The Abdus Salam
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Advanced School on Synchrotron and Free Electron Laser Sources and their Multidisciplinary Applications

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X-ray diffraction [from protein crystals]

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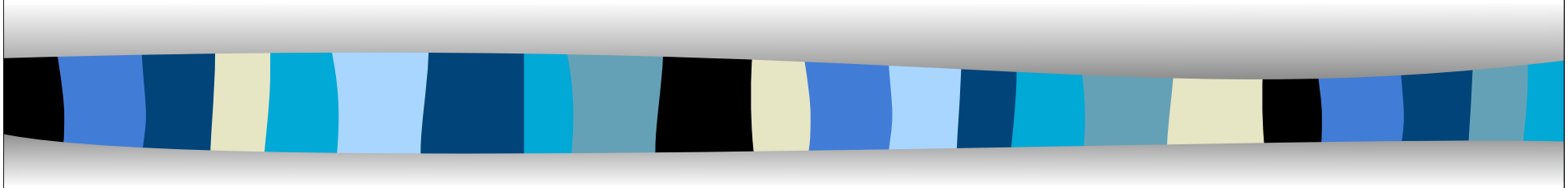
X-ray diffraction [from protein crystals]



Maurizio Polentarutti

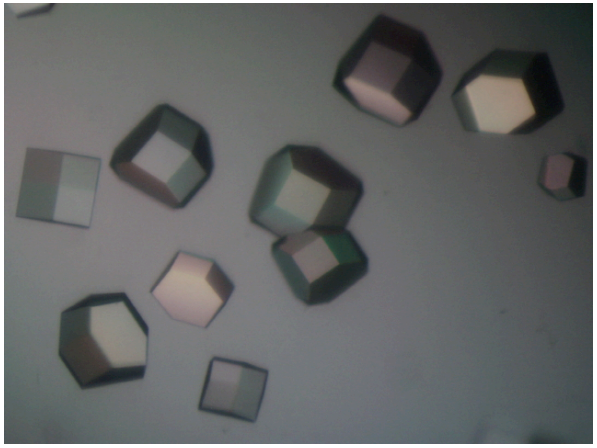
Elettra, XRD1 beam line

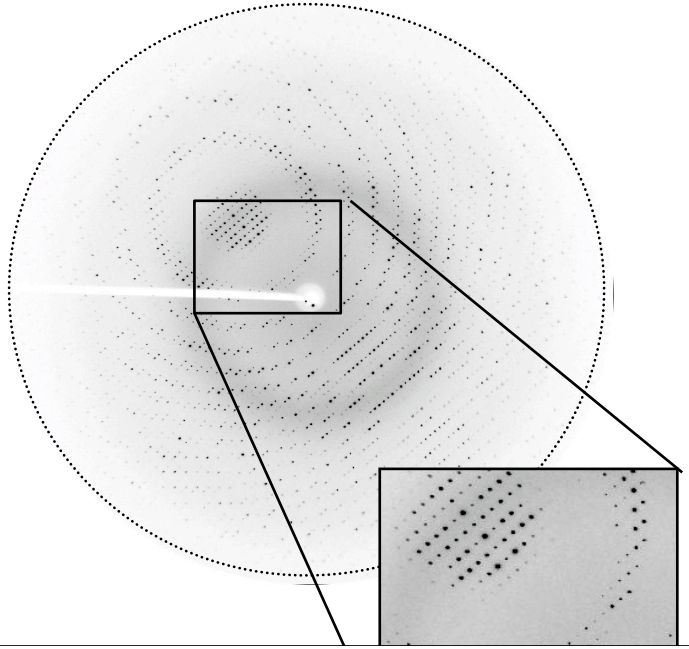
Layout

- Data collection (how the diffraction images should look and some examples from *real* life)
- The experimental set-up (key parameters, the samples and practical aspects)
- Data extraction and manipulation (programs, algorithms, data quality indicators)
- The missing data and the rest of the story (the phase problem and the model building)

Data collection

(what we want to collect and why, how the diffraction images should look and some examples from *real* life)





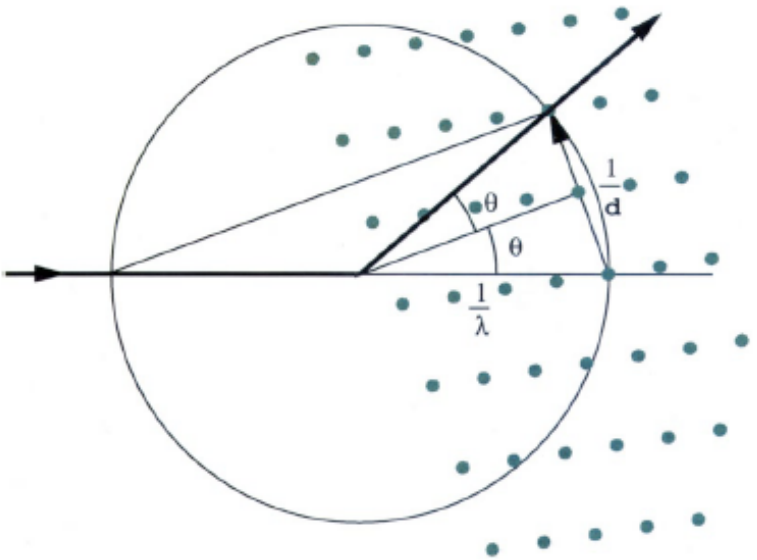
What we want to measure (and why)?

- The [protein] electron density equation:

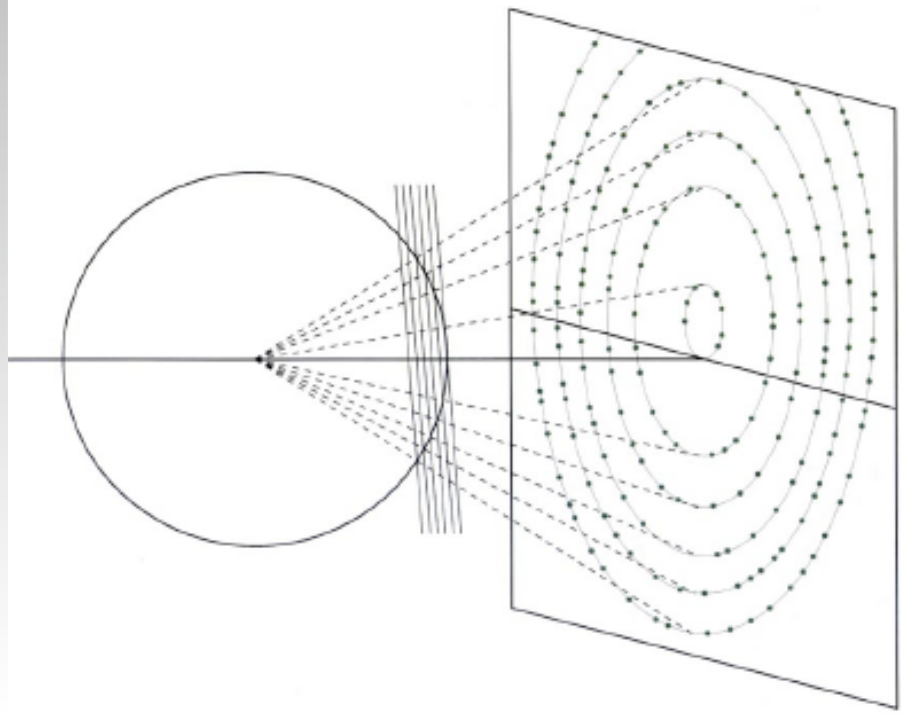
$$\rho(x,y,z) = \frac{1}{V_c}\sum_h\sum_k\sum_l \left|F(h,k,l)\right| \times e^{-2\pi i(hx+ky+lz)+i\alpha(h,k,l)}$$

- The integrated intensity for each reflection

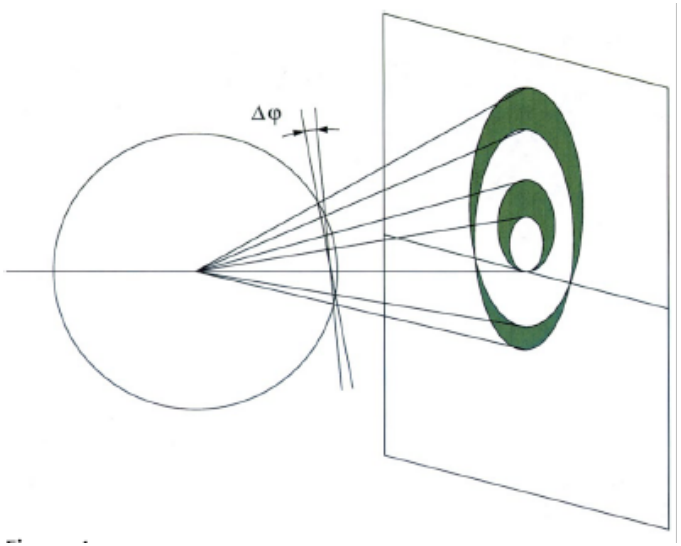
$$I(\text{int}, h\ k\ l) = \frac{\lambda^3}{\omega \times V_{\text{cell}}^2} \times \left(\frac{e^2}{mc^2}\right)^2 \times V_{\text{cr}} \times I_0 \times L \times P \times A \times \left|F(hkl)\right|^2$$



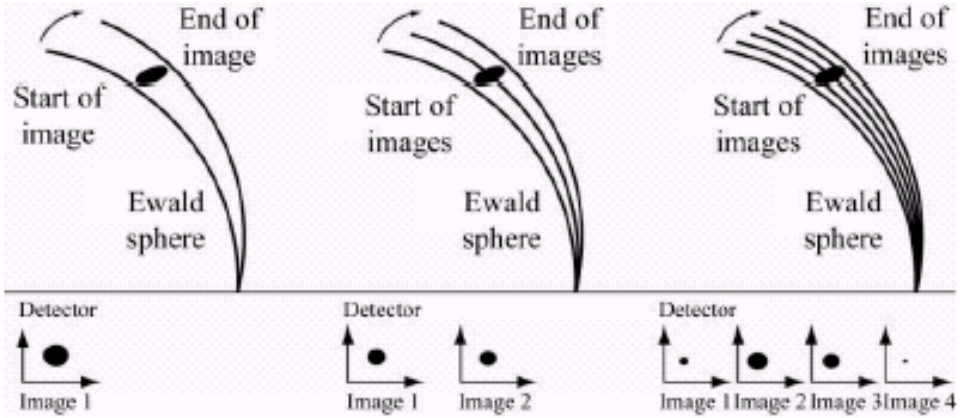
The Ewald construction. When the reciprocal-lattice point crosses the surface of the sphere, the trigonometric condition $1/d = (2/\lambda)\sin(\theta)$ is fulfilled. This is the three-dimensional illustration of Bragg's law $\lambda = 2d\sin\theta$



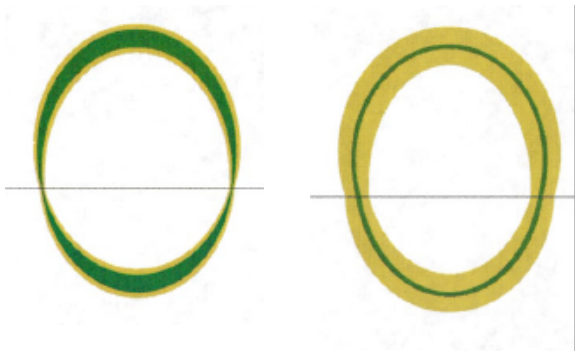
A still exposure with a stationary crystal contains only a small number of reflections arranged in a set of narrow ellipses.



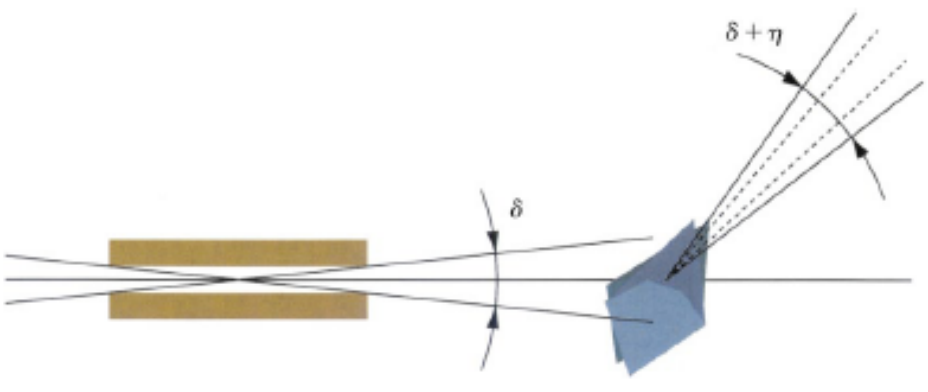
When the crystal is rotated, reflections from the same plane in the reciprocal lattice form a **lune**, limited by two ellipses corresponding to the start and end positions.



Real crystals are composed of small mosaic blocks slightly misoriented with respect to one another, which adds some divergence to the total rocking curve, that is to the amount of rotation during which an individual reflection diffracts.

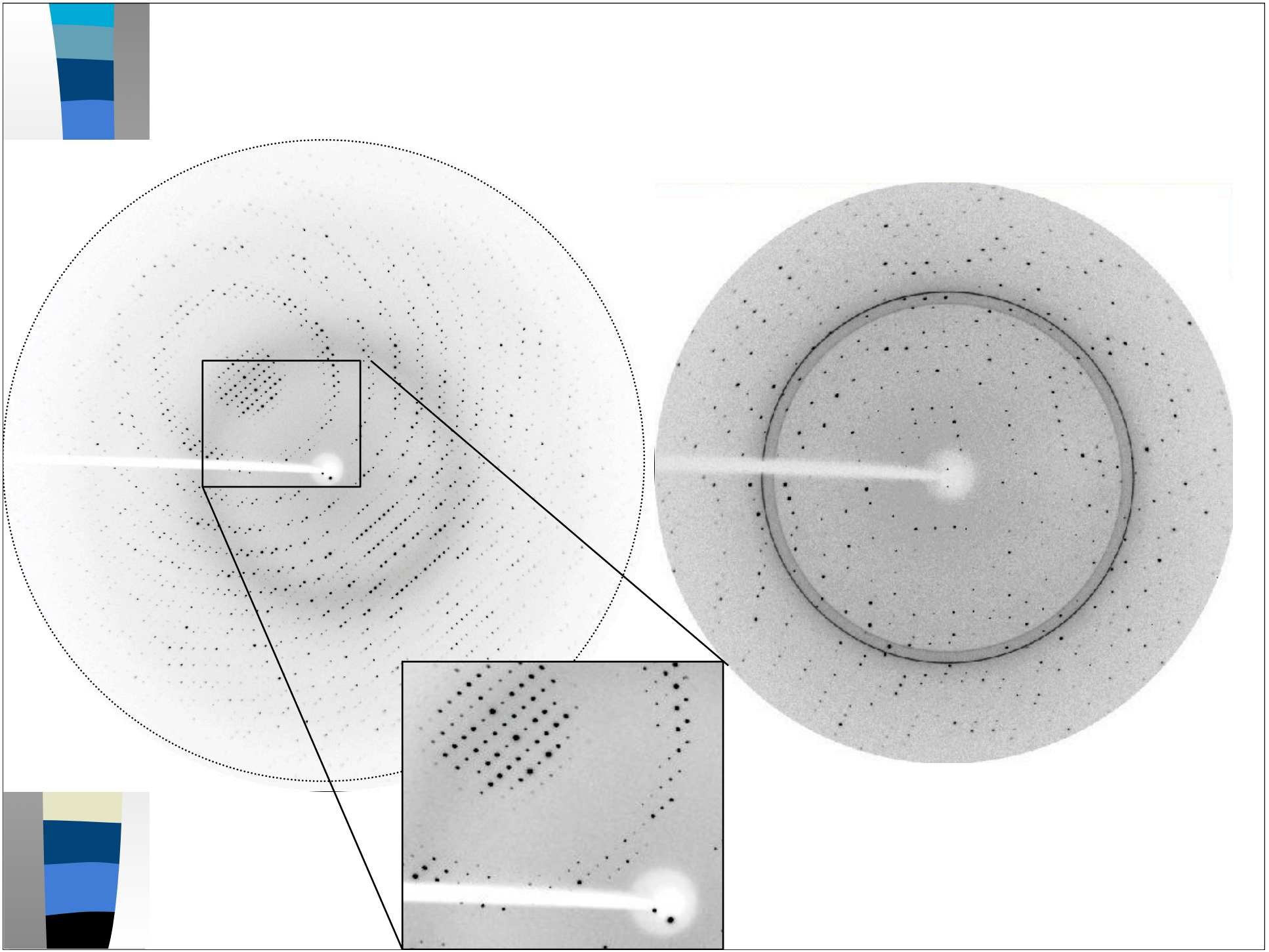


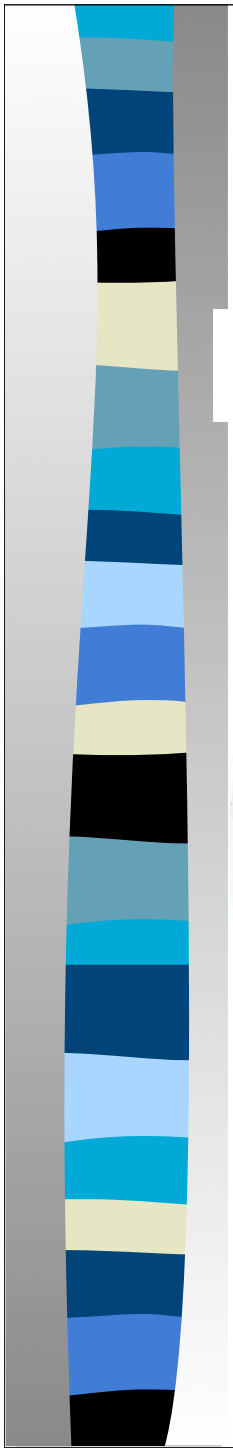
Low and High mosaicity lunes, with partially recorded and fully recorded reflections.



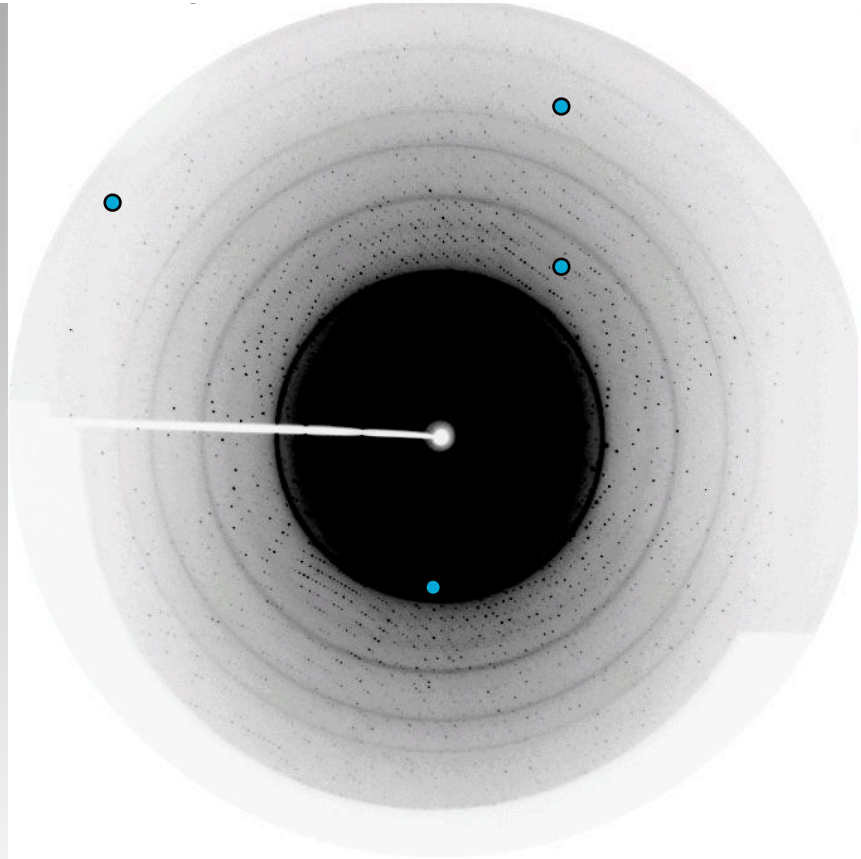
Schematic illustration of how beam divergence and crystal mosaicity combine to give the total rocking curve of the diffracted rays.

In addition, the X-radiation is monochromated to a defined narrow wavelength window and has a bandpass of the order 0.0002±0.001 at synchrotron beam lines; The wavelength bandpass effectively broadens the Ewald sphere (the radius depends on λ).

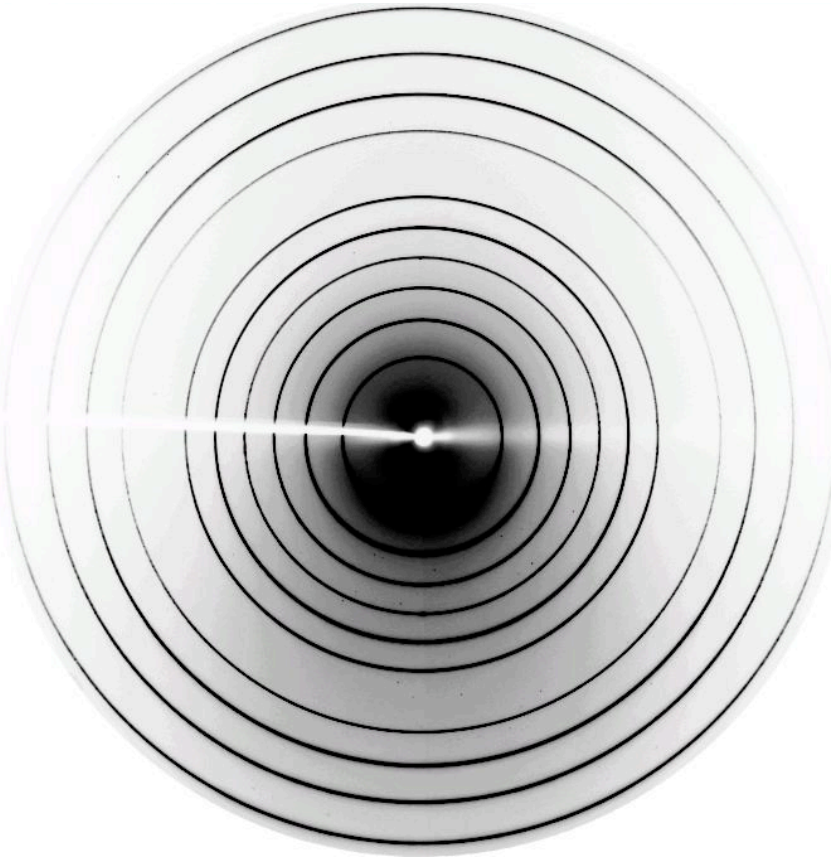


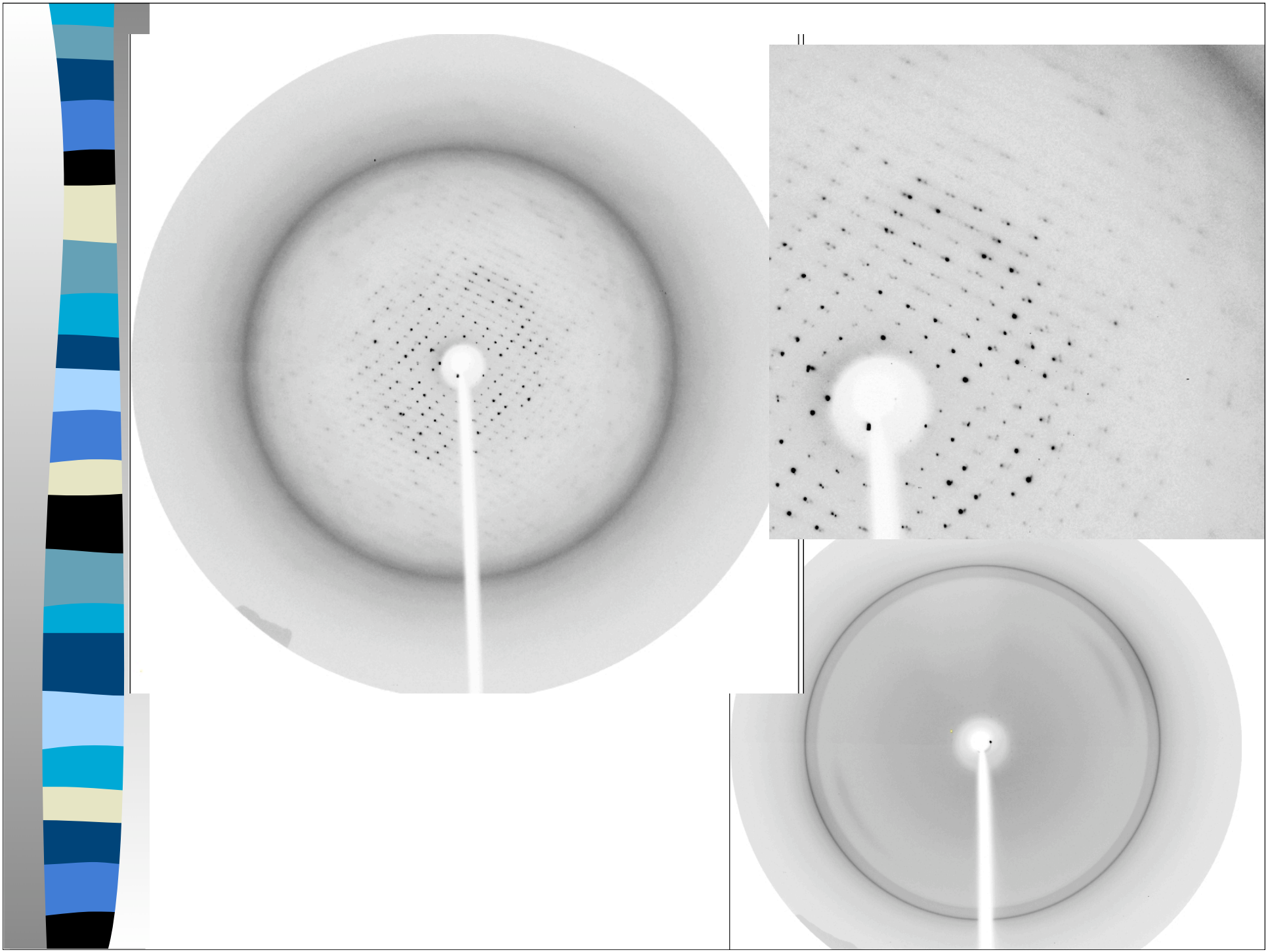


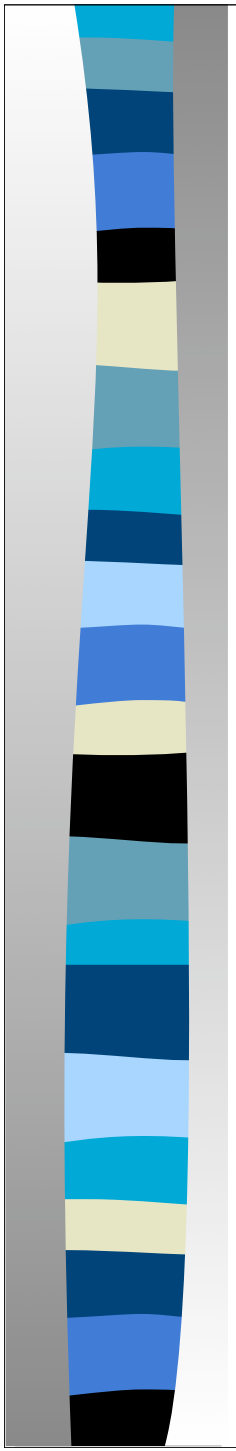
Protein (and bad cryosolution)



LaB_6 – policristalline powder
in capillary (not well-centered)







The experimental set-up

(key parameters, samples and practical aspects..)

- Common to all the PX beam lines/*home* labs
- Main components (in order of appearance):
  - *Slits (beam shapers)*
  - *Shutter (related to the time exposure)*
  - *Sample (protein single crystal)*
  - *Sample cooler system*
  - *Sample manipulator system (horizontal spindle axis)*
  - *Fluorescence detection system (beam lines only)*
  - *(Primary)-Beam stopper*
  - *Detector*
- Experimental key parameters

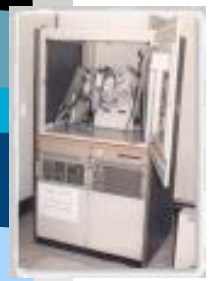
  Sample-to-detector distance, wavelength, detector surface, beam stopper position, sample macroscopic and unit-cell dimensions, sample orientation, detector angular position, sample rotation per image, exposure time.

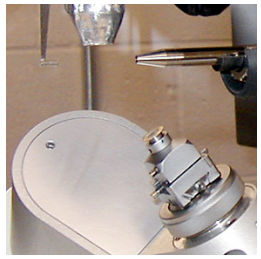
Cryo nozzle

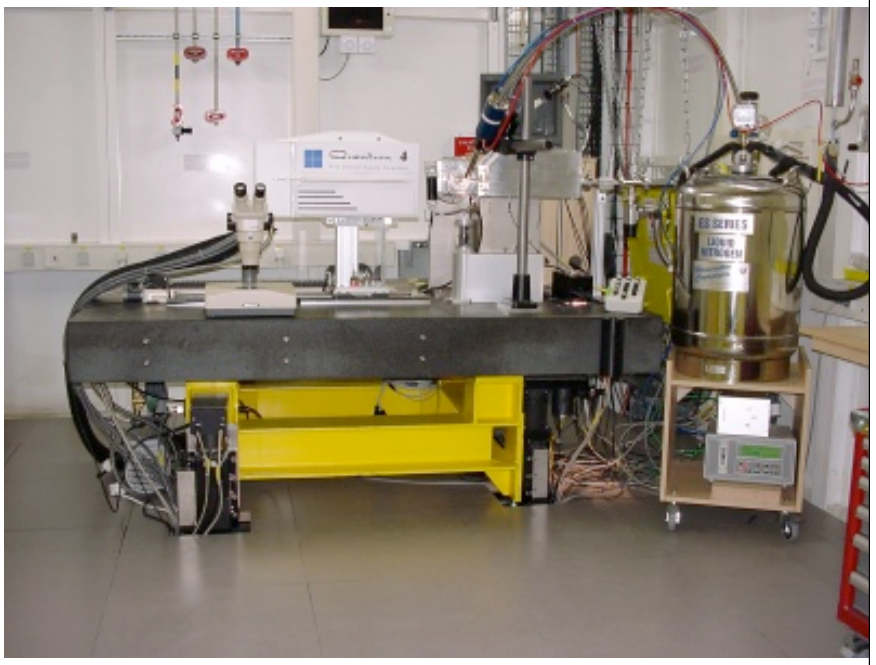
Vacuum Tube

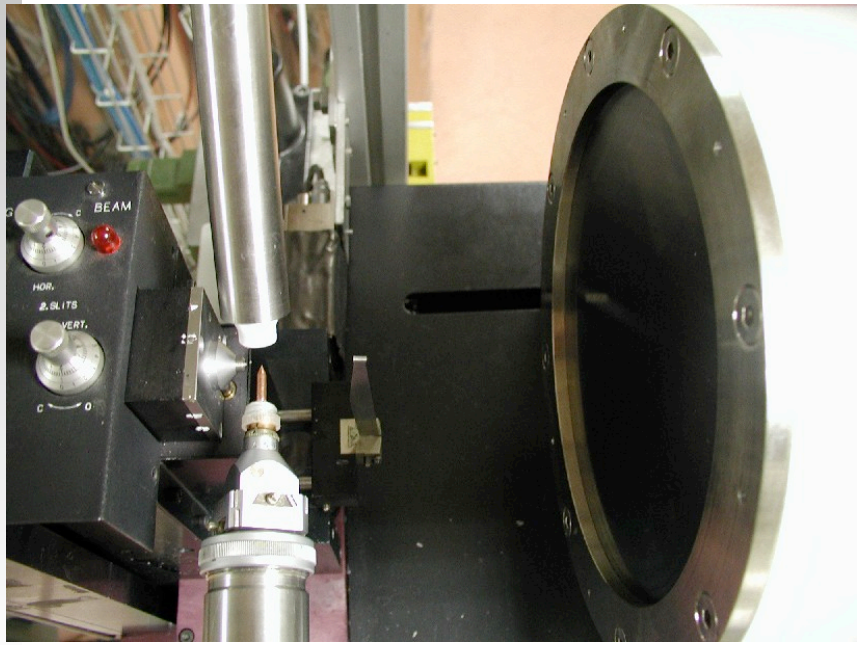
Collimators

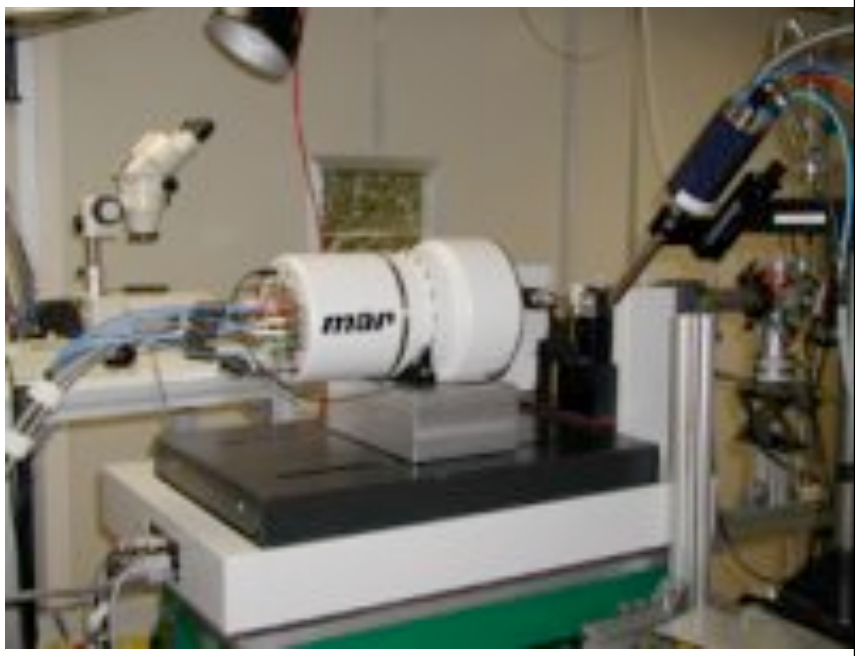
Area Detector








BEAM
HOR.
SLITS
VERT.



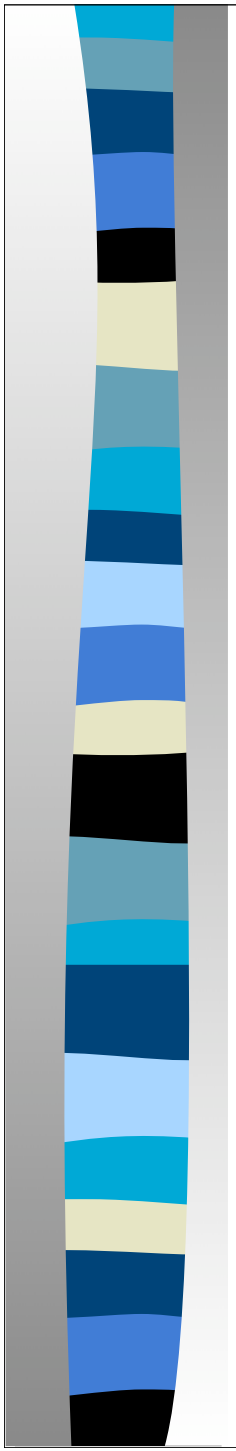
- How to choose the experimental key parameters?

Beam/sample size, sample-to detector distance, wavelength, detector area, beam stopper position, sample macroscopic and unit-cell dimensions, sample orientation, detector angular position, sample rotation per image, exposure time, sample diffracting power, min/max resolution

- Keep in mind Bragg's law: $2d\sin(\theta)=\lambda$ and

$$I(\text{int}, h\ k\ l) = \frac{\lambda^3}{\omega \times V_{cell}^2} \times \left(\frac{e^2}{mc^2} \right)^2 \times V_{cr} \times I_0 \times L \times P \times A \times |F(hkl)|^2$$

- Diffraction images are the product of a reciprocal space mapping
- The experiment is usually run in air → absorption and scattering
- Sample → unit cell and macroscopic dimensions, poor diffraction, diffracting power, mosaicity



Data extraction and manipulation

(programs, algorithms, data quality indicators)

Basic steps

- Image *integration (Denzo, Mosflm [ccp4], XDS)*

  Results: for each reflection (hkl), get a value and and error associated. Get the unit cell, the space group, the crystal orientation, effective resolution limit, refine the crystal to detector distance and detector angular positions.

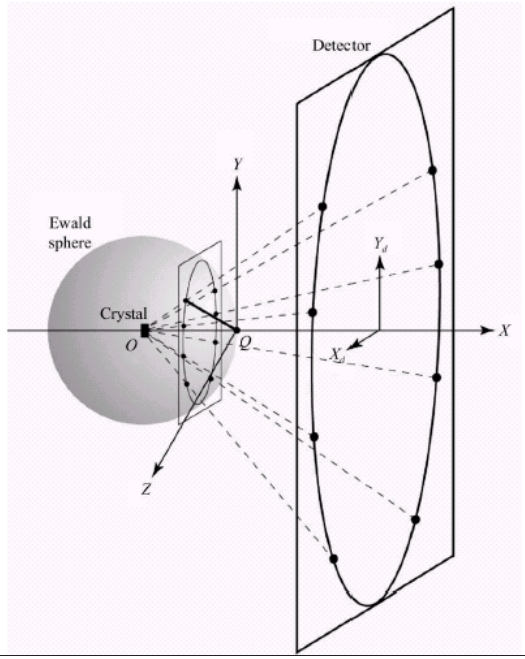
- Data *scaling (Scalepack, Scala [ccp4])*

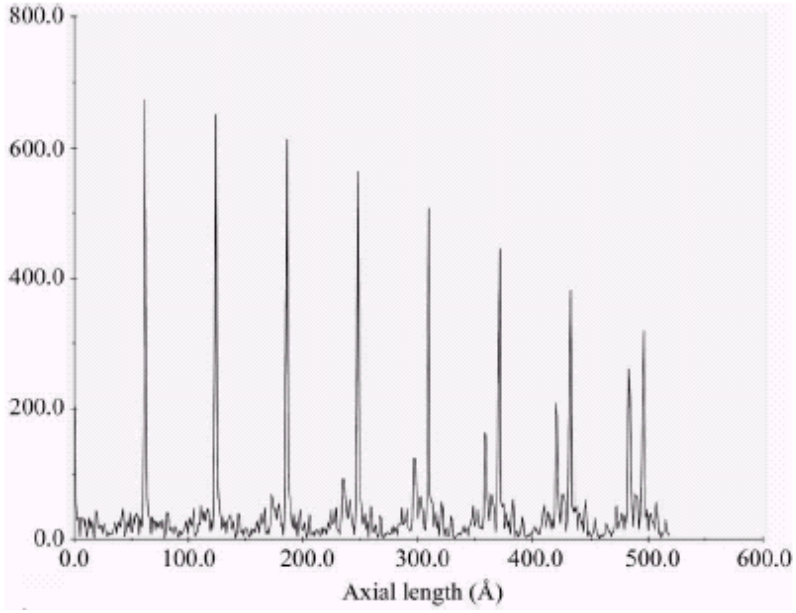
  Results: take into consideration the decay of the beam intensity, sample and air absorption, radiation damage, detector problems (spatial distortion, non-uniformity of response, time stability, bad pixels), changes in diffracting volume, estimation of data quality.

- *……solve phase problem (D. Lamba)*
- Electronic density *interpretation*
- Atomic-detail model building

How the *integration* works:

- If the members of a set of reciprocal-lattice planes perpendicular to a chosen direction **t** are well separated, then the projections of the reciprocal-lattice vectors onto **t** will have an easily recognizable periodic distribution.
- We consider about 7300 separate roughly equally spaced directions.
- The unit of the periodicity is obtained via a Fourier transform.
- The resultant unit cell is then reduced and analyzed in terms of the 44 lattice types (Burzlaff et al., 1992).





The *scaling* step:

Incident beam related factors

- Synchrotron
  - smooth decay of beam intensity
  - any discontinuities (e.g. beam injection) should be noted and included in scaling model
  - illuminated volume
  - shutter synchronization/goniometer rotation speed

Crystal related factors

- Sample absorption
  - diffracted beam absorption (shape dependent)
  - important for weak anomalous signal
- Radiation damage
  - can be significant on high brilliance sources
  - difficult to correct for
  - modeled as change in relative B-factor
  - extrapolation to zero dose

Detector related factors

- **calibration errors**
  - spatial distortion
  - non-uniformity of response
  - time stability
  - bad pixels

Miscellaneous factors

- unavoidable
  - zingers
- avoidable
  - beam stop shadow
  - cryo-stream shadow
  - should be dealt with at integration stage

Determination of scale factors

Scales are determined by comparison of symmetry-related reflections, i.e. by adjusting scale factors to get the best internal consistency of intensities. Note that we do not know the true intensities and an internally-consistent dataset is not necessarily correct. Systematic errors will remain

$$\text{Minimize } \Delta\Phi = \Sigma_{hl}\, w_{hl}\, (I_{hl} - 1/k_{hl}\langle I_h\rangle)^2$$

I_{hl} l'th intensity observation of reflection h

k_{hl} scale factor for I_{hl} $\langle I_h\rangle$ current estimate of I_h

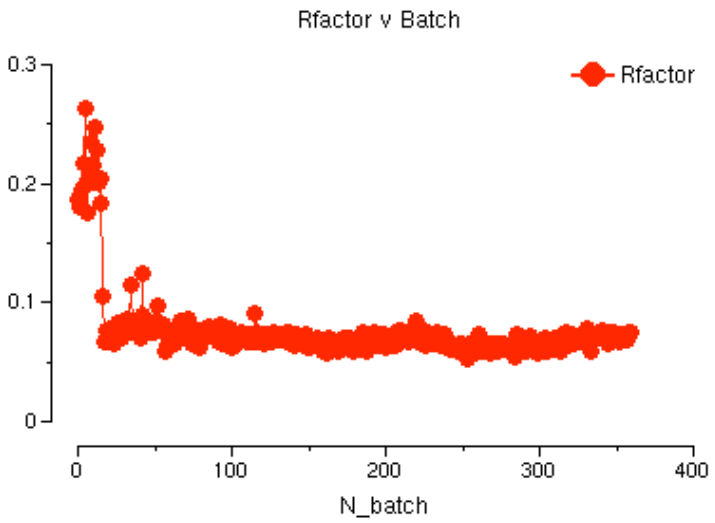
Data quality indicators

- Rmerge (Rsym) = $\Sigma \mid I_h - \langle I_h \rangle \mid / \Sigma \mid \langle I_h \rangle \mid$

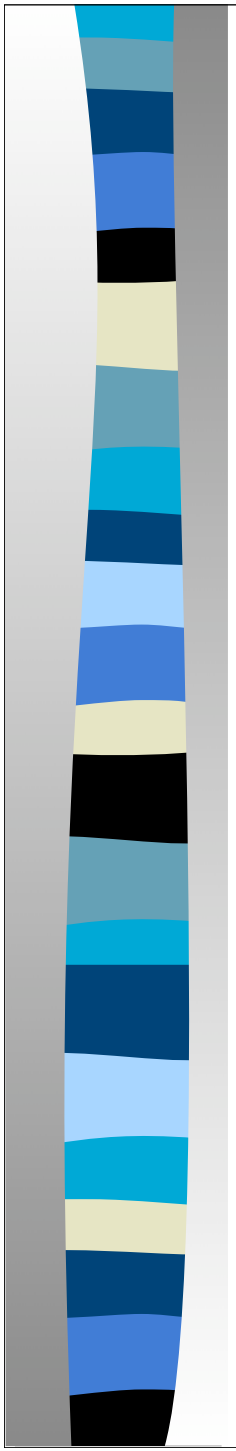
  Values: R ≤ 0.10 (10%)→Very good; 0.10≤R<0.20 →Suspect,

  R≥0.2 (20%)→ Bad !

Analysis of Rmerge against batch number gives a very clear indication of problems local to some regions of the data. Perhaps something has gone wrong with the integration step, or there are some bad images



- Here the beginning of the dataset is wrong due to problems in integration (e.g. poor orientation matrix in MOSFLM at start of job.)



The missing data and the rest of the story

(the phase problem and the model building)

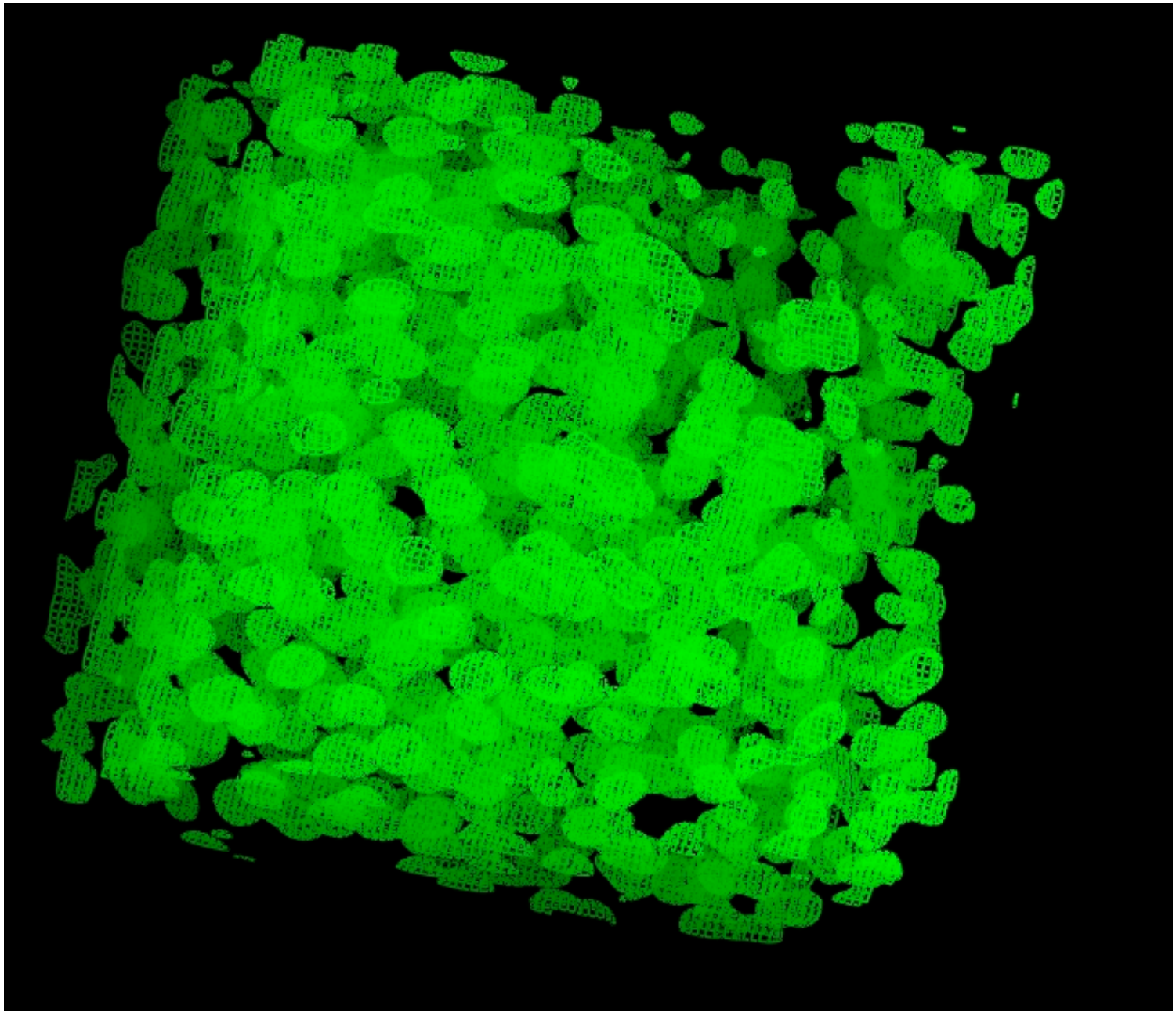
Why do we want to know the diffracted-beams intensities?

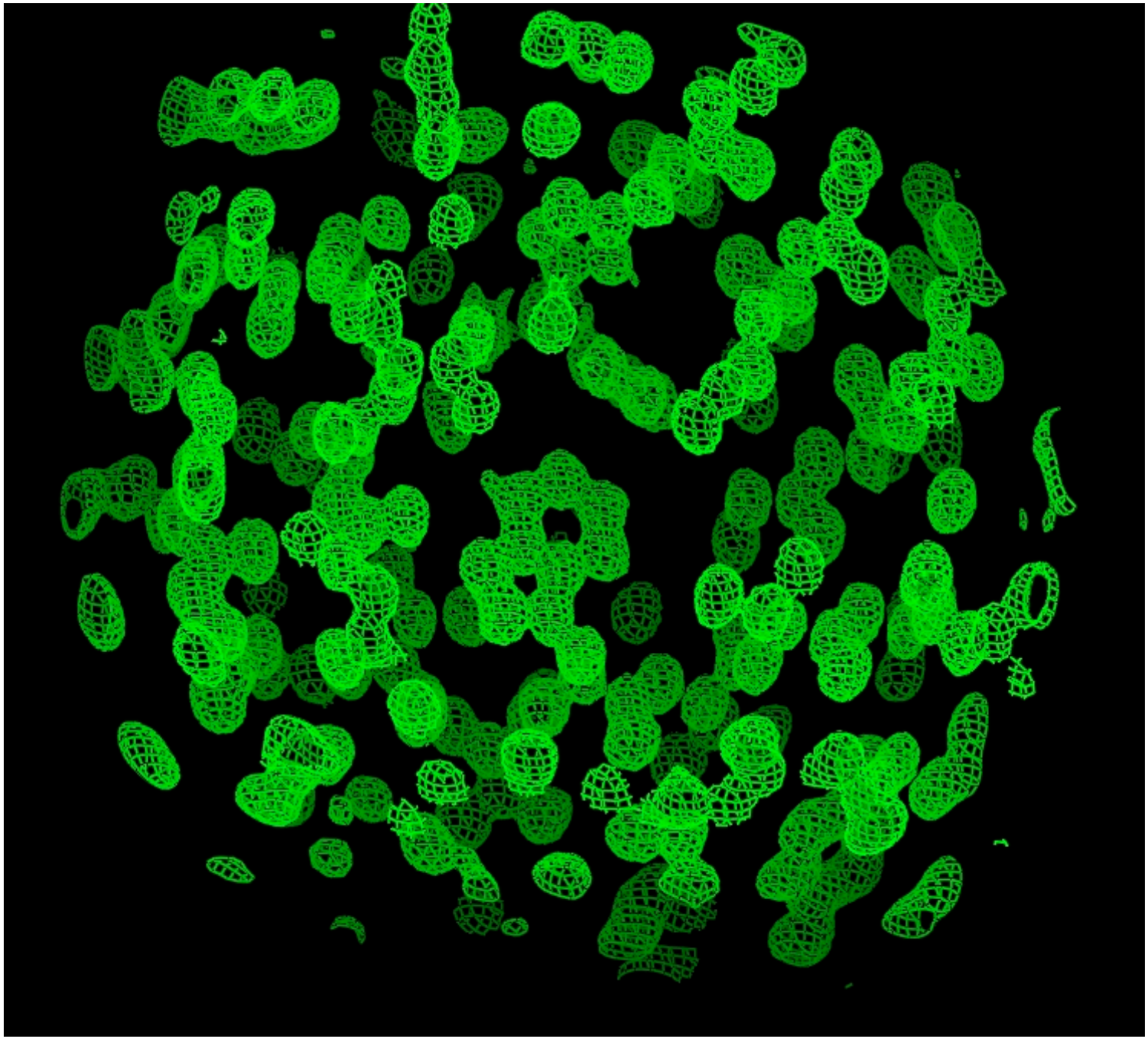
- The electron density equation:

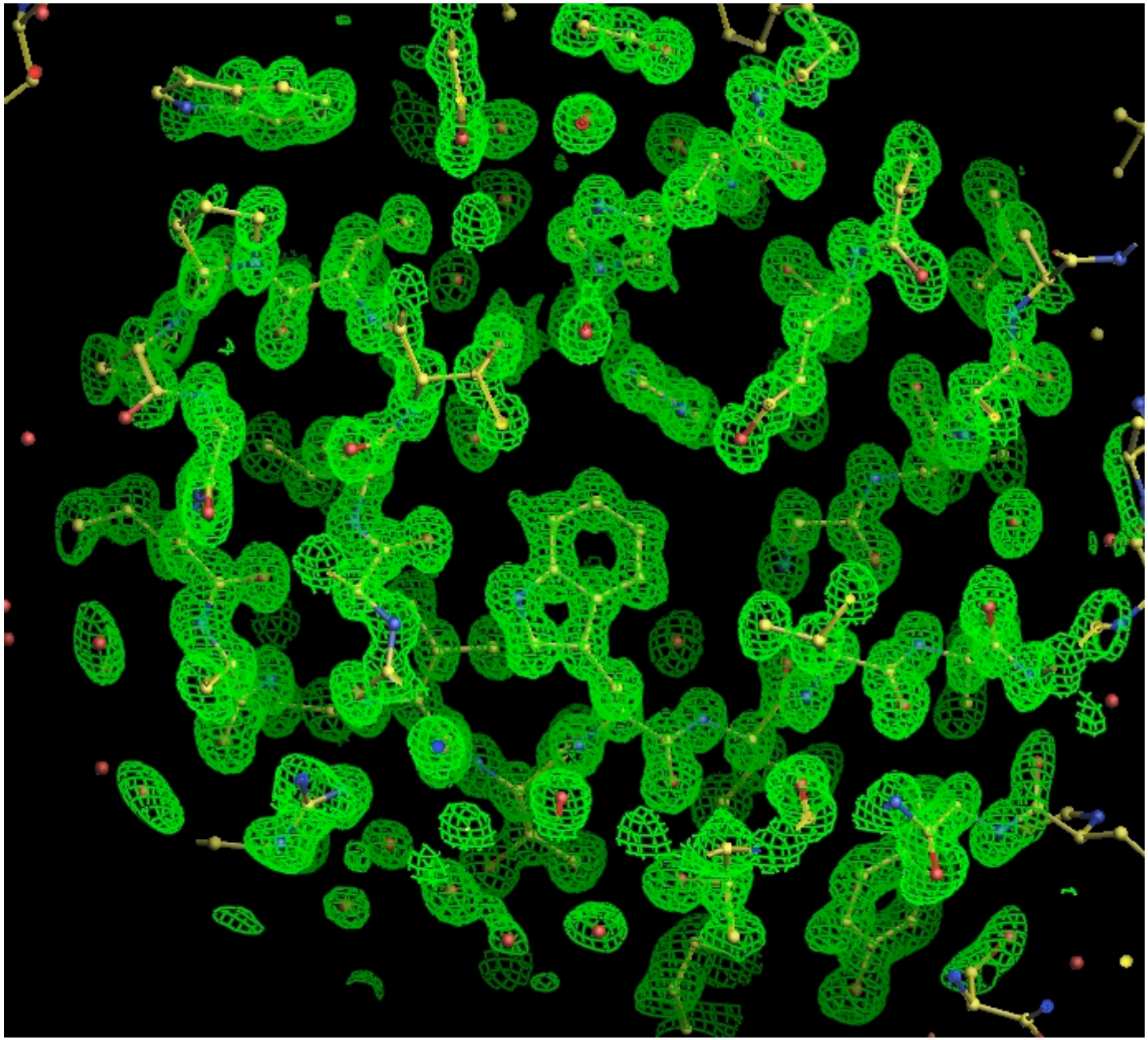
$$\rho(x,y,z) = \frac{1}{V_c}\sum_h\sum_k\sum_l \left|F(h,k,l)\right| \times e^{-2\pi i(hx+ky+lz)+i\alpha(h,k,l)}$$

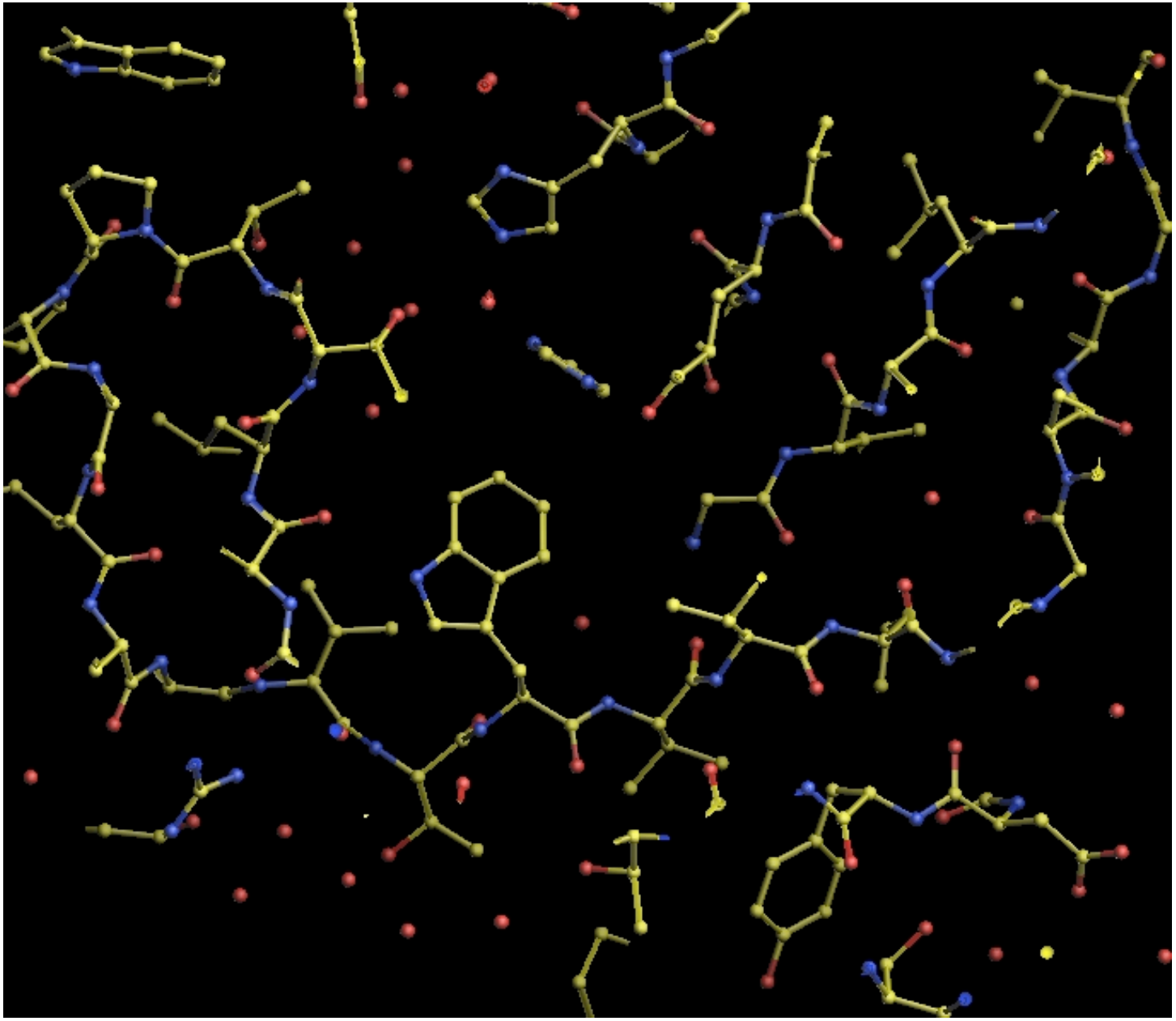
- The integrated intensity for each reflection

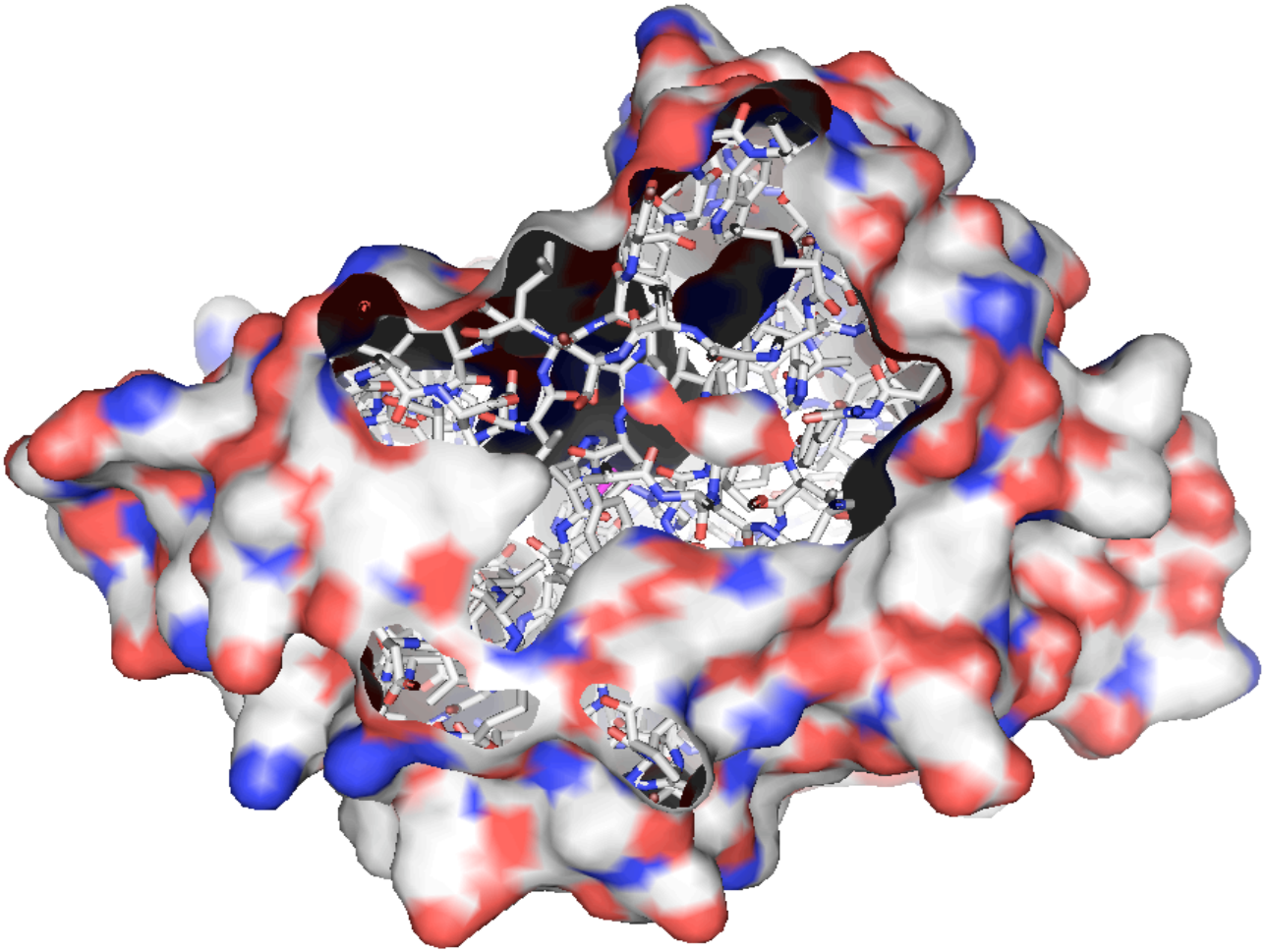
$$I(\text{int}, h\ k\ l) = \frac{\lambda^3}{\omega \times V_{cell}^2} \times \left(\frac{e^2}{mc^2}\right)^2 \times V_{cr} \times I_0 \times L \times P \times A \times \left|F(hkl)\right|^2$$











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- Principles of Protein X-ray Crystallography, J. Drenth, Springer-Verlag