Lesson 18

- Integration
- Scaling
- Other Corrections

An Overview

- Integration consists of changing the frame data into numbers—the intensity and the sigma of the intensity plus the hkl

- Must know where to look for spots –so crystal must be correctly indexed

- After integration must combine into a data set

  - Scaling the frames
  - Absorption correction
 - Merging
  - Formatting

Integration with HKL2000

- This is a very powerful integration program
- Works well for weak and strong data
- Works badly for "spread out" data
- Most widely used program for macromolecules

Parameters for HKL2000

- The only parameter after indexing that is needed is the size of the integration box.

- If the box is too small it may not contain all the pixels in the peak

- If the box is too large it may cause overlap and will not integrate

- The box can be made oblong and angled for a better fit.

- This must be done to cover $K_{\alpha 1}$ and $K_{\alpha 2}$ for Cu
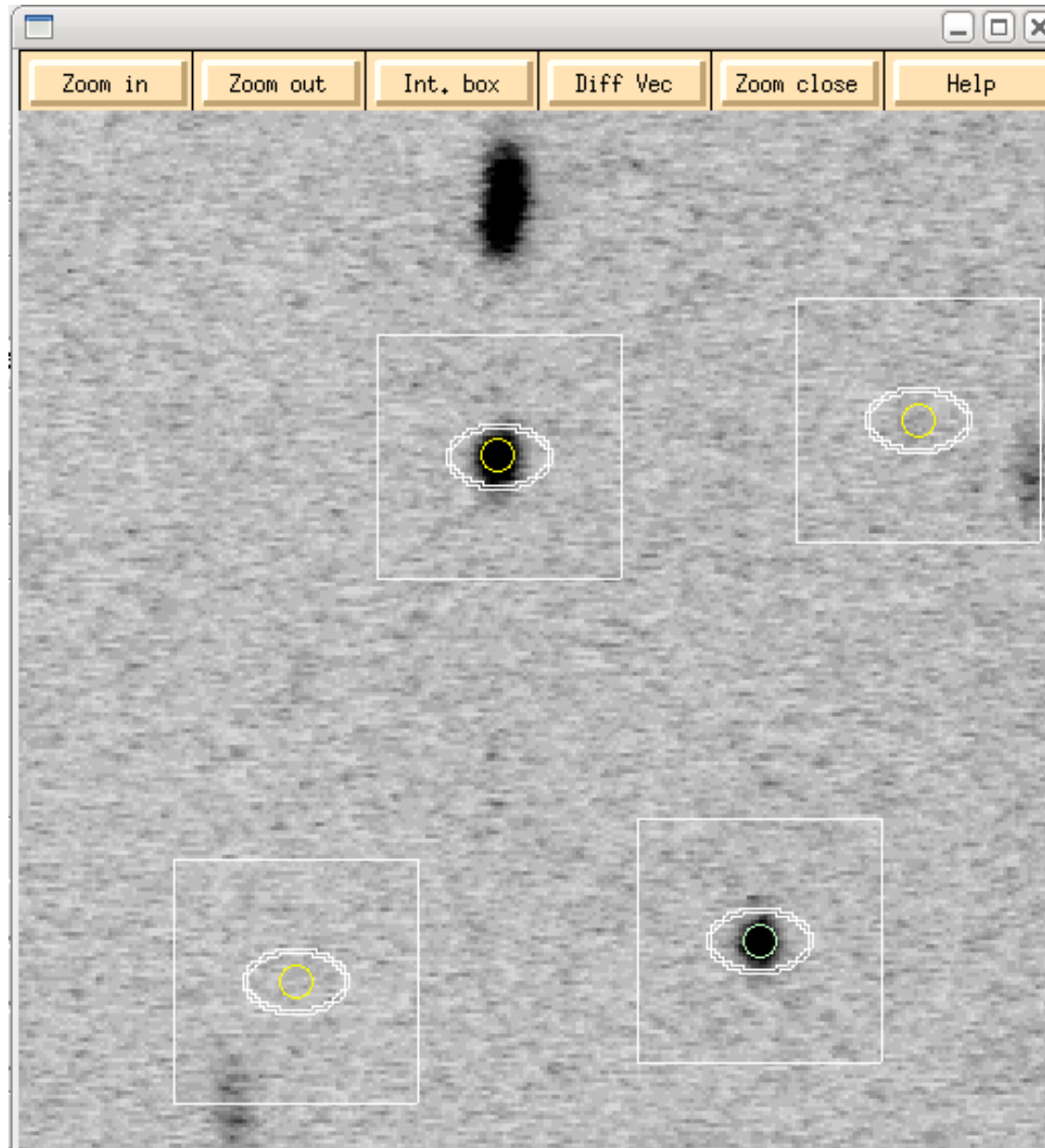
Good Spherical Boxes
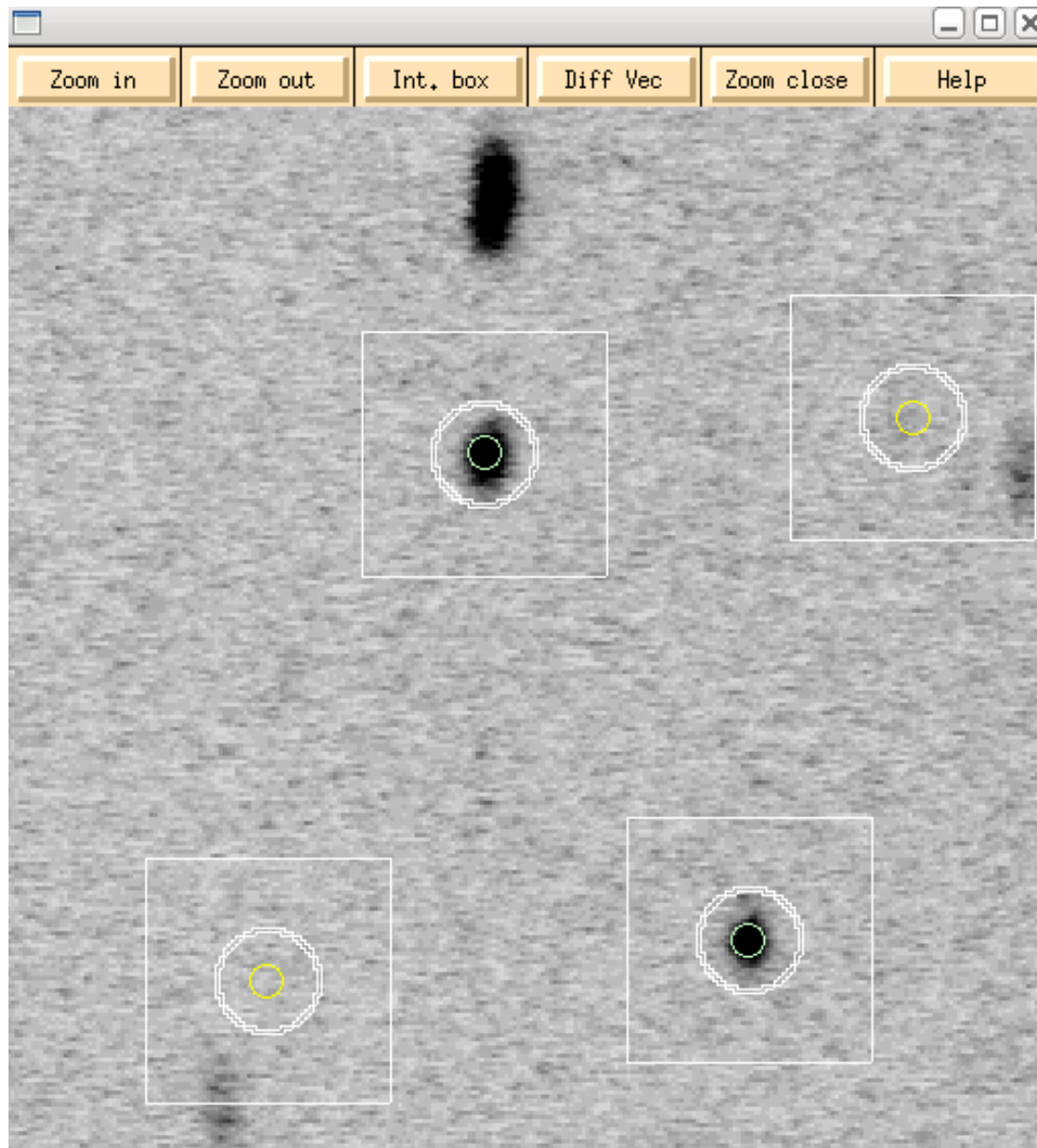
Overlapping Boxes
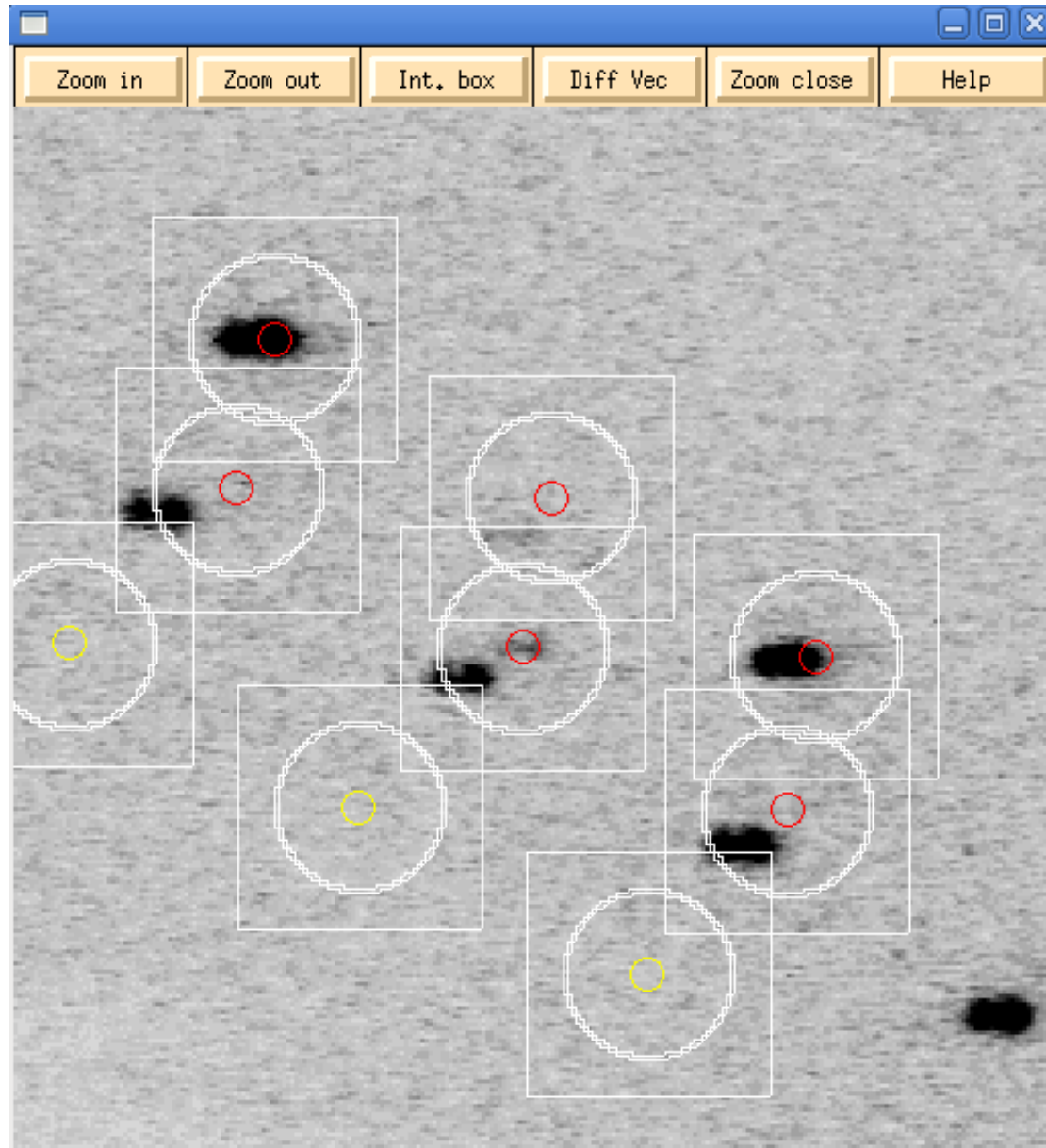
Oval Boxes at 90°

Integration

- Once a good size is determined then integration is run

- Some small number of overlapping boxes is OK

- The program displays various output both numerically and graphically

- The integration involves profile fitting.

Integration with CrystalClear

- In general the package fsProcess (the Japanese flag) is best for integration.

- Can switch from d*trek by pressing the flag

- Very good for broad or fuzzy spots.

- Not as good as HKL2000 for "good" crystals

- Very automatic

Integrating with fsProcess

- Simply click on integrate on the side panel

- It will select the box size automatically

- It will fail if the spots are not where they are calculated to be

  - Reindex with process

  - Try something else

Indexing Twins

- It is possible to have two or more single crystals making up the sample.--this is called non-merohedral twinning

- In this case one orientation matrix is needed to describe each twin component.

- Use the twin indexing part of crystal clear—the Swedish flag("There are certain fixed rules that one observes for one's own comfort. For instance, never be flippantly rude to any inoffensive grey-bearded stranger that you may meet in pine forests or hotel smoking-rooms on the Continent. It always turns out to be the King of Sweden." --Saki)

Twin Process

- Index the entire data set after data collection.

- Integrate the set

- There are two types of data points

    – Most points will belong to a single component

    – Some points will overlap

- Need to produce two data sets

    – One that has non-overlapped reflections for the most intense component (hklf4 file)

    – One with all the components and overlaps (hklf5)

Corrections

- The data measured are the intensity of the reflections (I).

- What is wanted is either F^2 or F—structure factors.

- F is the amplitude of the scattering vector.

- F^2 is proportional to I and two corrections must be applied to make them equal.

Lorentz-Polarization

- Earlier, it was mentioned that because the beam in not polarized the intensity will fall off by $(1+\cos^2(2\theta))/2$. This is the polarization correction p.

- In addition each spot spends different time in the diffracting position depending on the design of the instrument used

- This is corrected by the Lorentz correction L

- In the end $F^2=I/Lp$

- Called data reduction

Frame Scaling

- Each image will have a slightly different intensity
  - Movement of the crystal
  - Amount of crystal in the beam
  - Instrument factors
- These can be adjusted by looking at spots that overlap two images and then scaling so the profile is regular.
- This is called frame scaling
- Done by SCALEPACK in HKL2000 or scaling in Rigaku

Absorption Correction

- If the crystal absorbs the x-rays, this will effect the observed intensity and must be corrected.

- There are basically two corrections to be made

  – The absorption of the incoming beam

  – The absorption of the diffracted beam

- Obviously the amount of correction will depend on the shape of the crystal, its orientation, its size and the amount it absorbs the x-rays.

- The transmission factor is given the symbol µ and is a function of the contents of the cell.

Exact Corrections

- If the crystal is a sphere or a cylinder than the corrections can be calculated exactly.

- The spherical correction is only a function of the Bragg angle Θ while the cylinder is a function of Θ and the angle between the cylinder axes and the vertical.

- Many very highly absorbing compounds are ground to spheres so the exact correction can be applied.

Numerical Corrections

- It is possible to divide the crystal into little fragments. Then the distance of the incoming and diffracted beams can be calculated exactly.

- To do this a description of the crystal in terms of its faces and their size is required. The crystal must be face indexed

  – Sample must have well defined faces

  – Measured exactly

  – The Rigaku requires only an outline of the crystal and not faces.
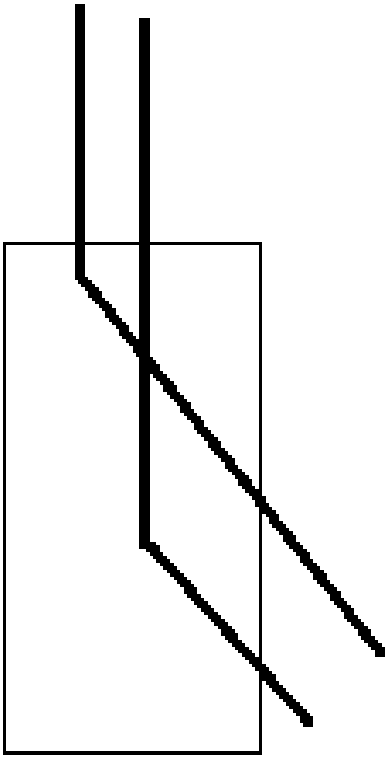
Numerical Corrections (Cont)

- Also need to know the orientation of the crystal.

- This is usually done by calculating the 6 direction cosines for each reflection. HKL2000 can produce a data file with direction cosines.

- The correction must be made before redundant data is merged (averaged). This is because each individual measurement was made at a different angle setting and has a different correction.

A New Approach

- First scale the data with HKL2000 using the direction cosines option.

    - This will prevent all scaling and corrections from being applied

    - The file will contain each collected datum.

- Calculate the crystal shape with EUHEDRAL.

    - Input is a SHELX file with only the cell constants and the absorption coefficient

    - Will interactively calculate the size and shape of the crystal

- Run PLATON

Empirical Corrections

- If there is sufficient redundant data than this can be used as the basis for an absorption correction.

- Each datum with the same hkl should have the same intensity.

- It is possible to fit a series to the redundant data that will provide an overall correction.

- Note this involves calculating an absorption surface which provides a correction for a reflection at a certain set of angles.

Approaches

- Use the setting angles (θ, ω, φ, and χ) to construct a trigonometric series. Used in the program CAMEL JOCKEY.

- Construct a series of spherical harmonics around the direction cosines.

- This is used in most modern programs for area detectors
  - Bruker SADABS
  - HKL2000 (absorption correction)
  - Rigaku empirical correction.

DIFABAS

- In the 70's it was suggested that the result of an absorption correction should make the observed and calculated data at the end of refinement be in agreement.

- Calculate an absorption surface based on the minimization of this difference.

- Obviously the validity of the correction depends on the validity of the calculated data and hence the model refined.

- Is this cheating?

Comparison of the Methods

- Difabs is the easiest to use as it requires no input. However, it can only be applied after the structure is fully solved. Absorption if great can actually make it impossible to obtain a model.

- Numerical is the most accurate if the faces can be measured accurately. Not always possible. The error in the correction will be the error in the crystal description.

- Empirical corrections require much redundant data. If there is not sufficient redundancy than the quality of the correction is questionable.

- Can apply corrections either as part of data reduction using the collection software or later if the data contains direction cosines.

HKL2000 to the rescue

- The program SCALEPACK which does frame scaling will apply a good deal of absorption correction.

- In addition a spherical harmonics correction can be applied at the same time to increase the efficiency of the correction.

- Experiment has shown this is equal or better than most empirical corrections and requires not great user input.

Merging

- The data must eventually be merged to combine redundant data into one unique set.

- This is done as part of scaling in HKL2000. The advantage is that obvious bad data can be eliminated at that point unless direction cosines are calculated

- No merging is done by CrystalClear.

- There is a program mergehklf5 that can merge and eliminate outliers.

It also is done as part of the refinement in SHELXL.

The agreement between equivalent data is given by Rint which typically for a good crystal is less than 0.05 but greater than 0.02.

The greater the value the worse the agreement for redundant data.

Reformatting

- The programs we will all use SHELX style data files.

- The actual file used is called an .hkl file as it always has .hkl as a file type.

- The program dform will convert HKL2000 data to Shelx format. This program is run usually automatically for Rigaku data.

- For accentric cells it may ask if you want to swap the axes. Always answer no.

Next Time

- Getting to an instruction file for calculations

- Keeping track of crystal details.