R&D Program at Quantum Wave Electron Microscopy Unit

T. Shintake, OIST 2011.10.11

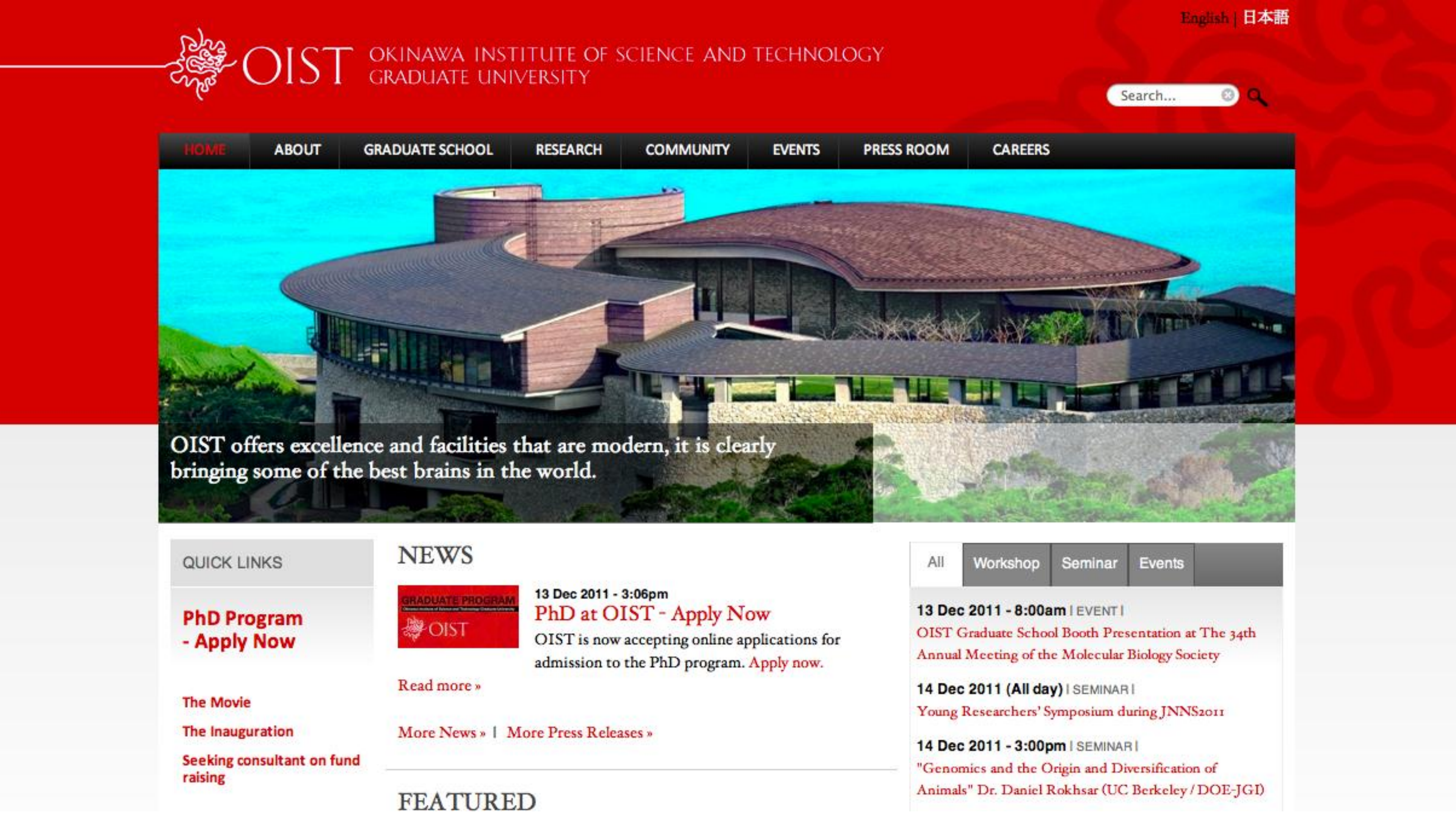
We are going to develop

"New Atomic Resolution Electron Microscope".

For biological sample: DNA, Ion-channel, surface structure of cells, and Membrane protein crytallorappy..

For material research: Li-ion battery, catalysis, ..

Tsumoru Shintake
shintake@oist.jp



Life Sciences, the Physical Sciences, and Mathematics.
To lay the foundation for the Graduate University, 44 Research Units.
220 Researchers.

X-ray FEL v.s. Electron Microscope

- X-ray FEL (SACLA at SPring-8, or LCLS at Stanford)
- Very large scale machine

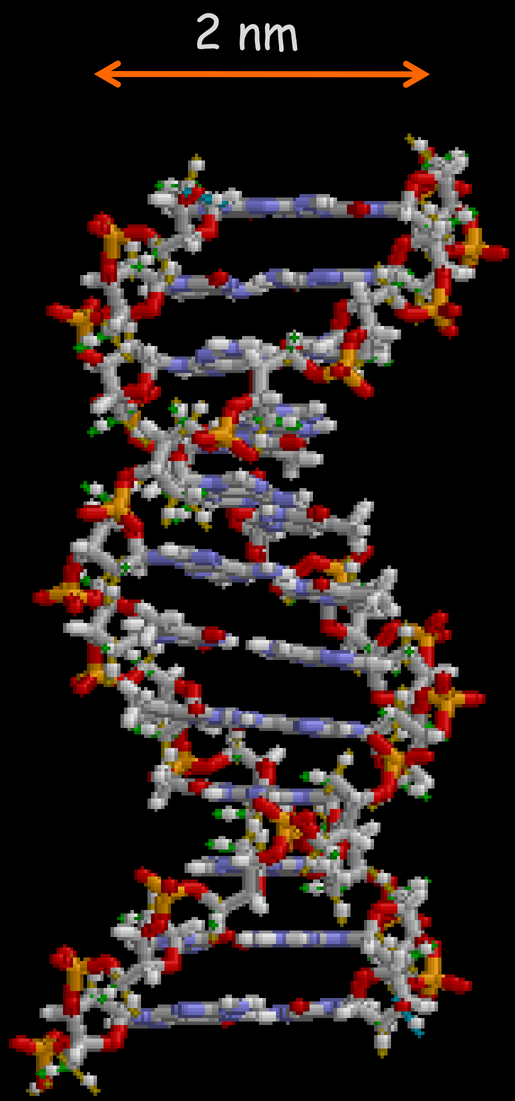
(> 1 km, 400 M$ cost)

- Short pulse (<100 femt-sec), intense X-ray flux (10^{10} photons /shot) illumination on sample.
- Image taking before sample is exploded.
- Flying sample (virus, nano-crystal)
- We do not need to care about vibration.
- X-ray photon and electron collision is based on "clean event", single scattering.

- Electron Microscope (SEM or TEM)
- Small scale machine (1 ~ 3 m high, 1 ~ 10 M$)
- Very weak electron beam.
- Image accumulation before sample is damaged (10 sec)
- We need to care about vibration.
- Electron to sample interaction is not clean event, there are many "multiple collisions" and "non-elastic collisions"
- → energy filtering is important.

Tsumoru Shintake
shintake@oist.jp

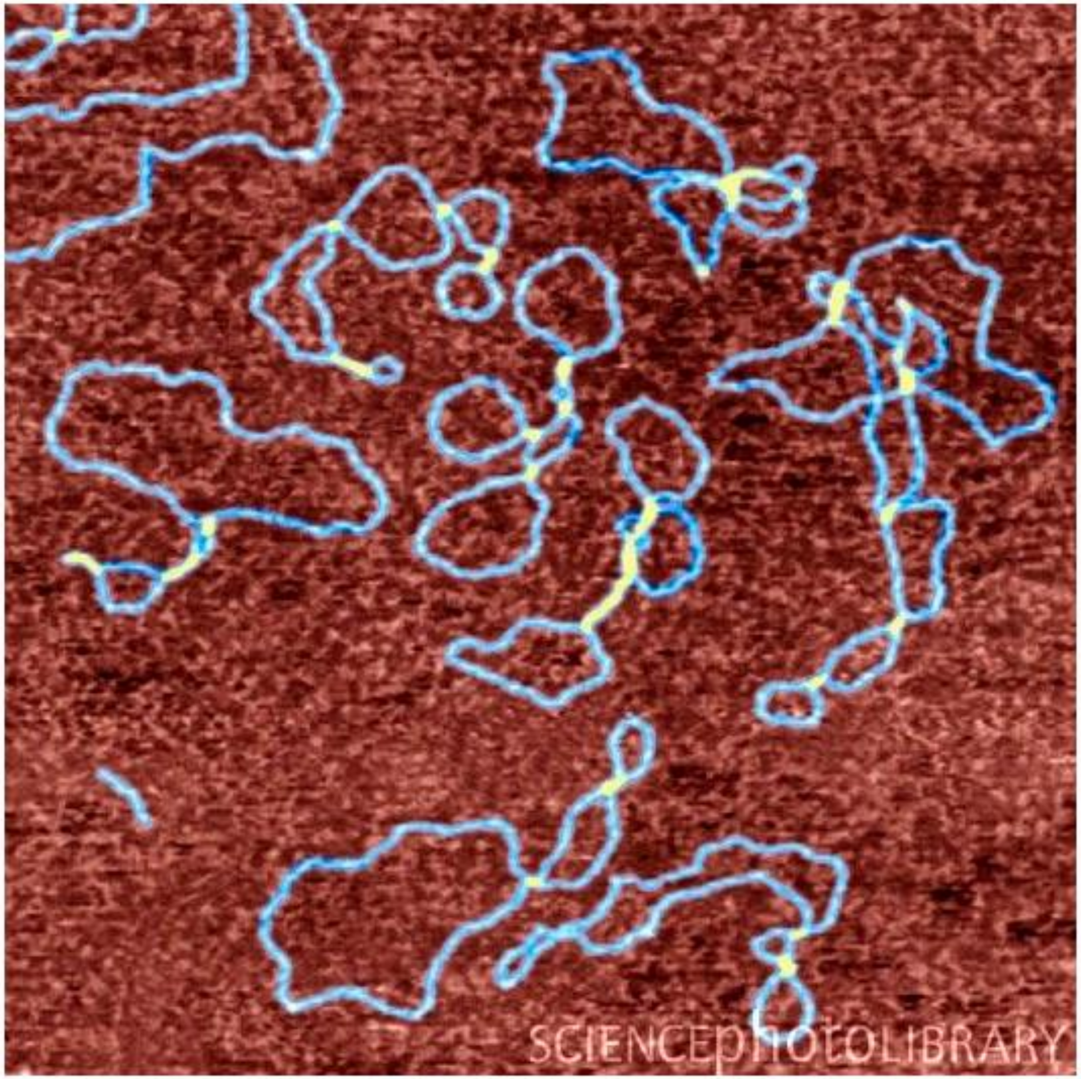
We want to see 3D DNA image



computer model of DNA
Gif animation image
copied from Wikipedia

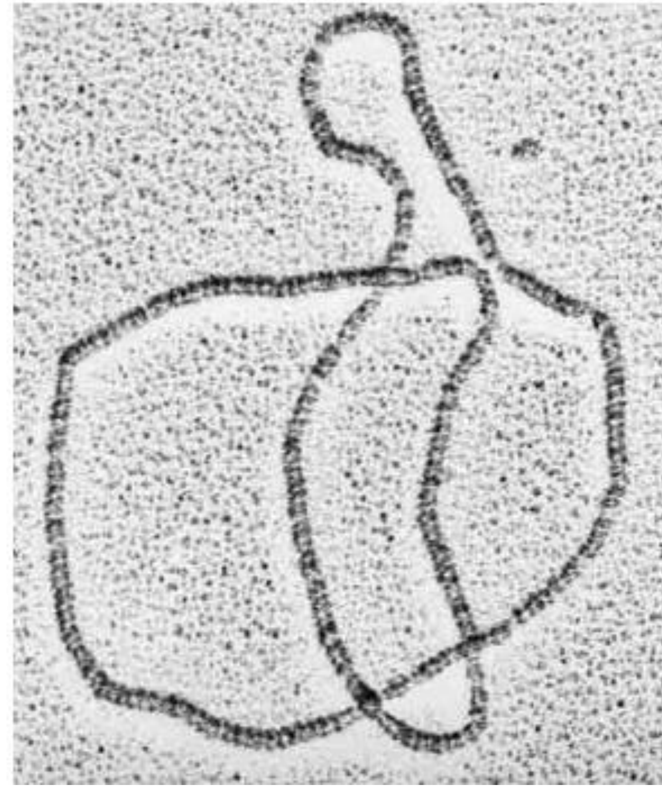
Tsumoru Shintake
shintake@oist.jp

CLOSE SCIENCEphotoLIBRARY



Anti-cancer drug binding to DNA, AFM

G255/0125 Rights Managed



Electron microscope image
Of DNA.
Double helix can not be seen.

Tsumoru Shintake
shintake@oist.jp

Required Resolution for DNA Structure Observation

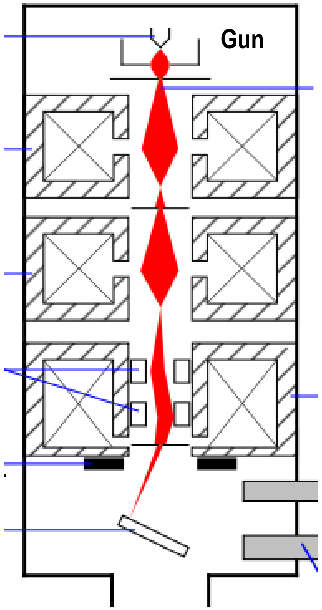
1 nm | 5 A | 2.5 A | 1 A | 0 A

Image cpied from Wikipedia

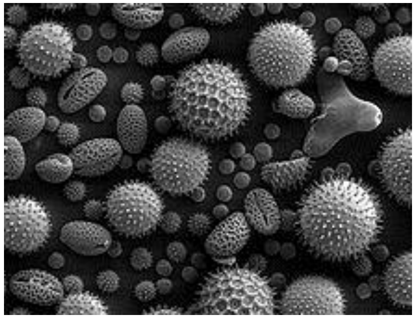
Tsumoru Shintake
shintake@oist.jp

SEM with Atomic Resolution

SEM
Scanning Electron Microscope



Copied from Wikipedia



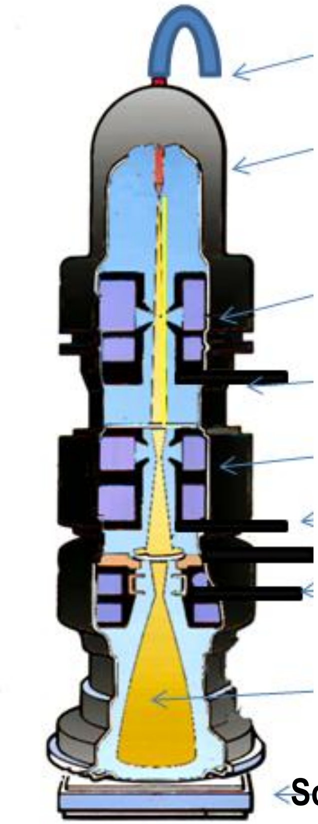
~ 10 Å
surface view

SEM++

- ~ 1 Å
- Surface View
- Low Sample Damage

S-TEM

TEM
Transmission Electron Microscope



Copied from Wikipedia

~ 0.1 Å for non biological sample with CS correction.
~ 5 Å for biological sample

Sample Damage

Tsumoru Shintake
shintake@oist.jp

Bio-imaging with conventional TEM

- In order to reduce multiple scattering, sample has been made thinner and thinner, and also electron beam energy has been raised to 300 kV, 1 MeV, even higher.
- de'Broglie wavelength is about 0.01 $\mathring{A}$.
- Resolution of TEM was limited by spherical aberration in objective lens. (CS correction technique improved resolution, energy filtering improved contrast)
- Contrast is low for biological sample → use staining technique (uranium, gold) → artifact problem
- Sevier same damage → cryo-sample → artifact problem

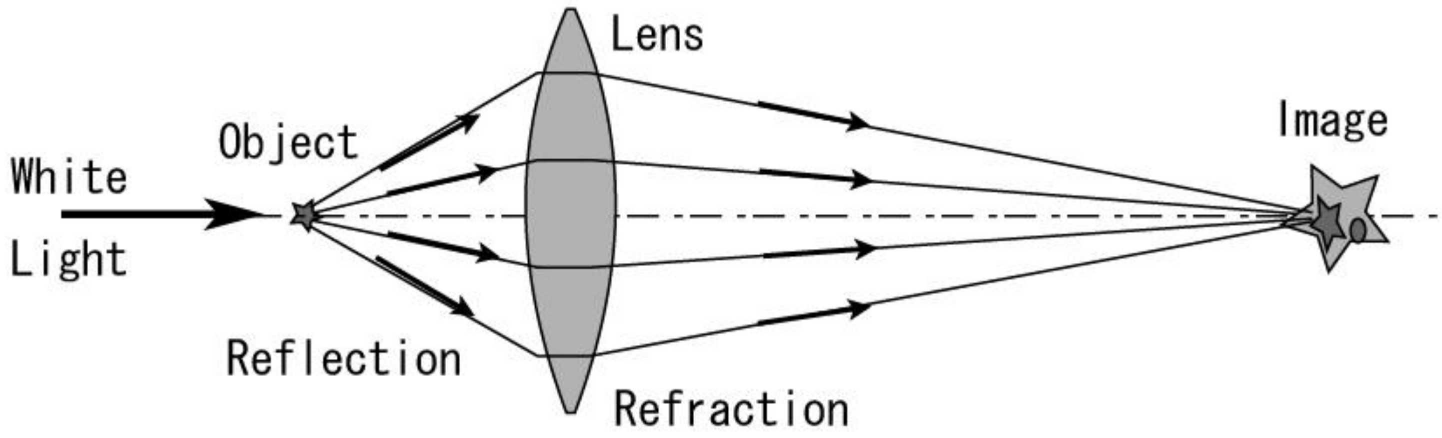
Tsumoru Shintake
shintake@oist.jp

Let's think to go lower beam energy.

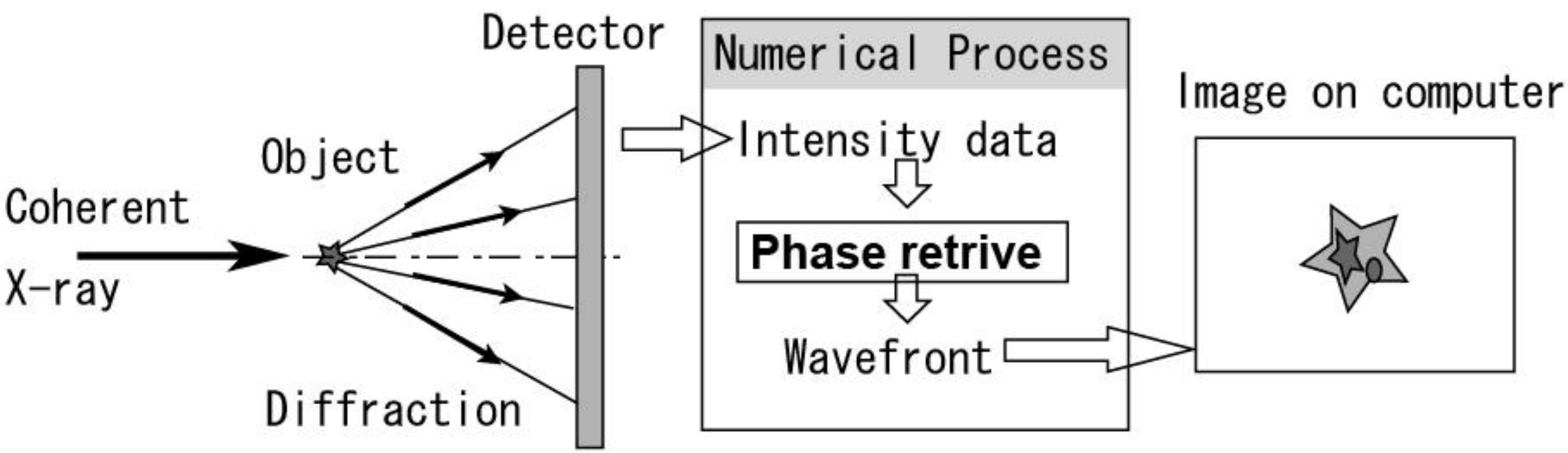
- Low energy electron does not go through sample.
- It is OK if we can observe the surface structure like SEM.
- Sample damage becomes lower → we do not need to cool down or free the sample → raw bio-sample can be observed (we may use fully hydrated sample) → less artifacts.
- We have to take "reflection signal" from the surface, thus the object lens can not be placed (no physical room), TEM optics can not be applied.
- "Remove Objective Lens" → lens less microscopy

Remove lens

Optical Microscope



Lensless Diffraction Microscope



Tsumoru Shintake
shintake@oist.jp

Holography

May 15, 1948 NATURE

A NEW MICROSCOPIC PRINCIPLE

By DR. D. GABOR
Research Laboratory, British Thomson-Houston Co., Ltd.
Rugby

IT is known that the spherical aberration of electron lenses sets a limit to the resolving power of electron microscopes at about 5 A. Suggestions for the correction of objectives have been made; but these are difficult in themselves, and the prospects of improvement are further aggravated by the fact that the resolution limit is proportional to the fourth root of the spherical aberration. Thus an improvement of the resolution by one decimal would require a correction of the objective to four decimals, a practically hopeless task.

The new microscopic principle described below offers a way around this difficulty, as it allows one to dispense altogether with electron objectives. Micrographs are obtained in a two-step process, by electronic analysis, followed by optical synthesis, as in Sir Lawrence Bragg's 'X-ray microscope'. But

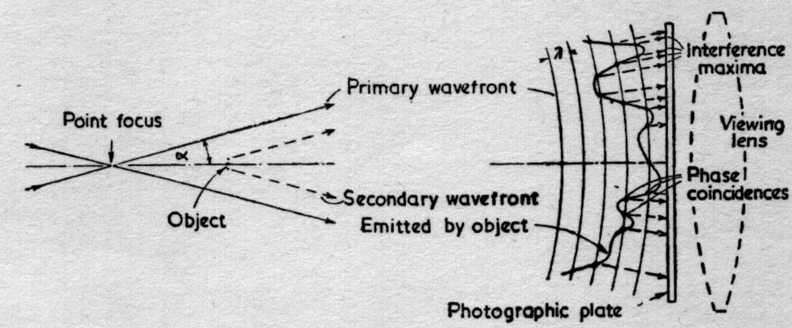


Fig. 1. INTERFERENCE BETWEEN HOMOCENTRIC ILLUMINATING WAVE AND THE SECONDARY WAVE EMITTED BY A SMALL OBJECT

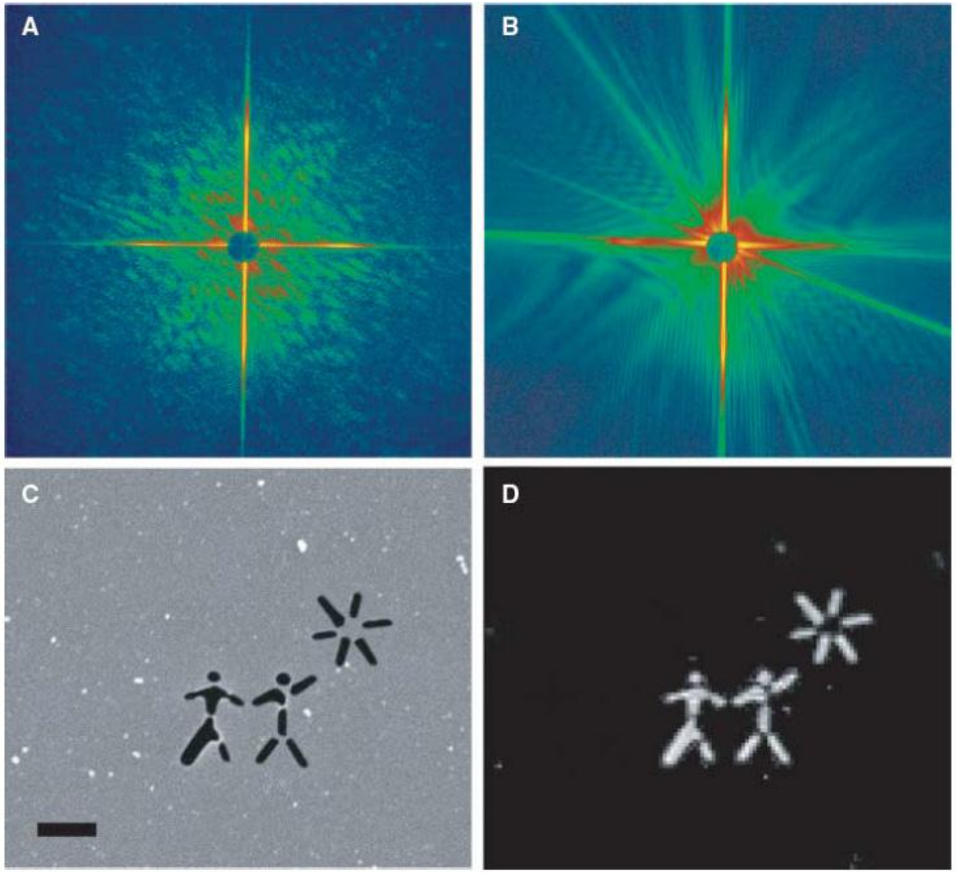
The Nobel Prize in Physics 1971
Dennis Gabor

Diffraction Imaging in X-FELs

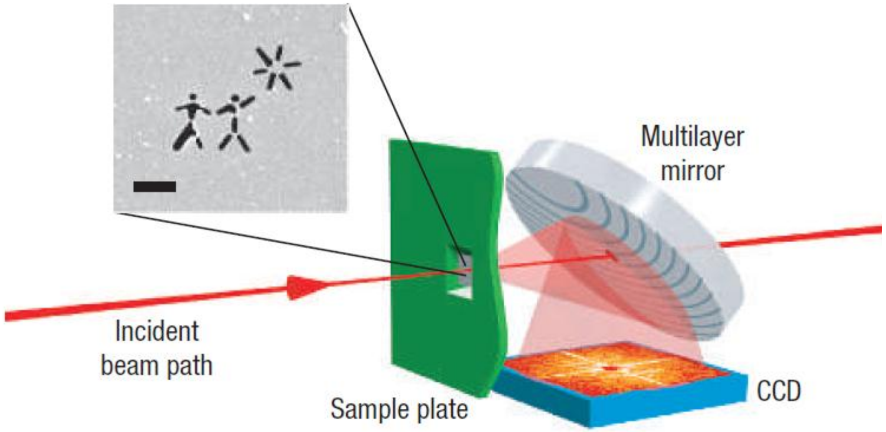
Pattern made by FIB on SiN4 membrane.
Illuminated by FEL beam at FLASH.
25 fs, 4 x10^{13} W/cm^{-2} pulse,
containing 10^{12} photons
at 32 nm wavelength

Phase retrieved image as D.

Chapman, Barty and Bogan 2006



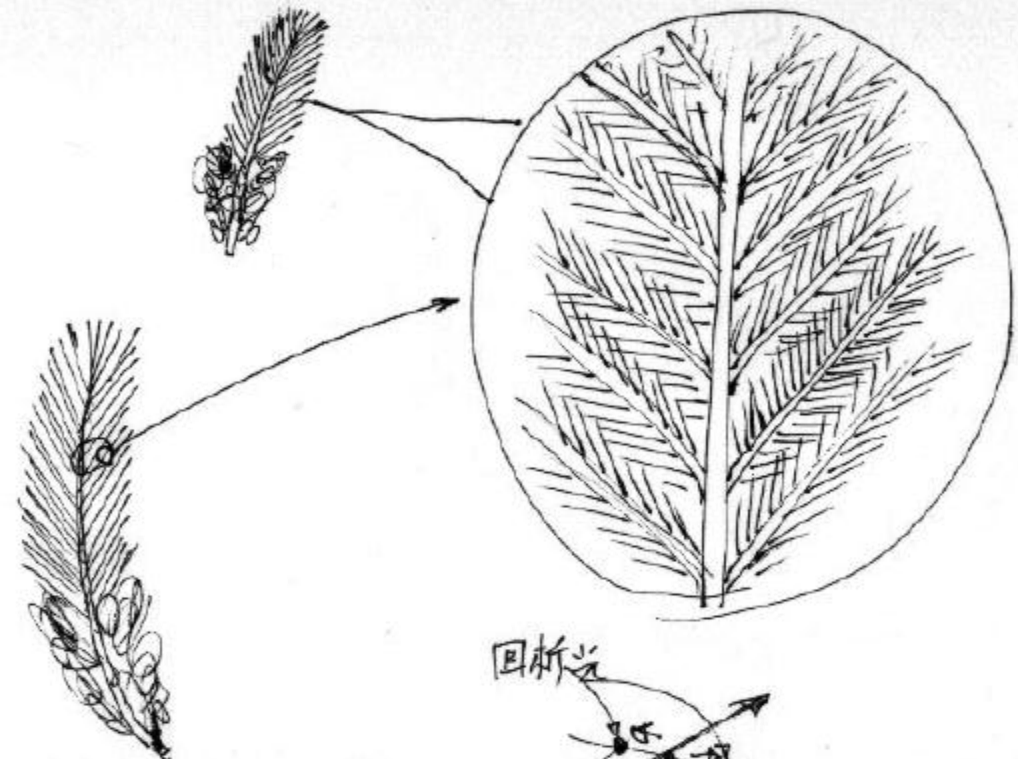
scale-bar 1 micron

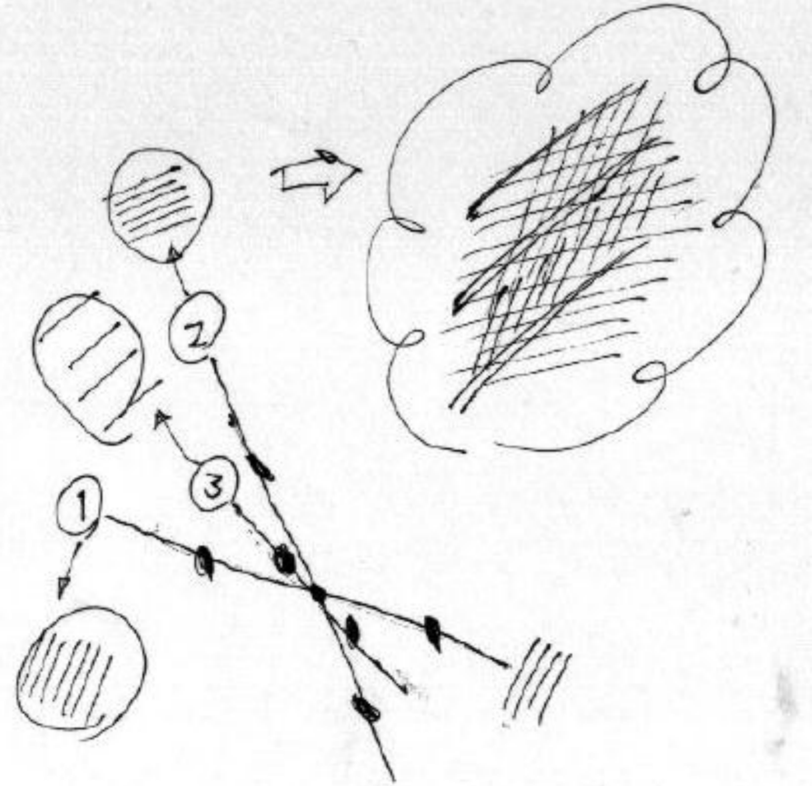


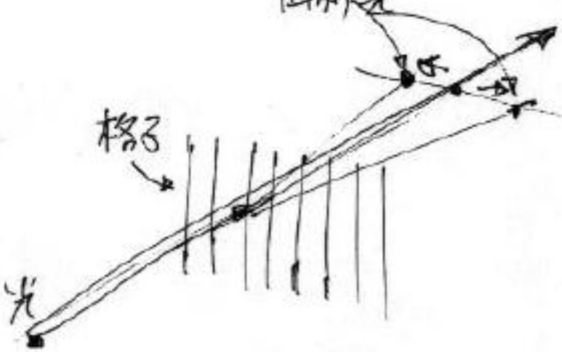
Tsumoru Shintake
shintake@oist.jp

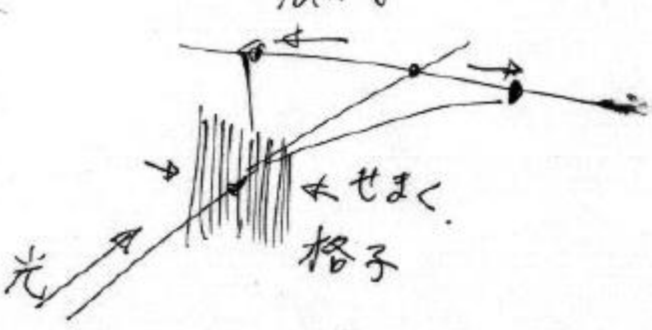
Shintake

鳥の羽根，回折



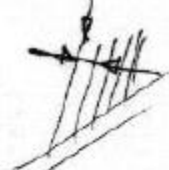


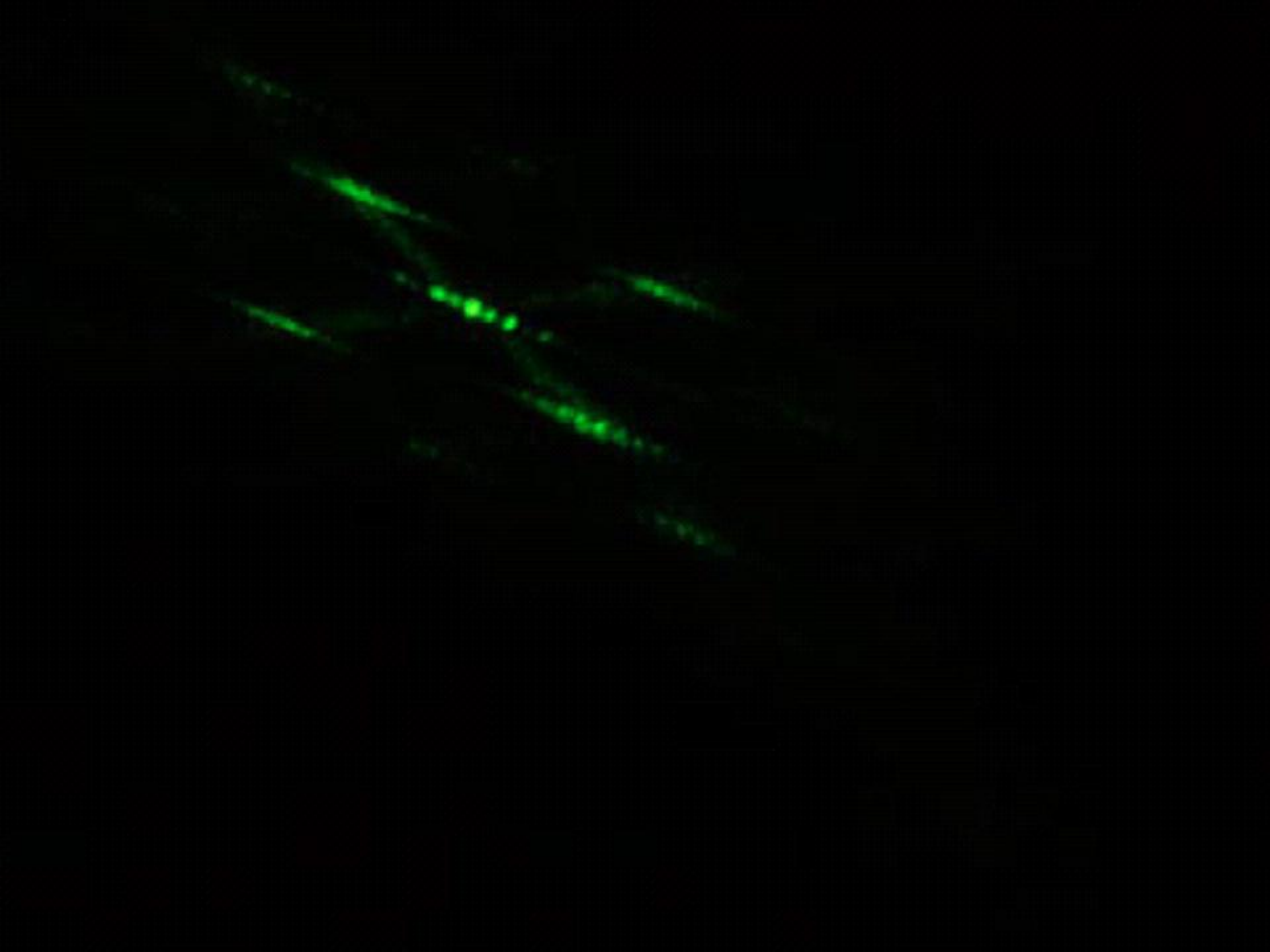


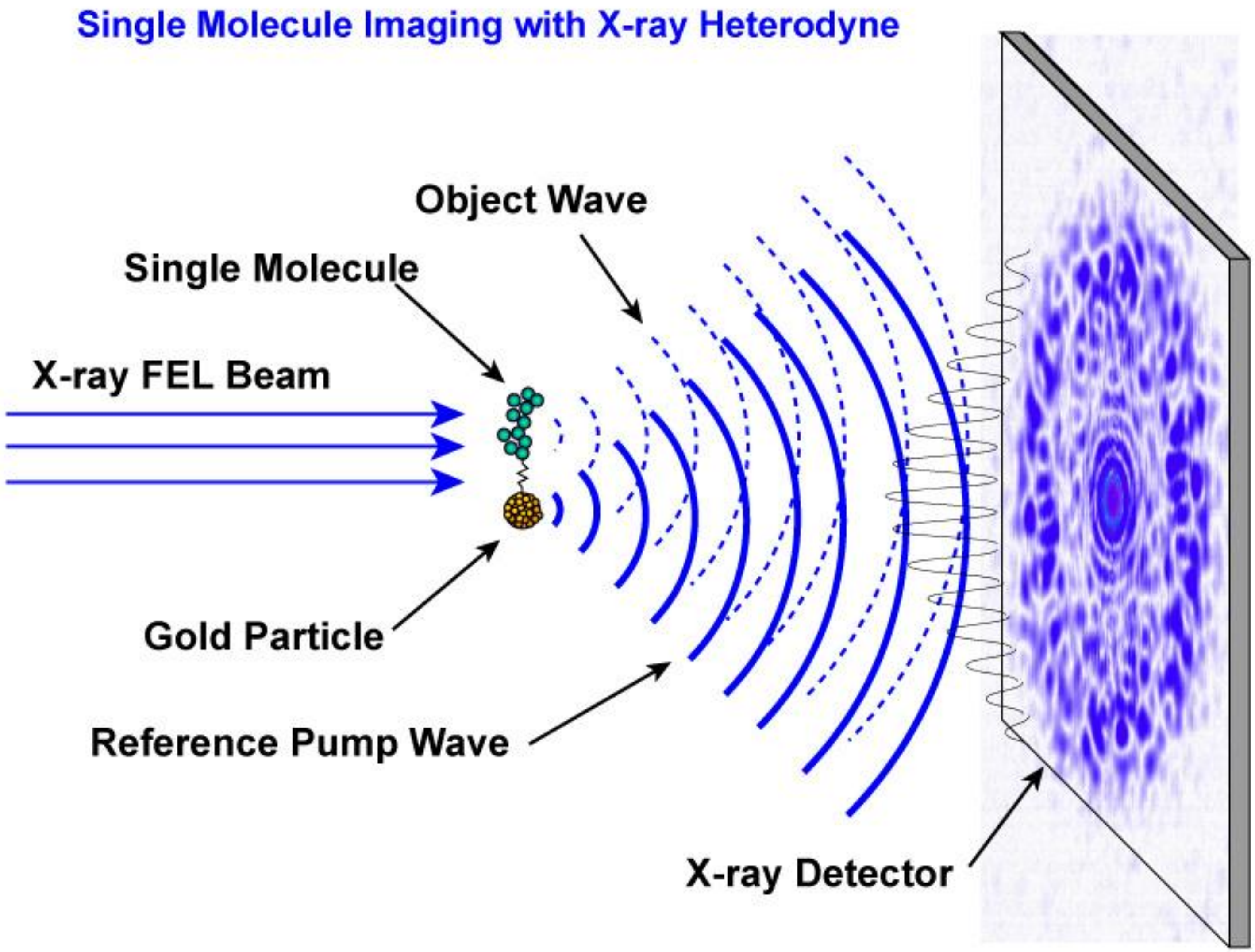


$$\theta = \frac{4\,cm}{1\,m} = 40\,mrad$$

$$P = \frac{\lambda}{\theta} = \frac{532\,nm}{0.04} = 13\,\mu m$$



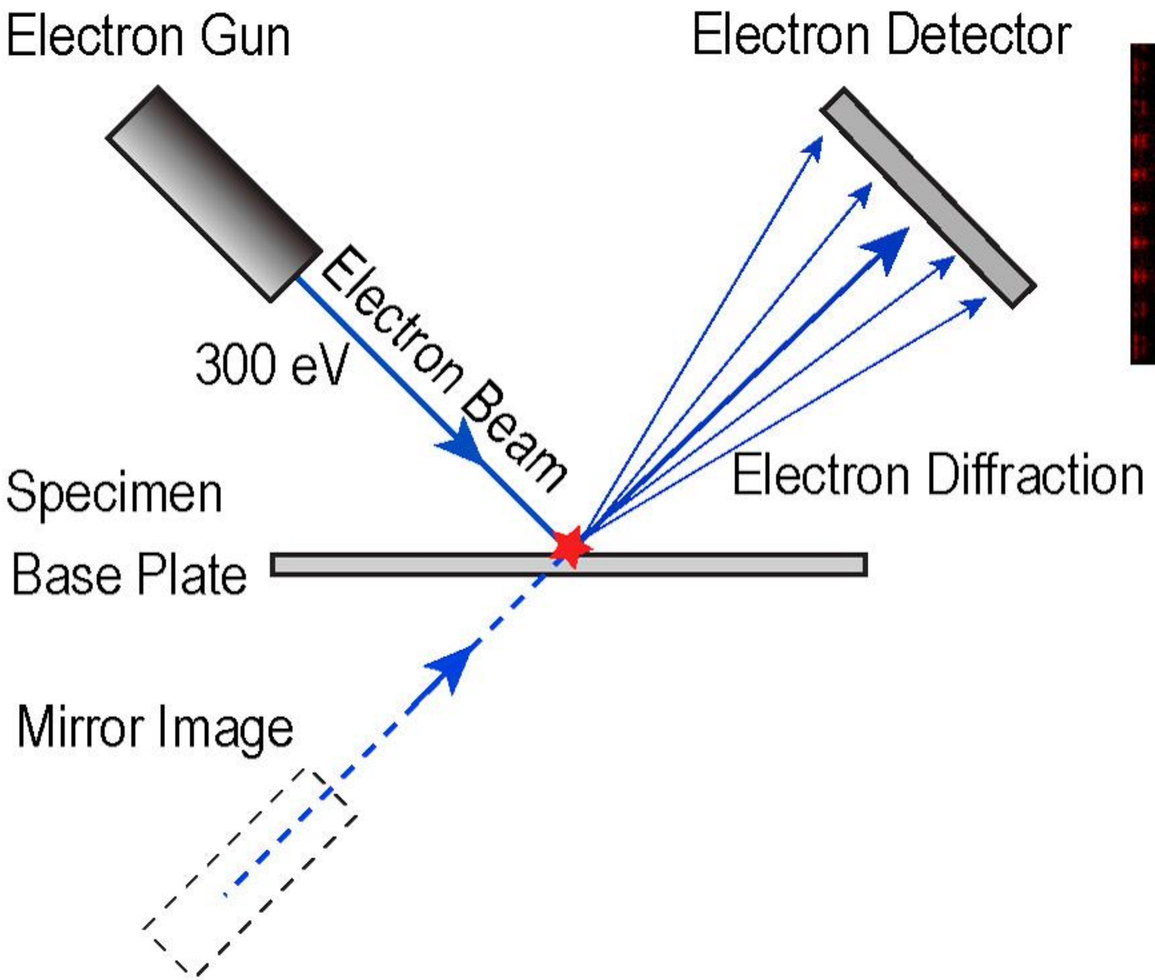


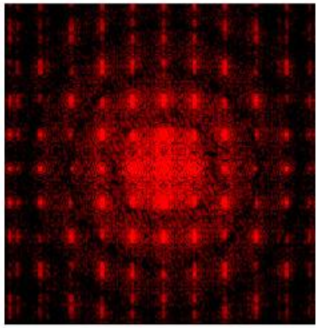


ons.

Shintake, T. 2008. Possibility of single biomolecular imaging with coherent amplification of weak scattering X-ray photons. Physical Review E 78: 041906

Diffraction Electron Interference





Tsumoru Shintake
shintake@oist.jp

To reduce sample damage → lower electron energy

- For atomic resolution microscopy, a prove wave has to be 1Å or shorter wavelength.
  - X-ray of 1 Å has 12 keV quantum energy (energy transfer is quantized at 12 keV)

$$E = \hbar\omega = h\nu = \frac{hc}{\lambda}$$

  - Electron of 1Å de'Broglie wave has only 150 eV kinetic energy.

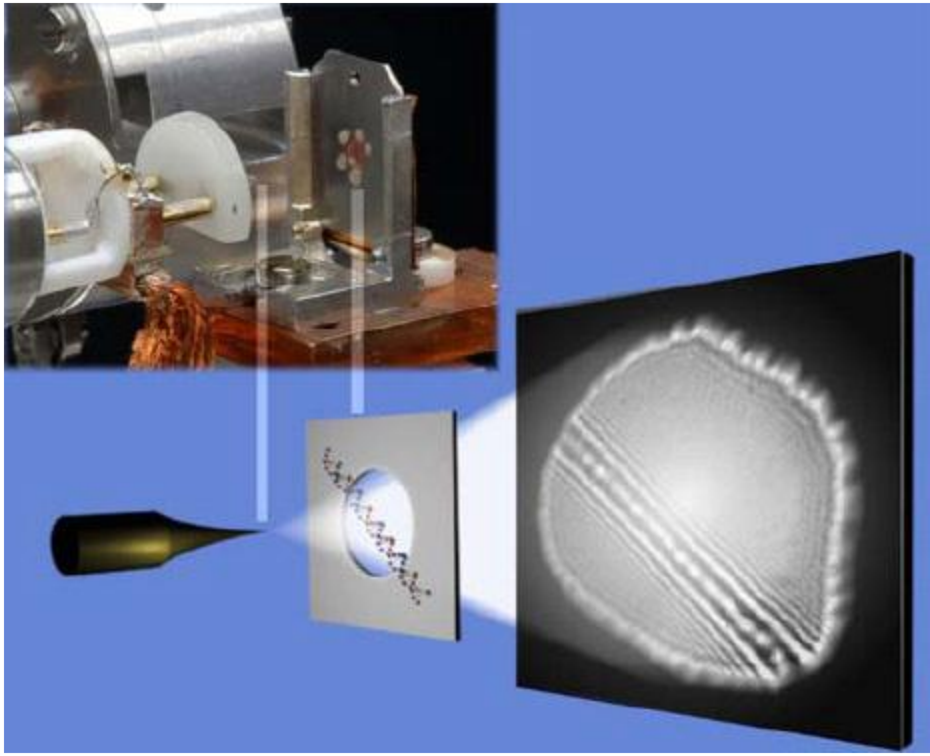
$$\lambda = \frac{h}{\sqrt{2mE}}, \qquad \lambda[\mathrm{A}] = \sqrt{\frac{150}{E[\mathrm{eV}]}}$$

→
  - Energy deposition of 150 eV single electron absorption is 80 times smaller than 12 keV single photon absorption.
  - 150 eV is 2000 times lower than 300 keV TEM.

Tsumoru Shintake
shintake@oist.jp

Low energy electron reduces sample damage.

LEEP: Low Energy Electron Point Source Microscope



- DNA molecule was exposed on coherent low energy electrons.
- 10 eV~ 300 eV, 200 nA
- DNA survived for a hour.
- 10^6 electrons/A^2
- at 260 eV damage started.

Matthias Germann, et al.
"Non-destructive Imaging of Individual Bio-Molecules", Nature 2009

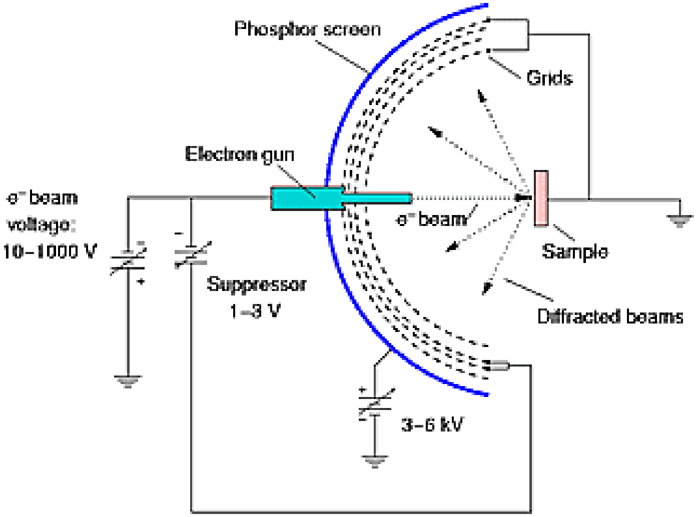
@Institute of Physics,
University of Zurich

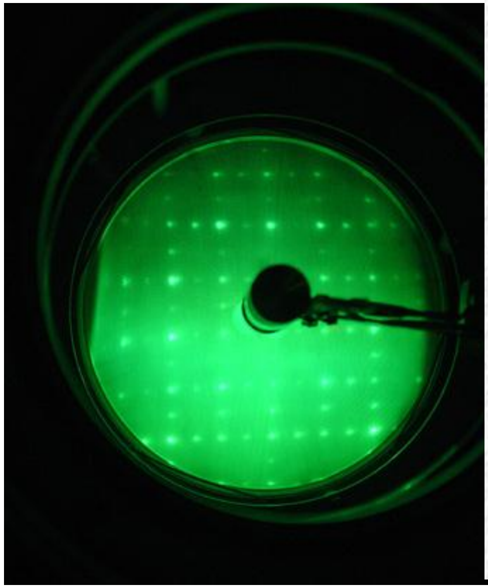
LEED : Low Energy Electron Diffraction

- LEED provides surface structure information through Braggs diffraction.

LEED-Gun

SPECS Inc.





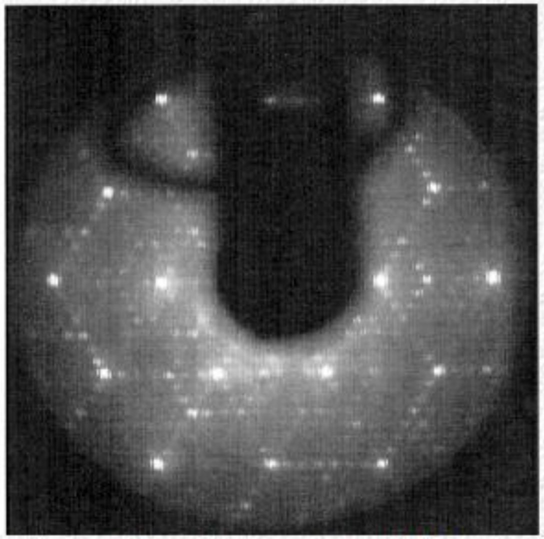
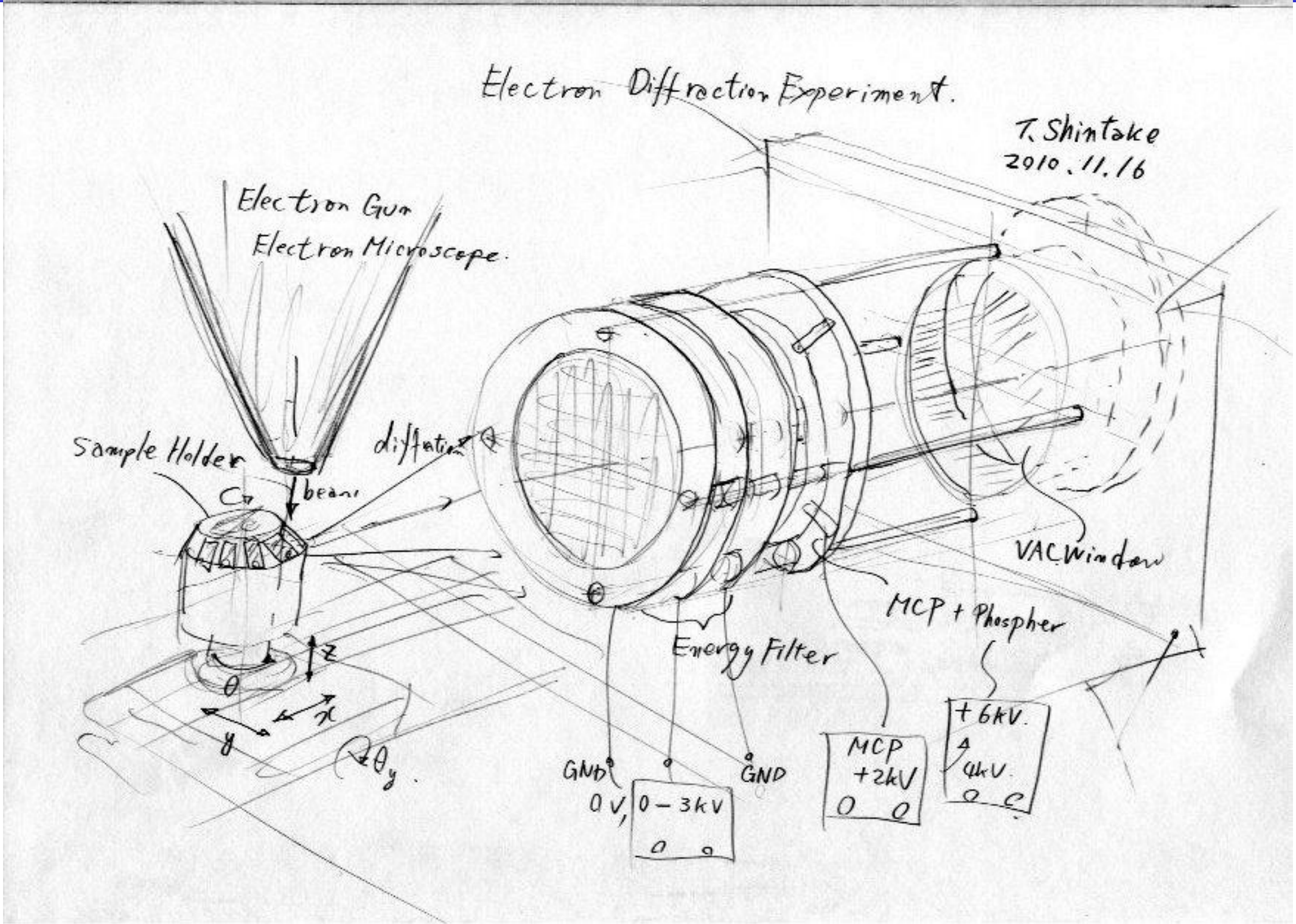


Figure 2.4: A LEED pattern for Si{111}7x7, at 100 eV. The dark object in the middle of the screen is the sample holder.

LEED pattern of a Si(100) surface.

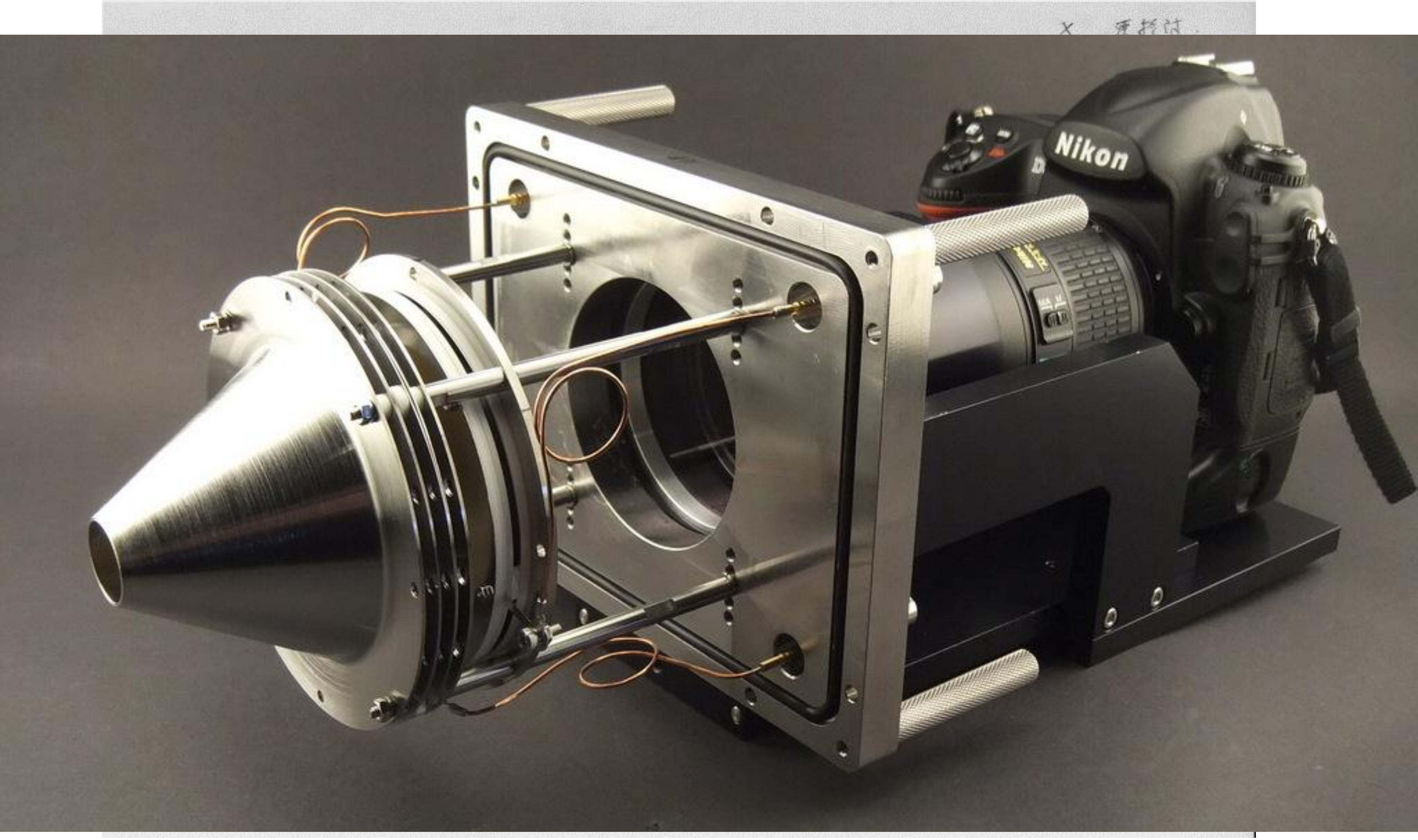
Tsumoru Shintake
shintake@oist.jp

Proof of Principle Experiment

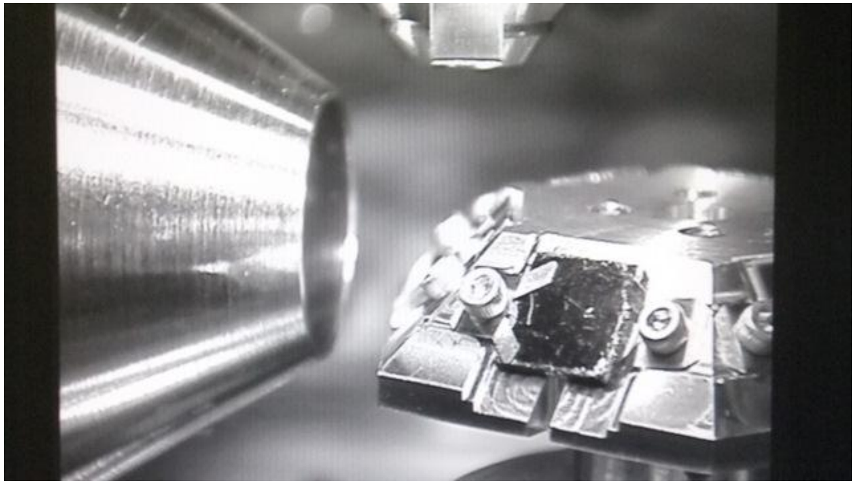


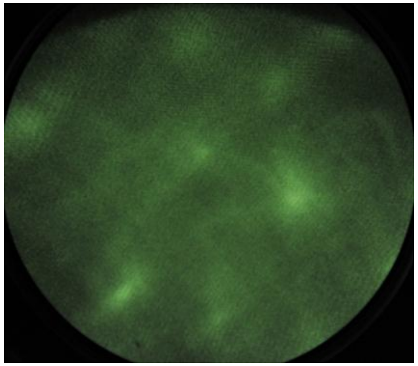
Tsumoru Shintake
shintake@oist.jp

Diffraction Electron Detector



Testing at SII Nanotech. 2011 Feb.



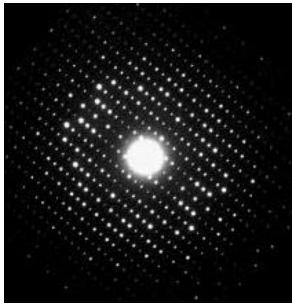


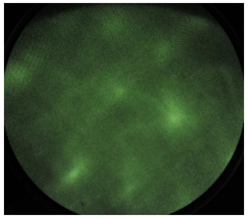
Diffraction from HOPG
(graphne)

Tsumoru Shintake
shintake@oist.jp

Finding Problems → Cure

- Diffraction from HOPG (Graphene) is not clear enough to go holography.
  - Contamination of carbon on electron beam results in poor diffraction.
  - Improve vacuum condition by ion-cleaner→ need space in front side→ transmission type will be better, less charge up, less contamination in backside.
- Resolution of MCP+P34 phosphor is poor and noisy. → Maybe it is due to secondary electron generation from energy filter grid → grid less design and CCD type detector.



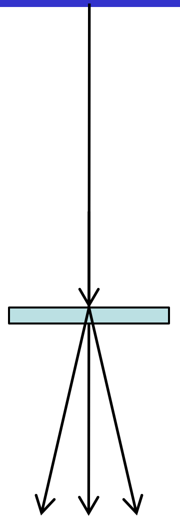


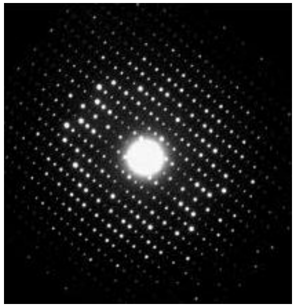
- 16 Megapixel resolution
- USB 2.0 interface
- 60 x 60 mm2 imaging area
- 1:1 external fiber optic
- 100% fill factor
- Scientific Grade 1 device

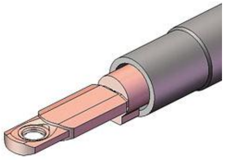
Tsumoru Shintake
shintake@oist.jp

Solution and Direction

- Change: Reflection type → Transmission type
  - This is mathematically simpler and easier.
  - Less path length in the sample material
    - Lower multiple reflections.
  - It requests a little higher electron energy
    - Need to care sample damage by cryo-system.
- It requests new configuration of SEM
  - Custom design → Need extra cost.
- Need to cool sample (electron energy is higher)
  - Cryo-sample holder cost.







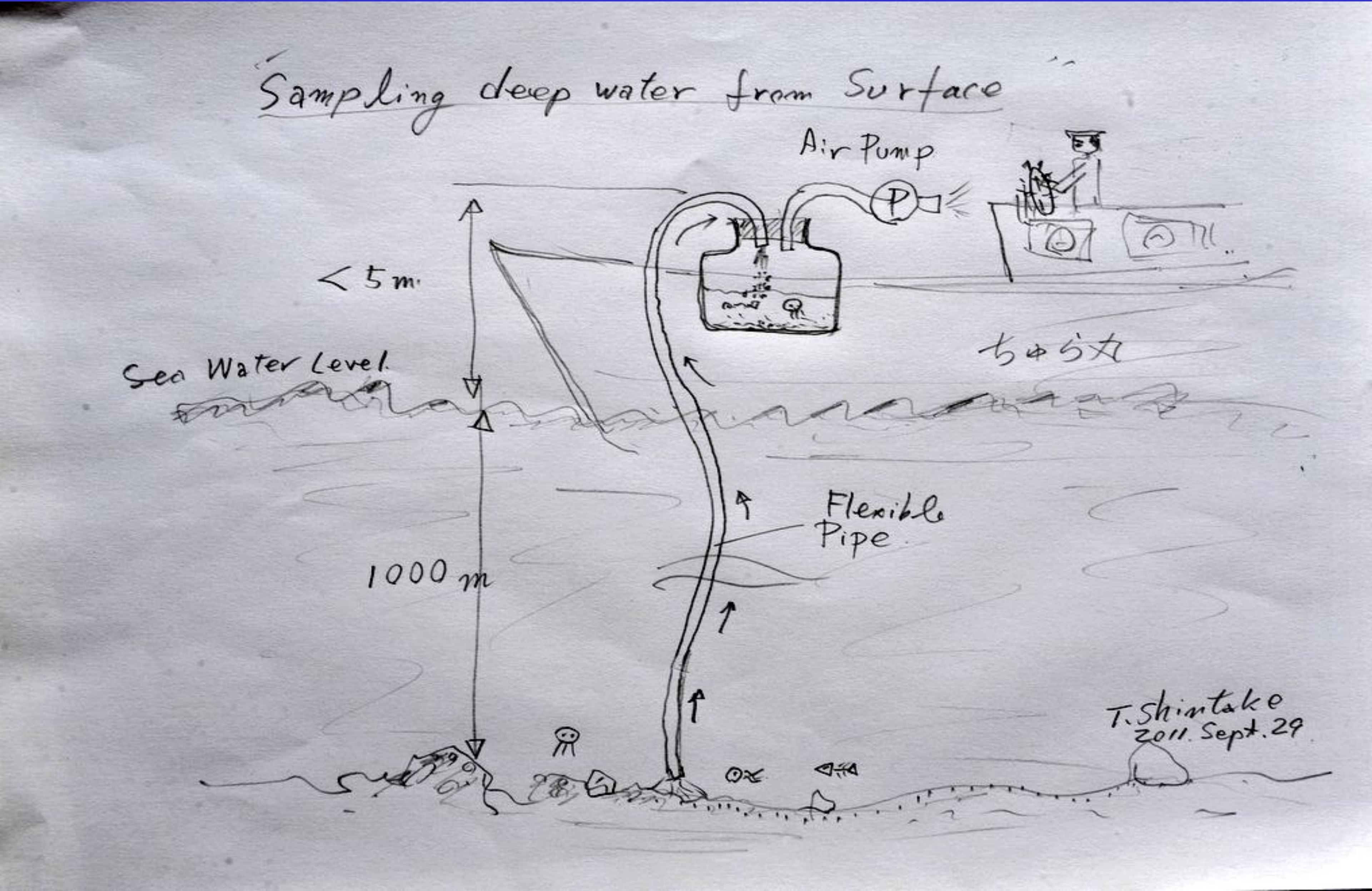
Tsumoru Shintake
shintake@oist.jp

R&D Program

2011	2012	2013	2014	2015
Proof of Principle Test Using Gemini SEM at SII	**Proof of Principle Test Using Merline SEM at OIST**	**First Dedicated Machine**		**For Biology**
HOPG, Au base	DNA, TMV	Reading DNA	Protein Nasno-Crystal	Bio-cell

Tsumoru Shintake
shintake@oist.jp

Proposal: New Project by Mitarai&Shintake Unit



Tsumoru Shintake
shintake@oist.jp

Under Sea "Element Map"

- Searching Minor Metals
  Minor metals: Ni、Cr、Co、Mn、W、Mo、
  Rare earth: Sc, Y, La, Ce, Pr, Nd, Sm, Eu

→ We detect by X-ray fluorescence (XRF)

SEA1200VX - High Sensitivity Element Monitor

- Searching petroleum
  far future, we go Natural energy
  but Short term (~30 years), we still rely
  on petroleum (probably, not nuclear energy).

Censo (Sicily), Italy

→ Seep gas (CH4, CnHn) detection
  by photo-absorption analyzer.

VA-3000/VS-3000 Multi-component Analyzer

→ Map with GPS

Tsumoru Shintake
shintake@oist.jp

We need efforts to teat CO2

Oil Field

Directly feed exhaust gas to bio-reactor ?

Power
Plant

Or, send back CO2 to deep sea water?
3000 m deep, form CO2 lake.

Tsumoru Shintake
shintake@oist.jp