PSD '20 -- Lecture 9

Coot

Manual refinement

Exercise 7

•Coot Tutorial

- Open Coot tutorial PDF file.

  https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/tutorial/tutorial.pdf

  (link on PSD website)

- Open Coot.
- Extensions / Load tutorial model and data
- Follow along with Prof Bystroff (finish on your own)
- We will do up to the end of 4.4 Blob 1.
- Save screenshots for Blobs 1, 2, and 3. Submit as Exercise 7.

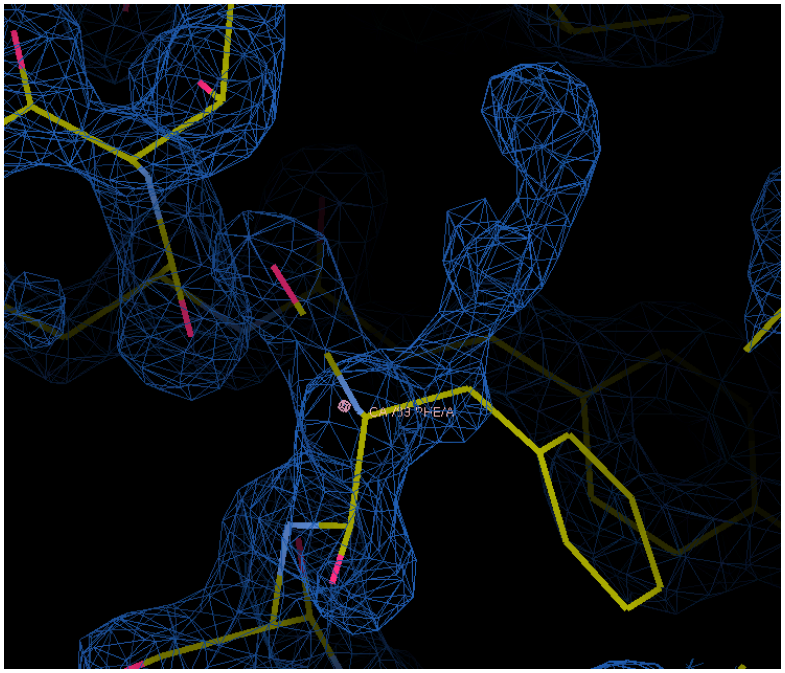
Coot

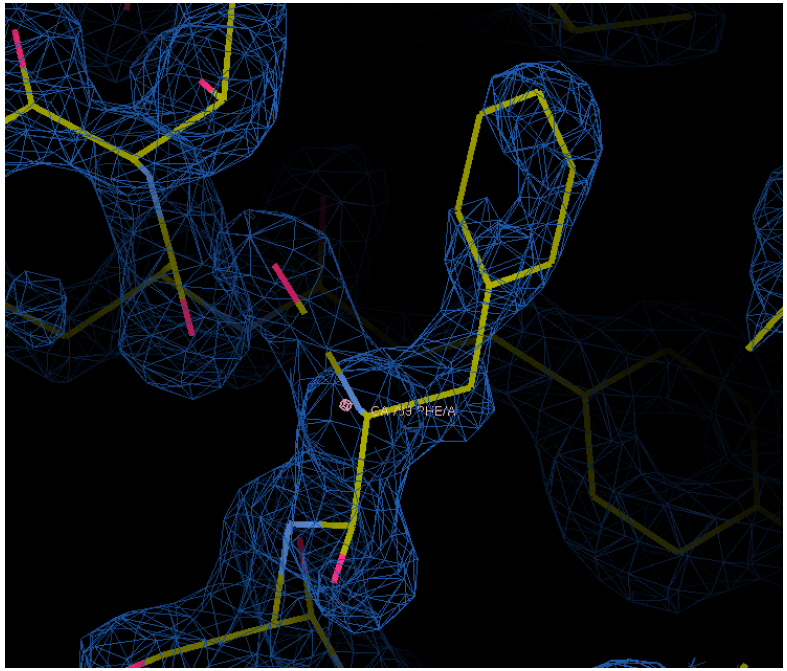
- Extensions / tutorial model and data
- Displays
- Moving around
  - mouse
  - cursor
  - spacebar
  - goto atom
  - i
  - o
  - mouse-left, right, middle, wheel

Rotamers

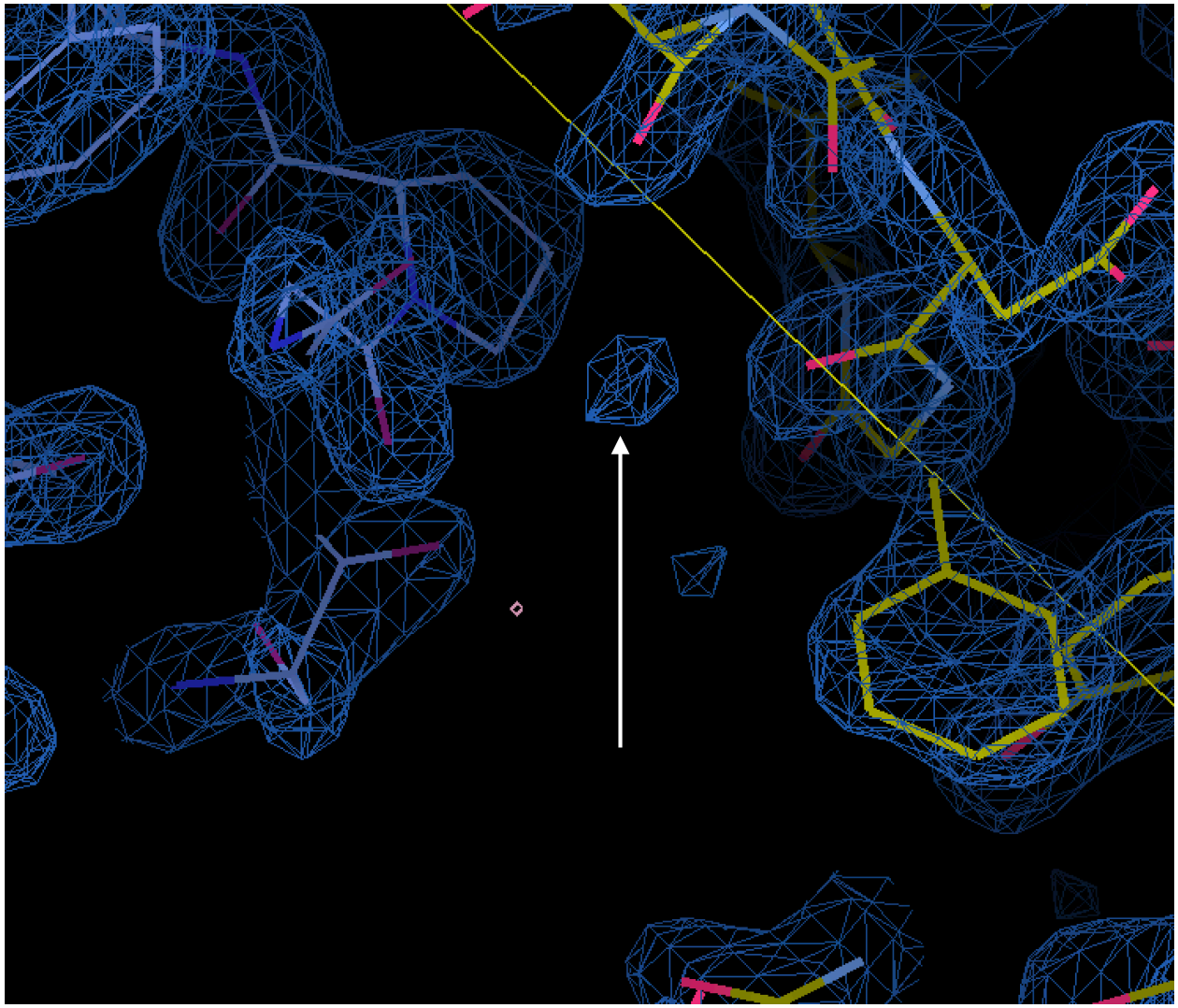
Sidechains adopt discrete positions (rotamer), due to energetic preference.

Use **Auto Fit Rotamer** (refine menu) so fit rotamer to density.





Crystallographic waters



Use Place Atom at Pointer (refine menu) to add as water

Symmetry and crystal contacts

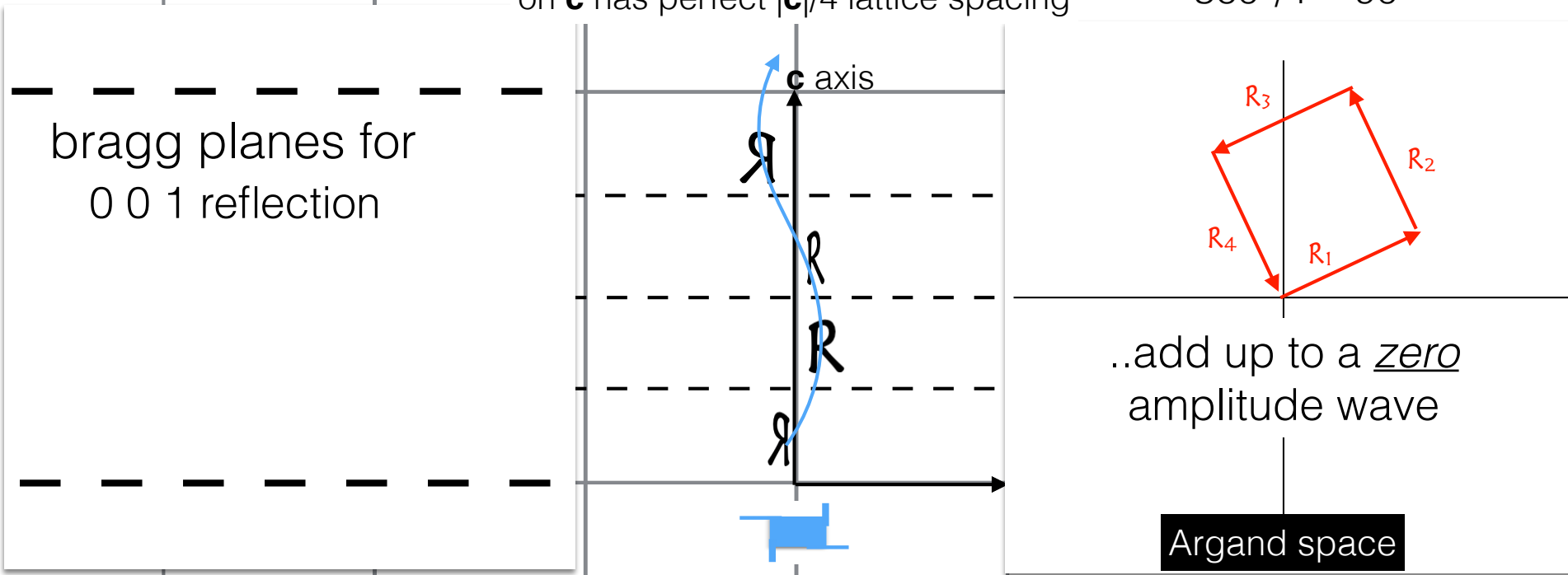
- Draw / cell and symmetry
- Master switch: Yes
- Symmetry by molecule: Display sphere, standard
- Show unit cell: Yes

Why are there systematic absences?

Absence of 001 in $P4_1$

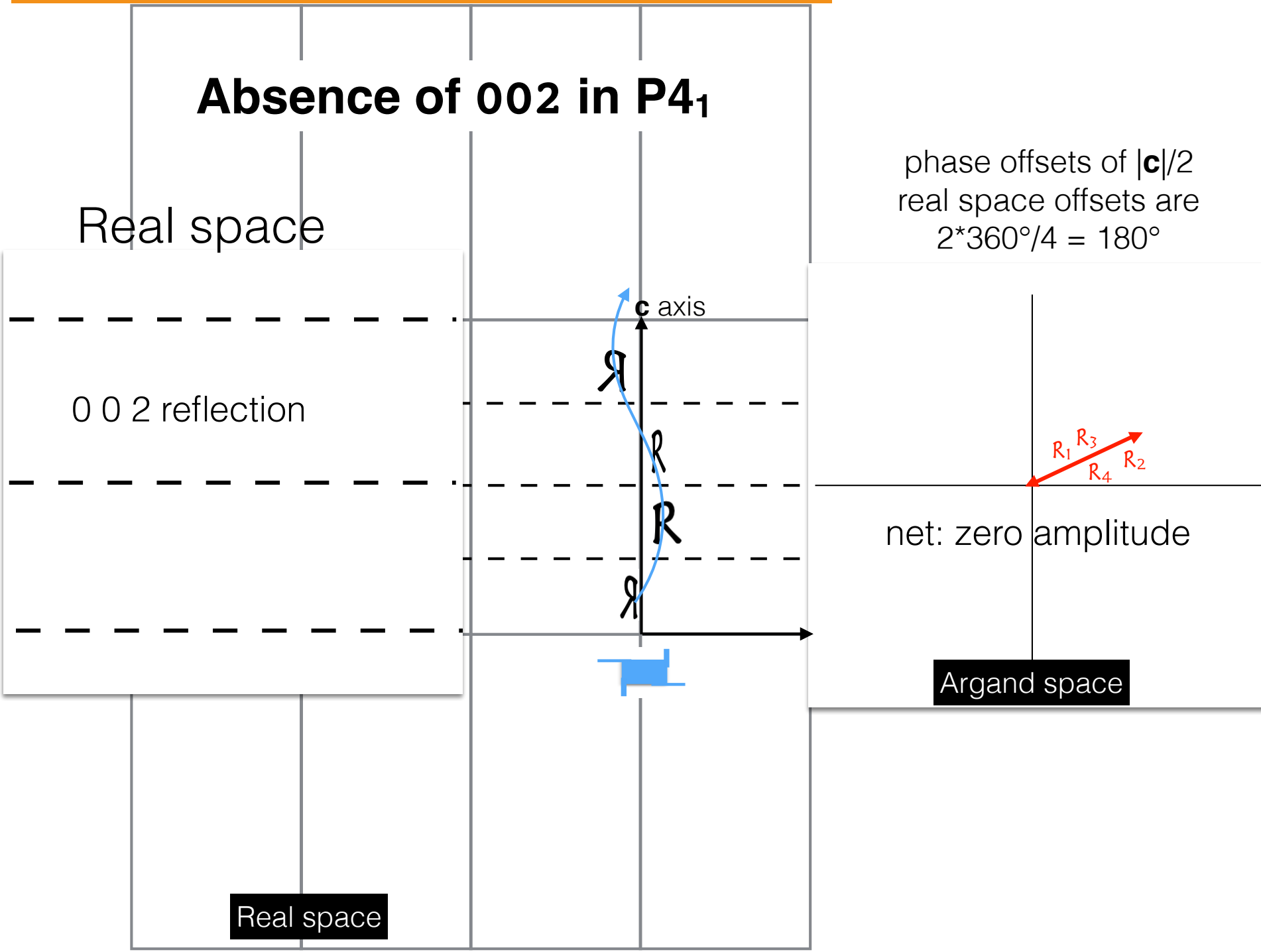
projection of molecule
on **c** has perfect |**c**|/4 lattice spacing

phase offsets of |**c**|/4
real space offsets are
360°/4 = 90°



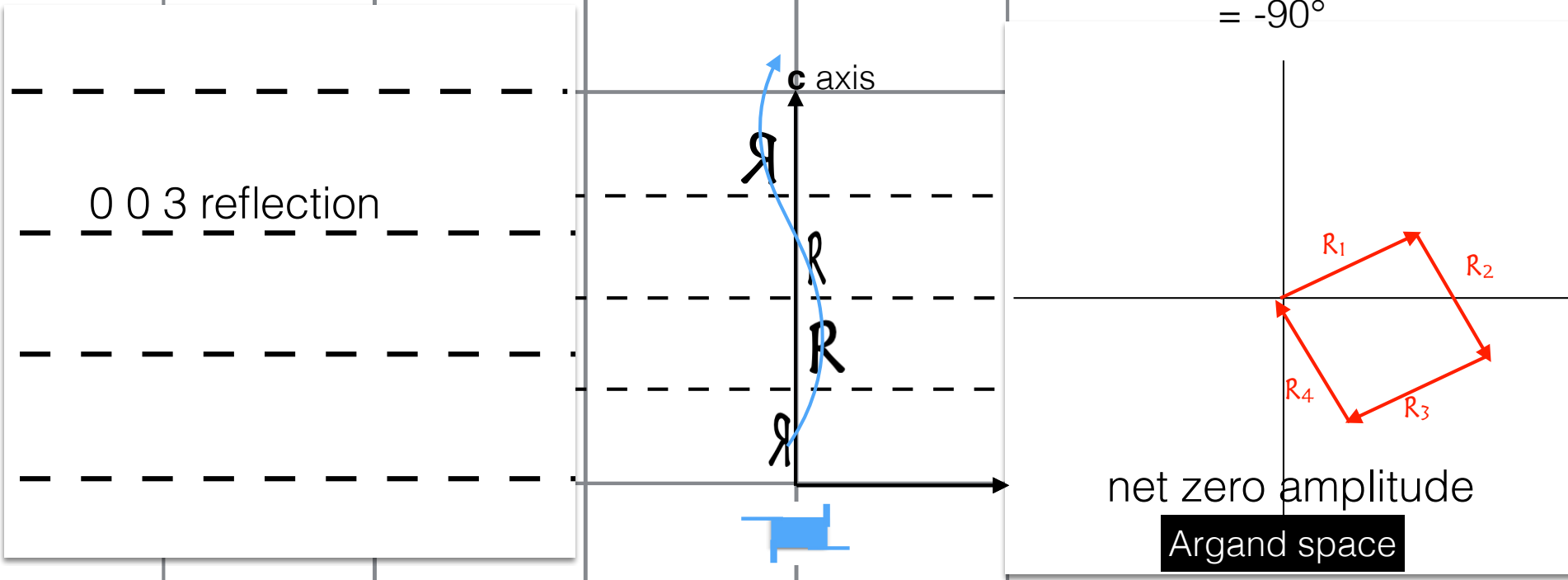
Why are there systematic absences?

Absence of 002 in $P4_1$

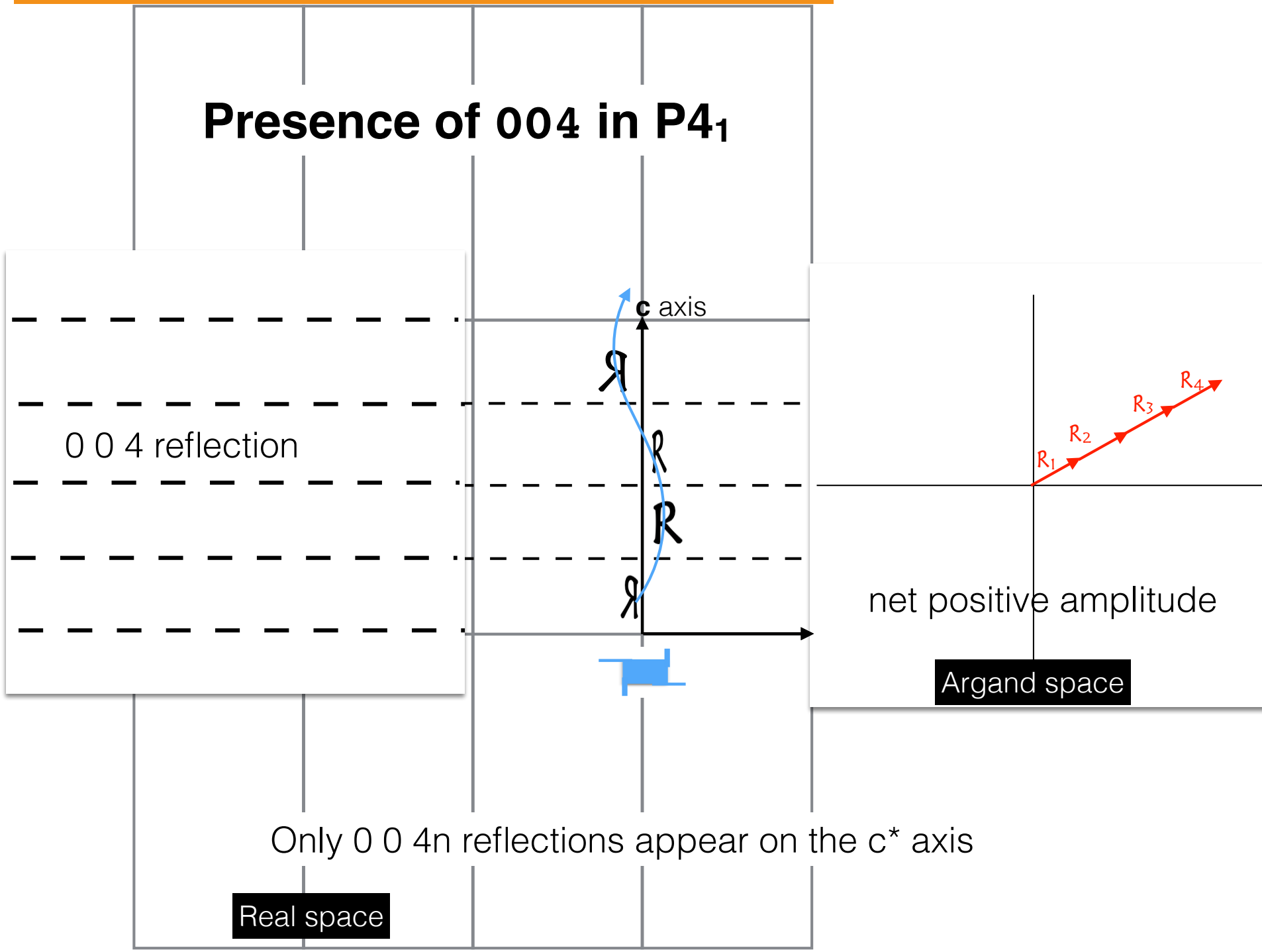


Why are there systematic absences?

Absence of 003 in $P4_1$



Why are there systematic absences?



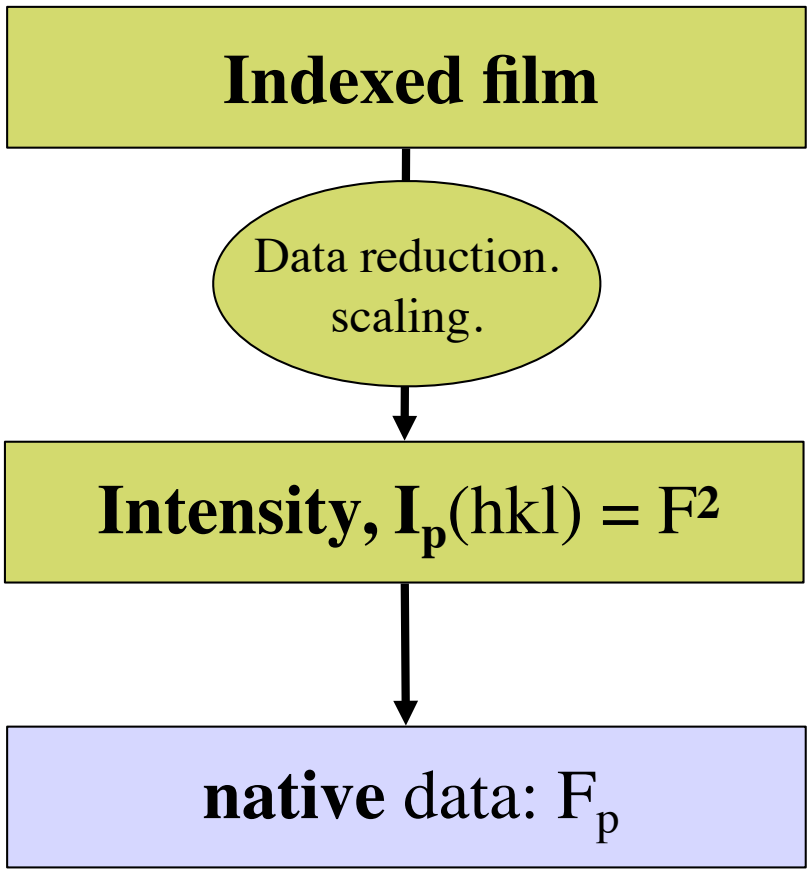
Exercise 7

Due Thu Nov 19

- Do Coot tutorial up to the end of Blobology
- For Blobs 1,2,3, follow the instructions. Take a screen shot at the end of each stage, showing the newly re-modeled blob.
- Paste the three images into a file and print (black/white is fine). Add comments as desired.

Summary flowcharts

From crystal to data



I is always a relative term

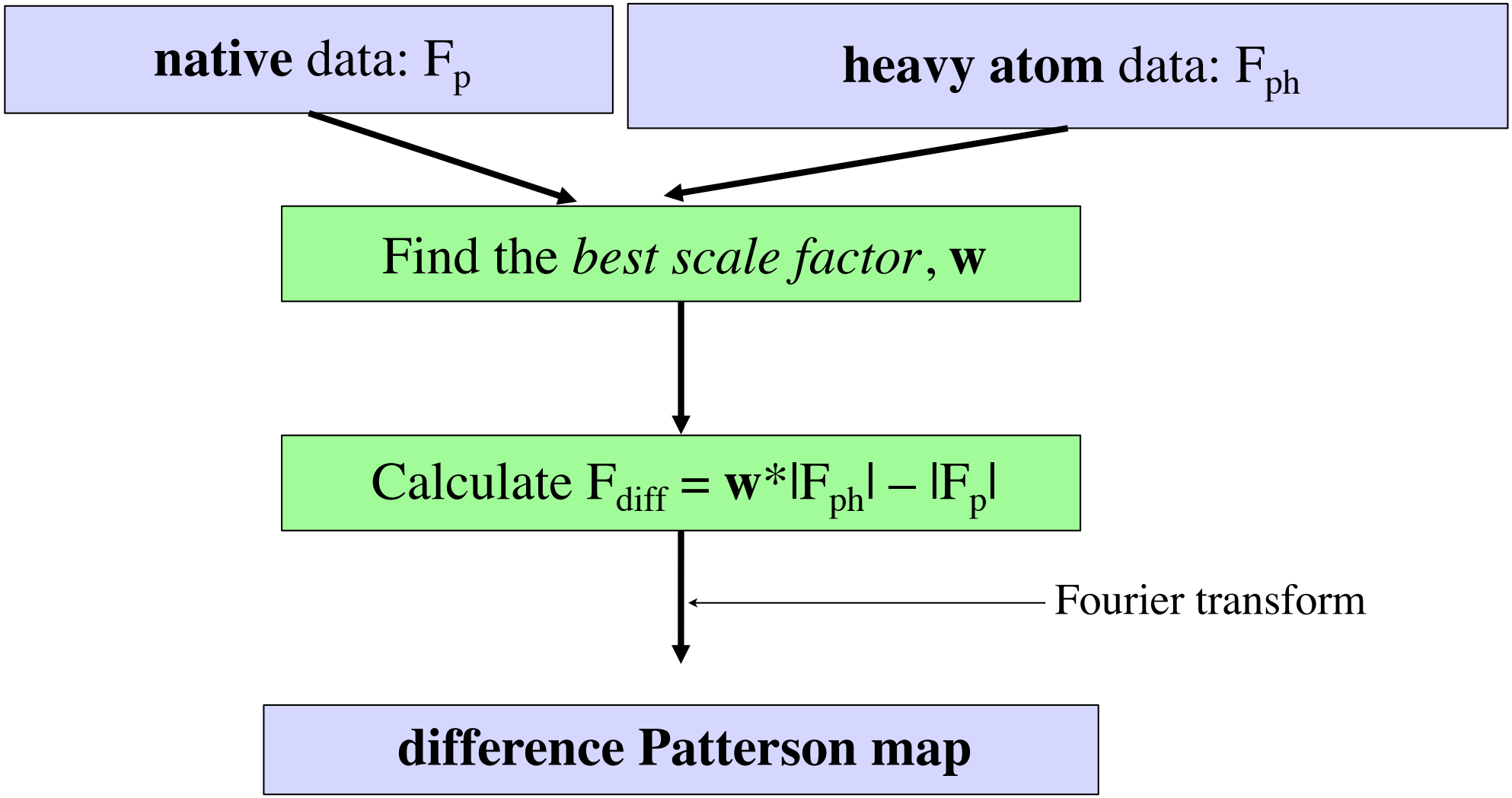
Bigger crystal, higher I

Better crystal, higher I

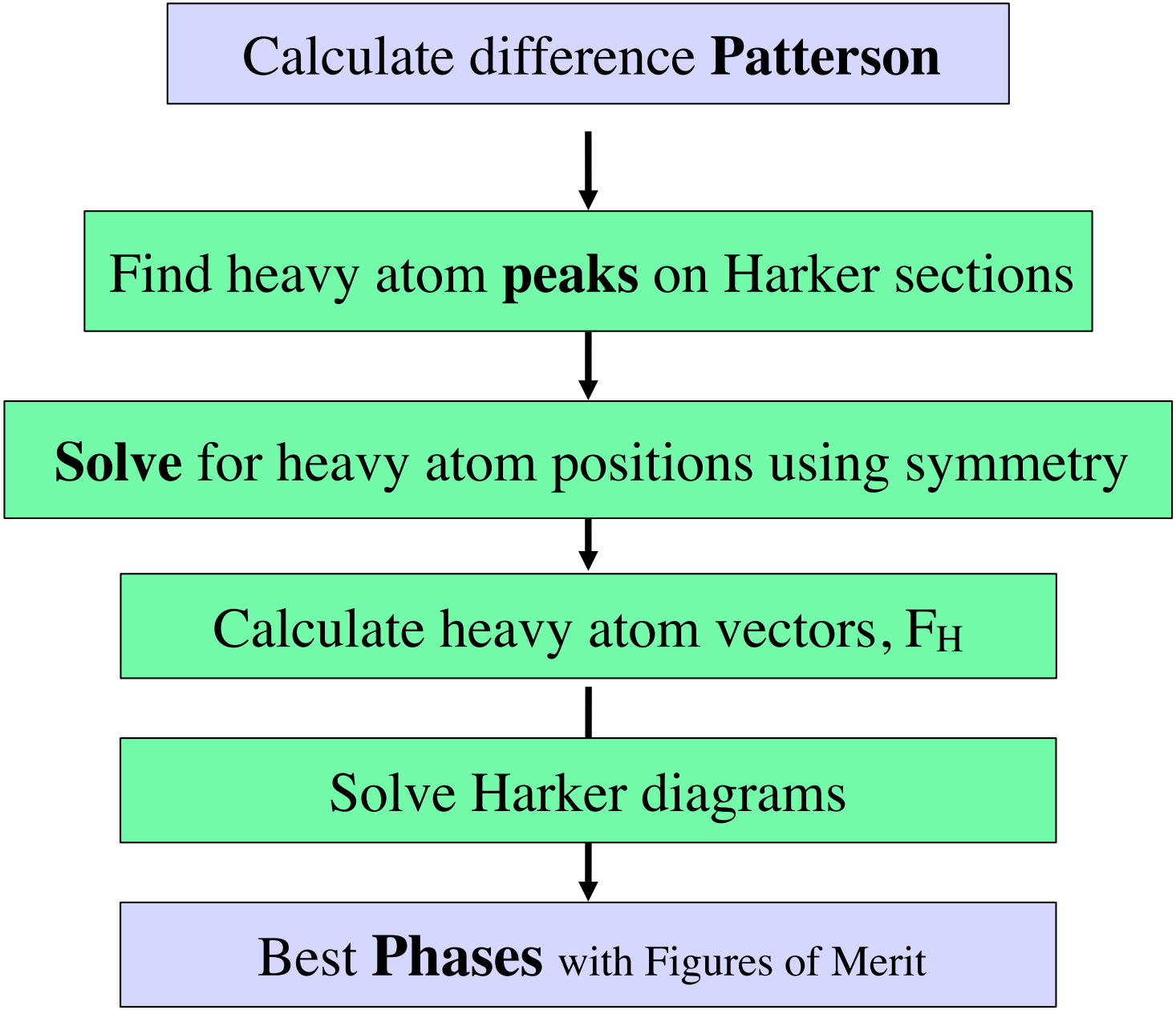
Longer exposure, higher I

More intense Xrays, higher I

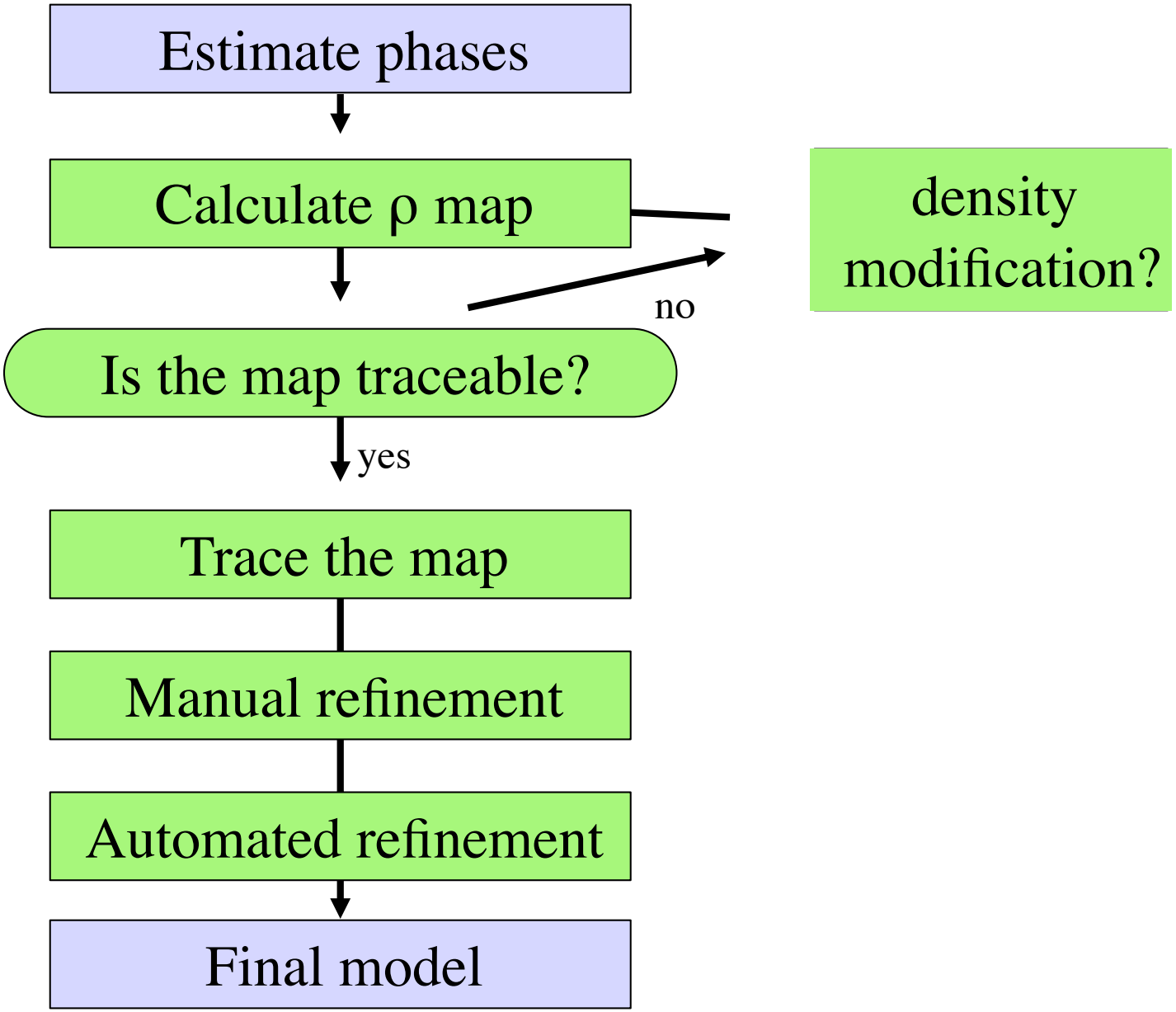
From data to Patterson map



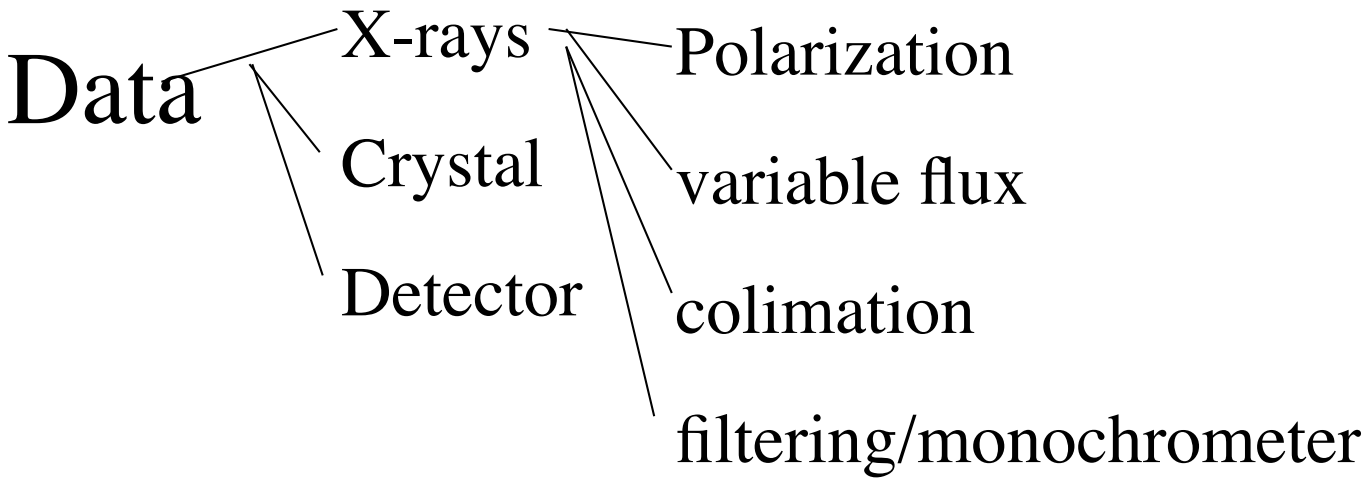
From Patterson map to phases



From Phases to Model



Sources of error in crystal structures



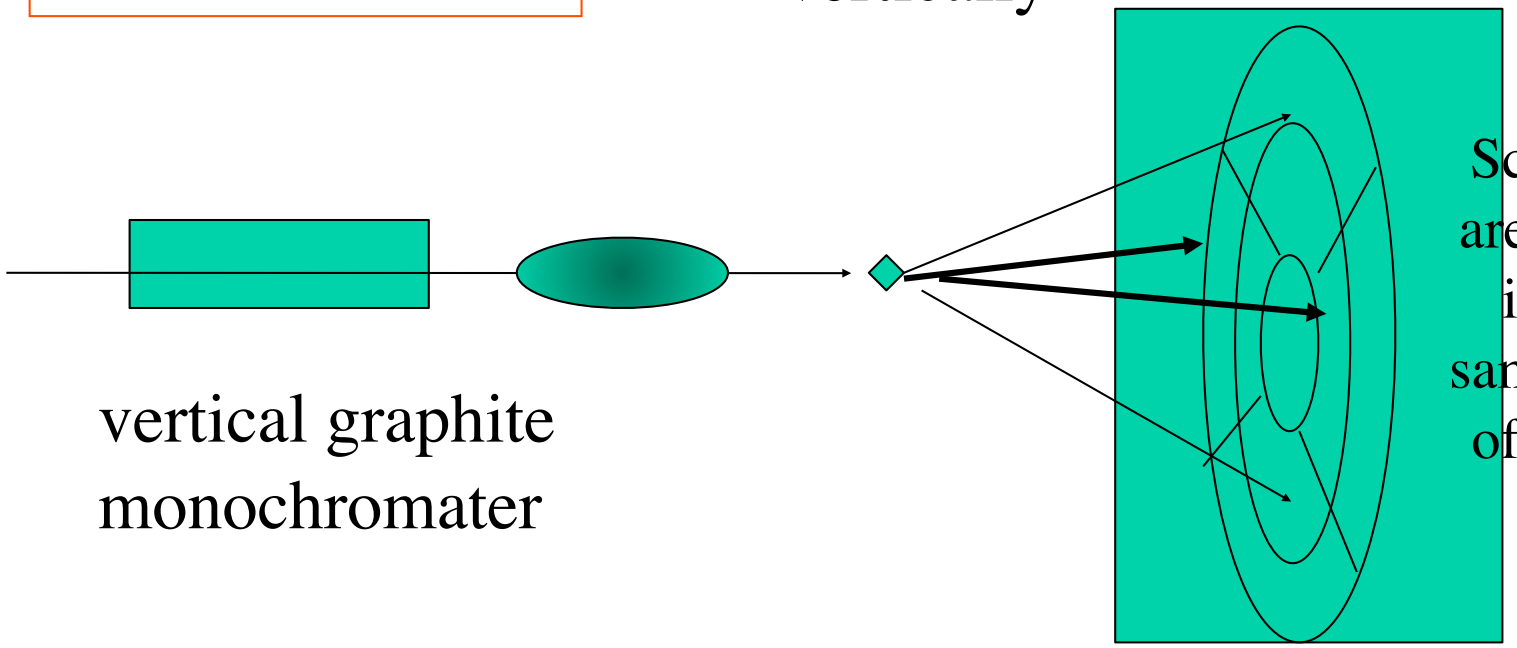
Model

Experimental sources of error

Polarization

weaker scatter vertically

Solution: zonal scaling.



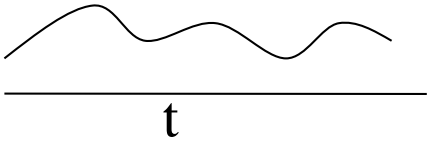
vertical graphite monochromater

Scale factors are calculated in evenly-sampled zones of reciprocal space.

horizontally polarized X-rays

Experimental sources of error

variable flux



A problem for *synchrotron X-rays*. Solution: Use an external flux meter + scaling.

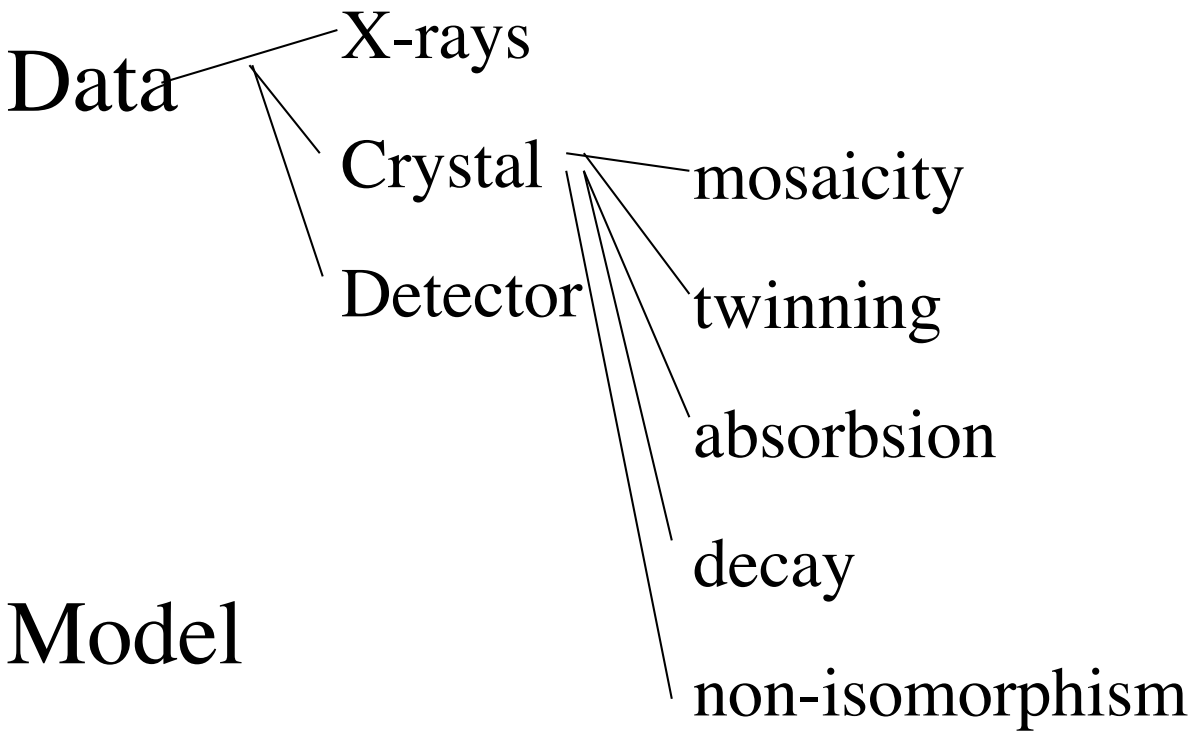
colimation

Wide beam means high background, large spots, spot overlap. Narrow beam means longer exposures, uneven exposure of crystal

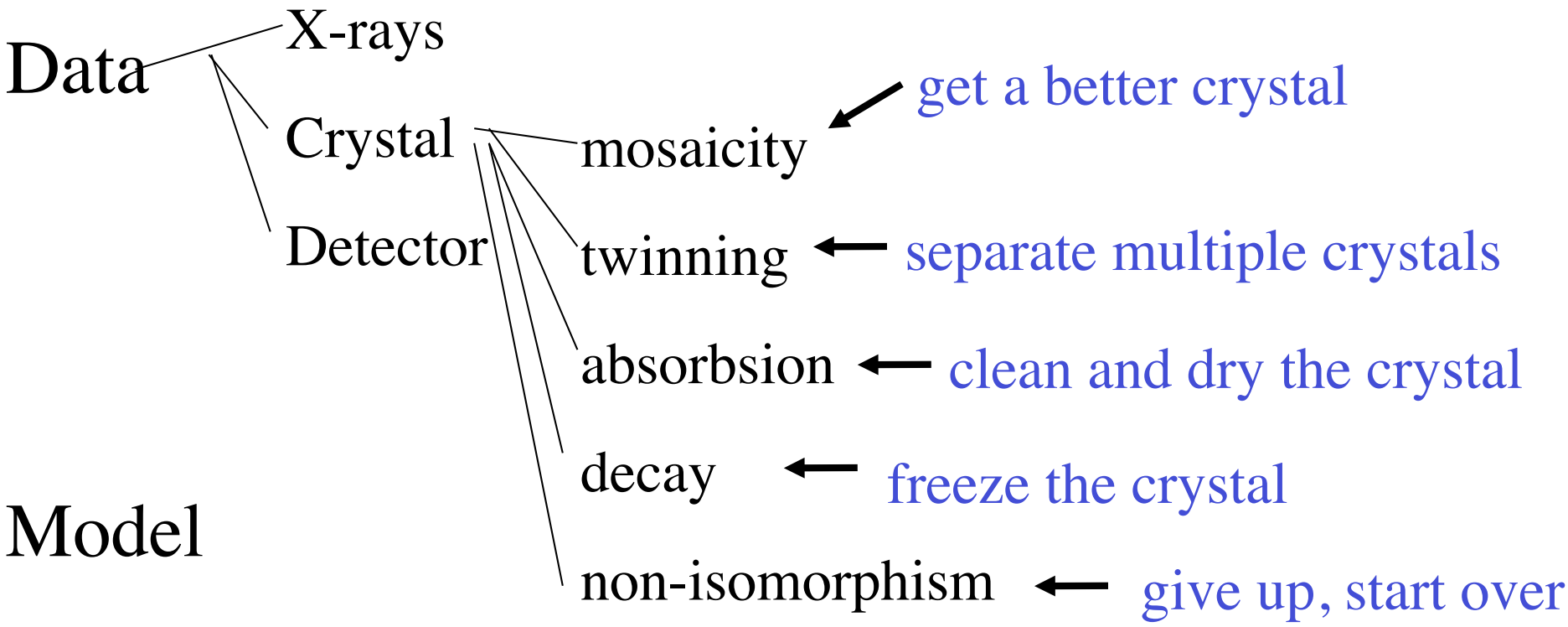
variable wavelength

Spots may be radially smeared. Solution: Use *monochromator*.

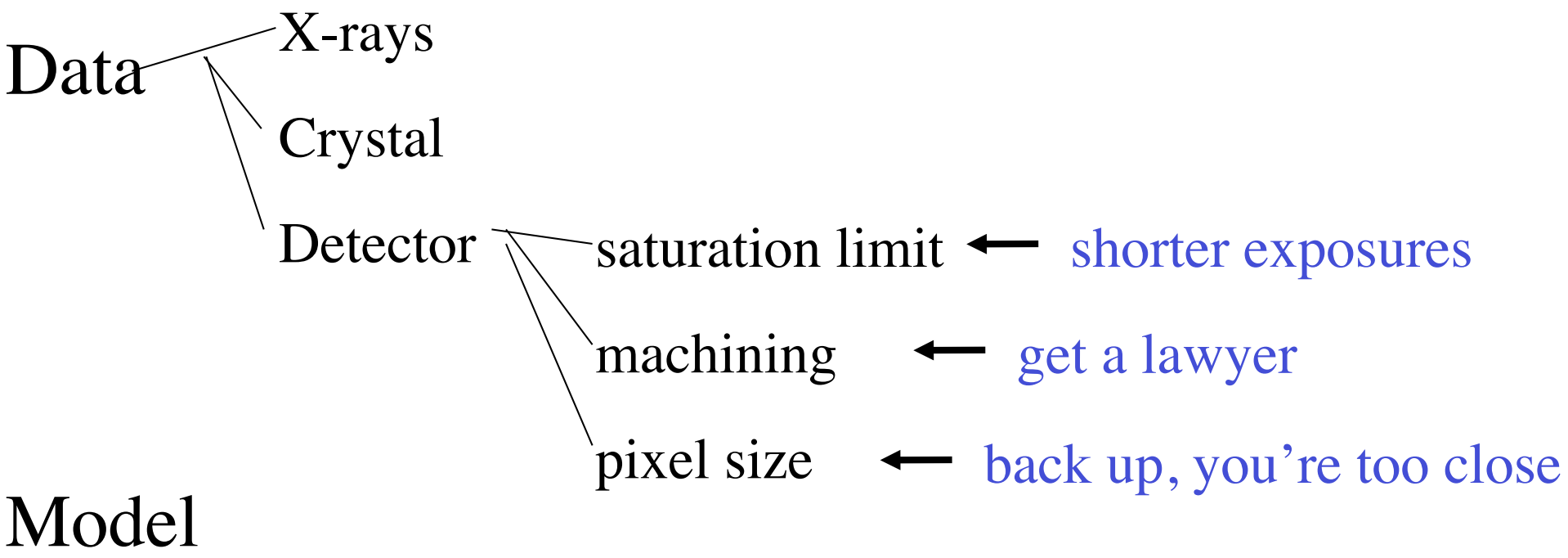
Sources of error in crystal structures



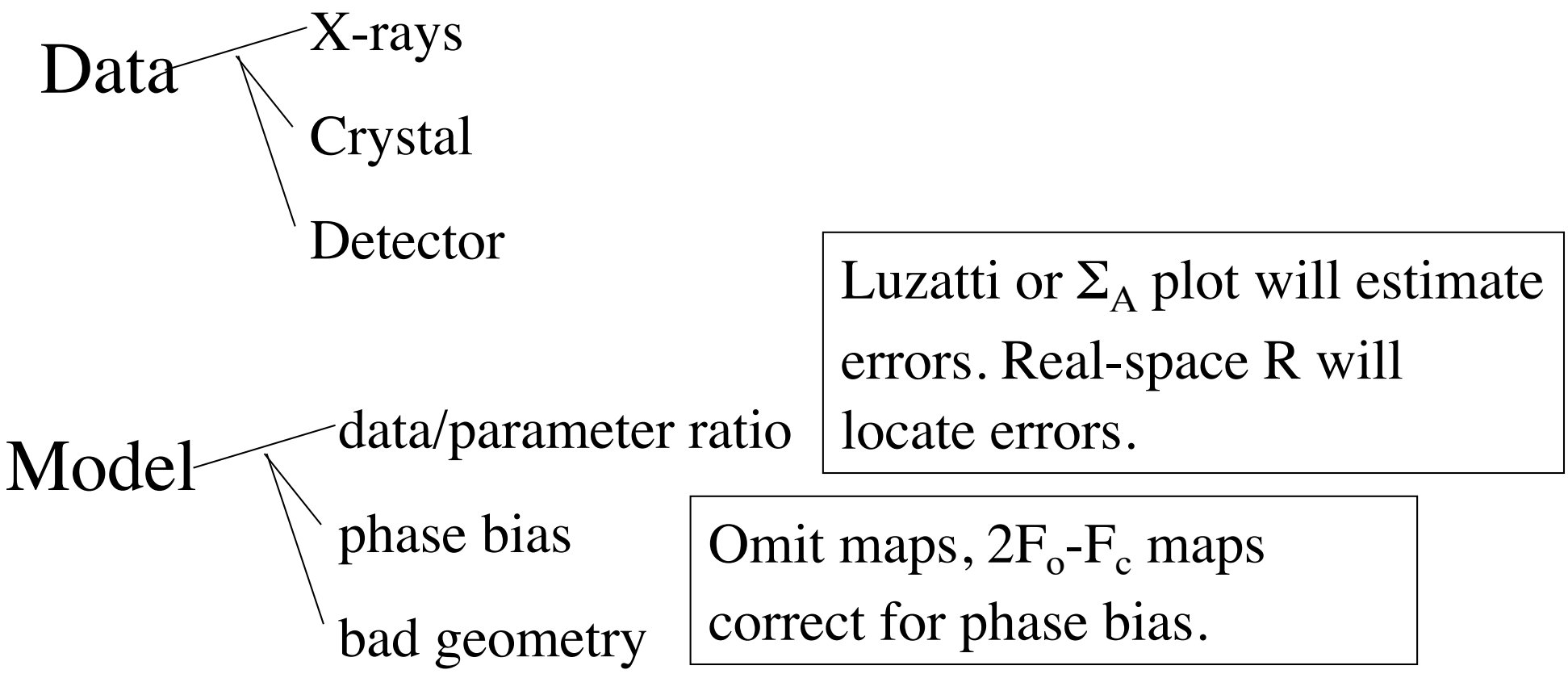
Sources of error in crystal structures



Sources of error in crystal structures



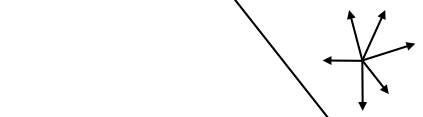
Computational Sources of error



Thought experiment

What is the phase error for a 4Å resolution reflection if the coordinate error is 1Å? (in a crystal with just one atom)

Positional uncertainty in the context of the Bragg planes gives the phase uncertainty of that atom's contribution to that reflection.



If the error in atomic position is 1Å,
and the Bragg plane separation is 4Å,
then the error in phase is (1Å/4Å)*360°=90°

Review

- systematic absences in reciprocal space are caused by lattice periodicity of the projected density in screw rotation (or glide plane)
- Sources of error are experimental and (mostly) computational
- Experimental error is reduced by scaling.
- Computational error is reduced by refinement.
- Using R-free avoids overfitting.
- R-free correlates with phase error.
- Phase error correlates with coordinate error.
- Therefore, R-free correlates with coordinate error.

Exercise 8 -- Reading a crystallography paper

Due mon Nov 23. Answer questions. Upload as PDF.

Download the PDF linked to "Ex 8 paper"
Read the section labeled "Structure Determination"
Explain: " All non-hydrogen atoms were refined anisotropically."
Read Table 1.
Answer: (1) What is the meaning of the second resolution range (in parentheses)?
(2) Why are there values in parentheses for the other items, such a R_{sym}?
(3) What is Wilson B?