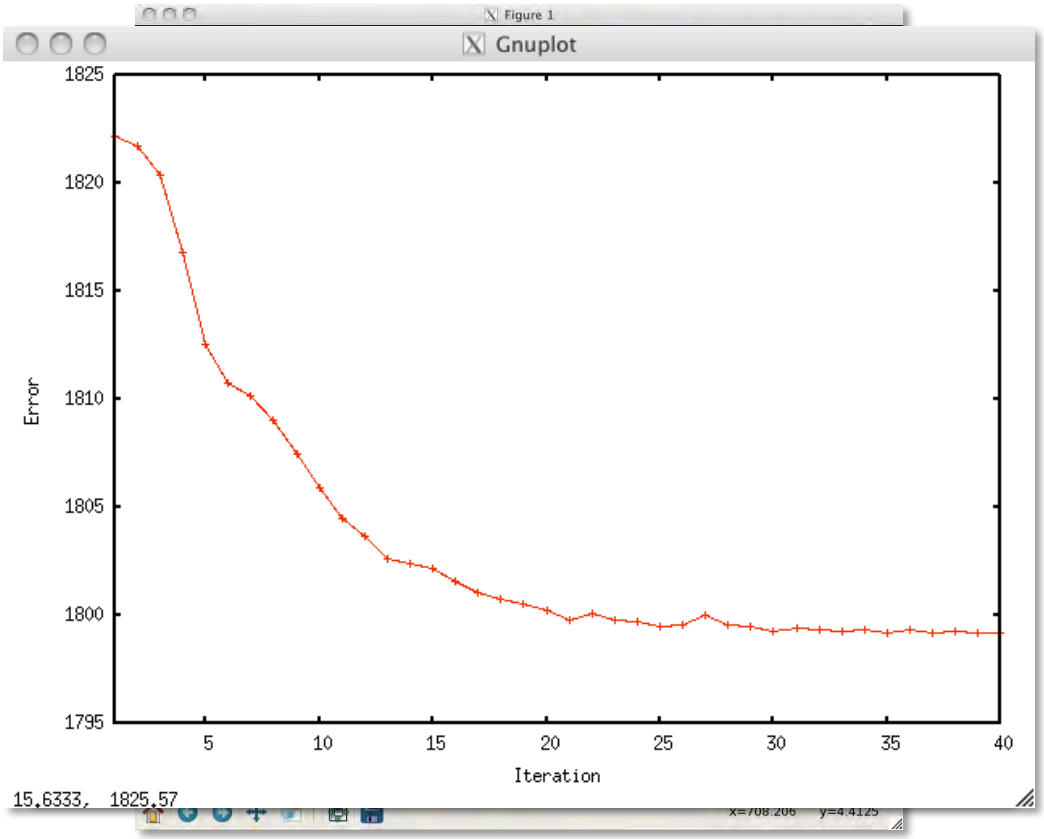
What we had and what we wanted

- Had:
  - Beamtime in 66 days
  - Two software developers
  - *pyana*
  - *LABELIT*
  - Computational Crystallography Toolbox (*cctbx*)
  - Sample data
- Wanted:
  - Processing suite for XFEL data

Understanding the CSPAD



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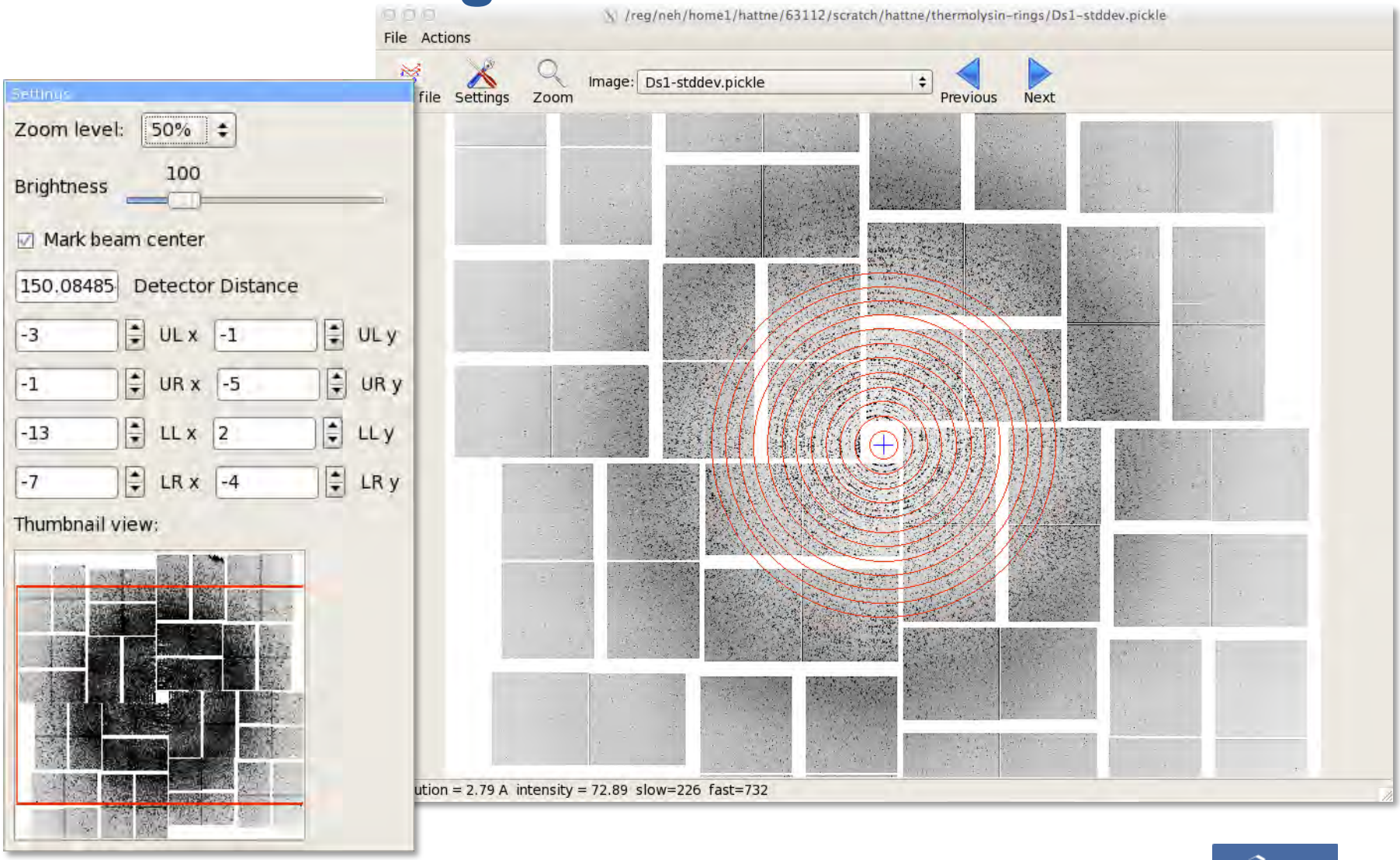
Understanding the CSPAD



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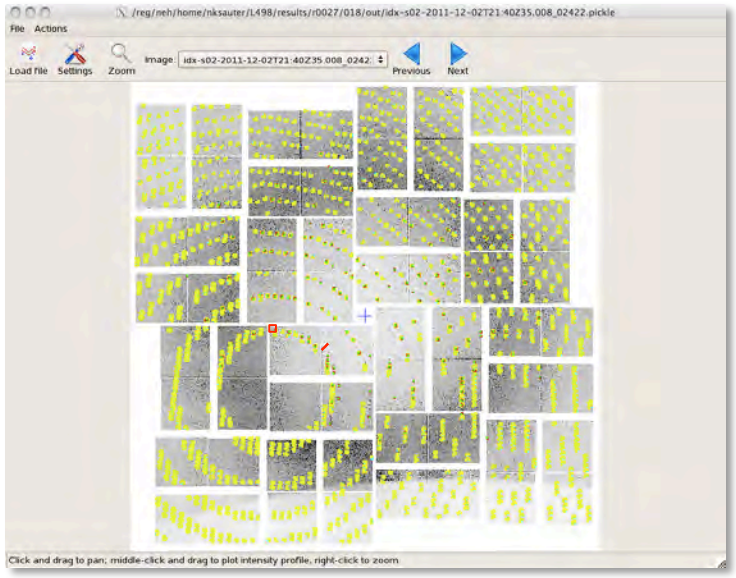
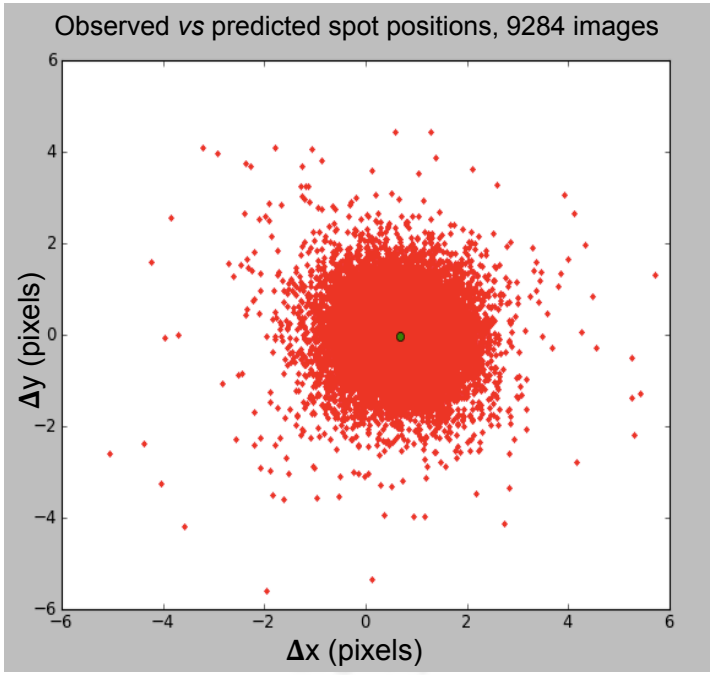
Fine detector calibration





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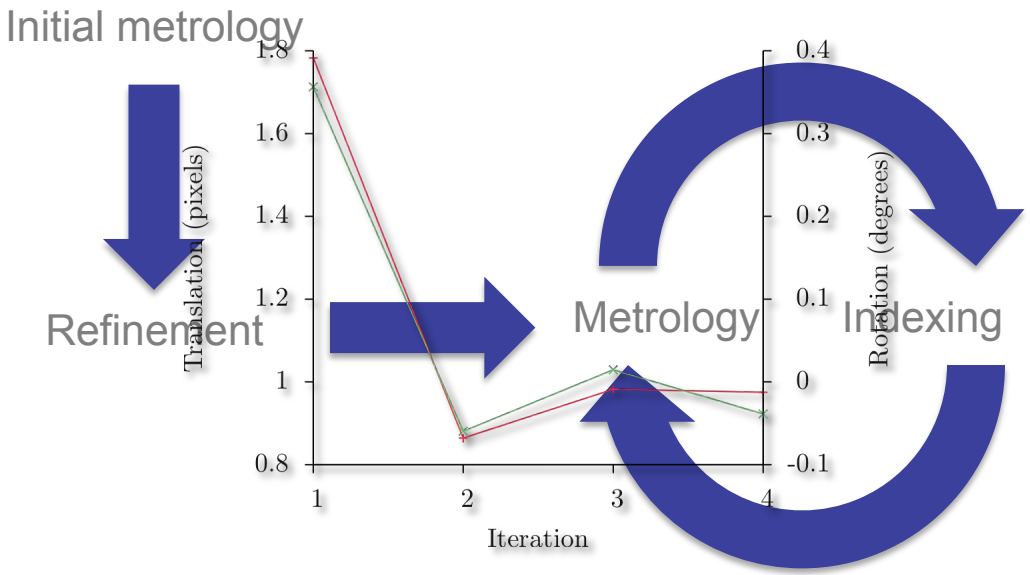
Metrology refinement March 2012



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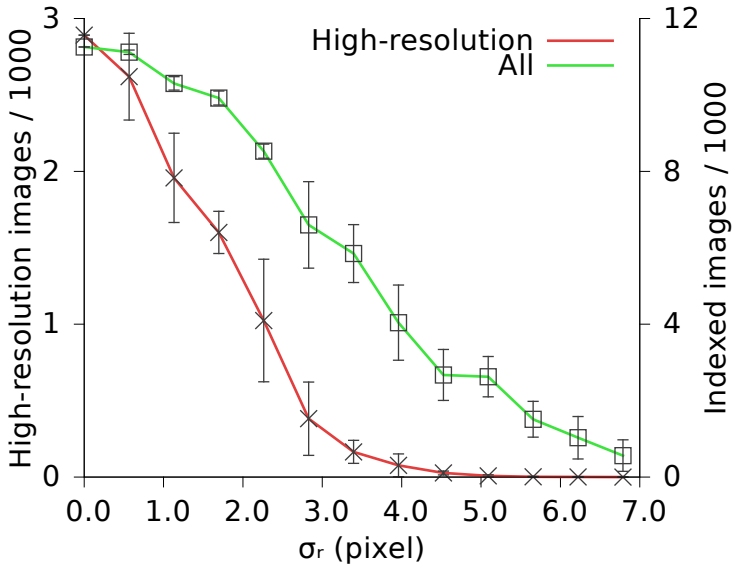
Importance of metrology



- Single-pixel metrology errors impact integration of high-resolution reflections

Hattne et al. *(2014) Nat Methods* ***11****, 545–548*

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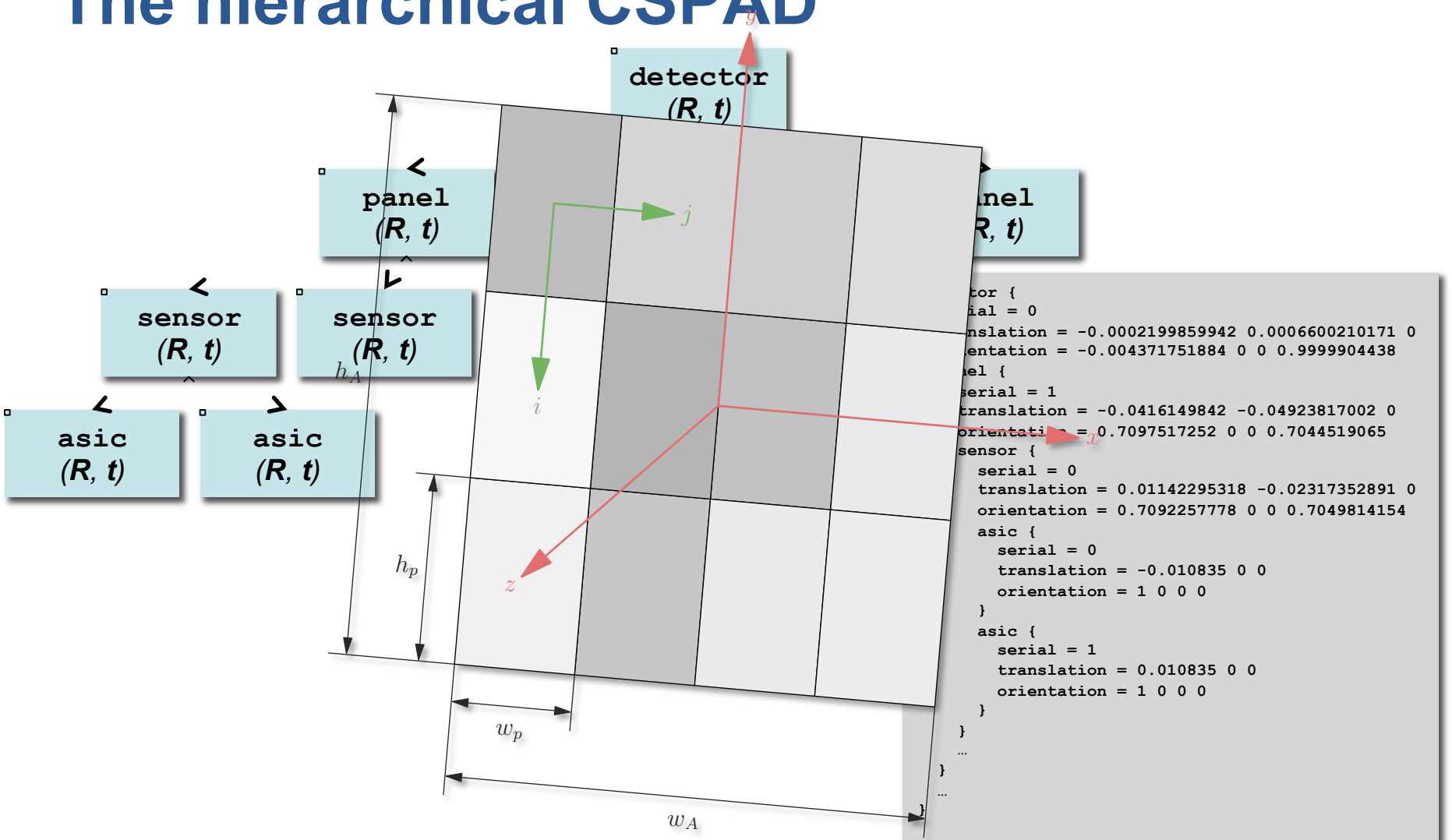
The hierarchical CSPAD



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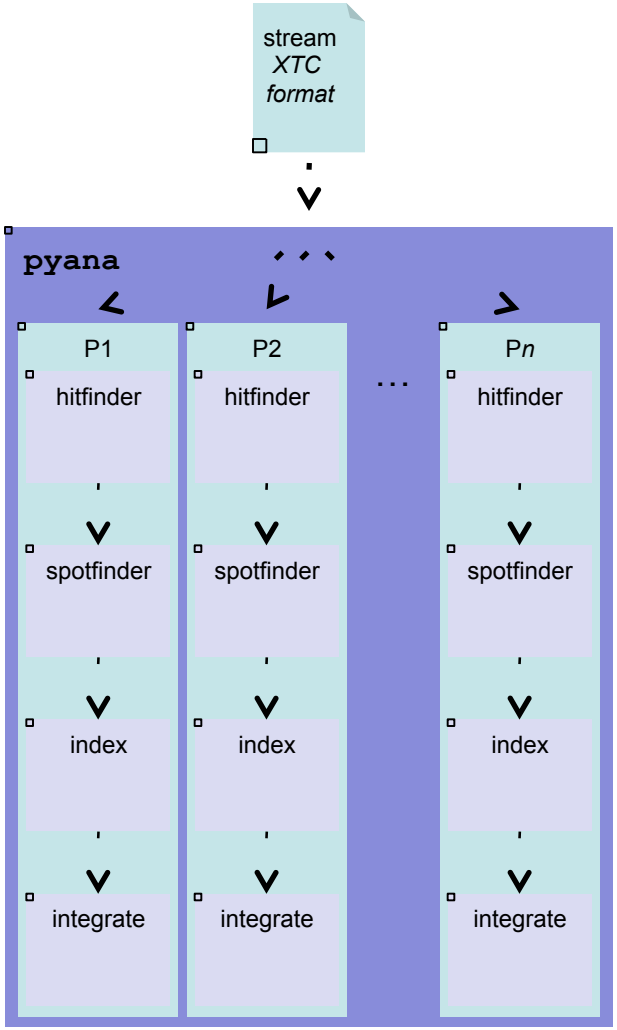
pyana multiprocessing

- Embarrassingly parallel
  - Every diffraction image can be indexed and integrated independently
  - More processors ⇒ faster processing
- *pyana* distributes events to subprocesses
  - Every process receives a non-deterministic subset of the events
  - Integrated intensities written to disk as they become available



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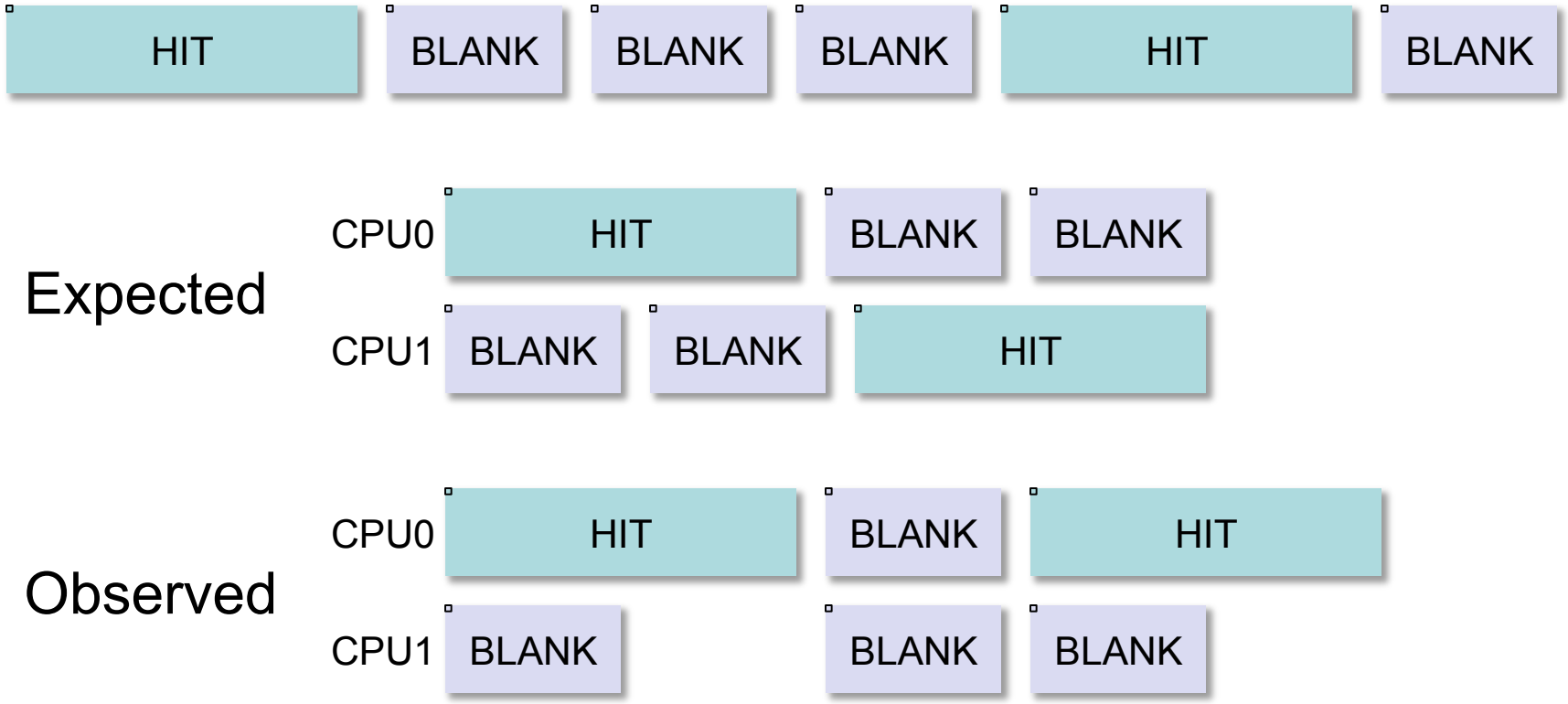
Early multiprocessing woes



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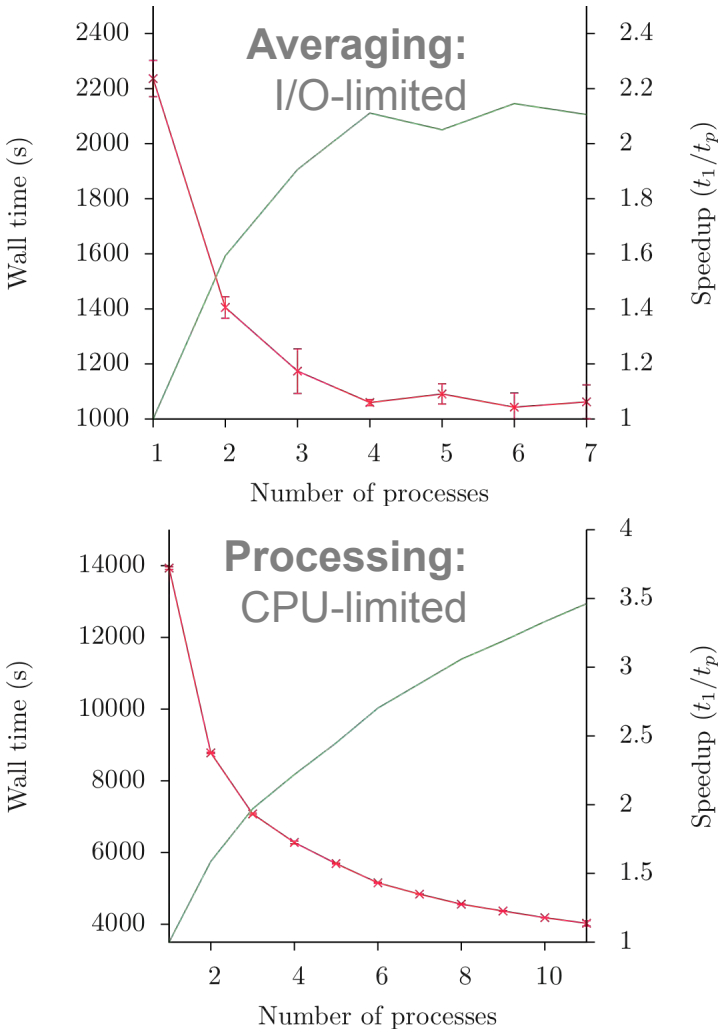
Data rates and volumes

- Processing time estimates
  - Hit-finding: 1 s / image
  - Indexing and integration: 10 s / image
  - 2% hit rate @ 120 Hz: 380 CPU seconds to process 1 s of raw data
- NERSC has thousands of cores
- But NERSC ≠ SLAC



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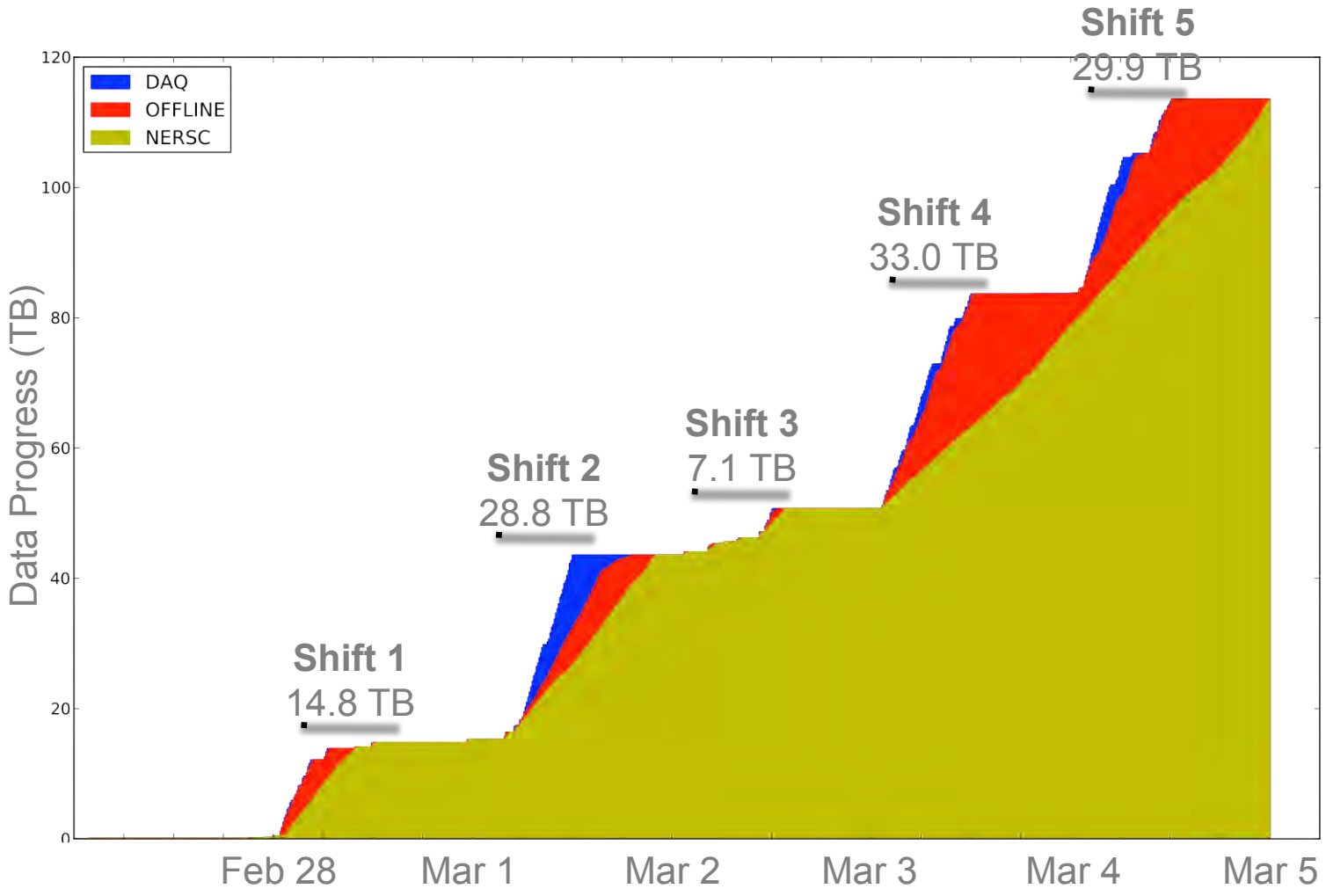
Data transfer SLAC➔NERSC



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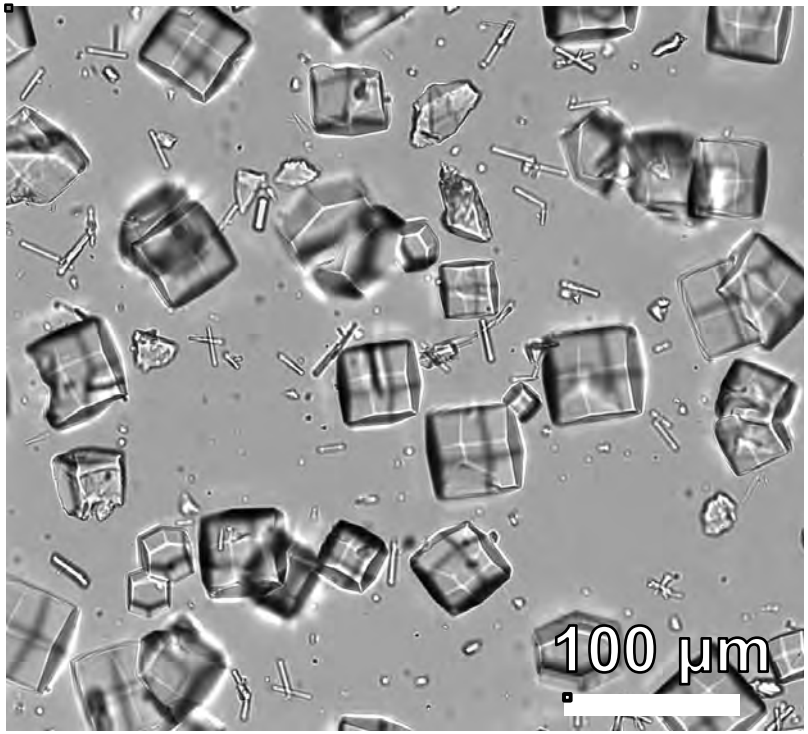
Electron diffraction

- Intense XFEL pulses enable diffraction measurements from tiny crystals
  - Collect diffraction pattern before onset of radiation damage
- Electrons interact with matter stronger than X-rays
  - But what about radiation damage?



Lysozyme microcrystals

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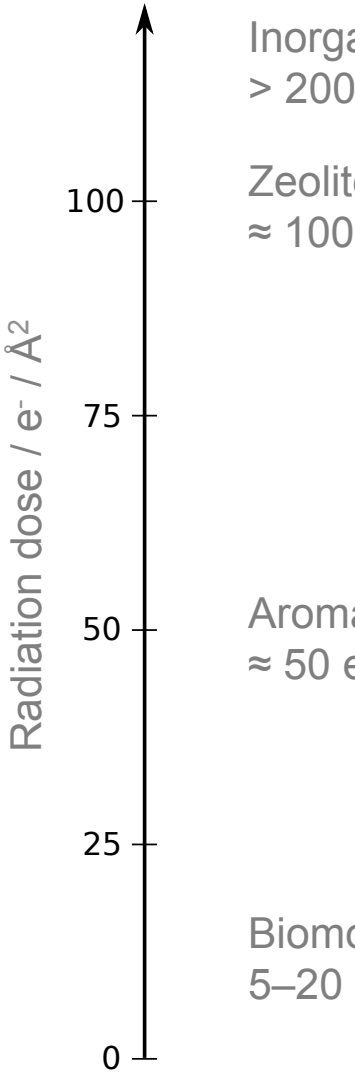
MicroED ultra-low dose diffraction

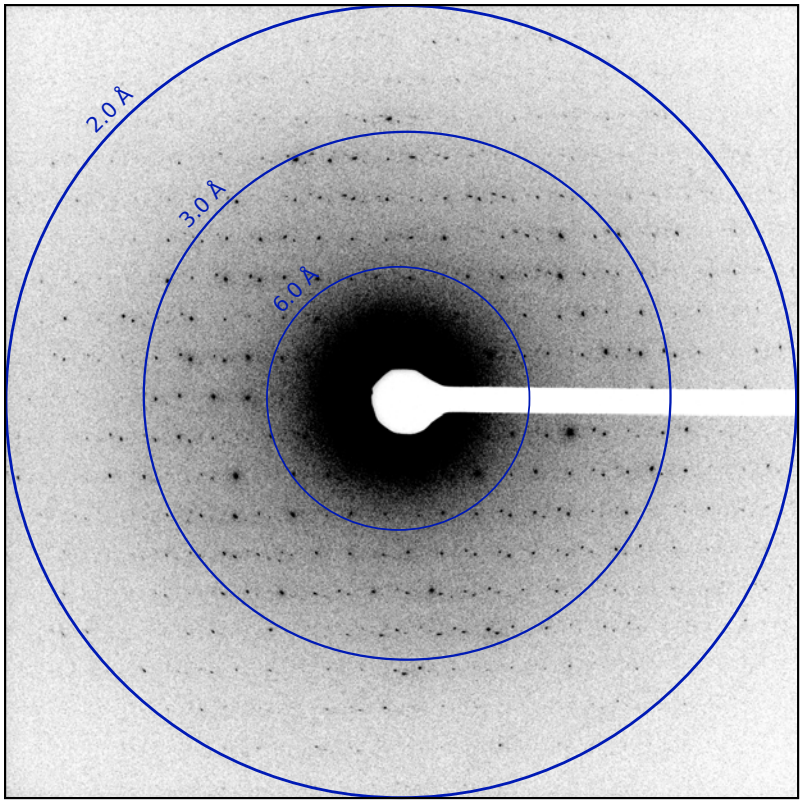


Inorganic materials
> 200 e^- / $Å^2$

Zeolites
≈ 100 e^- / $Å^2$

Aromatic polymers
≈ 50 e^- / $Å^2$

Biomolecules
5–20 e^- / $Å^2$



MicroED lysozyme diffraction pattern
Total dose: 0.1 e^- / $Å^2$

Shi et al. *(2013) Elife* **2**, *e01345*

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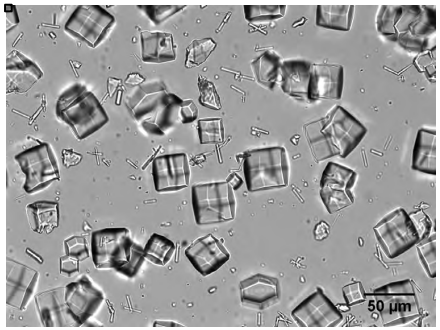
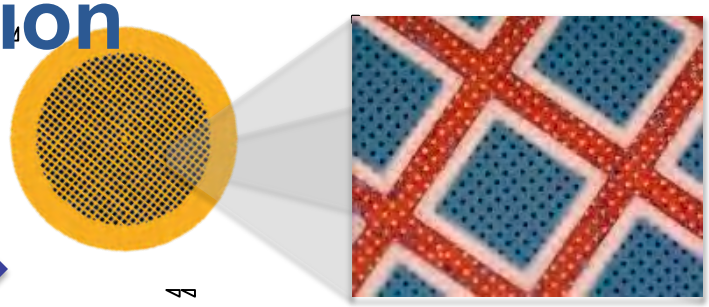
MicroED: sample preparation

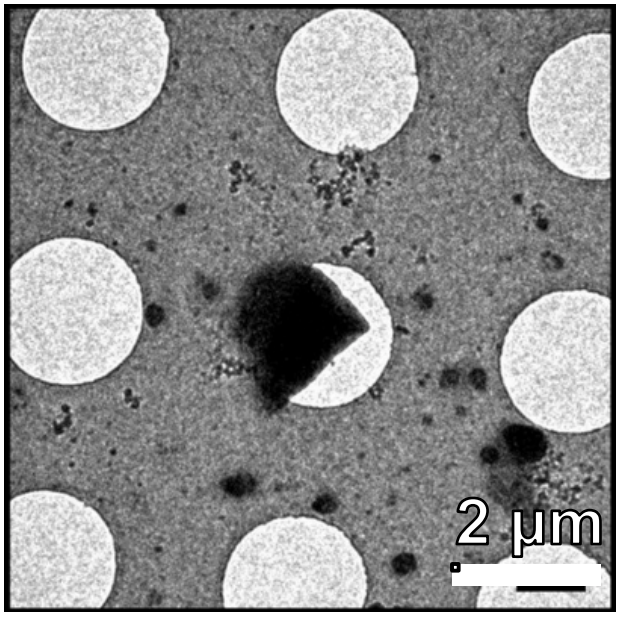


Deposit sample



Blot and freeze
Load in TEM

Screen microcrystals



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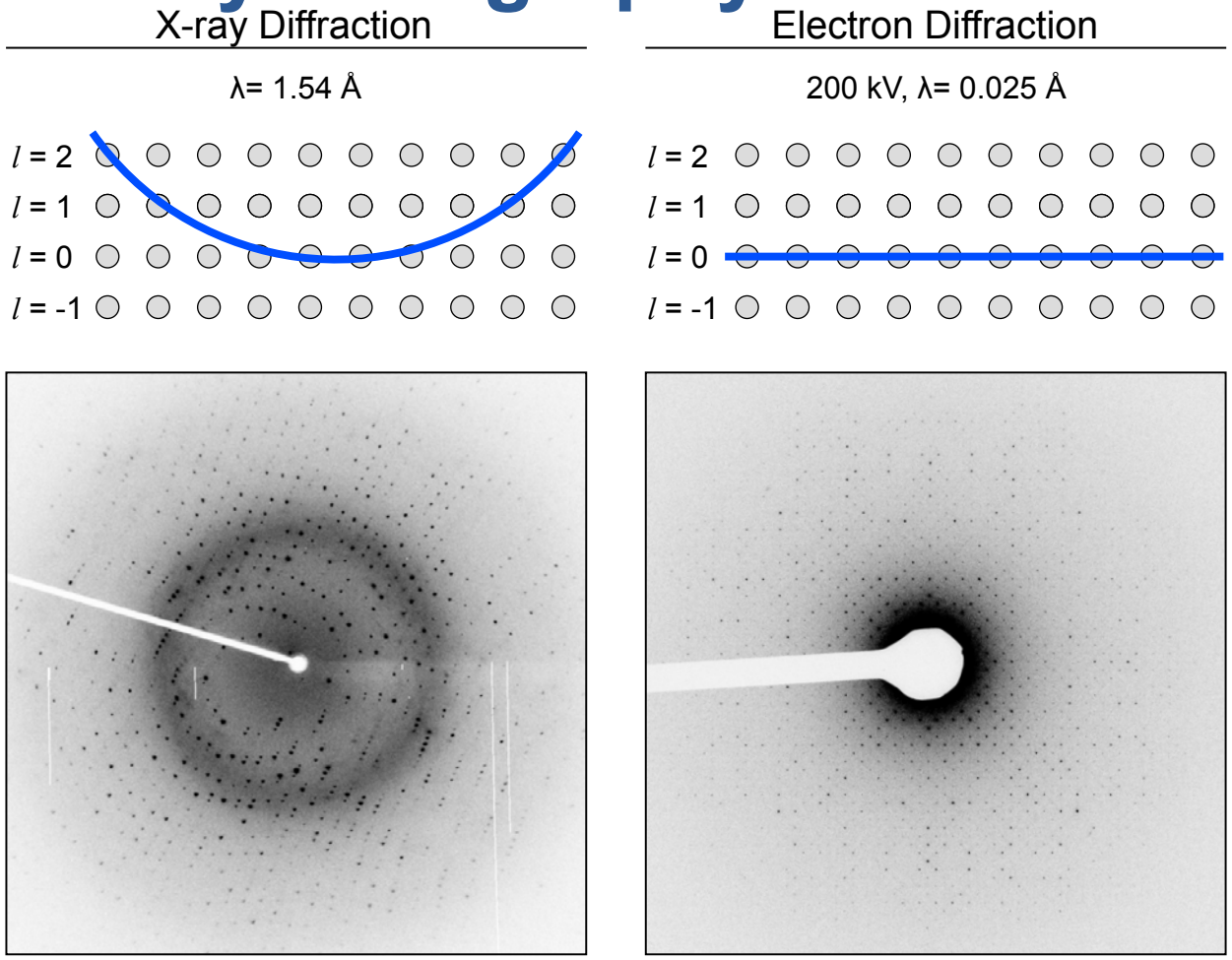
Electron crystallography



*Nannenga & Gonen (2014) Curr Opin Struct Biol **27**, 24–31*

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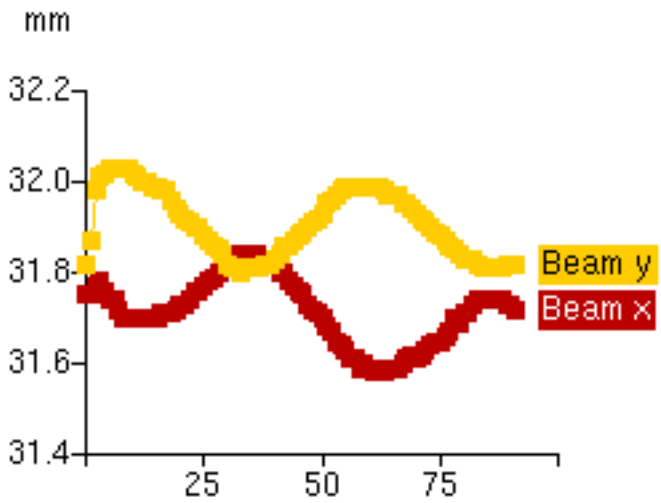
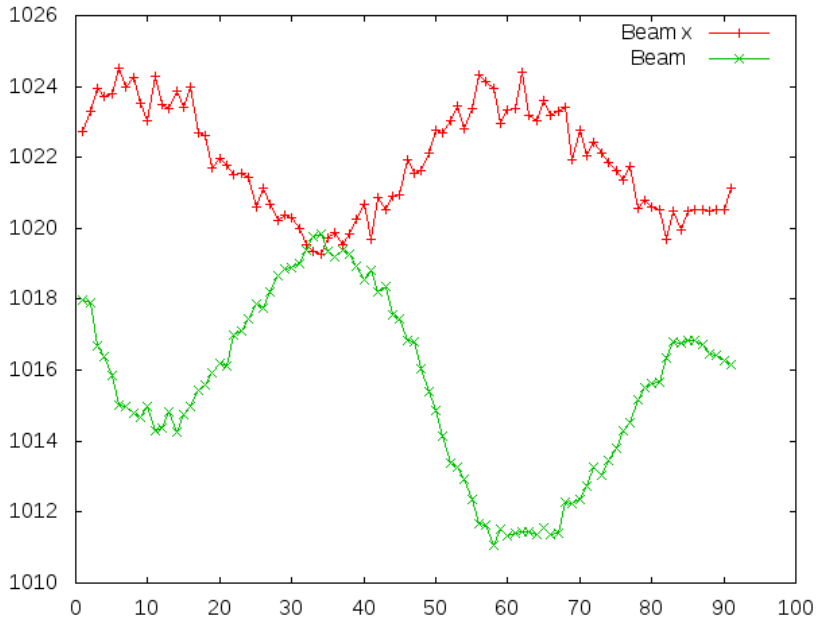
Beam center refinement





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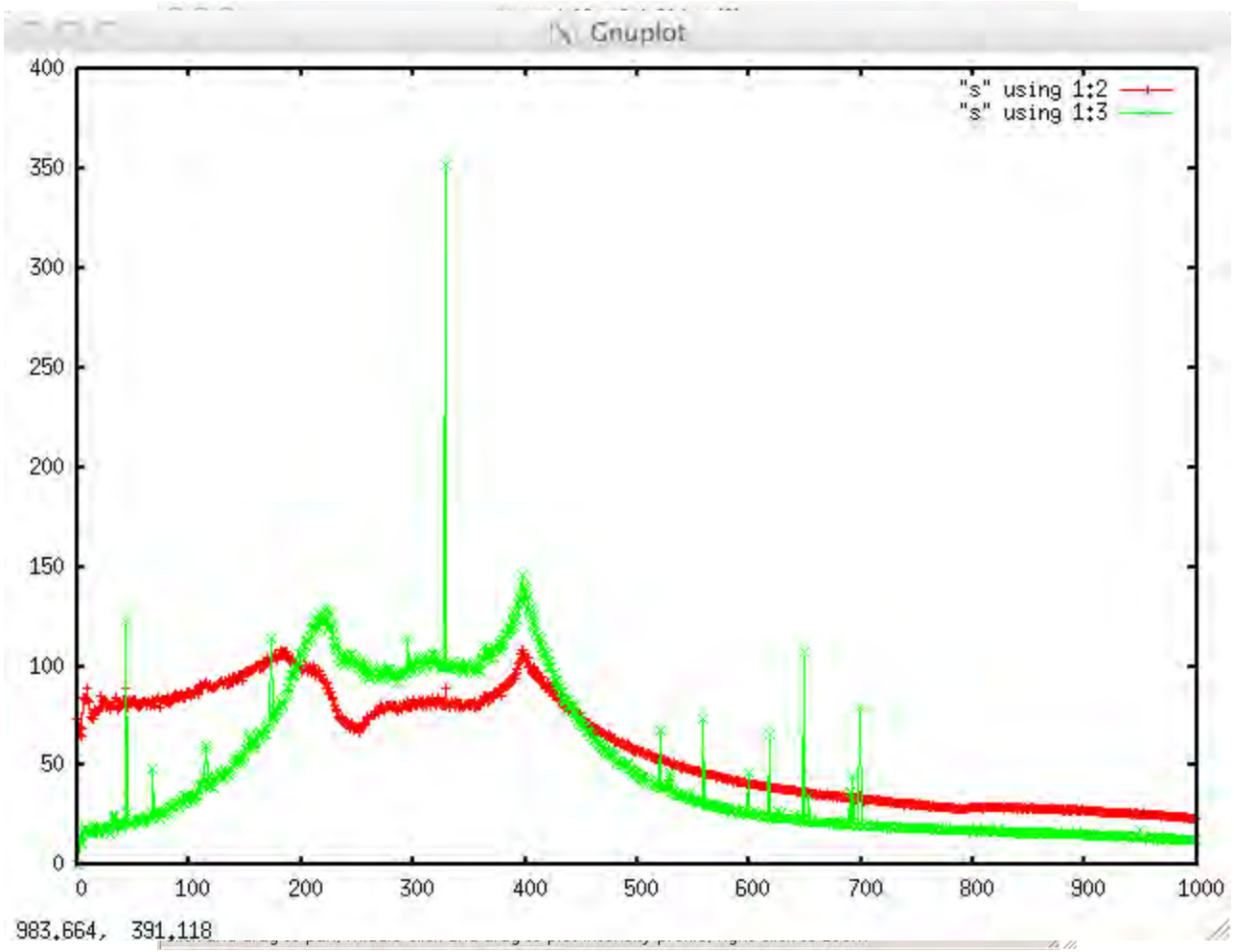
The idea



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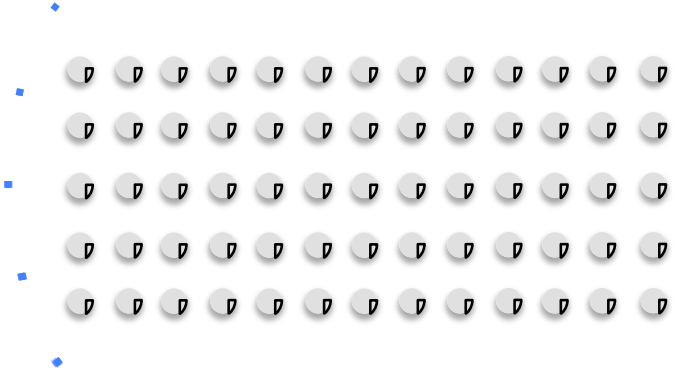
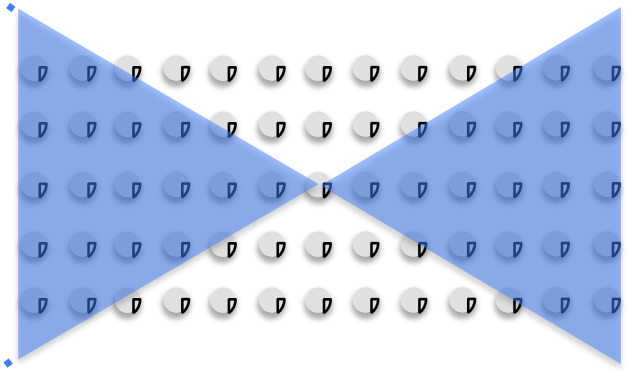
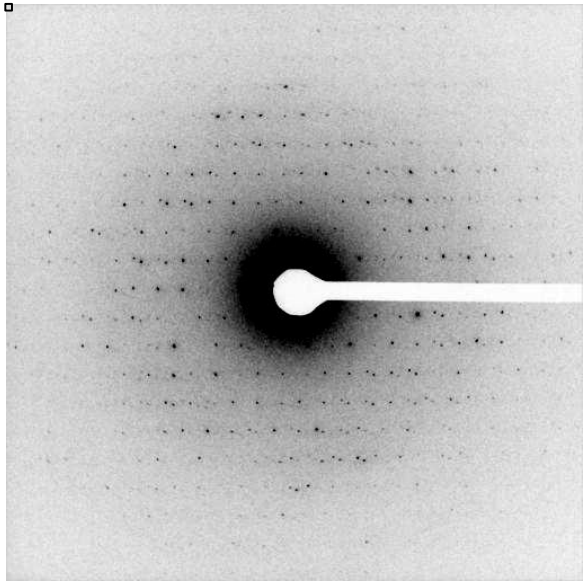
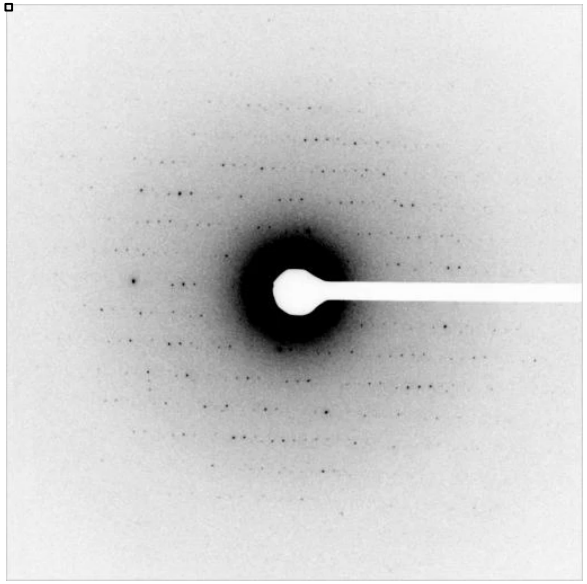
Continuous rotation data collection









Nannenga et al. *(2014) Nat Methods, in press*

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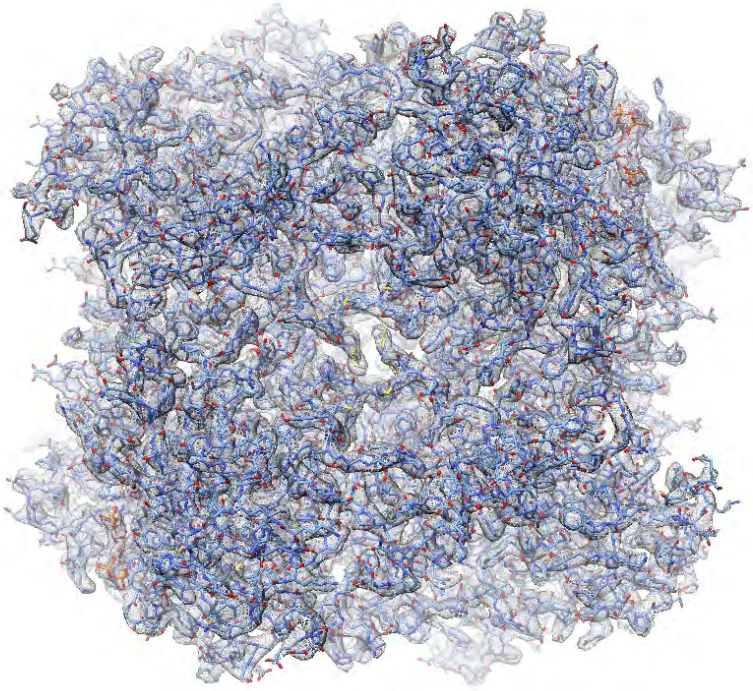
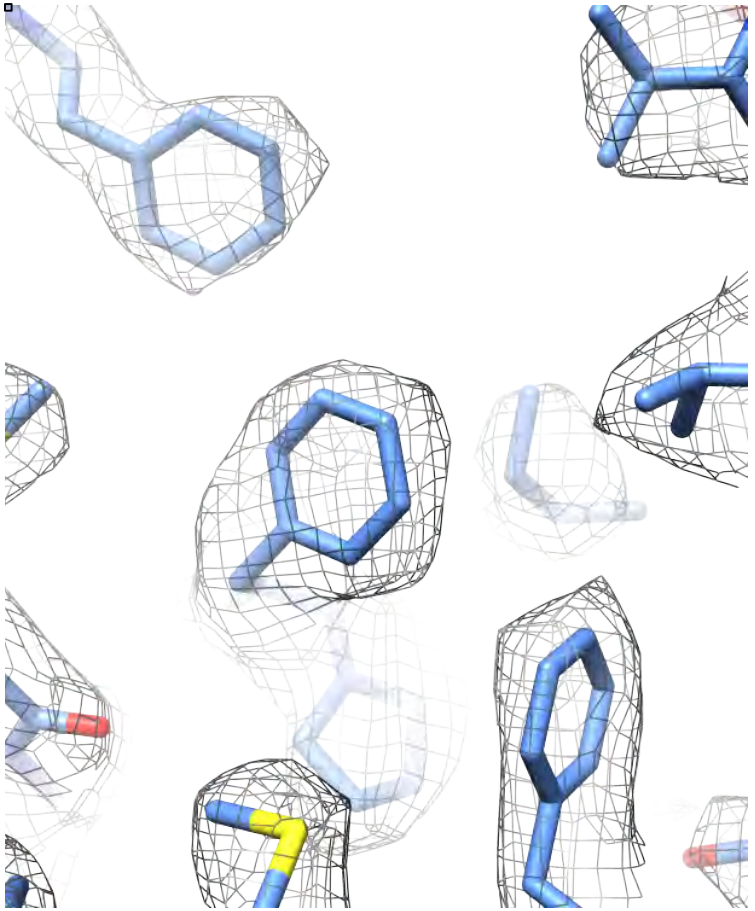
MicroED structure of catalase at 3.2 Å





Nannenga et al. *(2014) Submitted manuscript*

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XFEL *vs* MicroED

- XFEL = MicroED
  - Tiny crystals
  - Partial intensities
  - Finicky processing
  - Finicky hardware (detector, sample delivery)
  - Chunked streams
- XFEL ≠ MicroED
  - Room temperature *vs* cryocooled
  - Less demanding experiment
    - Cheap and fast, can process data on a desktop
  - ≈ 5 authors per paper

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