ESRF Detector Workshop, Grenoble, 13-14 February 2003

Detectors for Synchrotron X-ray Protein Crystallography

Soichi Wakatsuki

Photon Factory, IMSS, KEK, Tsukuba, Japan

Outline

1. Background: structural genomics and beyond
- New national project of Protein 3000
- Glycosylation and transport of proteins

2. Detector requirement for modern synchrotron X-ray protein crystallography

3. Detectors for future experiments: nano crystals/single molecule structual analysis using next generation X-ray sources: an attempt of HARP detector

PF

Currently 35 Structural Genomics Projects Worldwide

Internationl Structural Genomics Organization

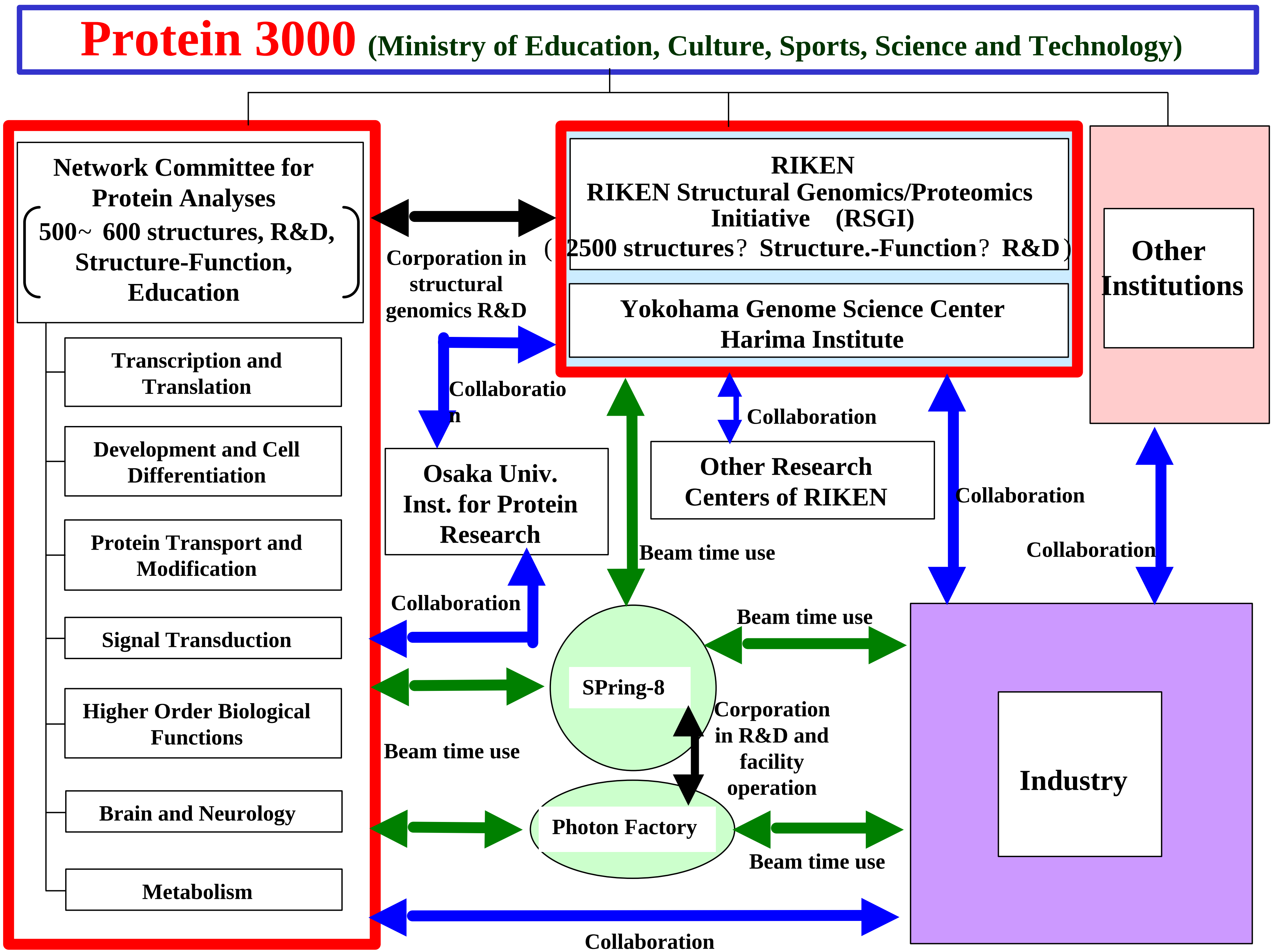
http://www.isgo.org

Structural Genomics and Proteomics Project list

-Worldwide Initiatives -

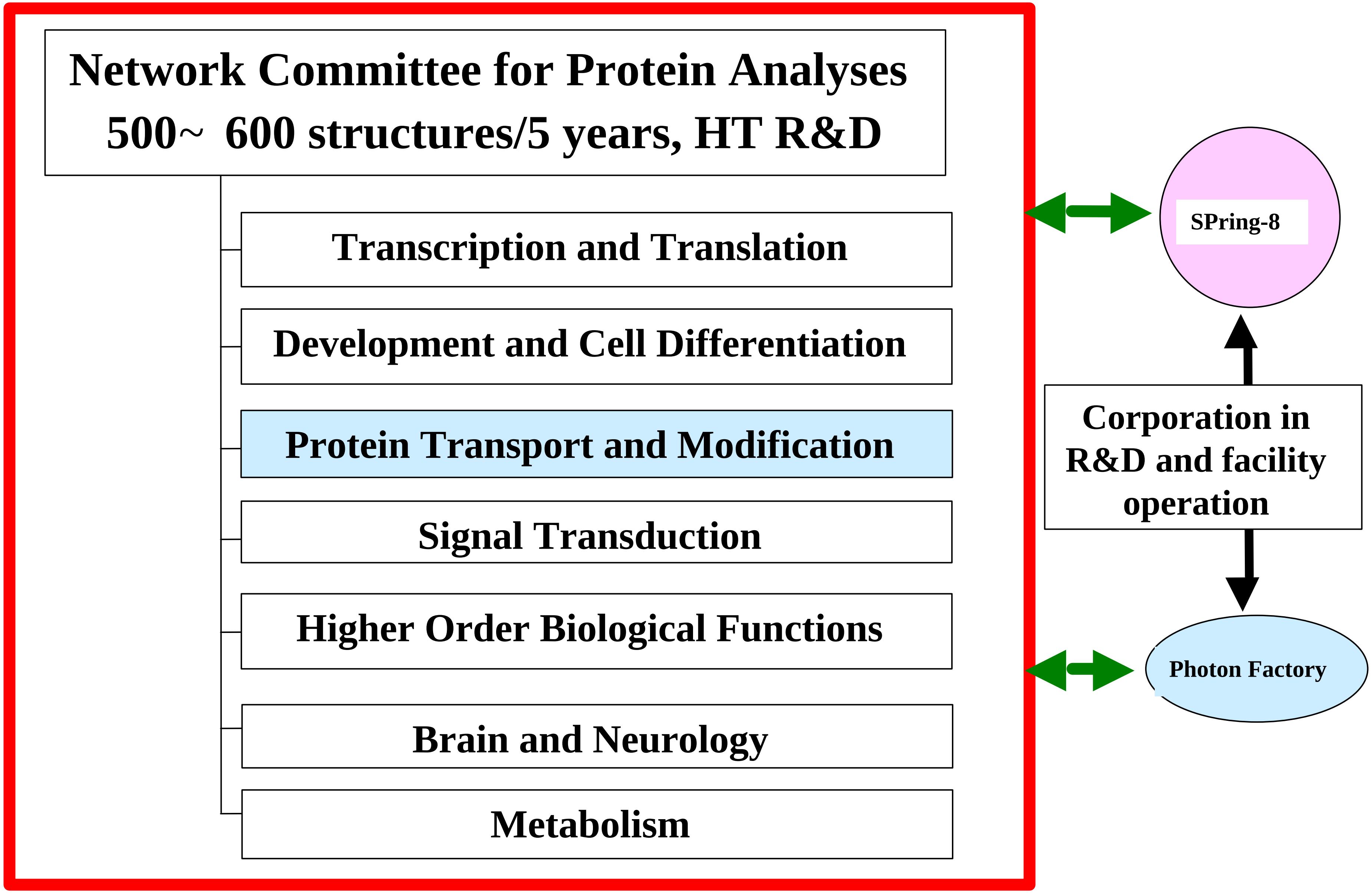
Australia / Canada / EU / France / Germany / Japan / Korea / Switzerland / UK / USA

Australia:	**3 planned**	**Canada**	**4**
EU	**1**	**France**	**4**
Germany	**1 & 1 planned**	**Japan**	**10**
Korea	**1**	**Switzerland**	**1**
UK	**3**	**USA**	**10**



Protein 3000 (Ministry of Education, Culture, Sports, Science and Technology)
Network Committee for Protein Analyses
(500~ 600 structures, R&D, Structure-Function, Education)
Transcription and Translation
Development and Cell Differentiation
Protein Transport and Modification
Signal Transduction
Higher Order Biological Functions
Brain and Neurology
Metabolism
RIKEN
RIKEN Structural Genomics/Proteomics Initiative (RSGI)
(2500 structures? Structure.-Function? R&D)
Yokohama Genome Science Center
Harima Institute
Other Institutions
Corporation in structural genomics R&D
Collaboration
Osaka Univ. Inst. for Protein Research
Other Research Centers of RIKEN
Collaboration
Collaboration
Collaboration
Beam time use
Collaboration
SPring-8
Beam time use
Corporation in R&D and facility operation
Beam time use
Photon Factory
Beam time use
Industry
Collaboration

8 Consotia: Target oriented structural genomics of Protein 3000 (FY2002-2006)



Tsukuba Structural Biology Consortium

Protein Transport and Posttranslational Modification

KEK, Photon Factory (X-ray)

Nagaoka Tech Univ (X-ray)

Showa Univ (X-ray)

Kyoto University (X-ray)
Pharmacology Department

Structural analysis
Functional analysis

Nagoya City Univ. (NMR)

Osaka Univ (MS)

Structural Genomics related R & D
Overexpression and purification
Large scale automate crystallization
Crystal handling robots
Microfocus MAD beam lines
Next generation area detectors
High-throughput structure determination
software etc.

JAERI, Nara (Bioinfo)

Kyoto University
Pharmacology Department

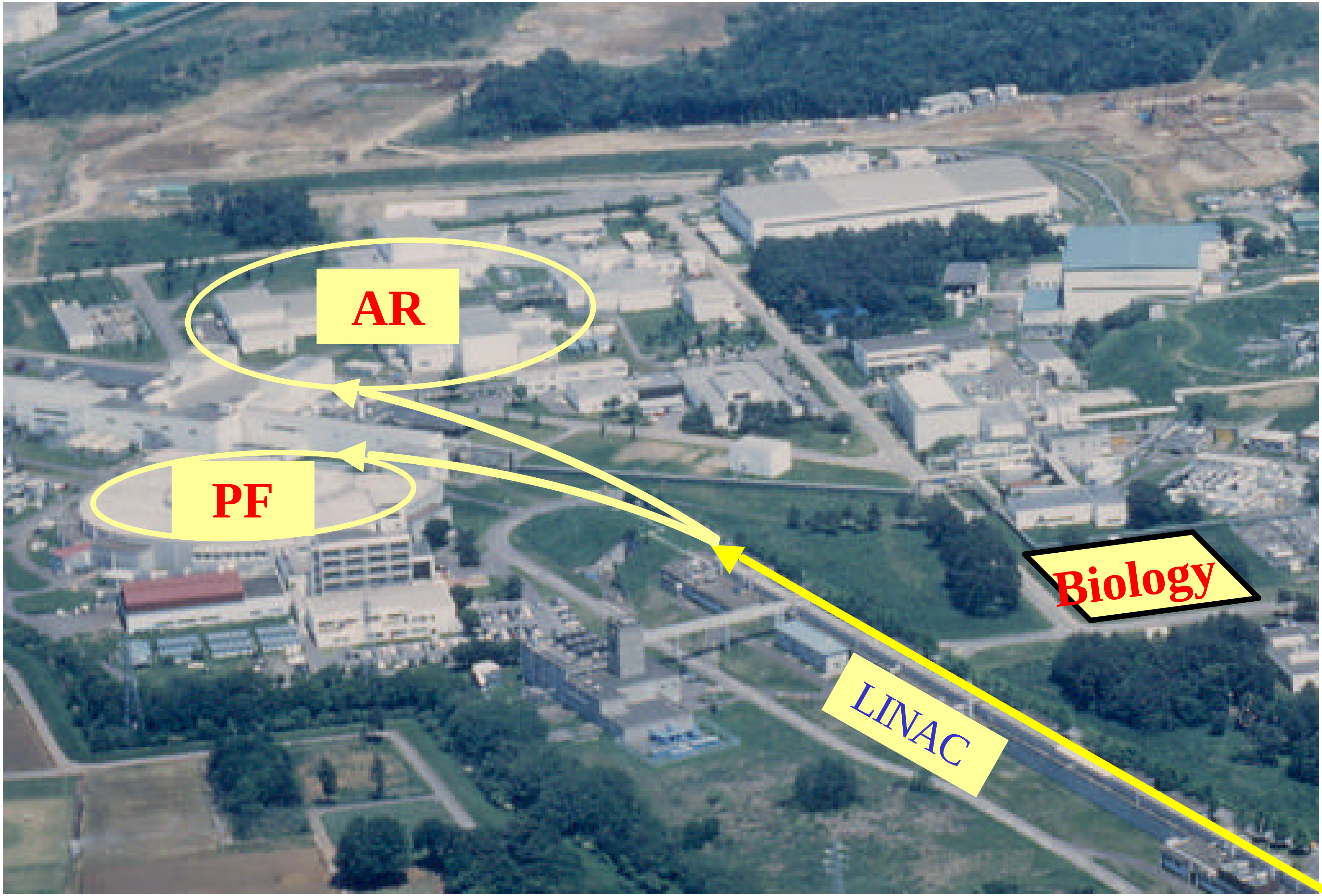
Okazaki, Natl. Institute of
Physiological Sciences

Riken, Membrane
Traffic Group

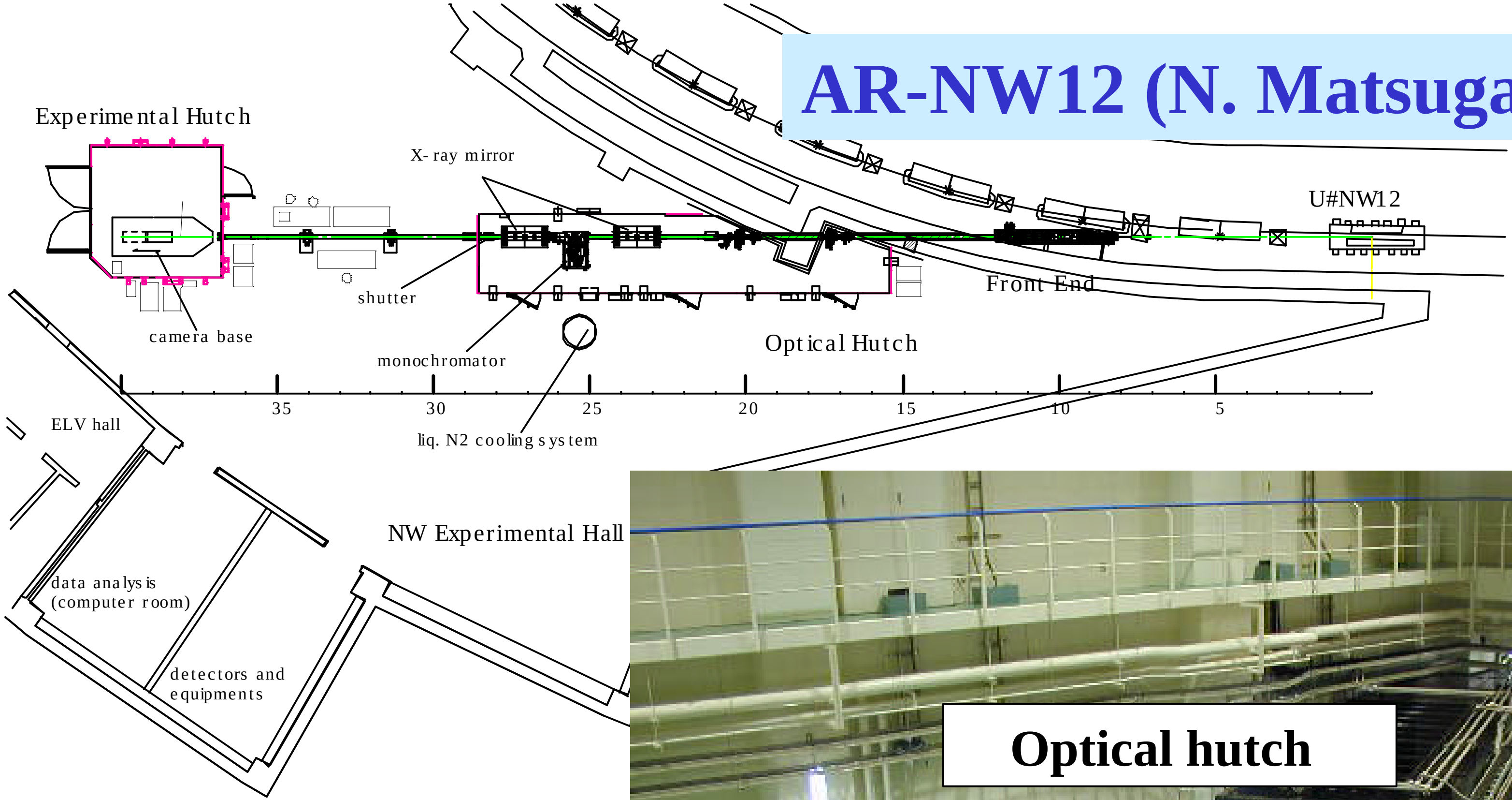
NAIST,
Molecular Biology Lab

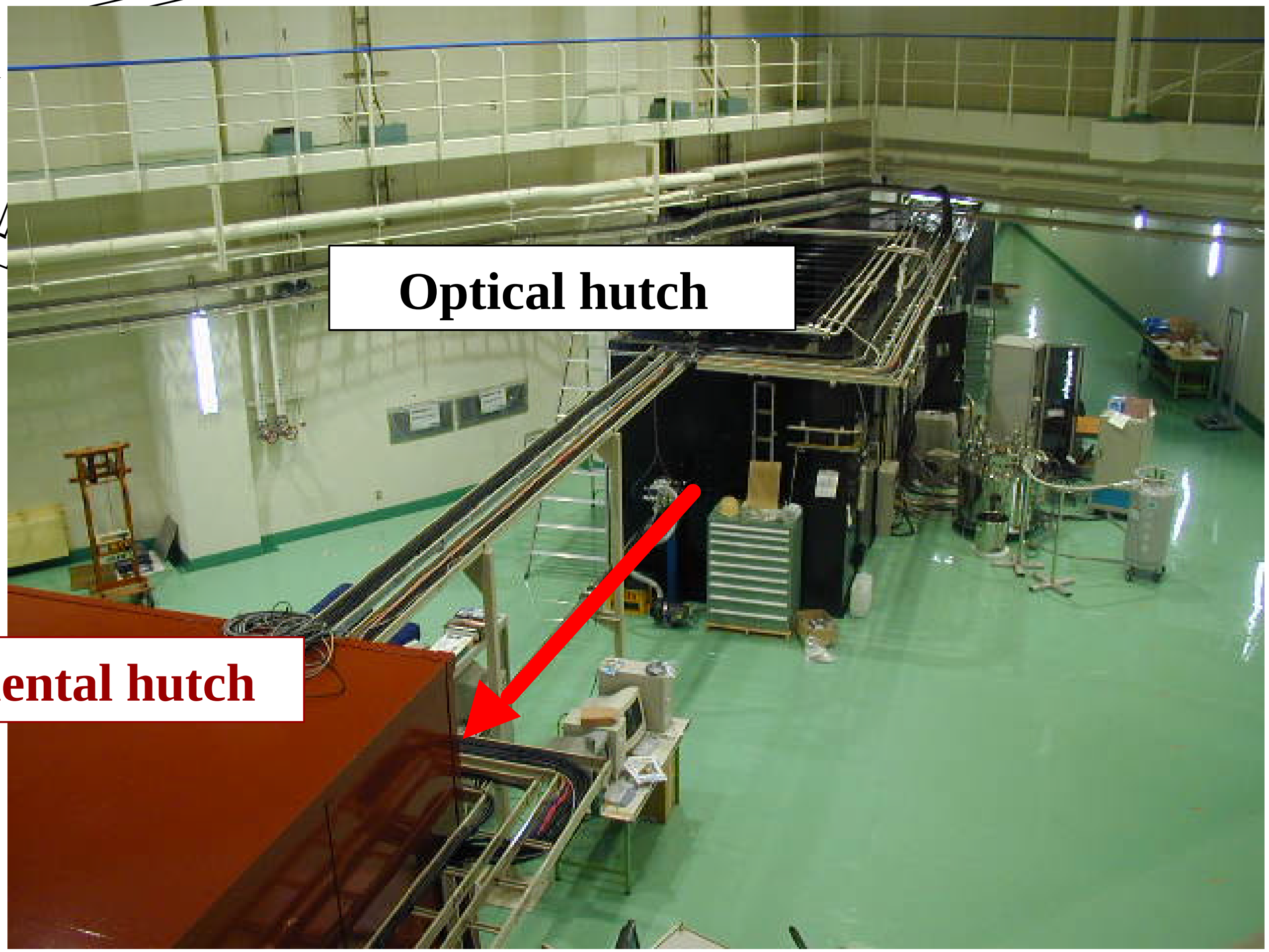
Osaka University
Medical School

Kanazawa University,
Pharma & Cancer Institute

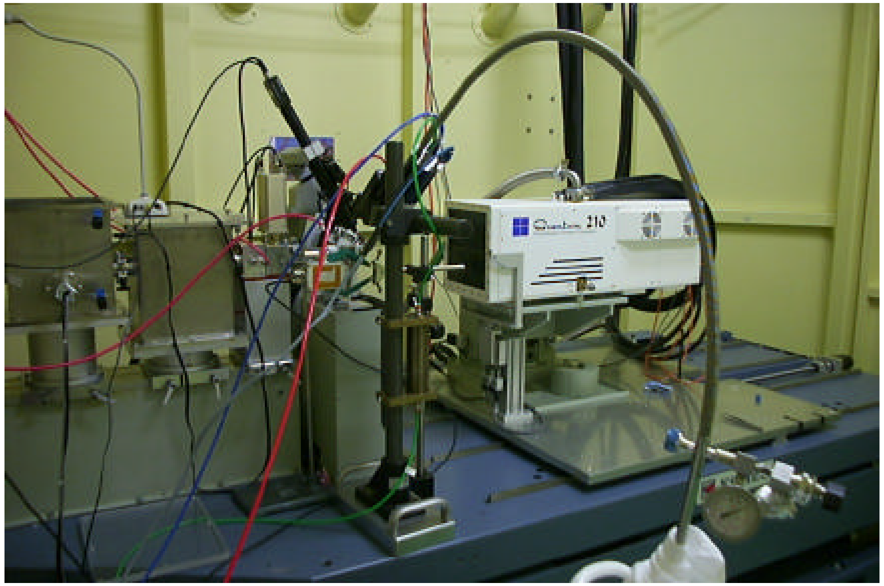

AR
PF
Biology
LINAC

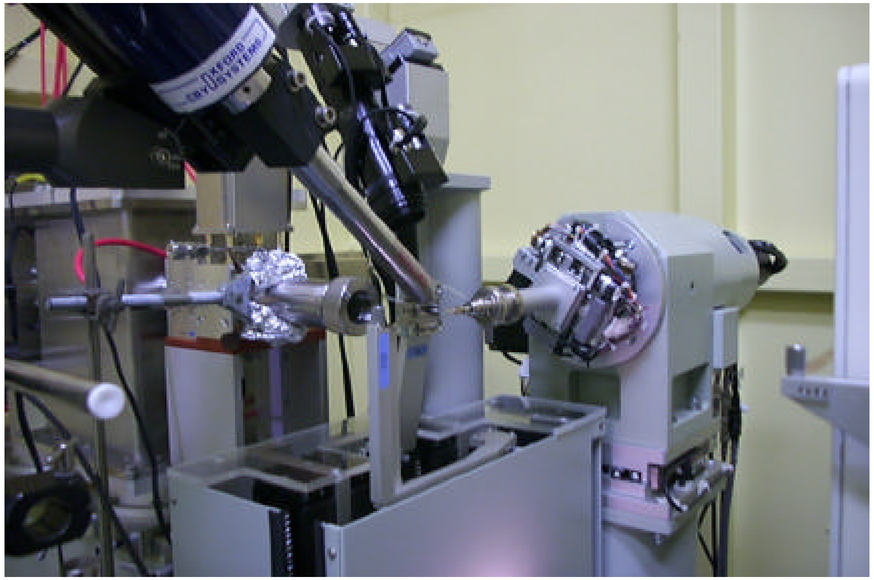
AR-NW12 (N. Matsugaki et al.)



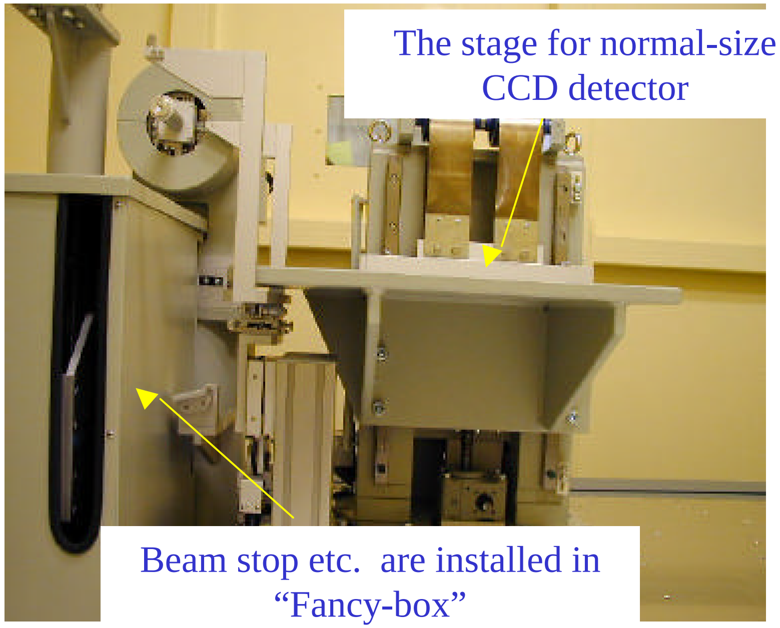


NW12 experimental table (N. Igarashi et al.)





Maximum sphere of confusion is 2.2 microns at the sample position

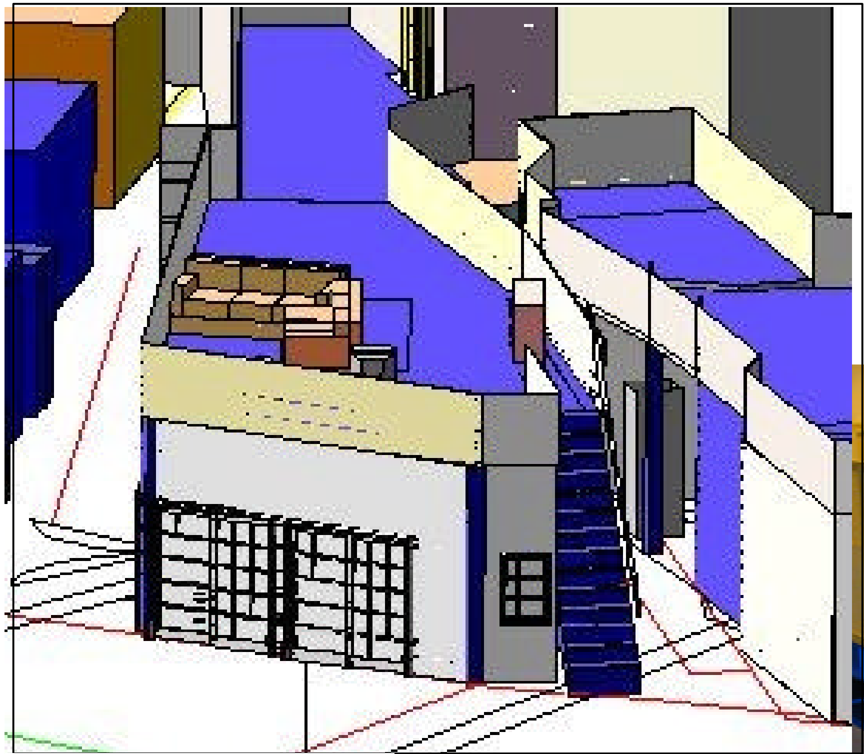


New MAD Beam Line BL5 (M. Suzuki et al.)

Multipole wiggler

6 x 10 [11] photons/sec through 200 mm x 200 mm

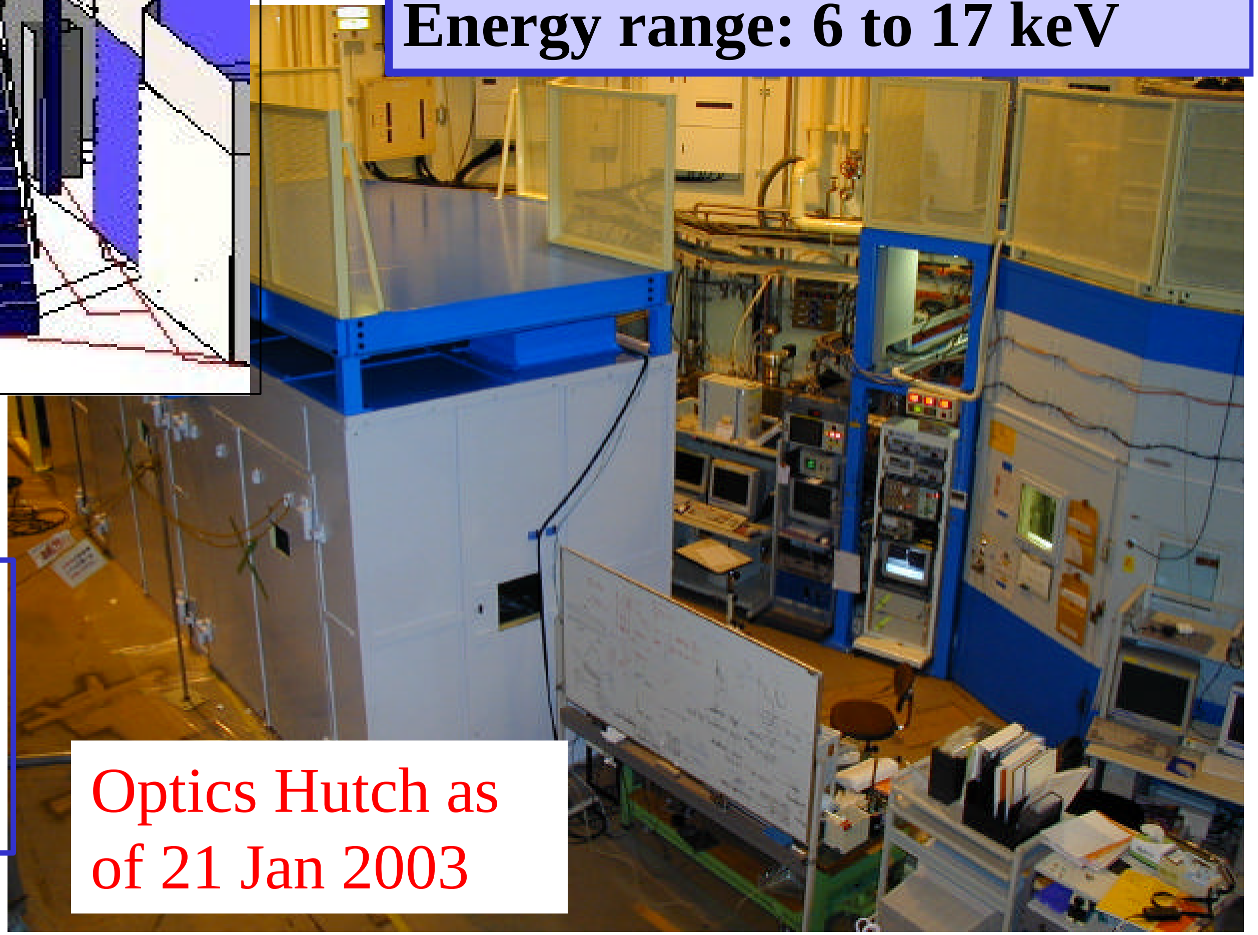
Energy range: 6 to 17 keV



Experimental Hutch

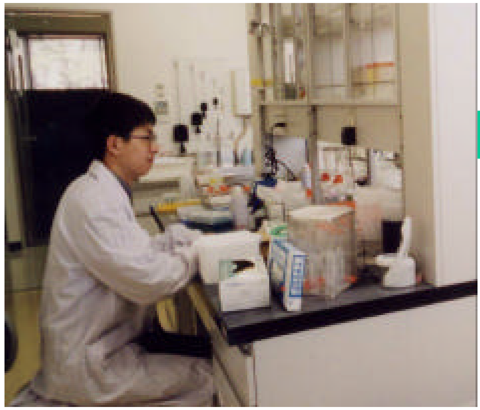
Detector size: ~300 mm

~30% of beamtime for SG projects



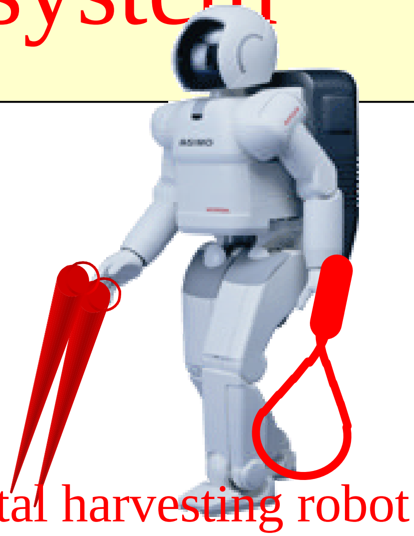
Optics Hutch as of 21 Jan 2003

Future: automated/integrated system

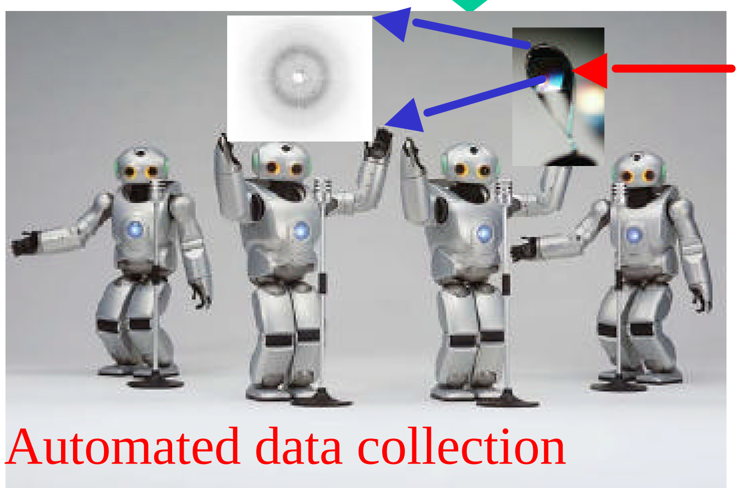


Expression and purification

Crystallization

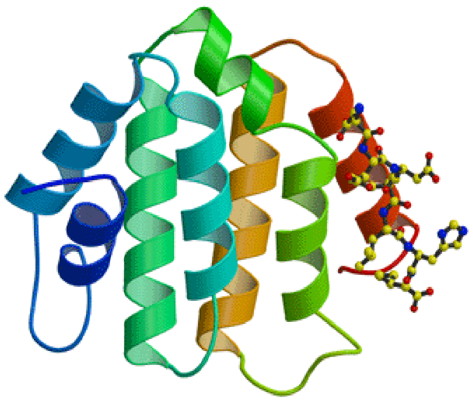


Crystal harvesting



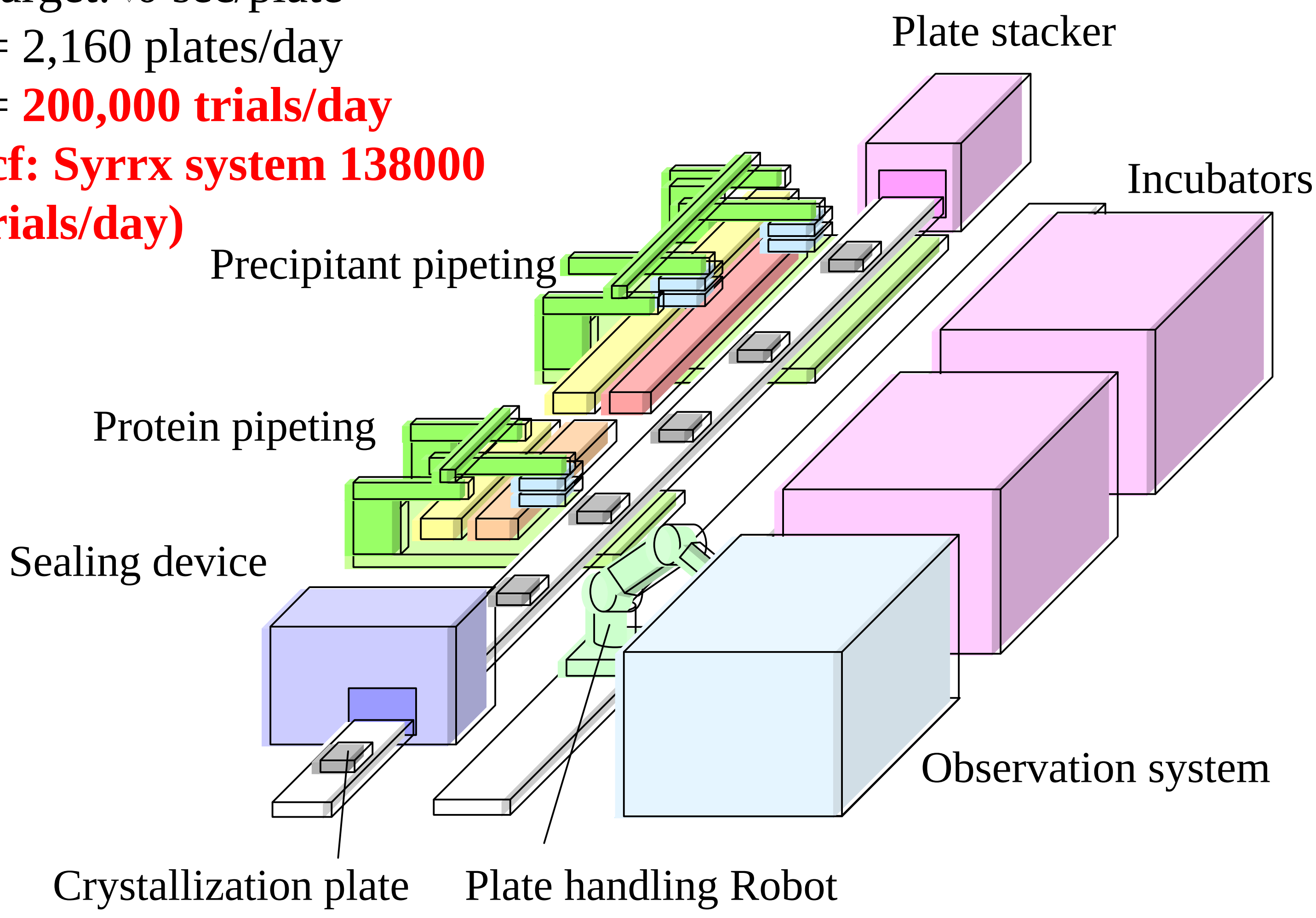
Mouting & data collection

Data analysis

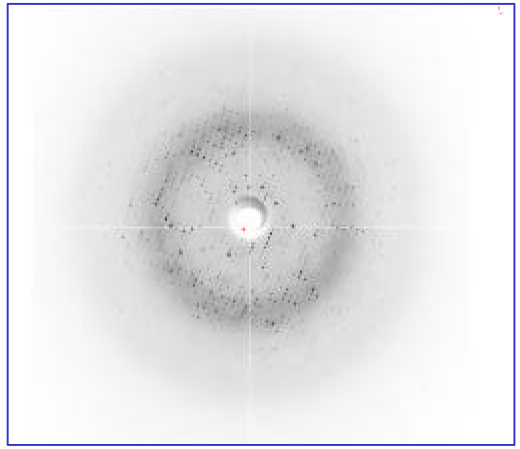


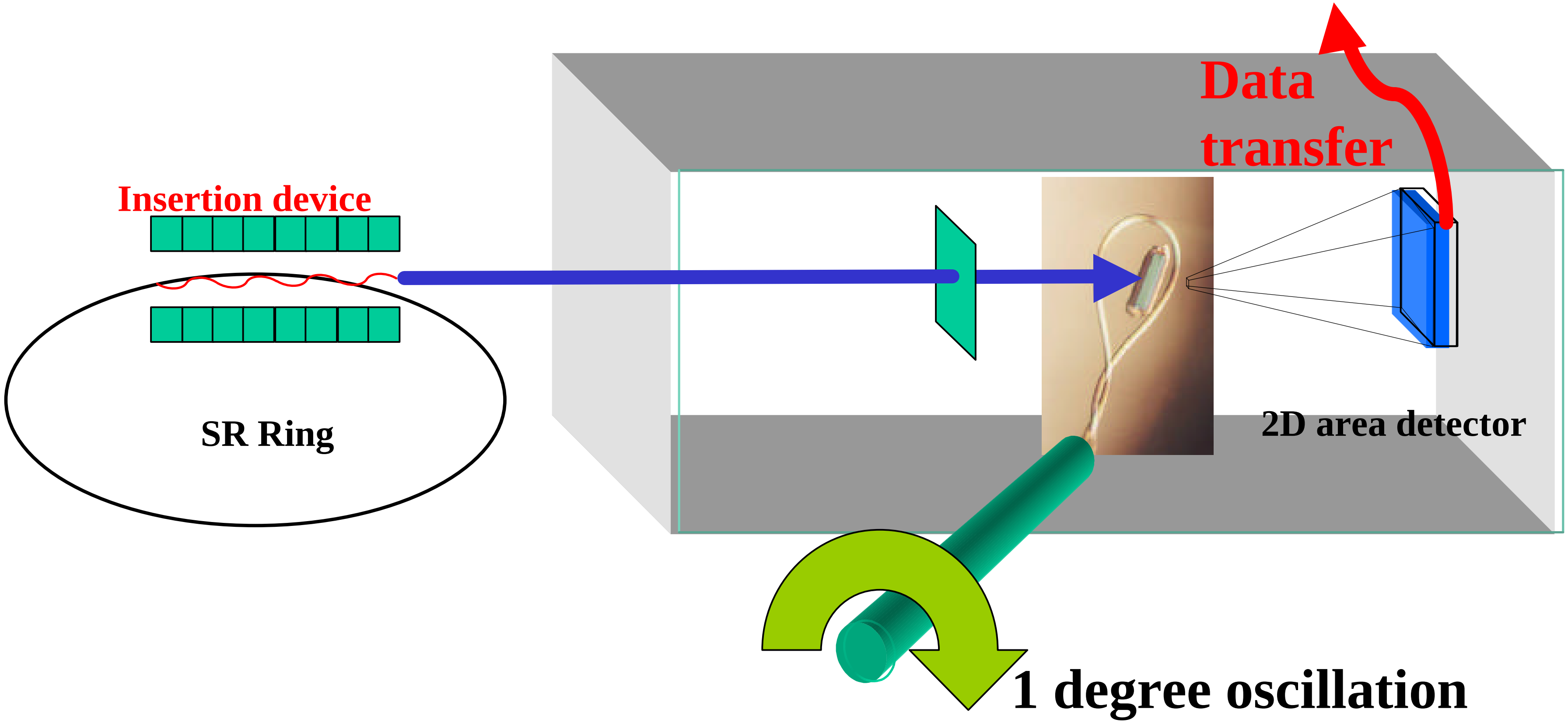
Protein Crystallization and crystal observation robot system to be finished by Autumn 2003

Target: 0 sec/plate
= 2,160 plates/day
= **200,000 trials/day**
(cf: Syrrx system 138000 trials/day)



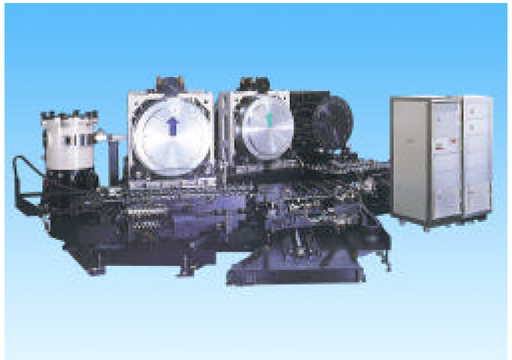
Oscillation method:
(PRESENT)
(eg. 90 X 1 deg oscaillation)



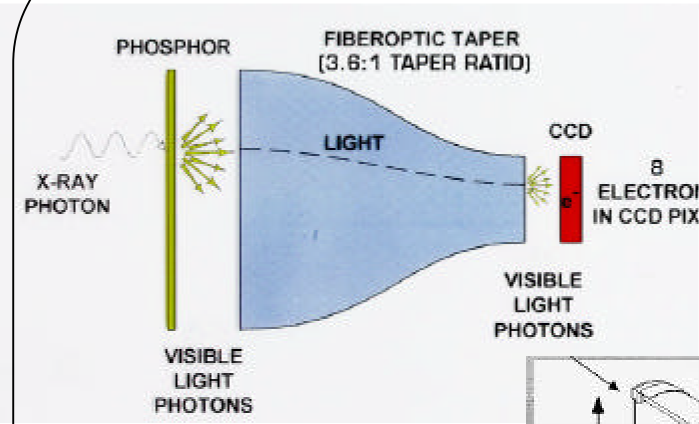


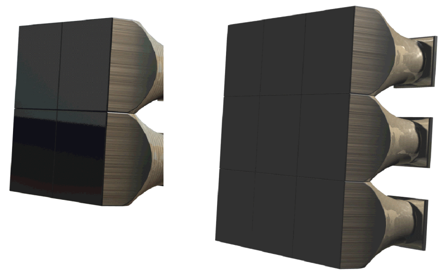
X-ray Area Detectors for Synchrotron X-ray Protein Crystallography

Imaging Plates

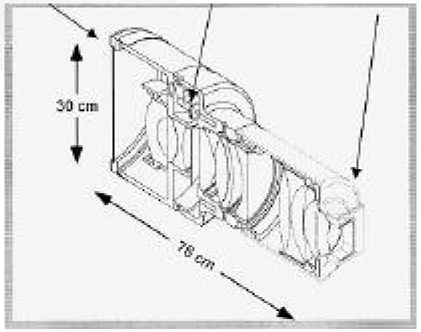


Tapered fiber optics CCD



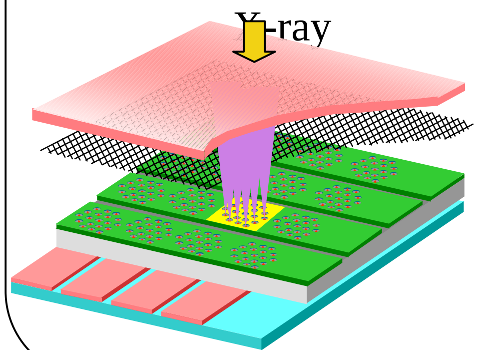


Lens-coupled CCD

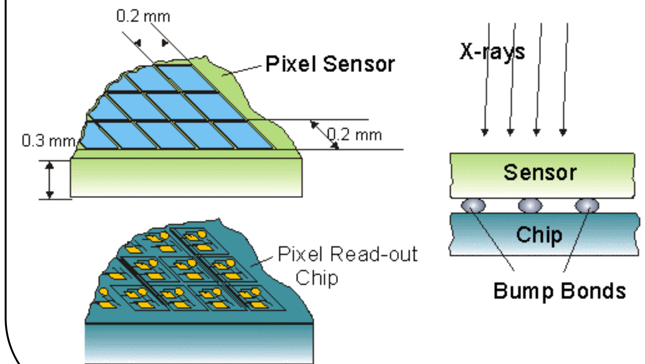


Flat Panel Detectors

HARP based Field Emitter Array (FEA)



Pixel Array Detectors (PAD)



X-ray Area Detectors for Synchrotron X-ray Protein Crystallographic Data Collection

Detectors	Pros	Cons
On-line imaging plates	Large dynamic range, large area	Slow readout (20 to 200 sec per image) -> poor duty cycle Relatively broad PSF Relatively inexpensive
Off-line imaging plates	Large dynamic range, large area	Slow read-out (20 to 200 sec per image) -> poor duty cycle Relatively broad PSF Cumbersome to handle
Tiled, tapered fiber optics CCD	Fast readout (0.3 to dozens of secs)	Limited dynamic range (~<16 bits) Expensive to cover large solid angle
Lens-coupled CCD	Large active area (300 mm diameter) Inexpensive	Has gone into market very recently, and not yet established. Large (1 m long) and heavy (100 kg)
Flat Panel Detectors	Inexpensive, very light (~7 kg) Large active area (easily 400 mm square) Fast readout (a few seconds)	Inherent problem of noise
Pixel Array Detectors (PAD)	Extremely good PSF Extremely Fast readout	Still under development Difficult to tile the components to cover a large solid angle
HARP based Field Emitter Array (FEA)	Extremely sensitive (800 X CCD) Large area and very fast readout Very good PSF	At the very first stage of the development

Detector Requirements for good PX data collection (BL scientists’ view)

- Large, fast, reliable and inexpensive
- Must be an integrated system
  - data acquisition and storage
  - data analysis
  - archiving
- Easy to maintain

Detector Requirements for good PX data collection (common and ideal)

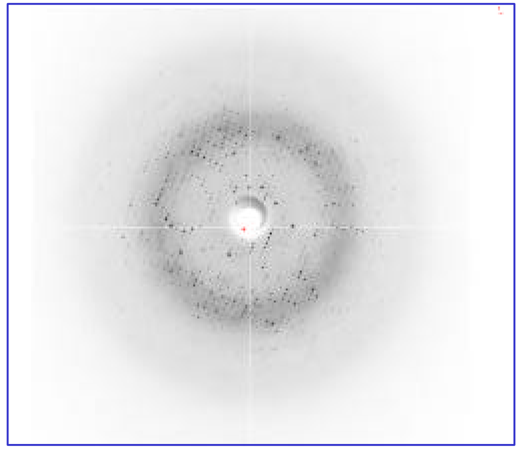
- Number of resoluion pixels: several thousands

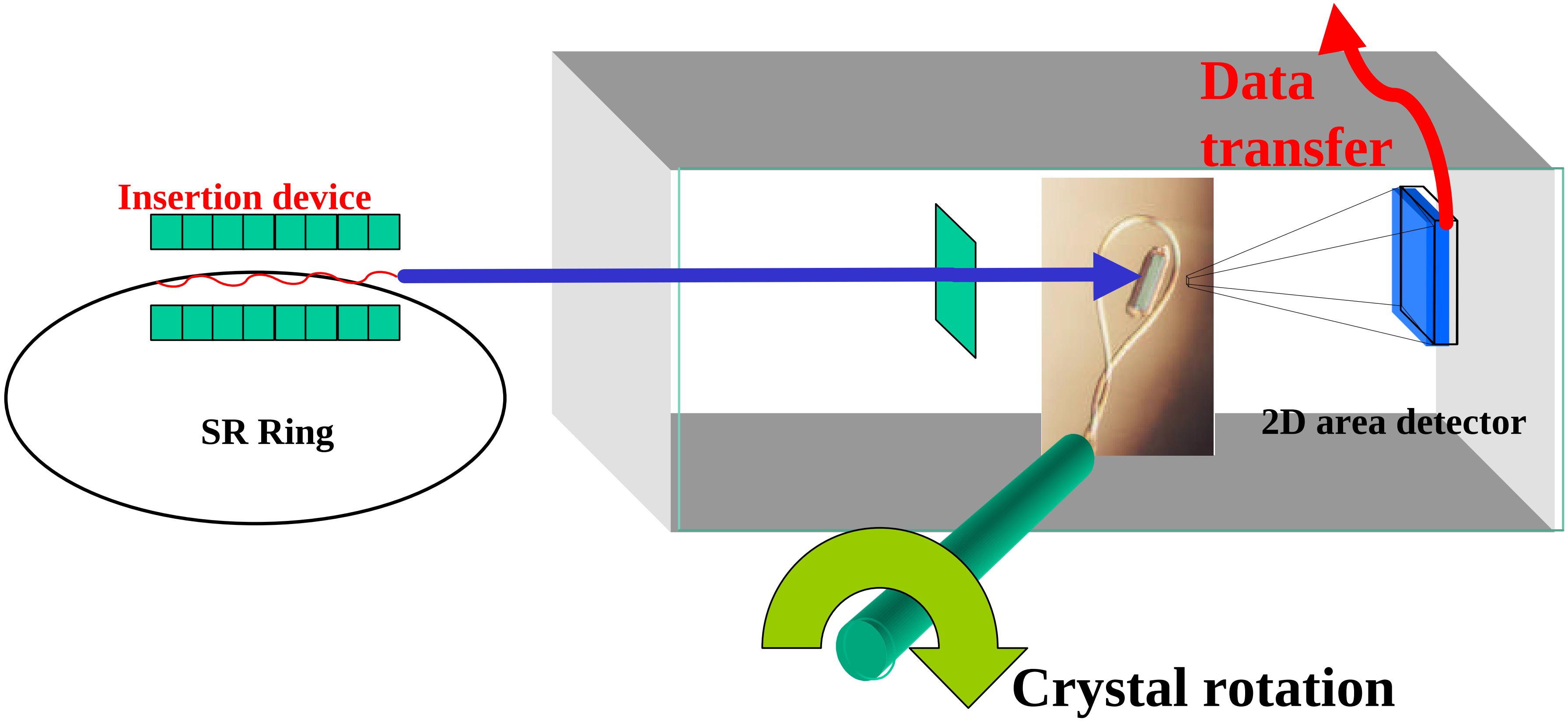
  No. of reflections across the edge

  = 2 * unit cell dimension / resolution limit

  = 100 to 500, 1000
- Active area size: 20 to 40 cm, 80 cm
- Sensitivity: photon counting
- Readout time: 1 to few seconds, 1~30 msec
  - Time to collect one data set: 1 min or less
- Noise level: should allow long exposure (~100 sec for very weakly diffracting crystals)
- Dynamic range: 10^4 or wider (high and low resolution)

Continuous rotation method:
(FUTURE)
(eg. 1 X 90 degs rotation)

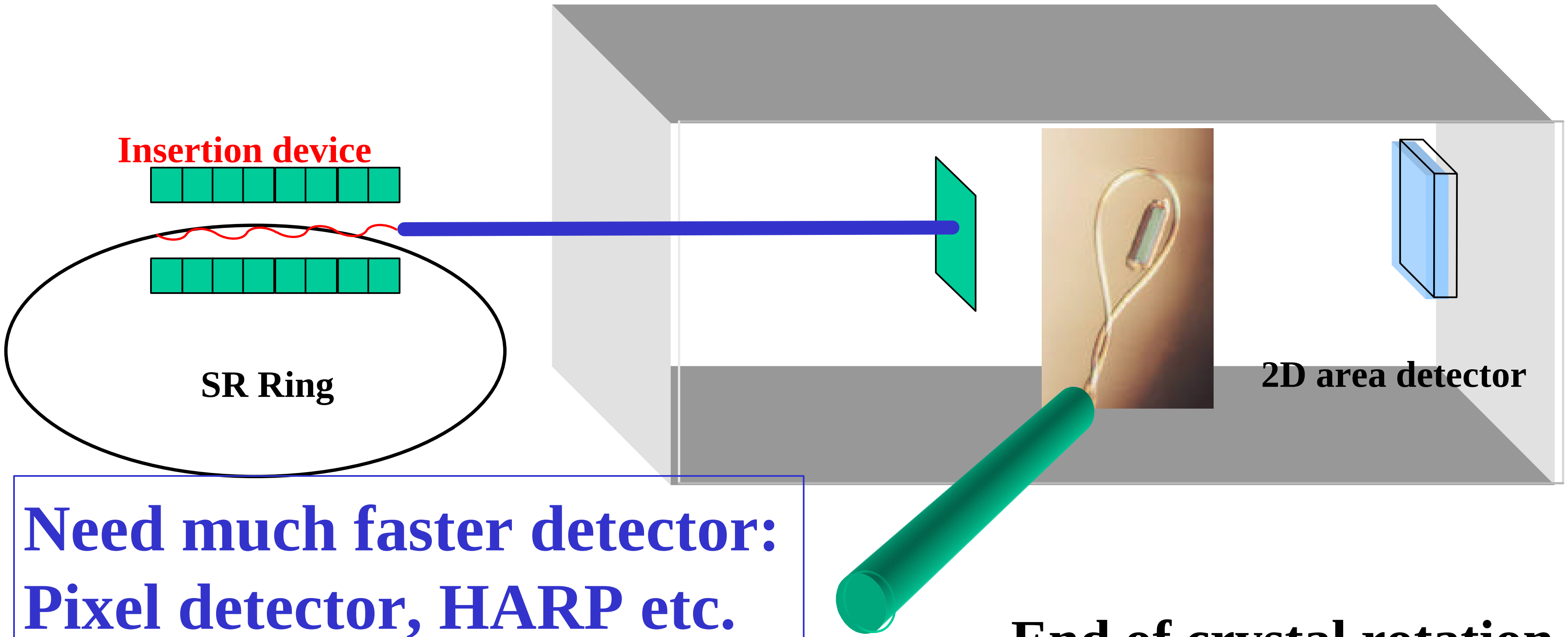




Continuous rotation method: (FUTURE)
(eg. single sweep of 90 deg rotation)

Complete

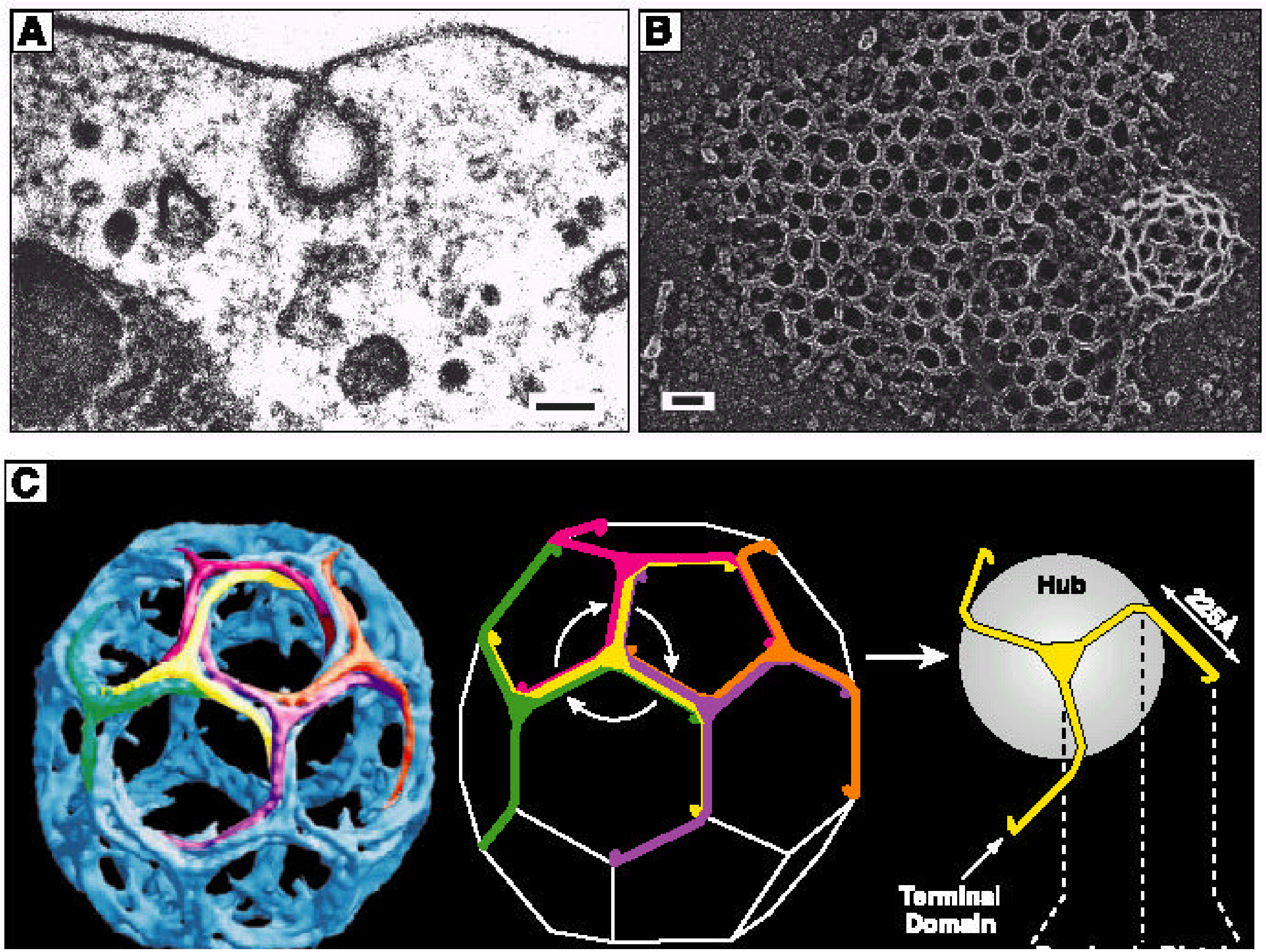
Dataset!



Need much faster detector:
Pixel detector, HARP etc.
+ 3D profile fitting

End of crystal rotation

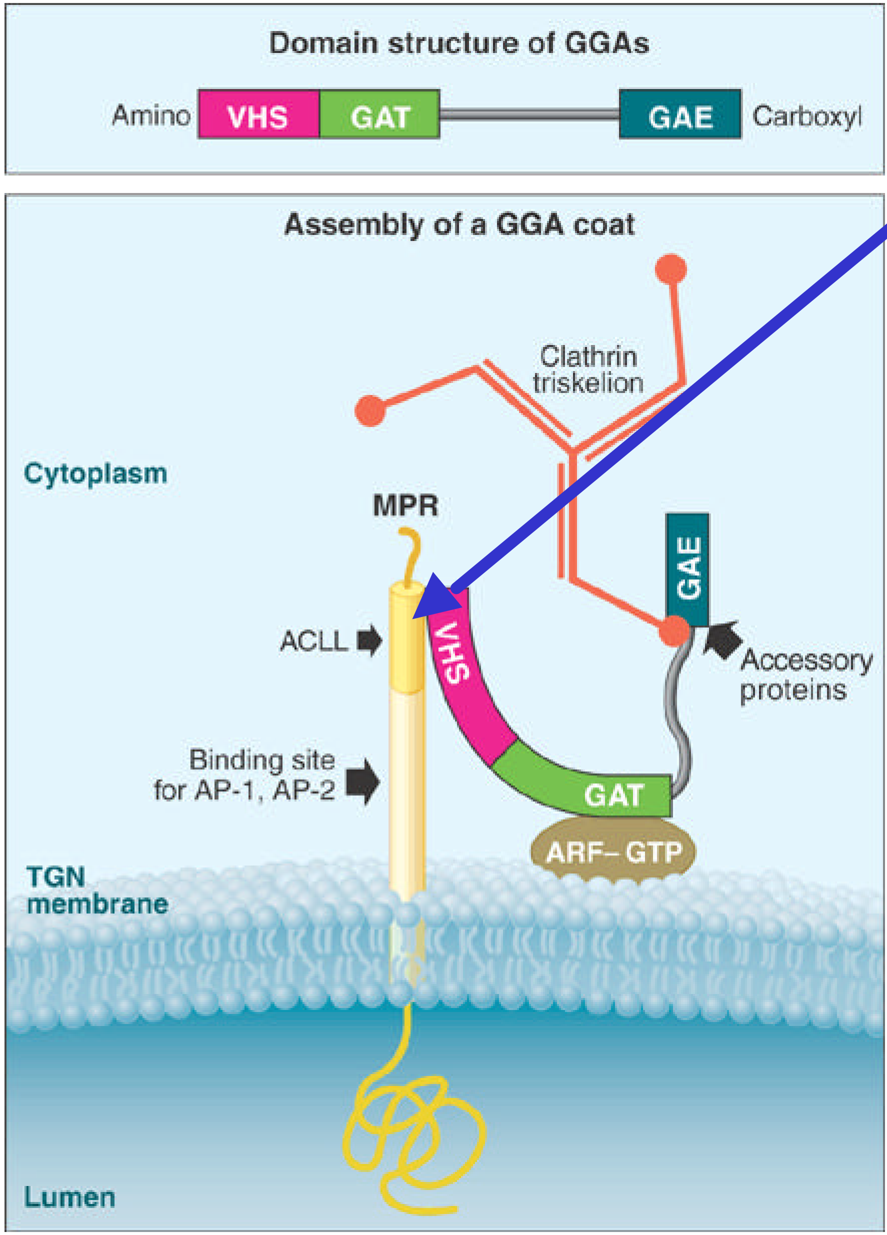
Future: nano crystals and single molecule structural analyses



M. Marsh and H. T. McMahon, Science, 1999, Vol. 285, 215

Clathrin movie by Allison Bruce, Harvard University

http://www.hms.harvard.edu/news/clathrin/



ACLL (acidic dileucin) motif

ACLL Peptides recognized by GGA1-VHS domain

LRP3	-MLEASDDEALLVC
CD-MPR	-EESEERDDHLLPM
CI-MPR	-SFHDDSDEDLLHI
Sort(WT)	-GYHDDSDEDLLE
Sort(DD/NN)	-GYHNNSDEDLLE
Sort(S/A)	-GYHDDADEDLLE
Sort(S/D)	-GYHDDDDEDLLE
Sort(DED/NQN)	-GYHDDSNQNLLE
Sort(LL/AA)	-GYHDDSDEDAAE

b-secretase -QHDDFADDISLLK

Red: acidic residues

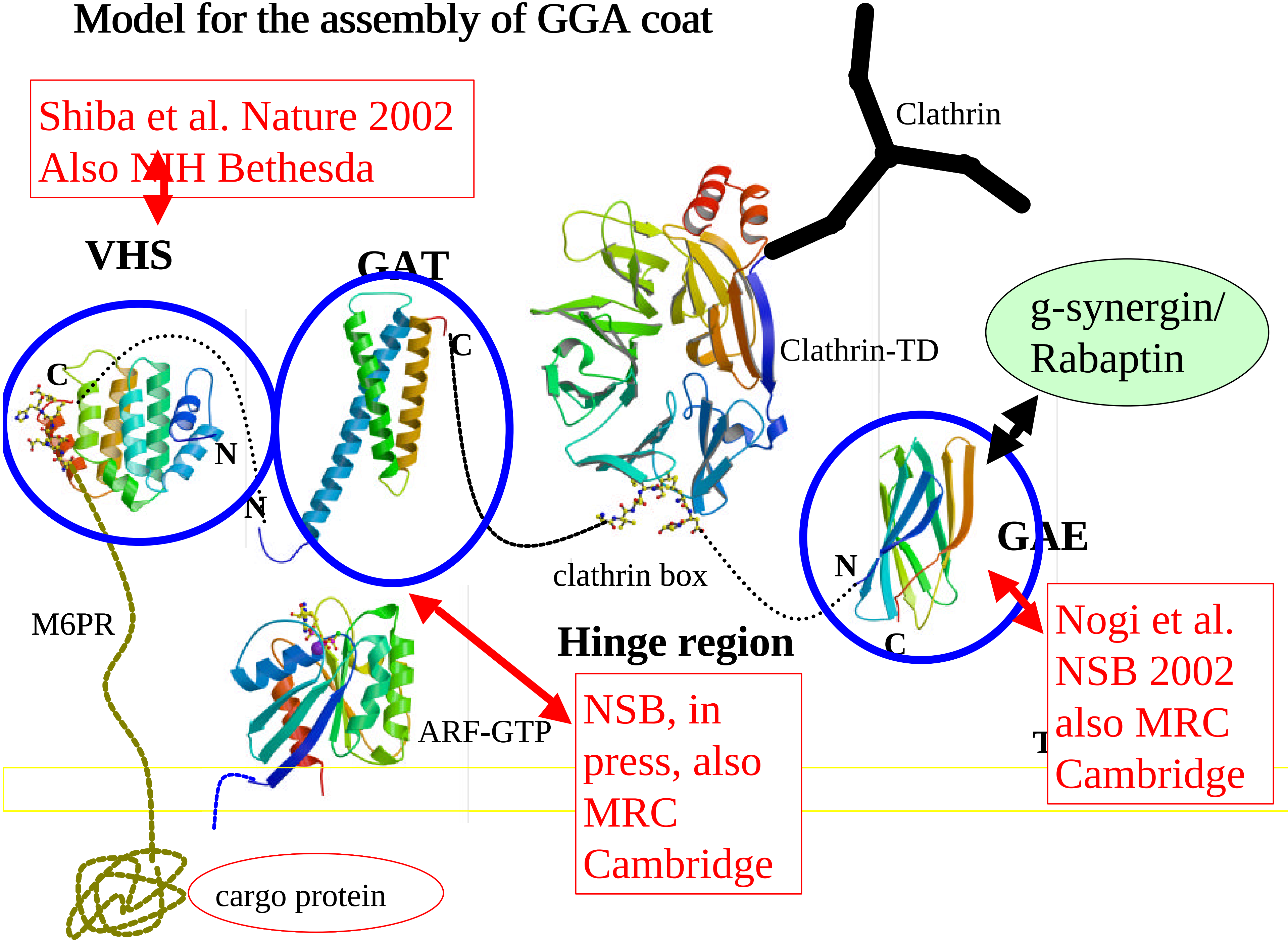
Blue: leucine pairs

Purple: serine residues that can be phosphorylated by CK-II

Takatsu et al, J. Biol. Chem. 276, 28541-28545

From S. A. Tooze, Science, vol. 292, 1 June, 2001

Model for the assembly of GGA coat



Energy Recovery Linac (proposal of KEK, Tsukuba)

Future of structural biology: Single molecule or nanocrystals structural analysis at atomic resolution

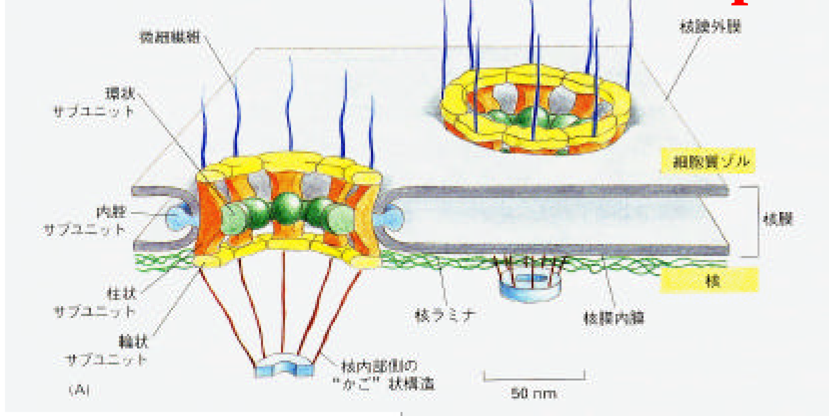
Properties

Low cost operation of multiple beam lines owing to the energy recovery

Brilliance: 1000 to 10000 times of the 3rd generation synchrotrons (ESRF, APS, Spring-8)

Very short pulse length： 100 femto seconds (1/100)

EX 1: Nuclear core complex



EX 2: Nano crystals 10 to 100 nm size

↓

Weak signal and many more images to collect

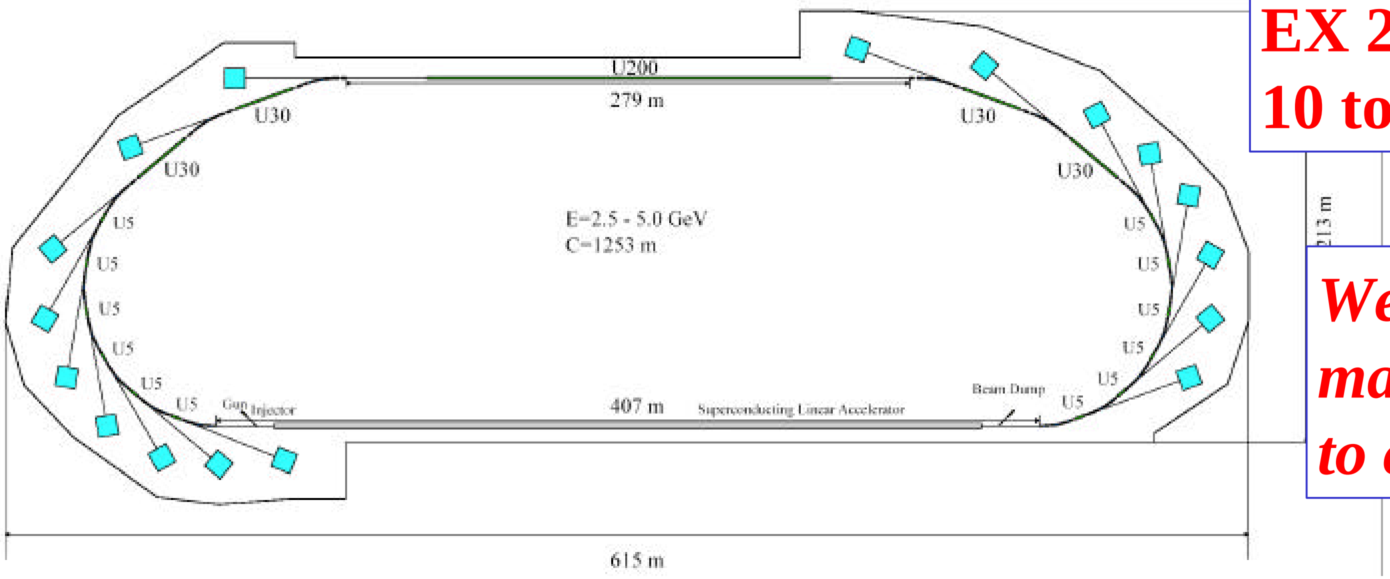


Image comparison of visible light HARP vs. CCD

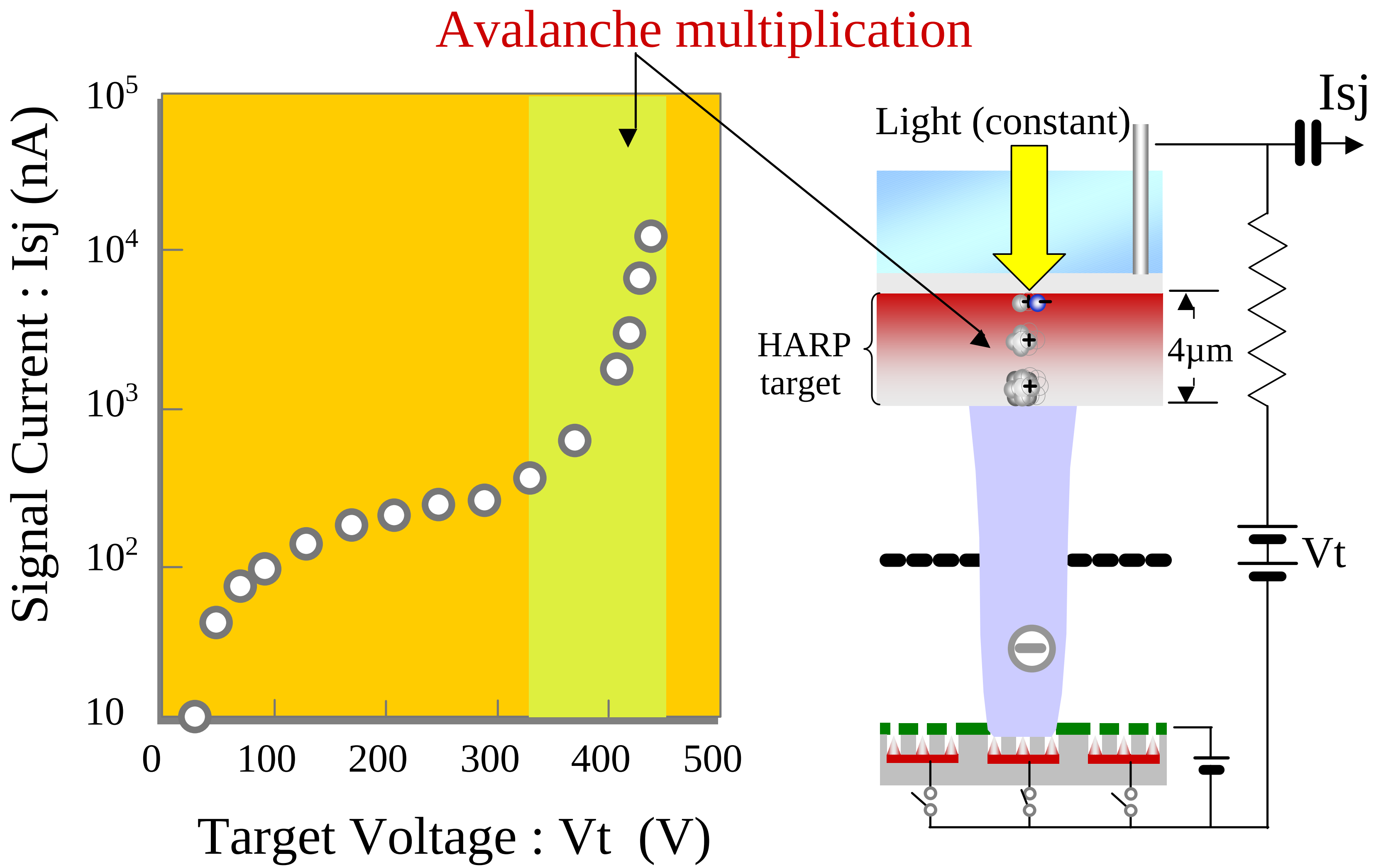
(a) HARP camera

(b) CCD camera(+12dB)

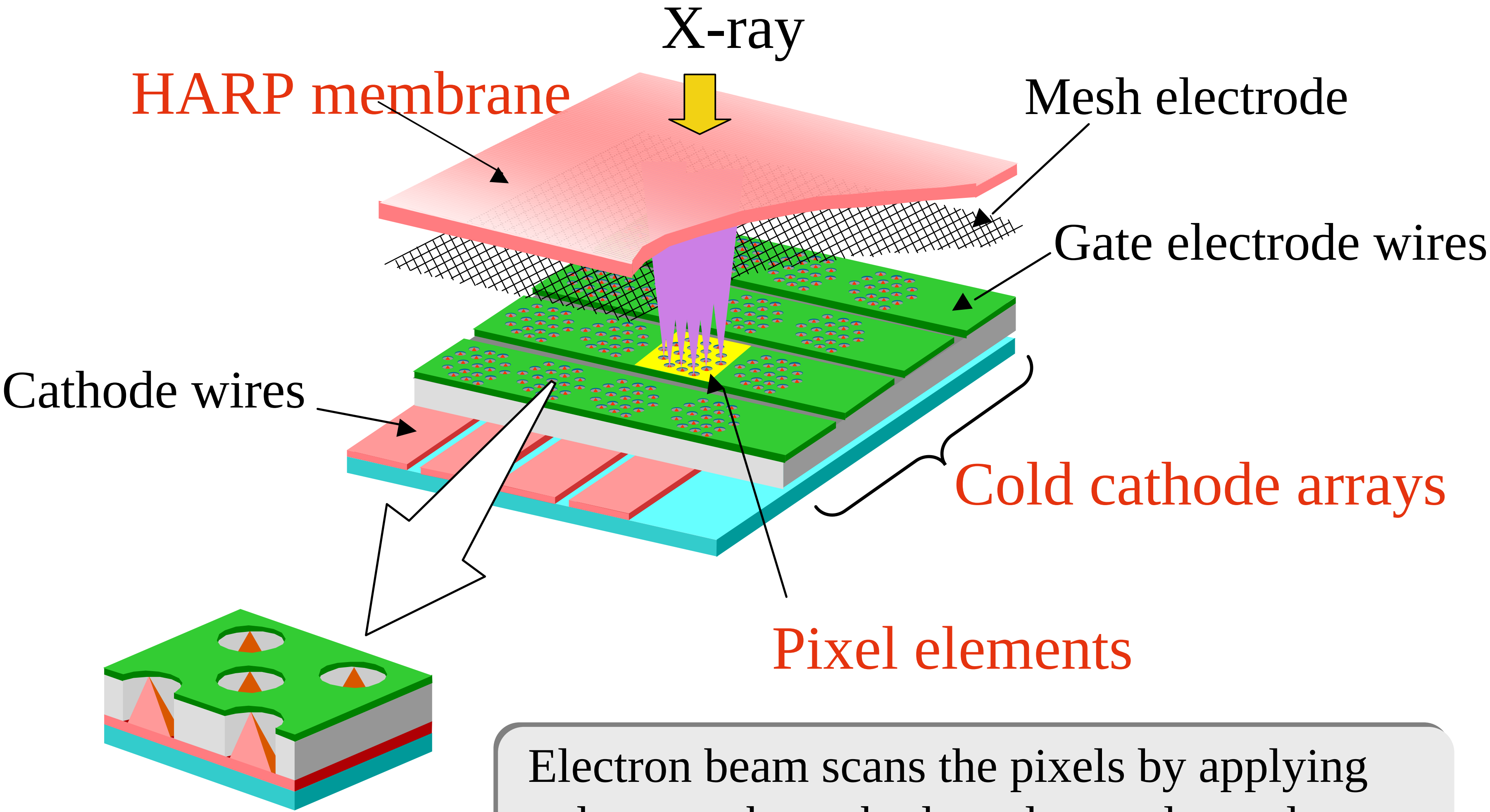
(0.3 Lux, F1.7)

Possibility of developing an X-ray detector with (1) much higher sensitivity compared to CCDs and (2) continous readout

Sensitivity



Cold cathode HARP area detector (Tanioka, NHK)



Electron beam scans the pixels by applying voltage to the cathode and gate electrodes

X-ray HARP Prototype 2001 with a preamplifier
(Mochizuki and Tanioka, NHK)

X-ray HARP 2001 prototype

130 mm (W) x 160 mm (H) x 247 mm (L)

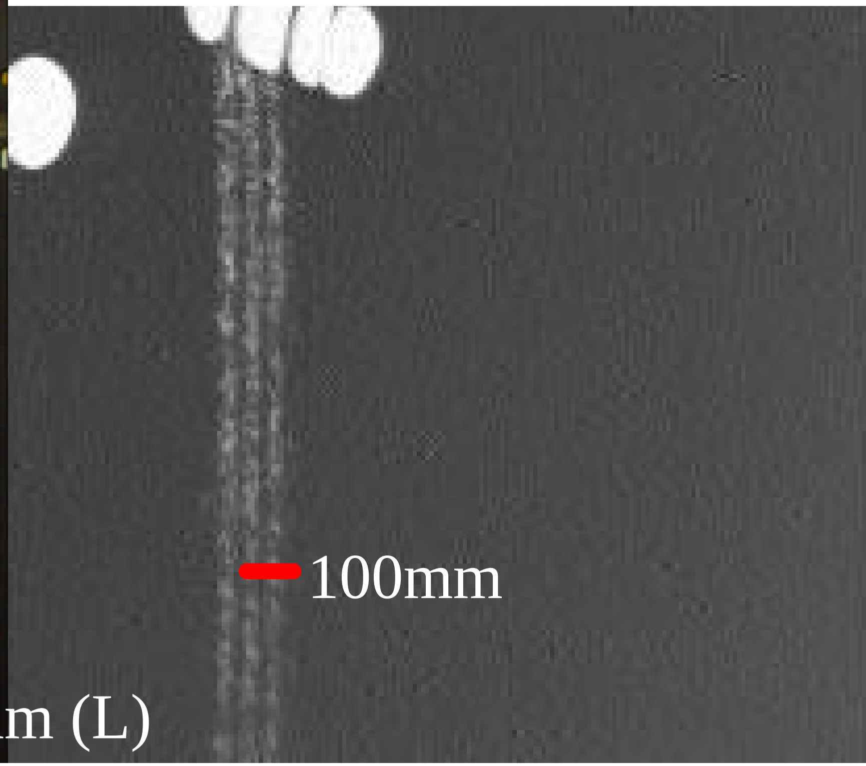
X-ray HARP 2002 model

Test on PF-AR NW2

(3,4 Dec 2002)

MTF 20 line pairs/mm

->spatial resolution < 25 mm



X-ray HARP camera (Prototype 2001) In collaboration with Mochizuki and Tanioka, NHK

Number of pixels: 500 x 500, 1000 x 1000, or 2000 x 2000

Spatial resolution: < 20 mm

Active area: f 25.4 mm (used area: 10 mm by 10 mm)

Frame rate: 90/sec, 60/sec, 30/sec, 15/sec, 7.5/sec

(Accumulation time: 33 msec ~ 8 sec)

X-ray HARP development

- Kenkichi Tanioka (NHK)
- Ryo Mochizuki (NHK Engineering Services)
- S. Kishimoto, K. Hyodo, Structural Biology Group, Photon factory, KEK

Micromanipulator, all-in-focus microscope

- Tamio Tanikawa & Kotaro Ohba (AIST, Tsukuba)
- Photron

PF Structural Biology Group

- Ryuichi Kato (Assoc. Prof.)
- Mamoru Suzuki (Research Assoc.)
- Noriyuki Igarashi (Research Assoc.)
- Naohiro Matsugaki (Research Assoc.)
- Masato Kawasaki (Research Assoc.)
- Masahiko Hiraki (Research Assoc.)
- Minora Nagai (Robotics technician)
- Tomoo Shiba (Post-doc)
- Shinsuke Hiramoto (Post-doc)
- M. Inoue (Ph.D. student)
- Y. Yamada (Ph.D. student)
- Leo Chavas (Ph.D. student)
- Yurii Gaponov Center-of-Excellence foreign scientist
- Seconded: two technicians and one engineer

GGA structures

Prof. Kazuhisa Nakayama
Kanazawa Univ.