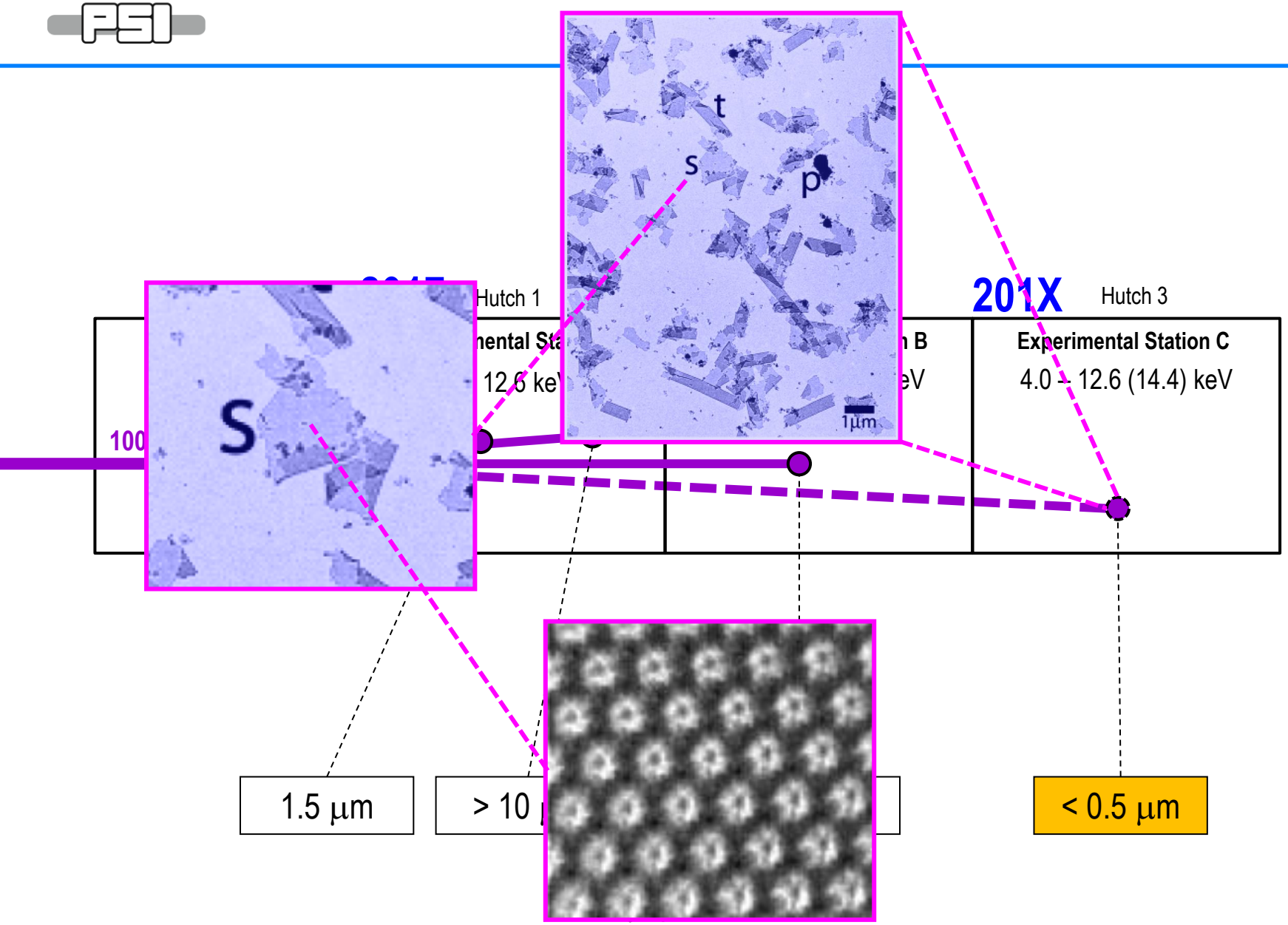
PAUL SCHERRER INSTITUT

Wir schaffen Wissen – heute für morgen

Achievements in 2D protein crystallography at the LCLS

Bill Pedrini
Paul Scherrer Institut


t
s
p
1µm
S
Hutch 1
201X
Hutch 3
Experimental Station C
4.0 – 12.6 (14.4) keV
1.5 µm
< 0.5 µm

Outline

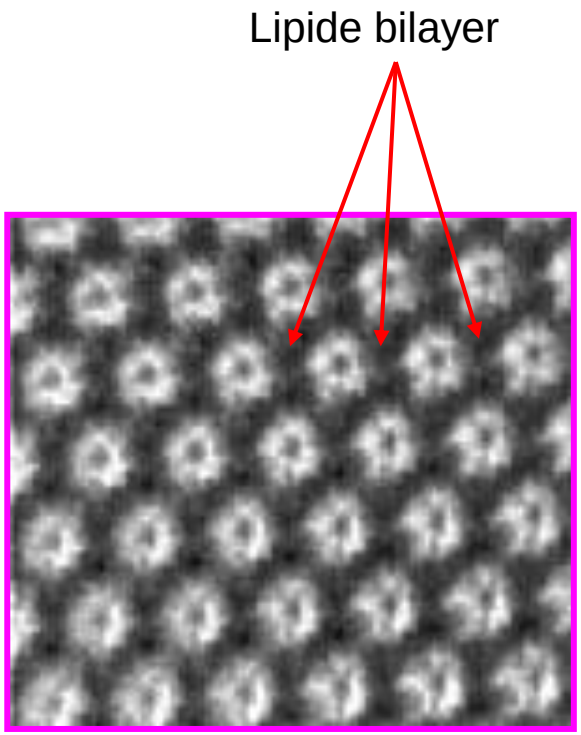
1. 2D crystals
2. 2D crystallography
3. Fixed target experiments at FELs
4. “Recent” results from LCLS experiments
5. Summary of the **facts**
6. Complementarity with cryo EM

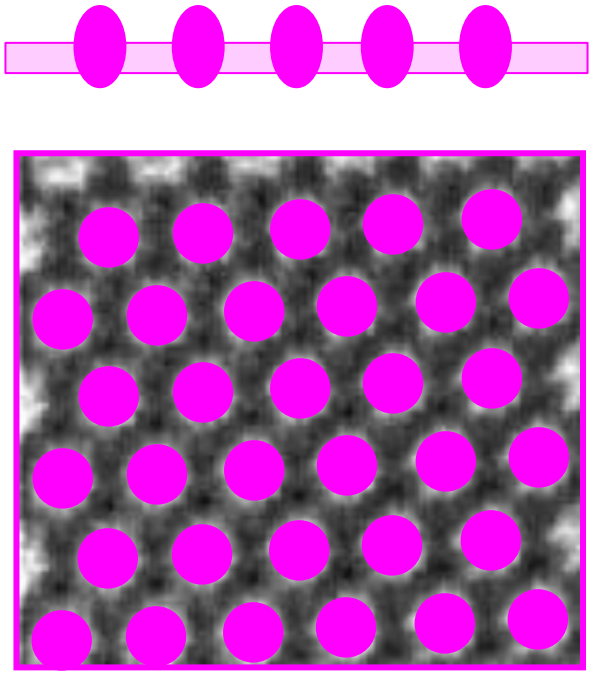
1.

2D crystals

Motivation for 2D crystals (I)

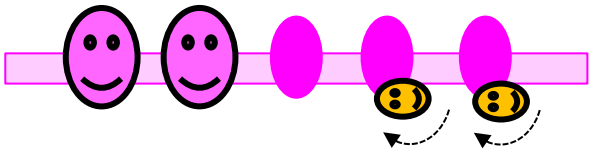
“(close to) native environment” for membrane proteins

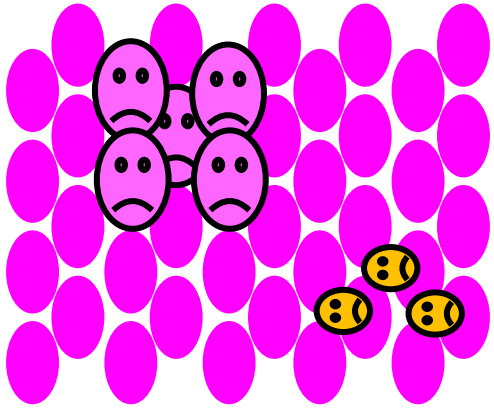




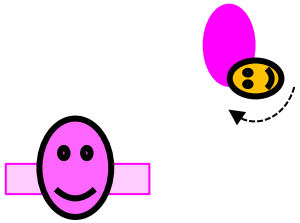
Motivation for 2D crystals (II)

2D crystal





3D crystal

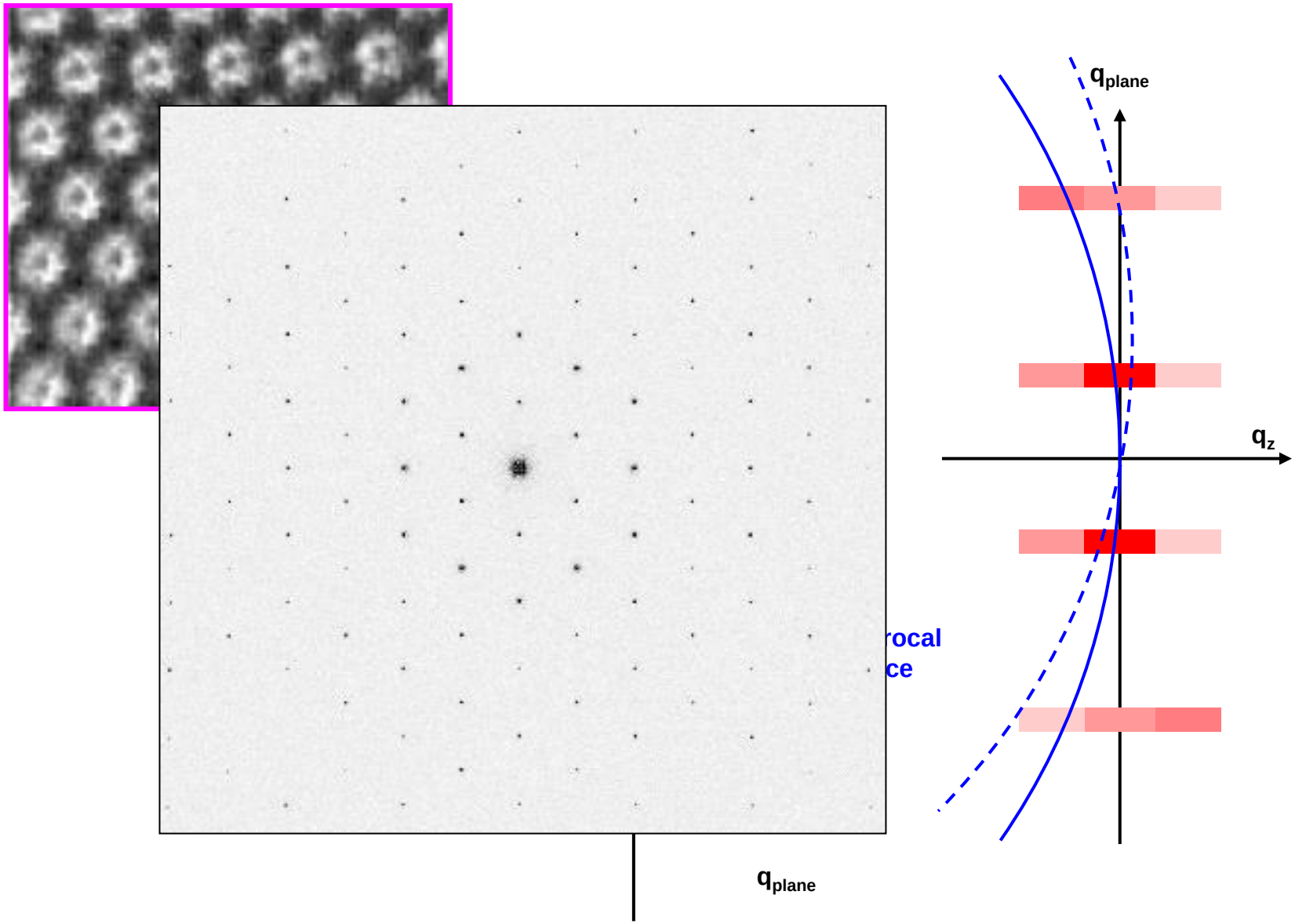


Single particle

2.

2D crystallography

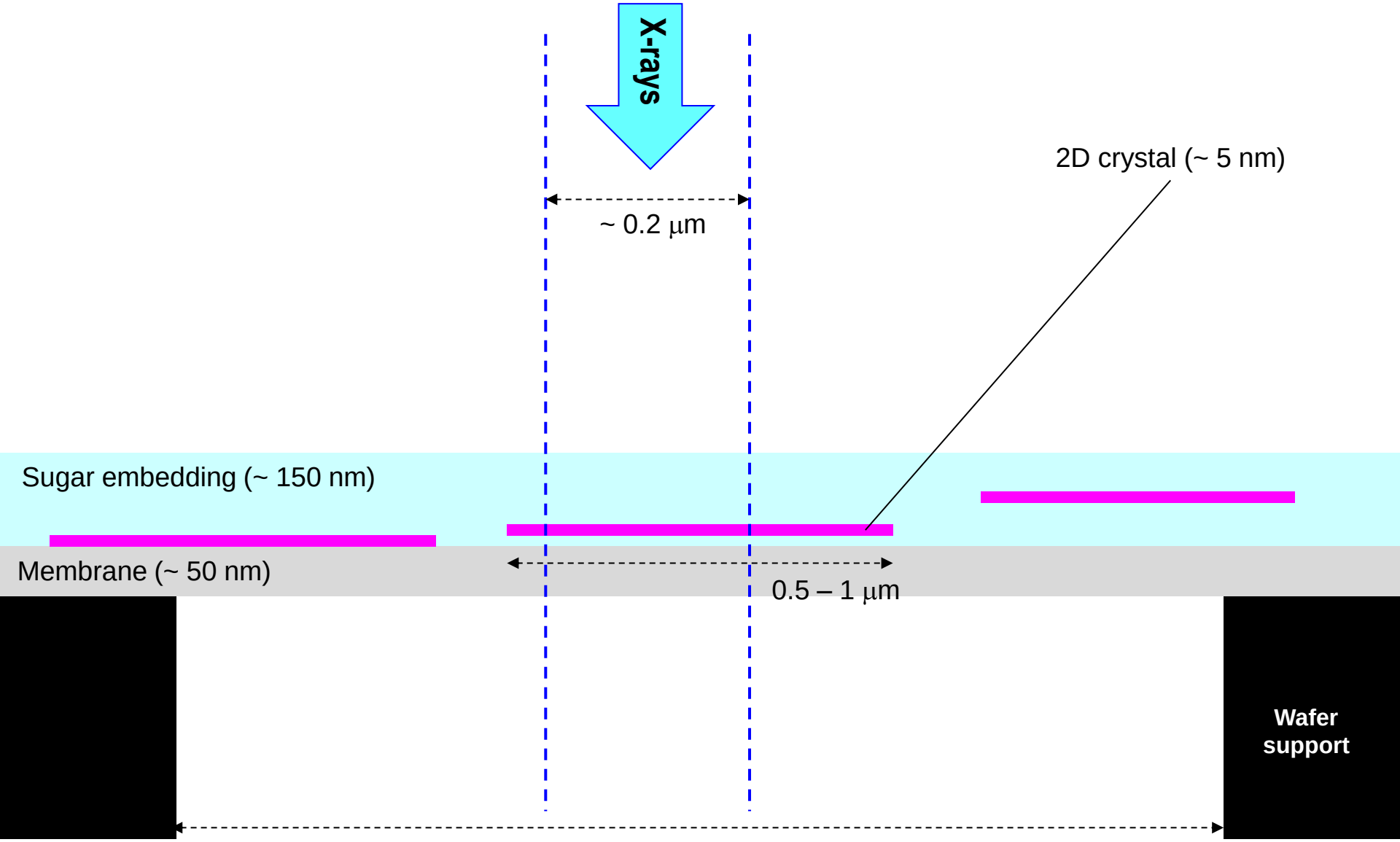
2D crystallography summarized



3.

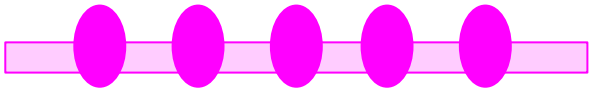
Fixed target experiment at FELs

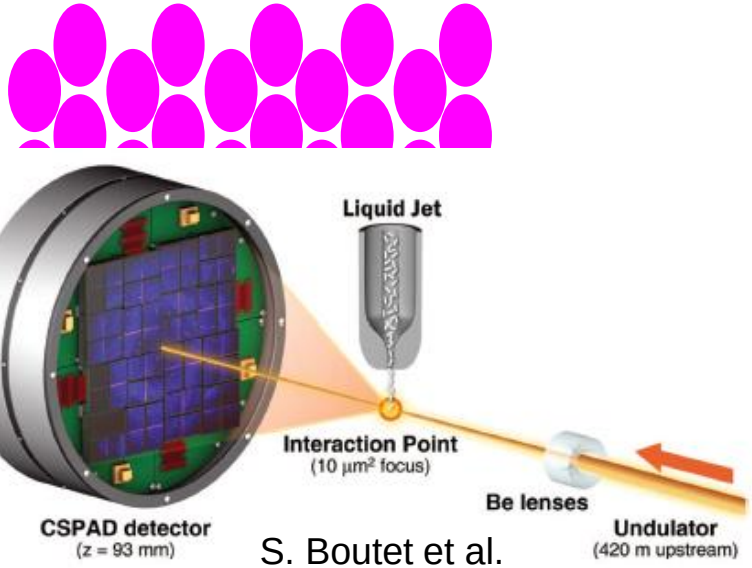
Sample


X-rays
~ 0.2 μm
2D crystal (~ 5 nm)
Sugar embedding (~ 150 nm)
Membrane (~ 50 nm)
0.5 – 1 μm
Wafer support

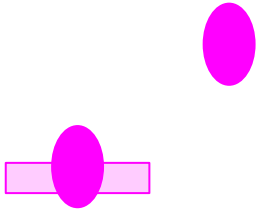
«Diffract before destroy»

2D crystal



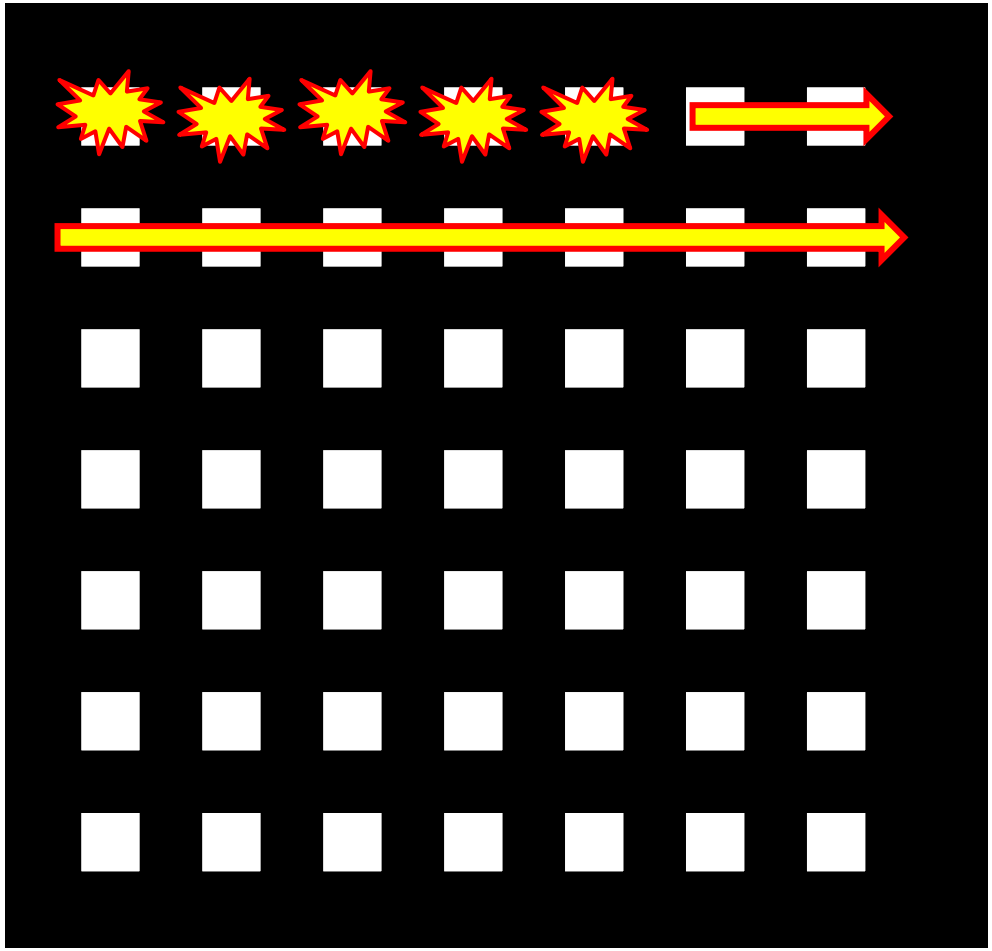


S. Boutet et al.
Science **337**, 362 (2012)



Single particle

Data acquisition



4.

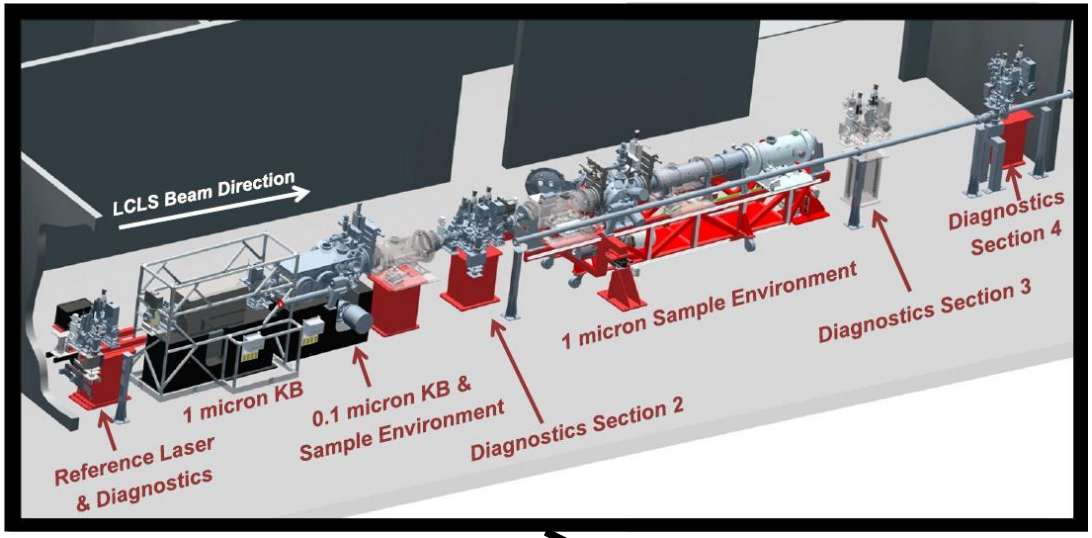
“Recent” results from LCLS experiments

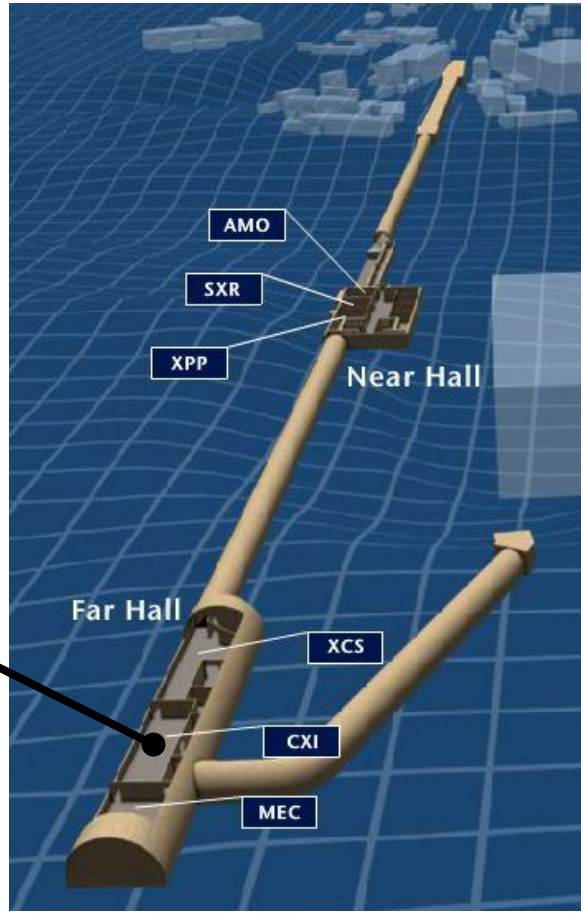
Acknowledgements:

2D crystal collaboration

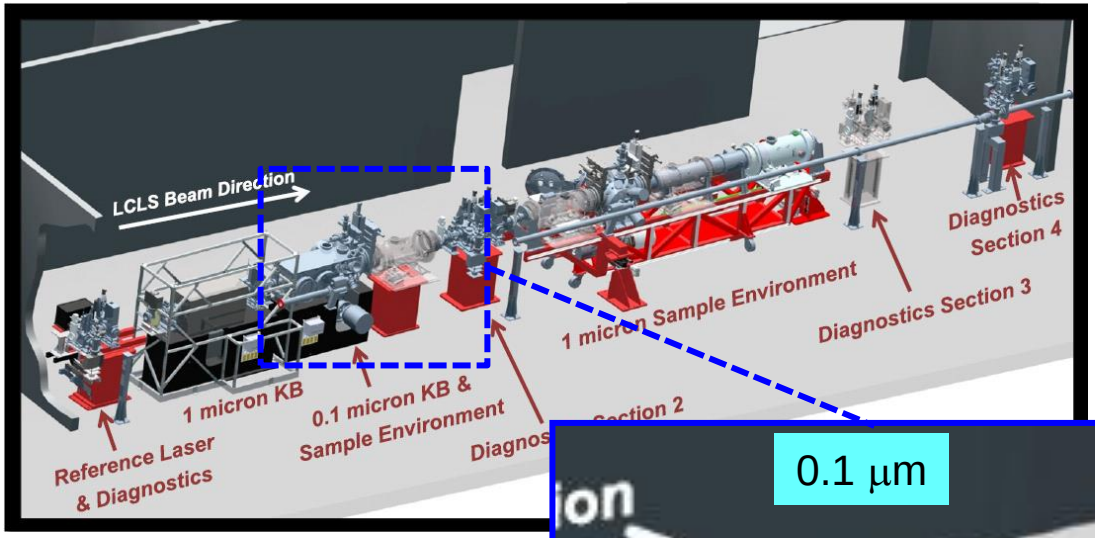
- **Lawrence Livermore National Laboratory:** **Matthias Frank**, Mark Hunter, Matthew Coleman, Henry Benner, Stefan Hau-Riege, Tommaso Pardini, Brent Segelke, Geoffrey Feld
- **Pacific Northwest National Laboratory:** James Evans
- **Arizona State Univeristy:** Nadia Zatsepin, John Spence, Christopher Kupitz
- **Boston University:** John Ogren, Kenneth Rotschild
- **CFEL:** Anton Barty, Richard Kirian
- **SLAC** (LCLS-CXI)**:** Sebastien Boutet, Garth Williams, Marc Messerschmitt
- **Paul Scherrer Institute:** Ching-Ju Tsai, Celestino Padeste, Guido Capitani, Xiao-Dan Li, Gebhard Schertler

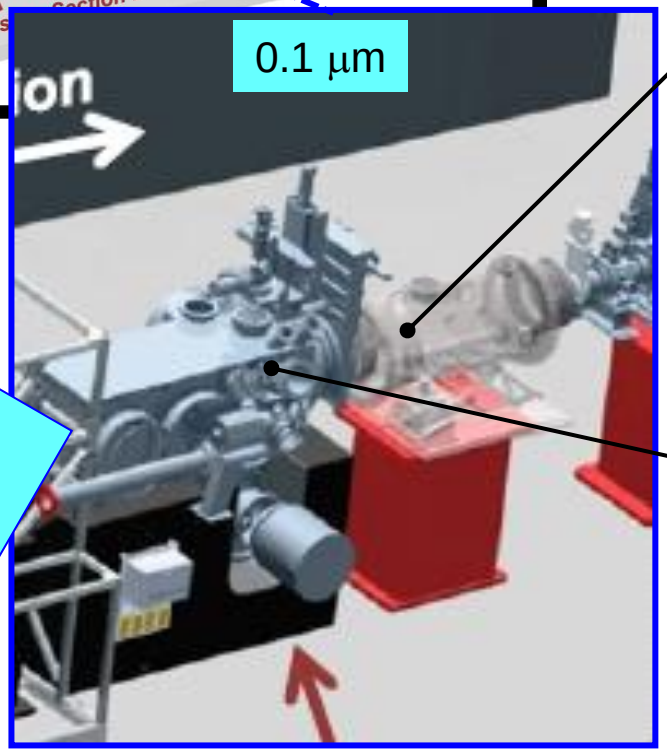
LCLS


LCLS Beam Direction
Reference Laser & Diagnostics
1 micron KB
0.1 micron KB & Sample Environment
Diagnostics Section 2
1 micron Sample Environment
Diagnostics Section 3
Diagnostics Section 4


AMO
SXR
XPP
Near Hall
Far Hall
XCS
CXI
MEC

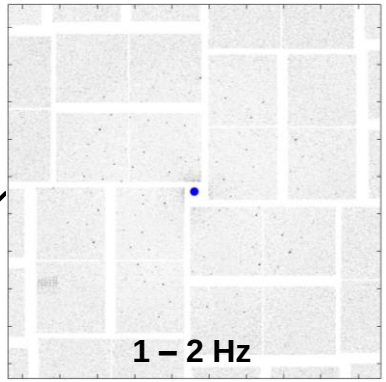
LCLS experiment

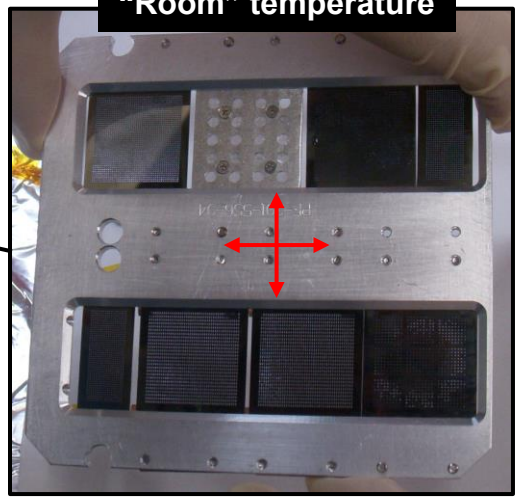




8.8 keV (1.4 Å)
~ 2-3 mJ

CSPAD 2D pixel detector
~ 20 cm distance





Sample holder

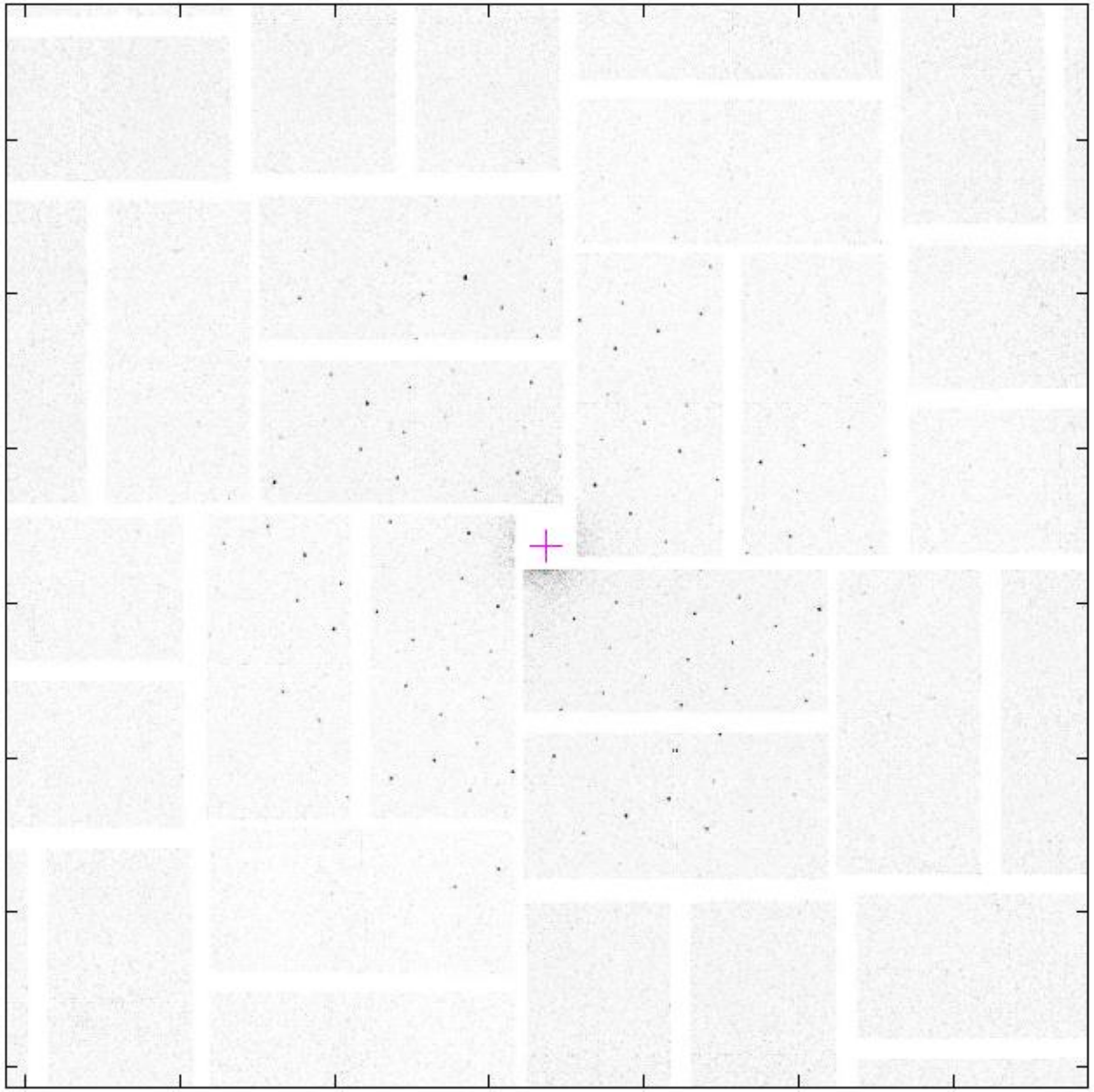
November 2013 data

bR D96N

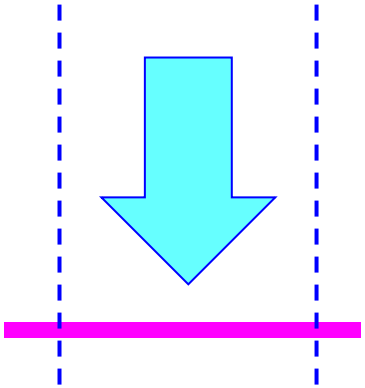
Run r0127:

Nr. of images:	968
Acq. Rate:	~1.5 Hz
bR mass:	«few» μg
Sugar:	0.5% glucose
Support:	20 nm Si_3N_4-membrane, 100 x 100 μm windows
Wavelength:	1.467 Å
Detector dist.:	0.235 m
Tilt angle:	0°
Illumination:	off
Pump laser:	off

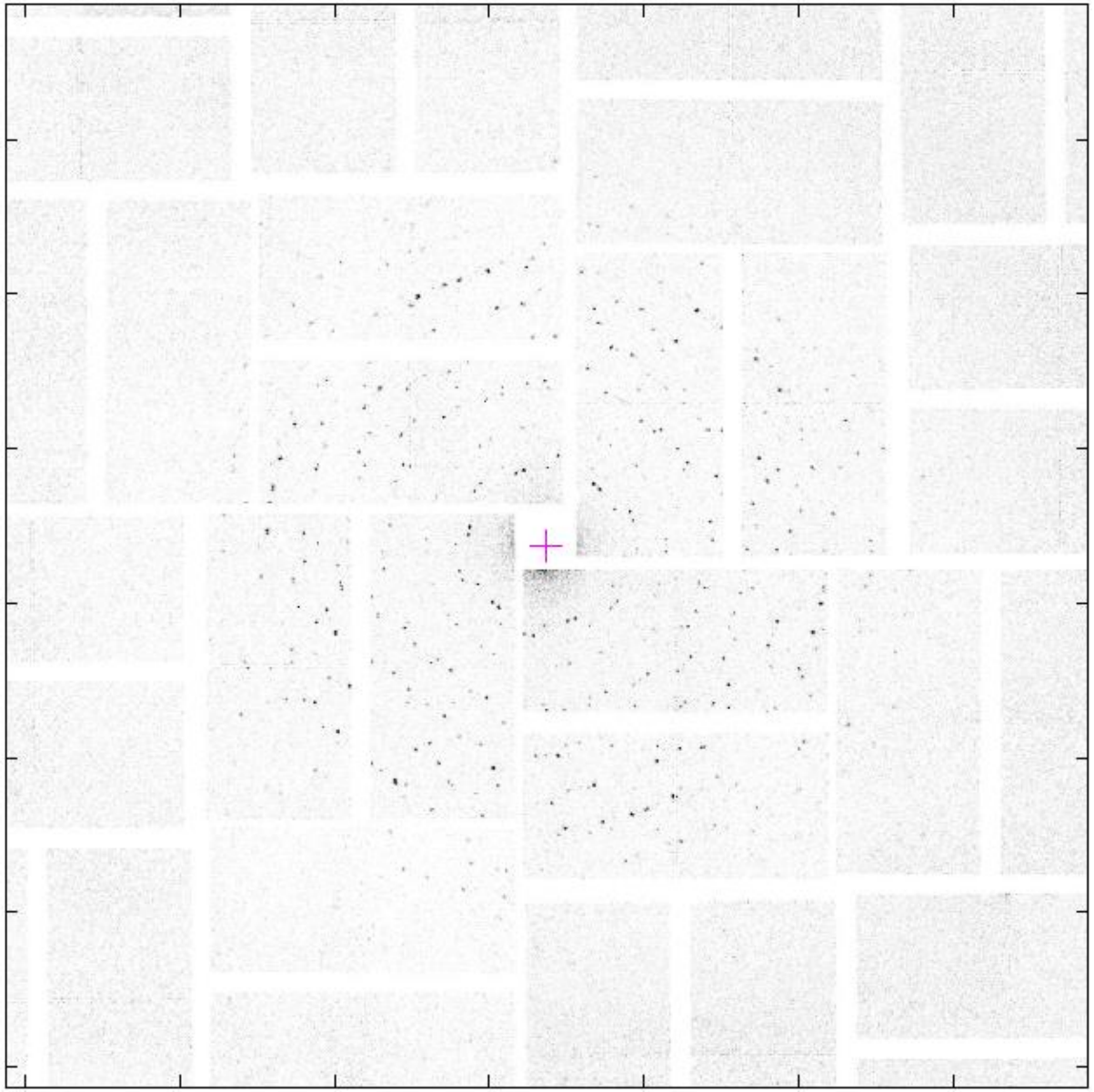
«Single crystals»



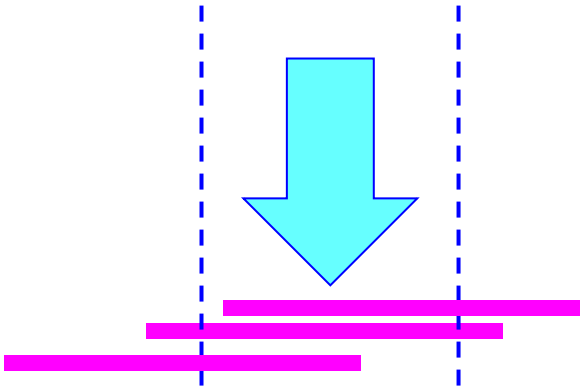
42 / 968 (4.3%)



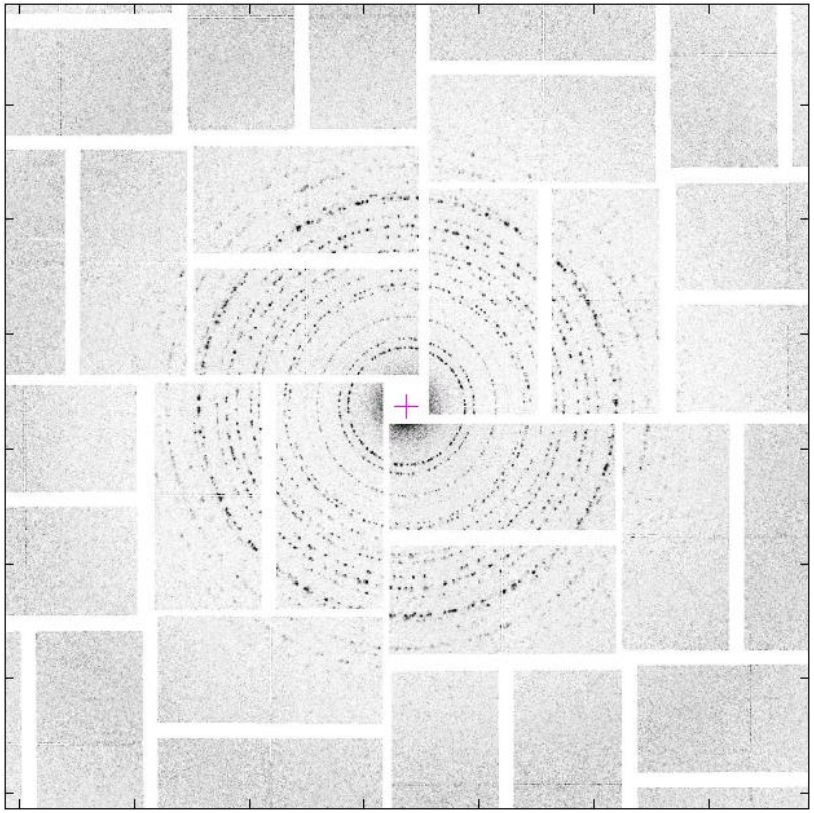
«Few crystals»

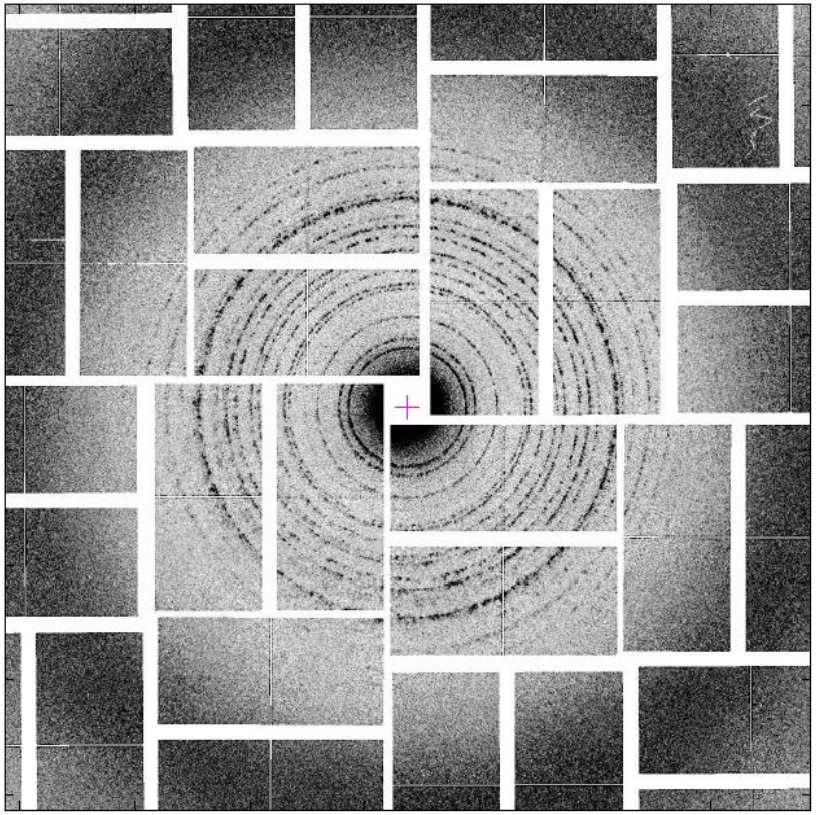


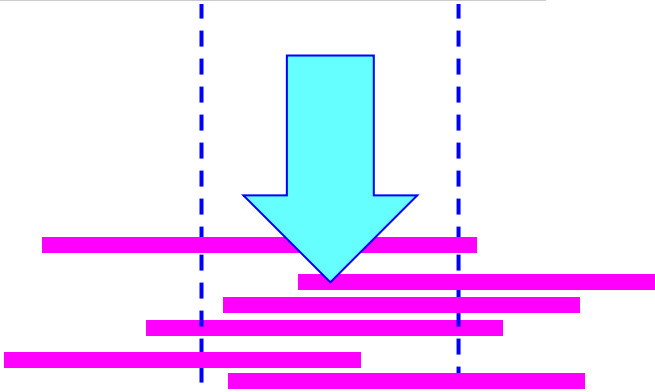
465 / 968 (48.0%)
(including single crystals)



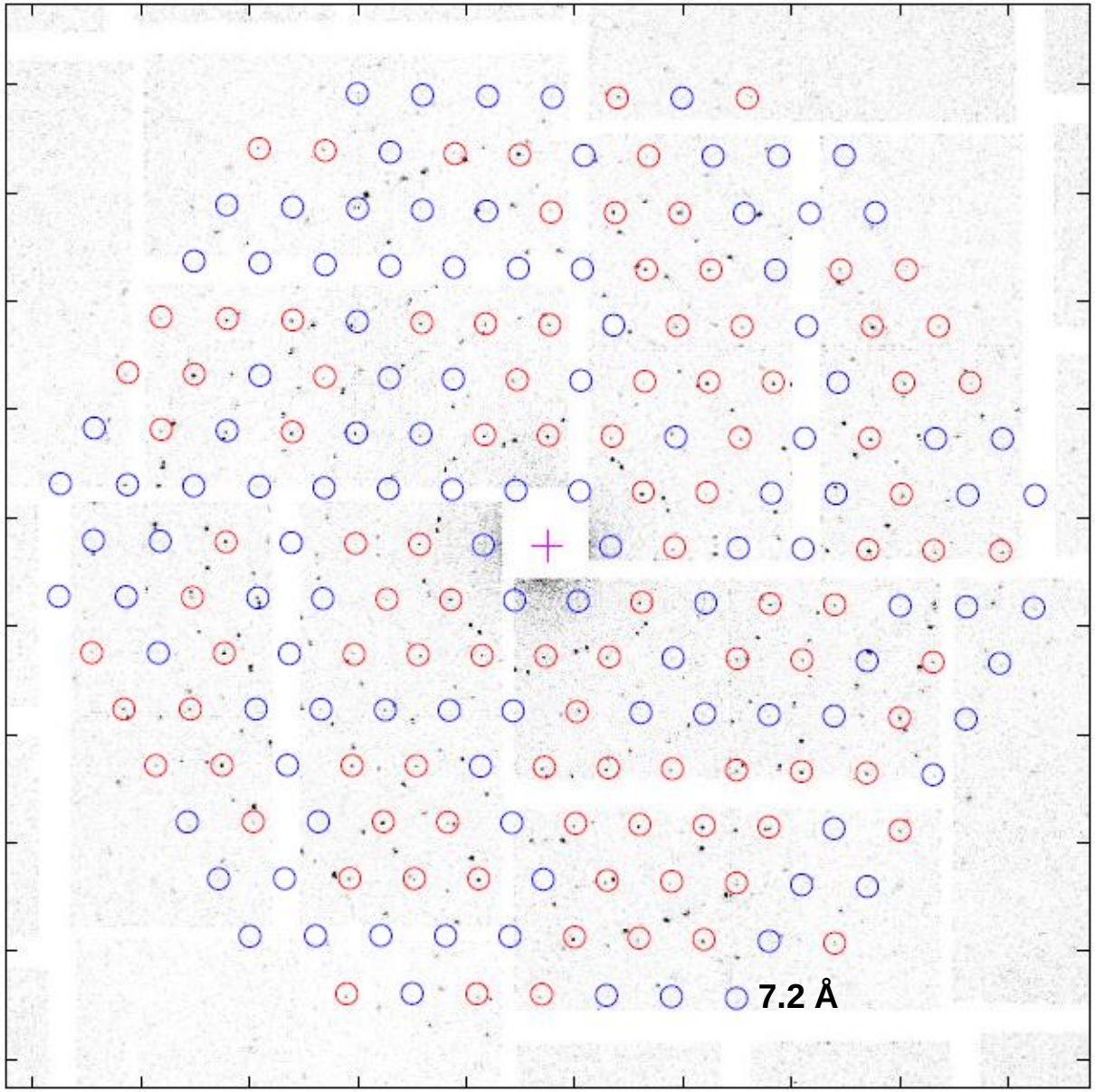
«Too many crystals»



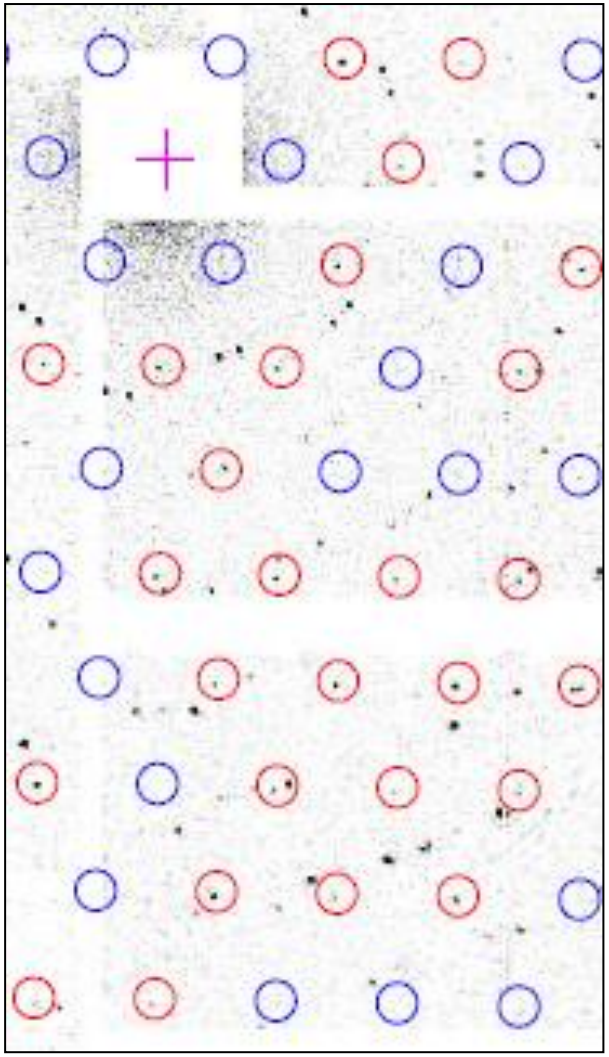


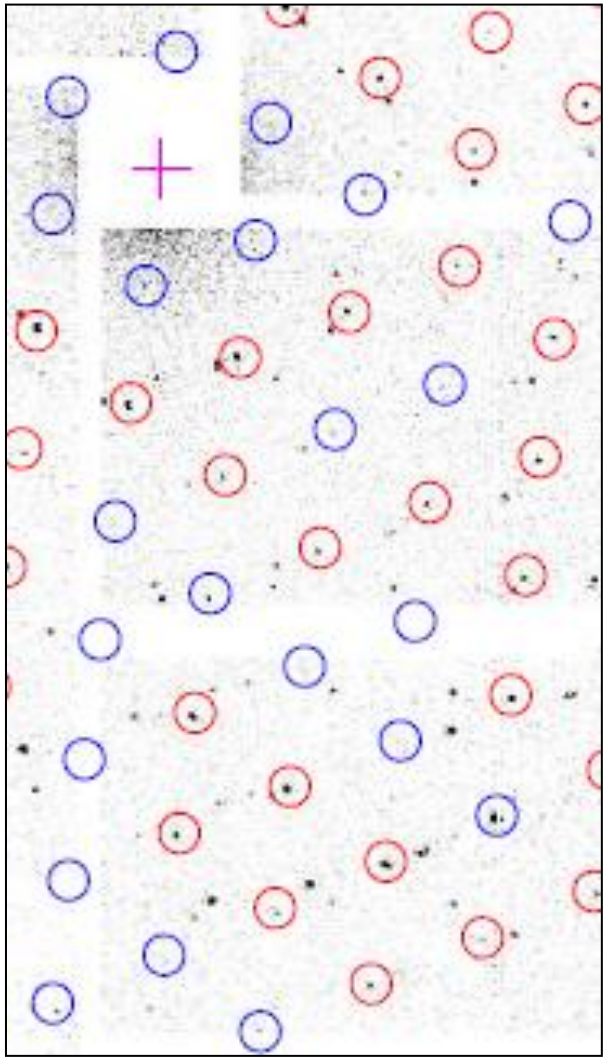


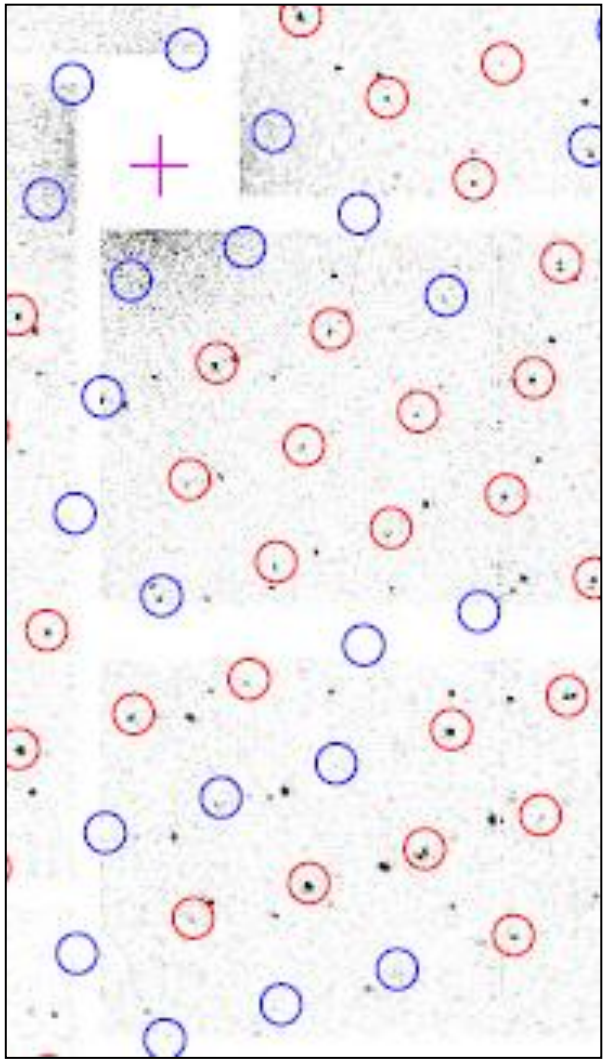
Indexing



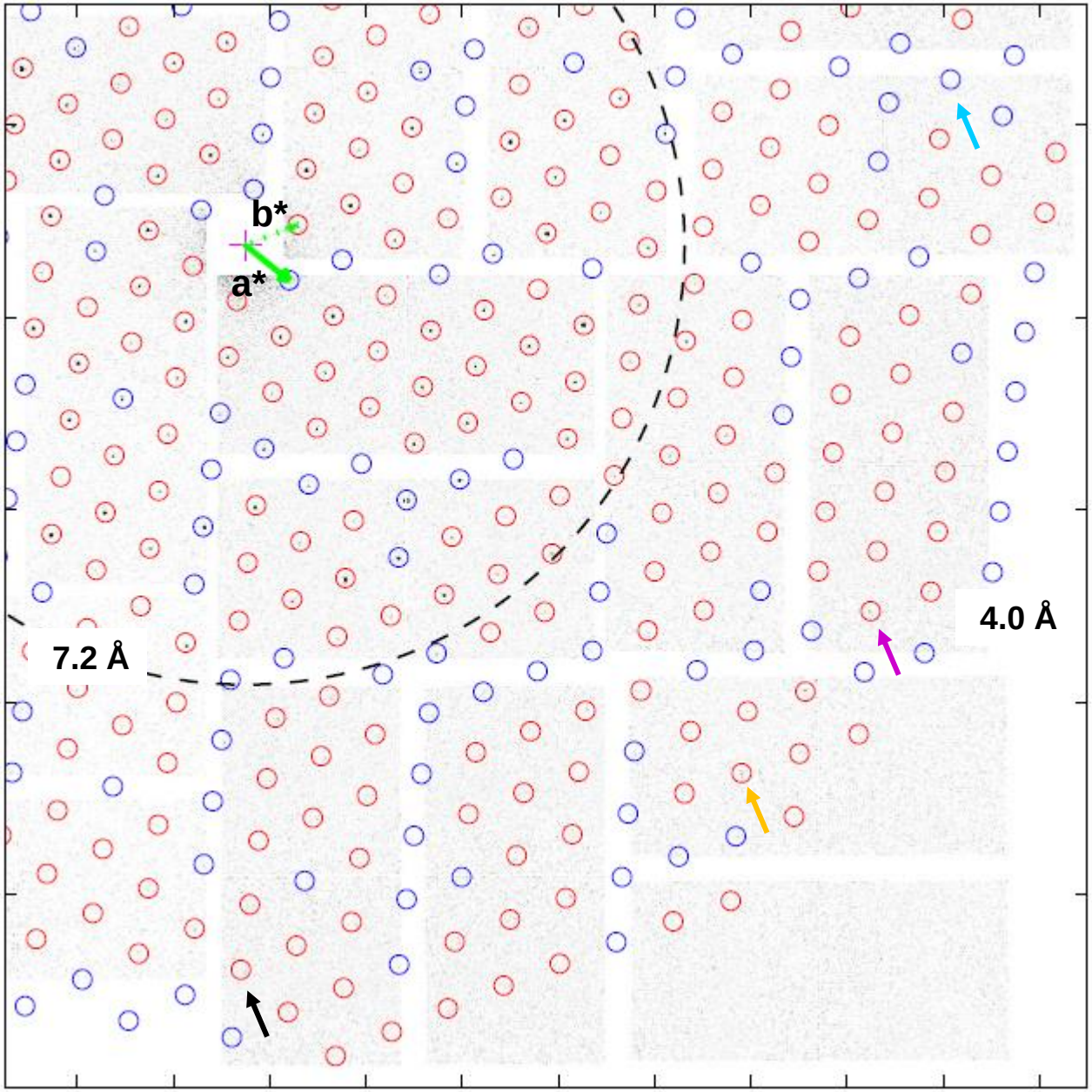
Indexing

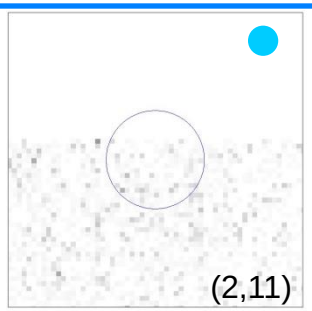


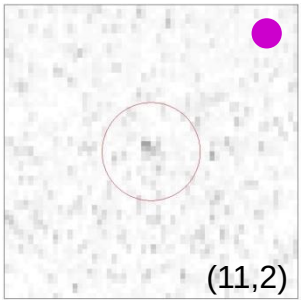


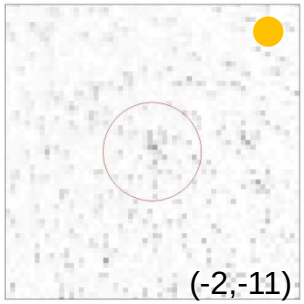


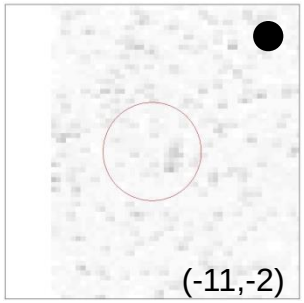
Peak position prediction



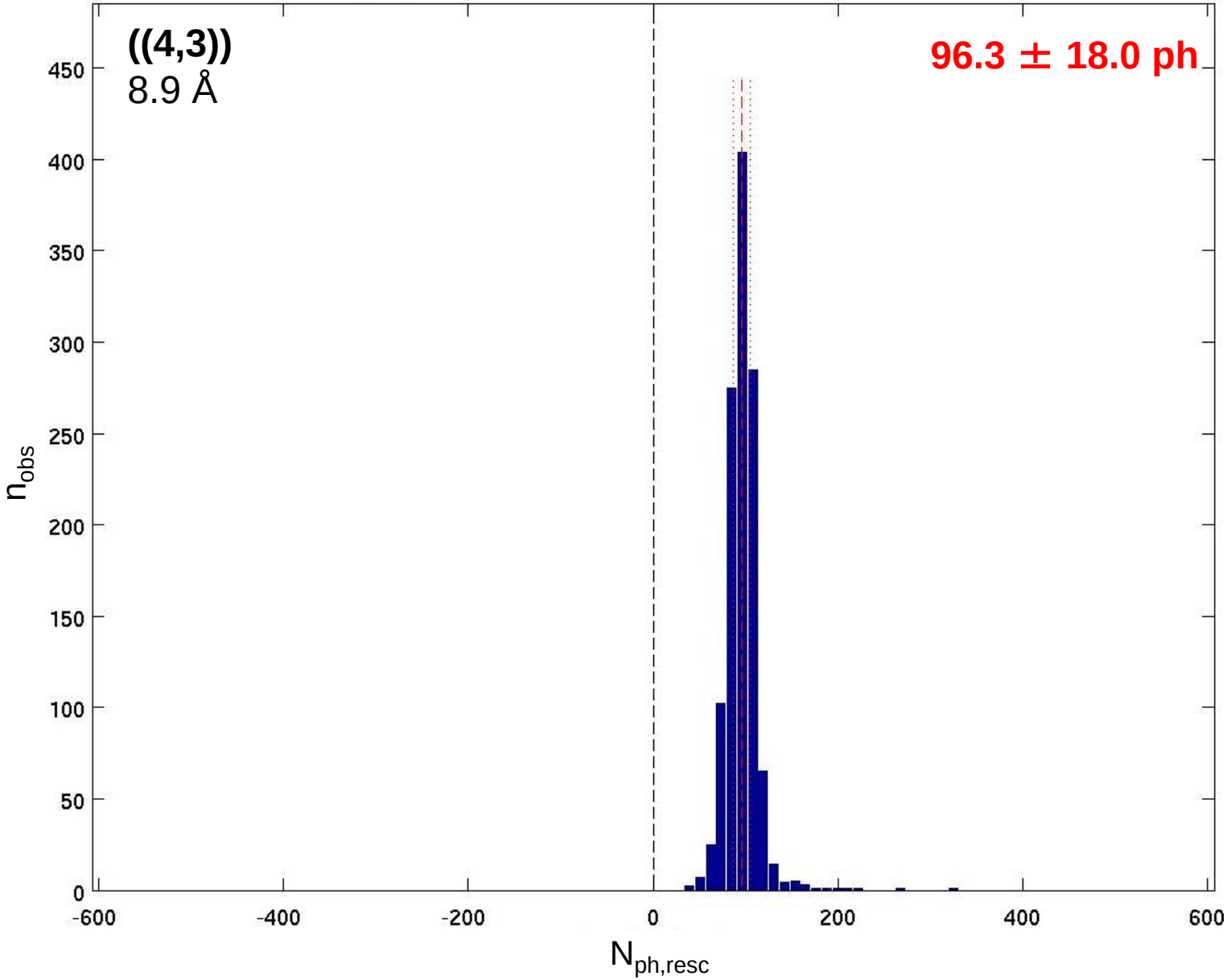




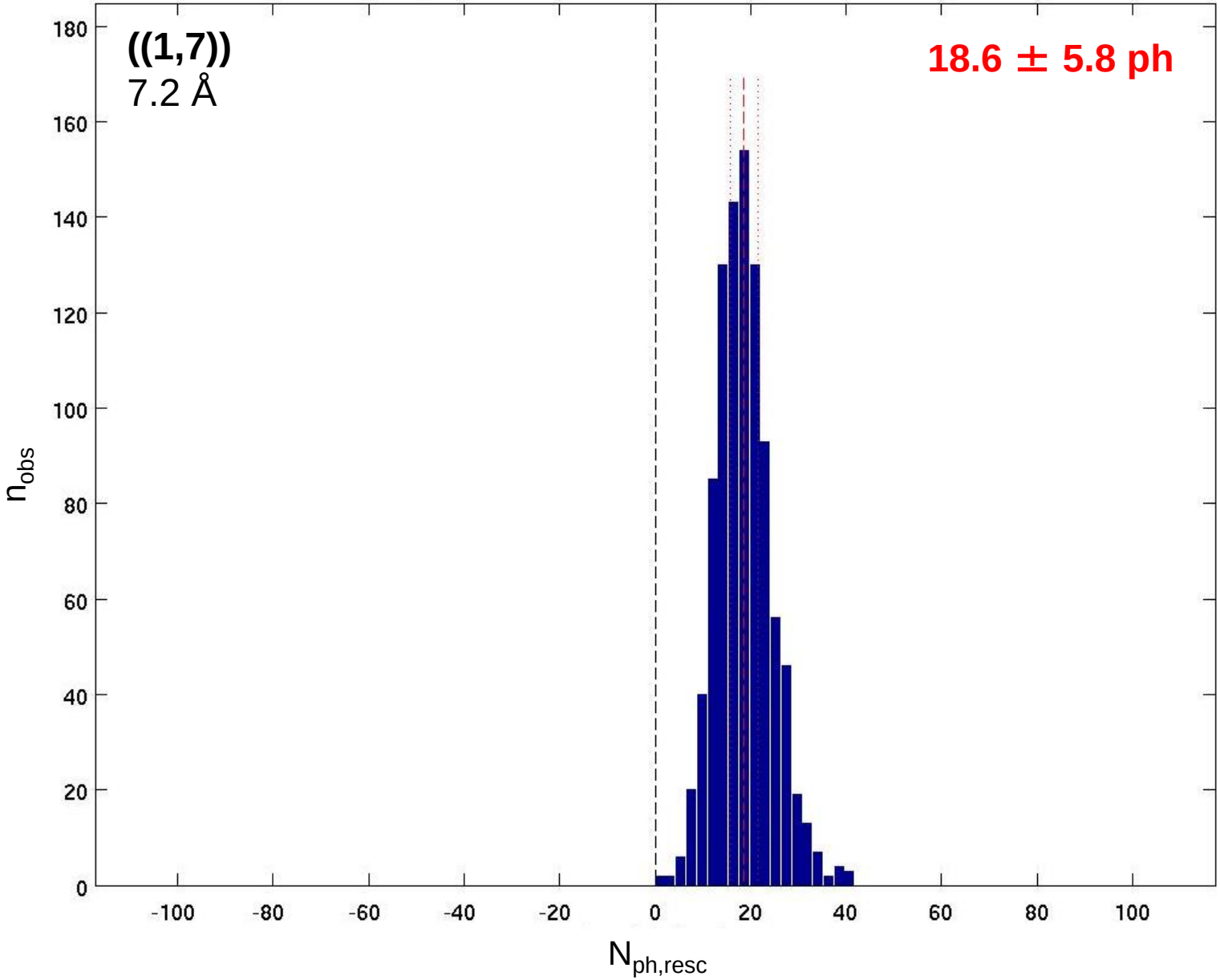




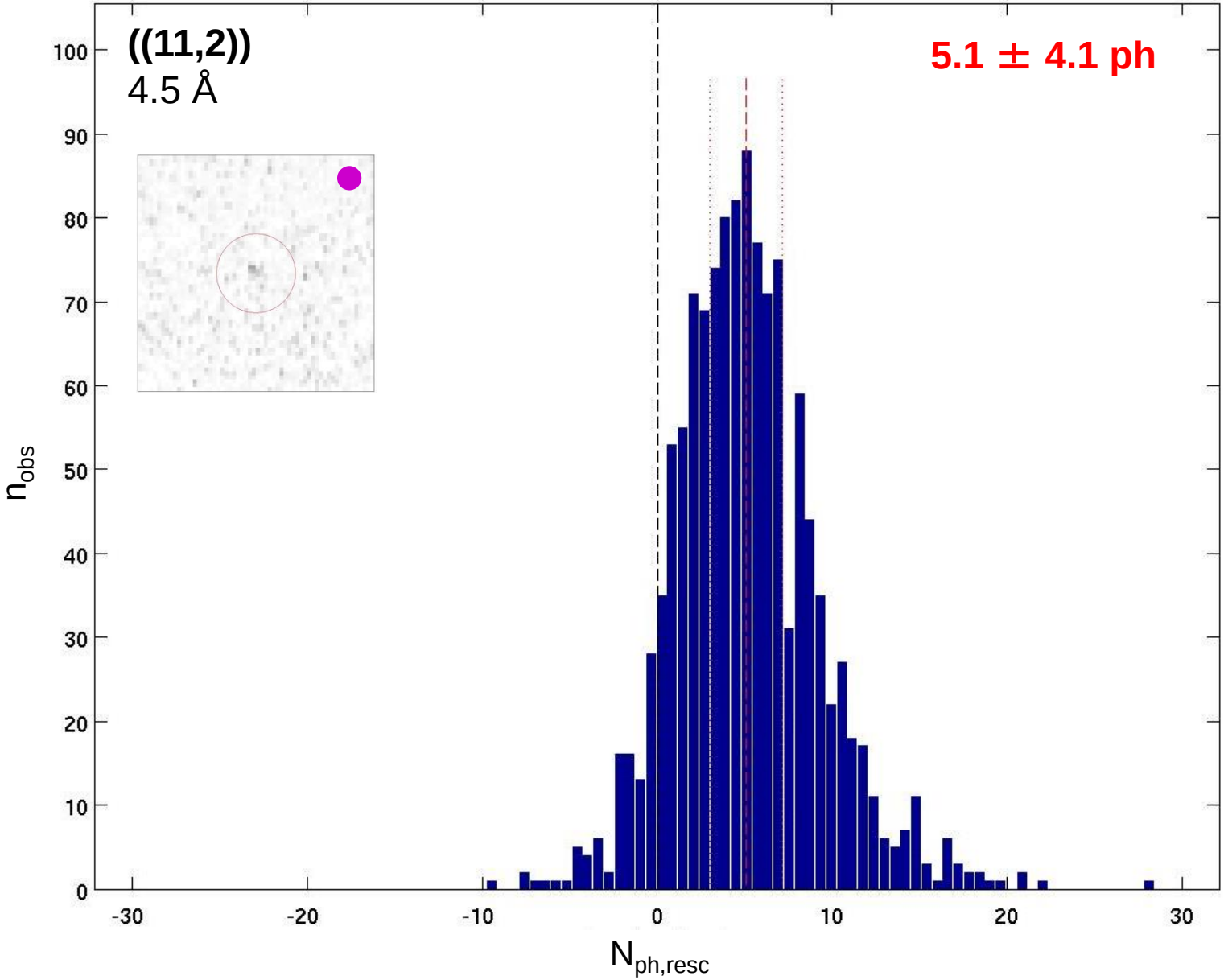
Rescaled reflection intensities



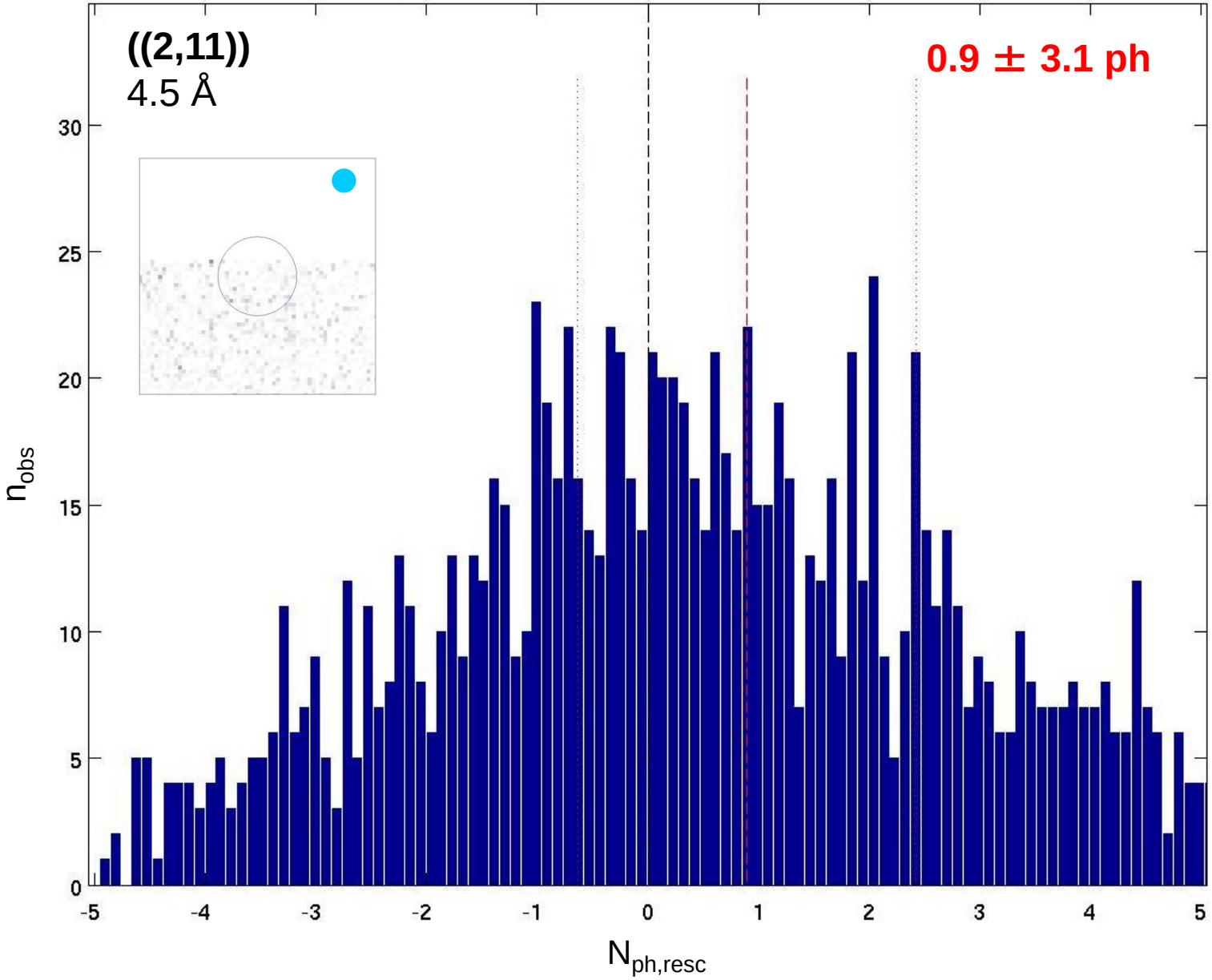
Rescaled reflection intensities



Rescaled reflection intensities



Rescaled reflection intensities



5.

Summary of the facts

Facts

- «Best» membrane protein 2D crystal
- 2D crystals stay flat
- 2D crystals survive in vacuum and at «room» temperature
- 2D crystals diffracted before getting destroyed
- Data acquisition is possible at > 1Hz
- «2-3 mJ»: 10 photons per peak at 7 Å, 1 photon per peak at 4 Å

- Tilted data analysis not yet implemented

Wishes

- Optimize everything: background, detector, analysis tools, …
- 10 times more photons on the sample
- Longrange crystal order at ~200 nm

6.

Complementarity with cryo EM

Complementarity

	PHASES **Electron microscopy (diffraction)**	NO PHASES **X-ray diffraction**
Traditional (radiation damage limited)	• Few tens of structures < 5 – 10 Å • Soon **3 Å** • Cryo conditions	Only powder
Ultrafast (diffract and destroy)	?	• **< 4 Å ???** • "Room" temperature / Pump-probe

THANK YOU