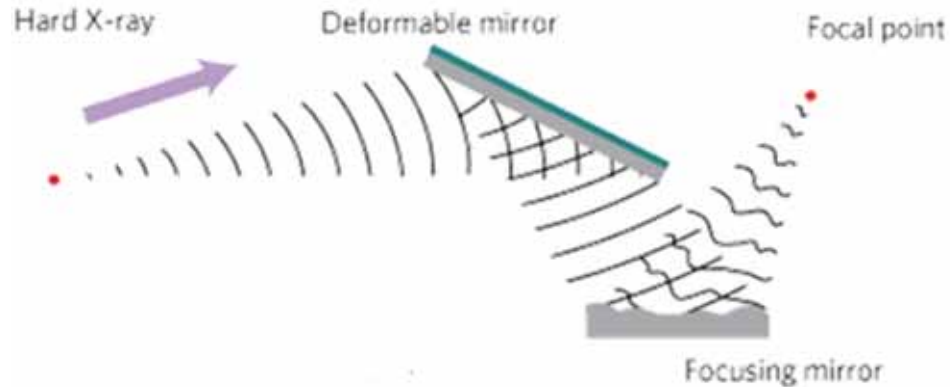$$\text{focal spot size} \sim \frac{\lambda}{\text{N.A.}}$$

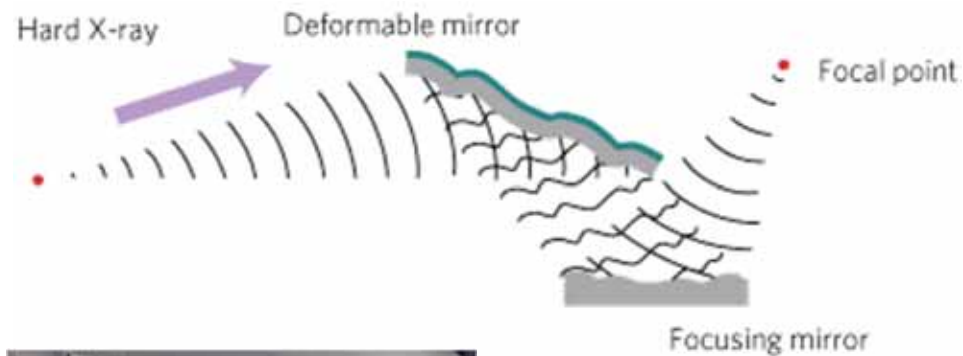ERL or URL:
Coherently Illuminate Focusing Mirrors without Loss

- Diffraction Limited Source both Vertically and Horizontally
- Long Beamline
- Stability

Adaptive Optical System for Hard-X-ray Focusing

in situ **determination of the wavefront error of X-ray focusing mirror**

Iterative phase-retrieval method using the intensity profiles around the beam waist

H. Yumoto, *et al.* , RSI **77**, 063712 (2006).

20 platinum/carbon bilayers

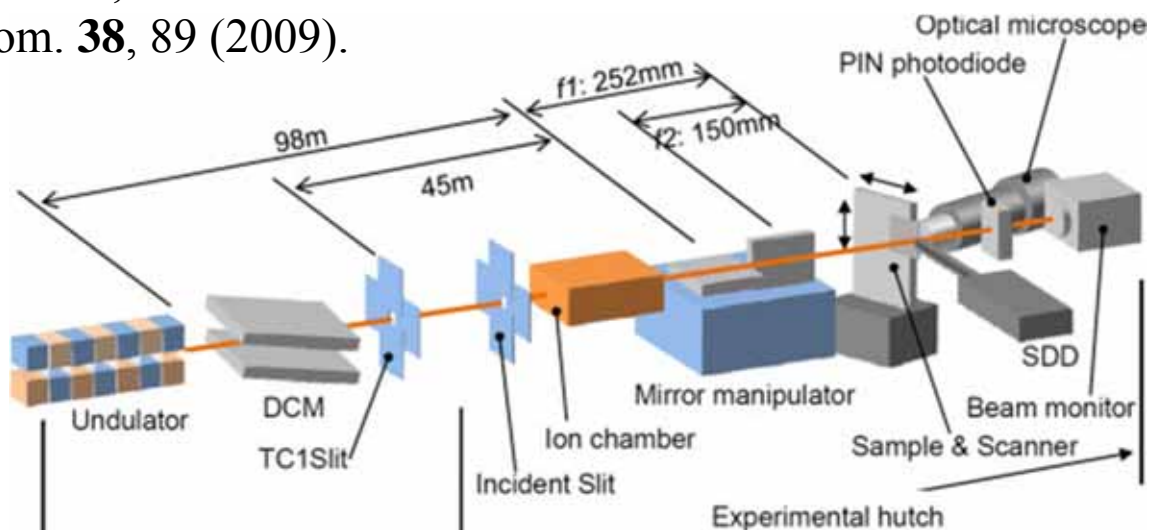**Adaptive optics to compensate figure error of the focusing mirror**

T. Kimura *et al.*, JJAP **48**, 072503 (2009).

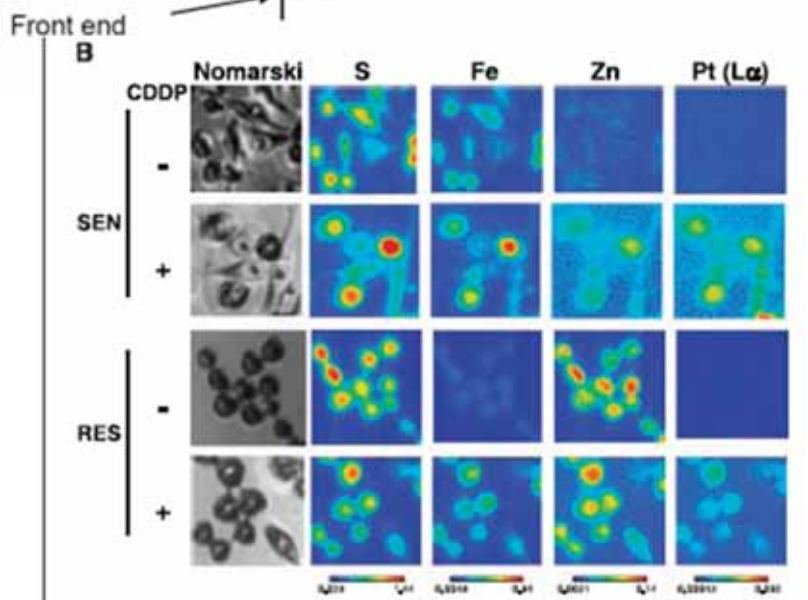H. Mimura *et al.*, Nature Physics **6**, 122 (2009).

Scanning X-ray Fluorescence Microscope

S. Matsuyama *et al.*,
X-Ray Spectrom. **38**, 89 (2009).

SPring-8
BL29XU EH2

M. Shimura *et al.*,
Cancer Research **65**, 4998 (2005).

Collaborators

- Hokkaido Univ.: T. Kimura, M. Newton

- National Institute of Genetics: K. Maeshima

- Osaka Univ.: Y. Takahashi, S. Matsuyama, K. Yamauchi

- Univ. Tokyo: H. Mimura

- National Center for Global Health and Medicine: M. Shimura

- JASRI: Y. Joti

- Univ. Tokyo: S. Takeuchi

- RIKEN SPring-8: Y. Bessho, T. Ishikawa

Summary

- Imaging Chromosome
  - X-ray diffraction microscopy enable high-contrast imaging for relatively thick samples.

  - 2D & 3D observation of unstained human chromosome

- Average Structure / Individual Structure
  - SAXS
  - cross-correlation

- Focusing X-ray
  - X-ray focusing down to 7 nm
    - long beamline
    - in situ determination of figure error of X-ray focusing mirror
    - Adaptive optics to compensate figure error