International Workshop: Ultra-fast Coherent Diffraction Imaging of Single Particles, Clusters and Bio-Molecules at the European XFEL

Uppsala, 20-22 November 2008

David van der Spoel and **Janos Hajdu**

Sunrise: 8:04 am Sunset: 3:07 pm Sun's altitude at zenith: 10.0°

http://xray.bmc.uu.se/spb/

PROGRAMME

I. PLANS FOR X-RAY FELs

- Status of the European XFEL, plans for the SPB instrument (Th. Tschentscher)
- Status of the CXI instrument at LCLS (S. Boutet)

II. THE SCIENTIFIC CASE FOR SINGLE PARTICLE CXI

- Cell and bio-particle experiments (J. Hajdu)
- Serial crystallography (U. Weierstall)
- Coherent diffractive imaging for protein-nano-crystallography (C. Kewish)
- A comparison of synchrotrons and FELs for diffraction from sub-micron samples (C. Nave)
- Structure determination of atomic clusters (Th. Möller)
- Coherent diffractive imaging of conformer selected and oriented (bio-)molecules (J. Küpper)
- Influence of radiation damage (S. Hau-Riege)

III. X-RAY DELIVERY AND INSTRUMENTATION

- Nano-focusing x-ray FEL beams (C. David)
- Ptychography at XFEL sources: wavefront and focal spot characterization (P. Thiebault)
- Targeting the XFEL Bulls-eye: Substrate-Free Sample Delivery (M. Bogan)
- Trapping particles for CDI experiments (G. Grossmann)
- Area detectors for CDI experiments (H. Graafsma)

IV. DATA ANALYSIS

- Classification of a million noisy, random orientation single molecule diffraction patterns (G. Bortel)
- Single step orientation (A. Ourmazd)
- Reconstructing objects from noisy and randomly oriented diffraction patterns (V. Elser)
- Image reconstruction from dynamical objects (F. Maia)

V. WORKING GROUPS

Photographs: Daniel Larsson
http://xray.bmc.uu.se/~larsson/SPBworkshop2008/

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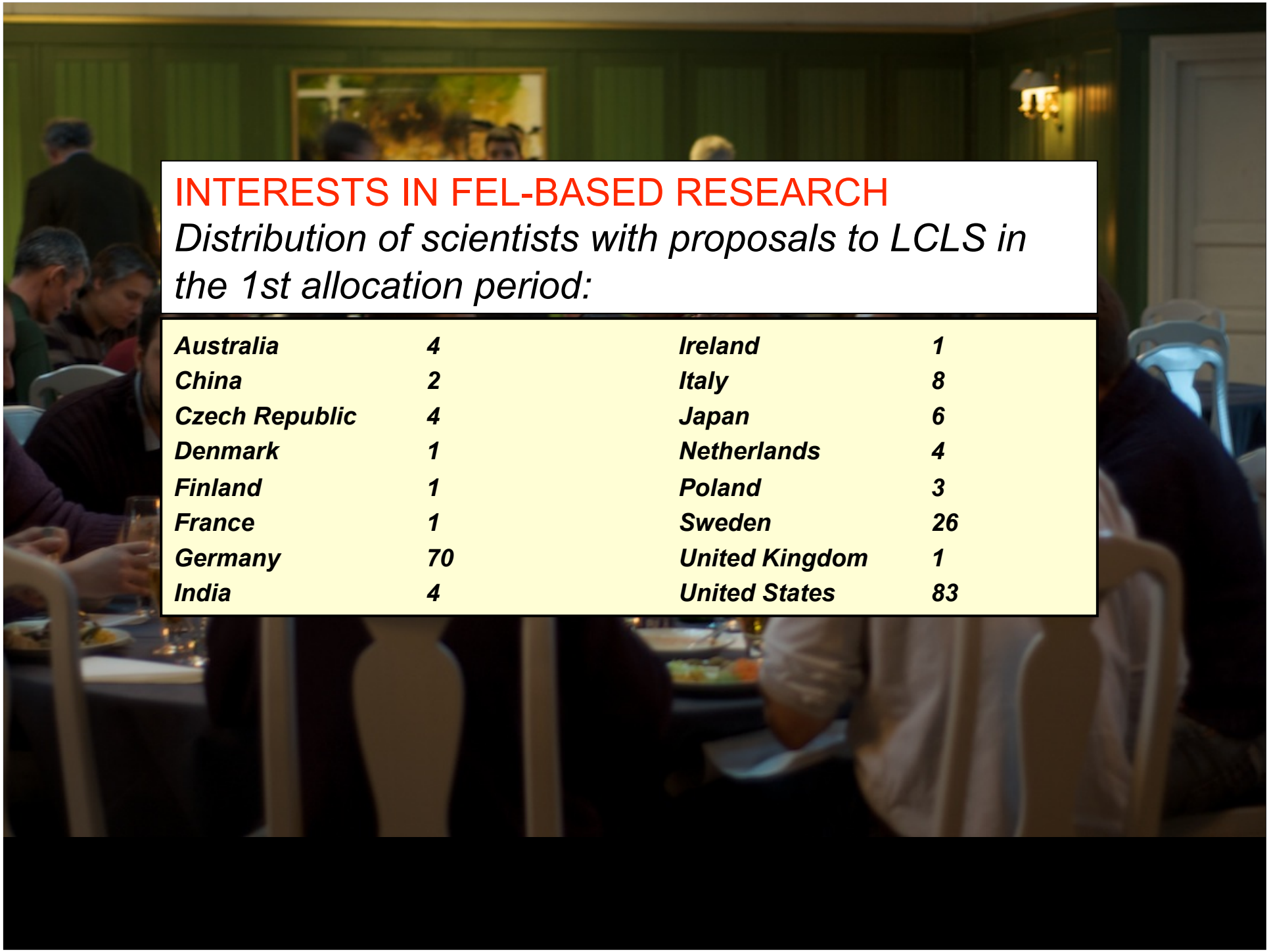


INTERESTS IN FEL-BASED RESEARCH

Distribution of scientists with proposals to LCLS in the 1st allocation period:

Australia	***4***	***Ireland***	***1***
China	***2***	***Italy***	***8***
Czech Republic	***4***	***Japan***	***6***
Denmark	***1***	***Netherlands***	***4***
Finland	***1***	***Poland***	***3***
France	***1***	***Sweden***	***26***
Germany	***70***	***United Kingdom***	***1***
India	***4***	***United States***	***83***

MEETING with the RESEARCH COUNCIL: SWEDISH INTERESTS in FEL-BASED SCIENCE

Uppsala 19-20 November 2008

PROGRAM S. Werin, V. Ziemann, M. Larsson, D. van der Spoel

1. **FLASH, XFEL** and **FERMI@Elettra**
2. **THE VIEW OF THE RESEARCH COUNCIL ON FELs** L. Börjesson
3. **EXPERIENCE WITH LINAC-BASED LIGHT SOURCES:**
   - **The SPPS collaboration**
   - **User experiments at FLASH - results**

   6 talks
4. **IN-KIND CONTRIBUTIONS** T. Tschentscher, M. Altarelli, T. Andersson

   8 talks on proposed in-kind contributions from Sweden

CONVENTIONAL TOOLS: PDB on 20/11/2008 had 54,298 entries

1 46,417 X-RAY XTAL

2 7,576 NMR

3 204 EM

4 101 OTHERS

Problems with conventional imaging in biology:

(1) Inability to obtain high resolution structures for non-reproducible objects of any type

(2) Problems with large reproducible objects

(3) Problems with crystallography
- Resolution depends on crystal quality (1D, 2D and 3D cryst.)
- Does it crystallise at all?

(4) Problems with the 4th dimension

Ultra-fast coherent scattering carries promise here

WHAT ARE THE BIG QUESTIONS?

Life. What lies ahead is to derive a 4-dimensional understanding of *living* systems across multiple levels of biological organization within a quantitative framework of structural systems biology.

New light sources will provide *technical foundations* for structural studies, and we expect biology to grow substantially through these developments.

Ultra-fast coherent diffraction imaging is probably the only method to study ***living cells*** at high resolutions.

Aim

Structure determination of single particles, atomic clusters, bio-molecules, viruses, cell organells, living cells

Main techniques proposed:

1. Coherent x-ray imaging of single injected particles/cells/etc.
2. Tomography/holography
3. Time-resolved structural studies

Application areas:

i. Atomic clusters and nanocrystals
ii. Biomolecular nanocrystals
iii. Two-dimensional crystals of macromolecules
iv. Closed nanoclusters
v. Single nano-particles
vi. Living cells
vii. Time-resolved structural studies

Single Molecule X-ray Imaging

One pulse, one measurement

3. Nanoscale manipulation

Biomolecule/particle injection

4. Data collection

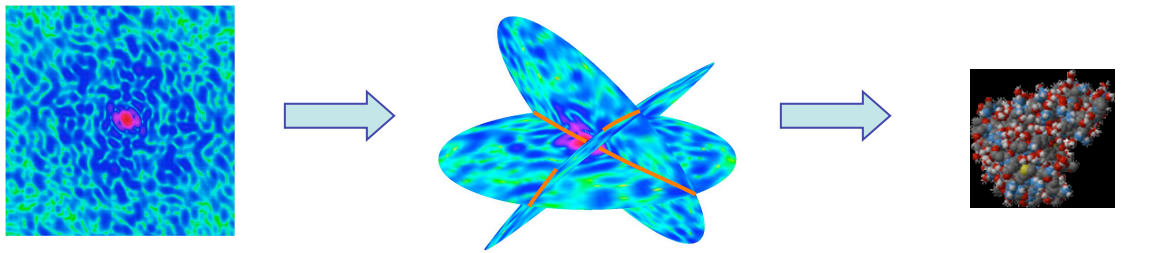
2. X-ray Laser Development

Noisy diffraction patterns

X-ray laser pulse

Combine many measurements

1. Theories of damage and imaging



Data frames

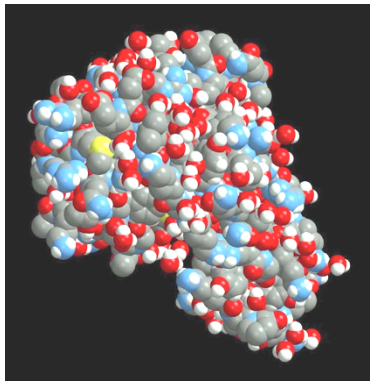
Combined data set

Reconstruction

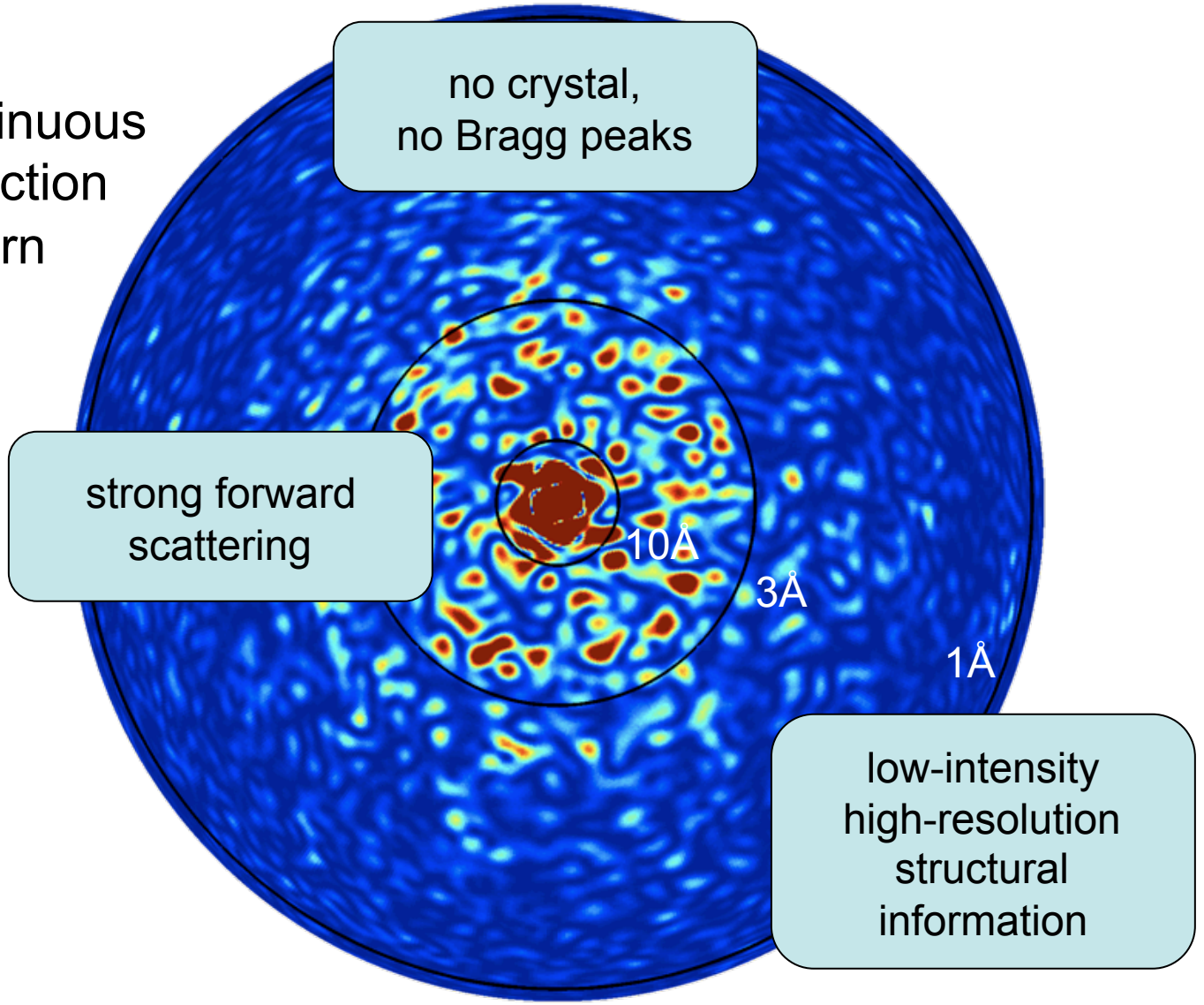
5. Data processing, phasing & reconstruction

THIS DIFFRACTION PATTERN WILL BE MODIFIED BY RADIATION-INDUCED CHANGES IN THE SAMPLE

Continuous diffraction pattern



Lysozyme

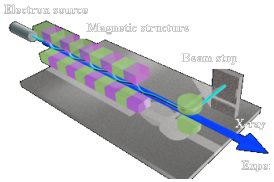


A spherical slice through the 3D reciprocal space – the Ewald sphere

WORKING GROUPS

SIMULATION OF SIGNAL FORMATION AND RADIATION DAMAGE (Gyula Faigel)

SIMULATE THE EXPERIMENT FROM THE POINT WHERE THE PULSE HITS THE SAMPLE TO THE DETECTOR



Source

Incident beam

Sample

Detector

RADIATION DAMAGE
CHANGES SAMPLE
CHANGES DIFFRACTION PATTERN

AGENDA

Overview of damage models

Molecular Dynamics model (Zoltán Jurek, Budapest)
Hybrid model (Nicusor Timneanu, Uppsala)
Boltzmann transport model (Beata Ziaja, DESY)
Hydrodynamic model (Stefan Hau-Riege, LLNL)

Signal formation

Signal formation in single-particle XFEL imaging (Stefan Hau-Riege, LLNL)
Effect of signal degradation and noise on classification (Miklós Tegze, Budapest)

There is a general agreement between the results of all models

ACTION PLAN

Need to **refine damage models** and derive **better estimates for hot cross-sections and ionisation potentials**.

Need to develop better models to simulate **soft x-ray experiments** (complicated due to inverse bremsstrahlung and high-field effects).

Put emphasis on **extended MD calculations** (more detail, good for planning experiments)

SET UP AN INTEREST GROUP TO CO-ORDINATE WORK
(under XFEL umbrella)

Common benchmarks, literature database. Experimental data sharing. Develop modular software for accurate predictions for planning experiments.

DATA ANALYSIS AND ITS NEEDS (David van der Spoel)

BIO-IMAGING DATA

Data Rate of Bio-Imaging Experiment		
	LCLS	EU-XFEL
Rep Rate	120	5000
Detector Size (Megapixel)	1-4	1-4
Intensity Depth (bit)	32	16
Data Rate (Gigabit/s)	4-16	80-320
Data Production (Pb/day)	0.04-0.16	0.9-3.5

POINTS TO CONSIDER

DATA REDUCTION

- COMPRESSION
- INCREMENTAL DATA PROCESSING
- INTELLIGENT DATA ACCEPTANCE / FILTERING,
- AUTOMATIC CORRELATION WITH OTHER DATA (ion and electron TOF / VMI)

DATA STORAGE AND PROCESSING ISSUES

- SHOULD THERE BE A CENTRAL DATA PROCESSING FACILITY @EU-XFEL?
- HOW LONG SHOULD DATA BE STORED?
- GRID COMPUTING
- PARALLEL FILESYSTEMS, DATA FORMATS
- ALGORITHMS FOR DATA PROCESSING
- COMPUTER POWER, SCALING OF ALGORITHMS
- SOFTWARE DEVELOPMENT: A MODULAR APPROACH

BENCHMARKING IMAGE RECONSTRUCTION

- SIMULATIONS: ALIGNMENT, CONFORMATIONAL HETEROGENEITY AND RADIATION DAMAGE (AUTOMATICALLY)
- RECONSTRUCT TO AS GOOD RESOLUTION AS POSSIBLE (AUTOMATICALLY)
- MAP UP THE EFFECT OF NOISE

http://xray.bmc.uu.se/spbwiki

CONCLUSIONS

1. DATA STORAGE AND ANALYSIS IS **FEASIBLE** AT VARIOUS LEVELS DEPENDING ON POLICY AND MONEY

2. **COLLABORATION** WITH LCLS WILL BE MUTUALLY BENEFICIAL

3. IMAGE PROCESSING AND RECONSTRUCTION: THERE ARE SOME **VERY PROMISING** ALGORITHMS, WHICH NEED TO BE TESTED ON REAL DATA (FLASH, LCLS)

4. SET UP AN **INTEREST GROUP** TO CO-ORDINATE WORK (CCP4)

INSTRUMENTATION (Henry Chapman)

Source and Beamline

Single particle imaging is dominated by noise (counting statistics of quanta)
- scattered counts per Shannon pixel is proportional to λ^2
- number of incident photons per pulse fluence proportional to λ

6 keV (0.2 nm) is 8 times better than 12 keV (0.1 nm)
3 keV (0.4 nm) is 64 times better than 12 keV (0.1 nm)

We should work at the longest wavelength that supports the desired resolution
This requires highest angles possible on detector

e.g. 2 theta = 60°: d = λ

$$d = \frac{\lambda}{2 \sin \theta}$$

Problem: SASE 1 is fixed at 12 keV

Source and Beamline

Longer mirrors required for longer wavelength

State of art mirrors are 75 nm focus at 15 keV (Rev. Sci. Instrum. **79** 083104 (2008))

Assume largest *reproducible* objects are about 0.2 micron

Require 5, 2 micron focus (f = 20 m) and 0.1 micron focus (f = 1 m)

don't work too far out of focus

There could be a desire to use unfocused beam (aligned molecules)

There will be a need for perfect optics (zone plates) even if inefficient

- need an estimation of the effect of variable wavefront error on reconstruction

Need a longer, wider, and taller hutch

For nanocrystals (with conventional analysis) need spectrum for every shot

Need a dedicated laser

Sample delivery and manipulation

Many potential particle injection methods
- aerodynamics lensing of aerosols (e.g. with cell sorter)
- electrospray ionization and trapping
- synchronized droplets
- pulsed molecular beams, quantum-state selected

Common chamber

Test setup nearby

Isolate beamline mirrors and CCD from sample

Some injectors could replenish sample at MHz

Require tunable laser / laser ports
Require hit diagnostics, such as TOFS / VMI

Fixed bio samples (on TEM grids or larger arrays) require cryogenic stage
Sample chamber could be an SEM (allows larger grids of samples)
- ⋆ Xradia is developing a large-travel cryo stage
- ⋆ BESSY XRM has guts of an FEI

Require two sample chambers (ideas from LCLS)

Detectors

AGIPD detector is designed for SASE1 (12 keV)

- 200 micron pixels
- 200 stored frames
- linearity can be calibrated

There may be a use for photon counting detectors operating at pulse frequency (e.g. for aligned molecules)

Number of pixels required: limited by the bandwidth $N = \frac{2s\lambda}{\Delta\lambda}$ $\frac{w}{d} = \frac{N}{2s}$

N < 2000 for unmonochromatised

N > 2000 for monochromatised

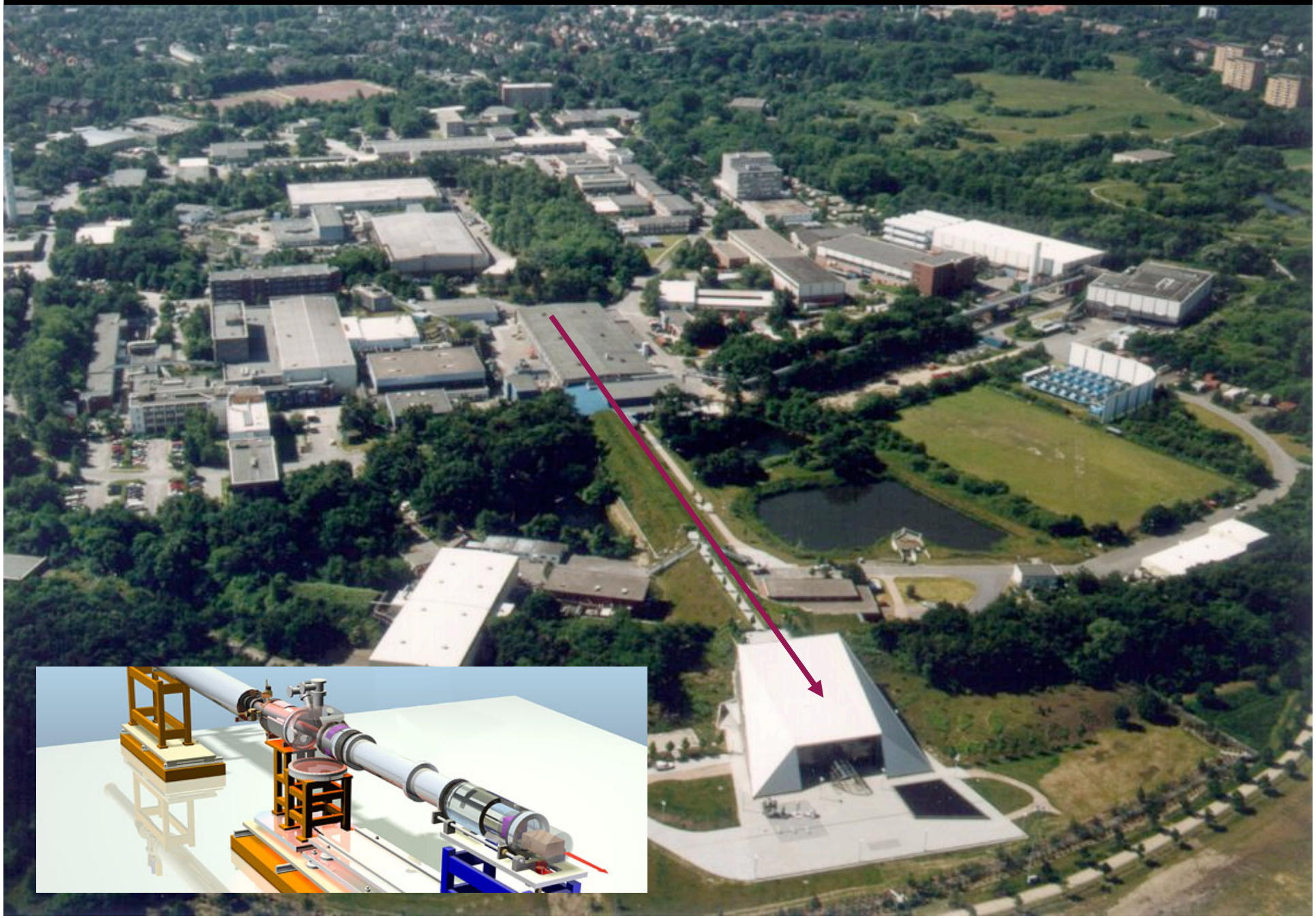
500 x 500 for 0.3 nm resolution of (75 nm)^2 object s=1 (not safe)

2k x 2k for 0.2 nm resolution of 0.2 micron object s=1

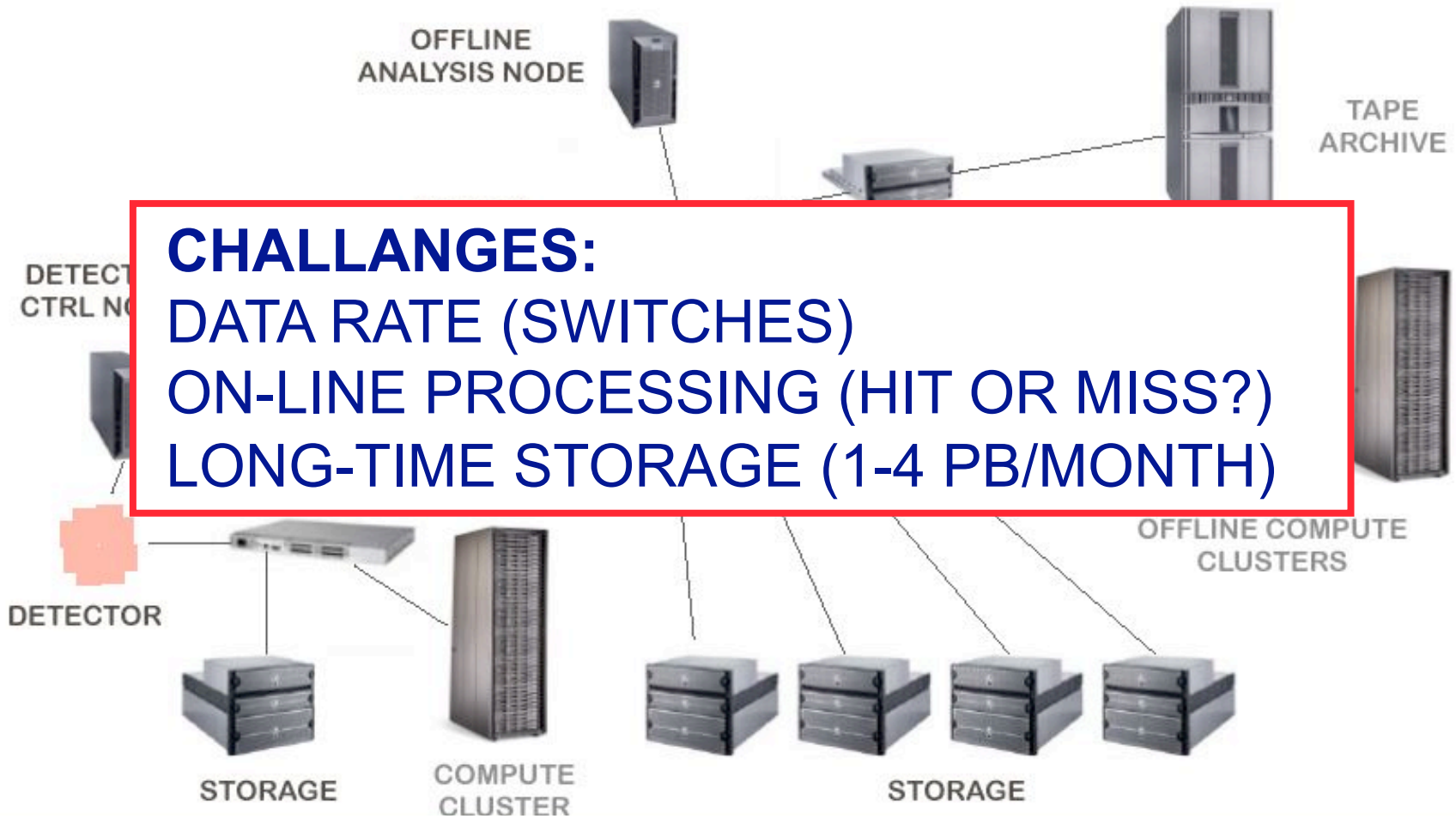
2k x 2k for 0.4 nm resolution of 0.2 micron object s=2 (safer)

2k x 2k for 0.1 nm resolution of 0.05 micron object s=2 (safer)

Future: Build on experience gained at FLASH and LCLS



SYSTEM INFRASTRUCTURE



CHALLANGES:

DATA RATE (SWITCHES)

ON-LINE PROCESSING (HIT OR MISS?)

LONG-TIME STORAGE (1-4 PB/MONTH)

IMAGE FROM YIPING FENG, SLAC