

An Analytic Absorption Correction for Protein Crystallography.

W. Armour & G. Evans & M. Basham

Diamond Light Source



Introduction.

What are we trying to achieve?

- We aim to develop methods to apply analytical absorption corrections to macromolecular diffraction data.
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- We aim to develop methods to apply analytical absorption corrections to macromolecular diffraction data.
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Why are we trying to achieve this?

- This will allow data collection strategies optimised for collection rates and coverage to be used, rather than ones designed to minimise absorption effects.

A hard problem.

- To calculate an absorption correction we must solve the transmission integral.

$$T = \frac{1}{V} \iiint_V e^{-\mu(p+q)} dV$$

- T is the transmission coefficient, V is the crystal volume, p & q are the ingoing and outgoing paths, dV is a volume element.



A hard problem.

- Because one ingoing path gives rise to many outgoing (diffracted) paths we have to deal with a one-to-many function.
- Hence any method that attempts to combine the paths will result in a loss of information since we have no idea how many photons took which of the possible routes.

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- Current analytic methods can only deal with the crystal and have to neglect the surrounding mother liquor.
- We have now developed working models in 2D and have promising 3D analytic models based on spherical waves, matrix methods or Lebesgue integration .
- Unfortunately though, all of our 3D models have errors associated with them.



A Numerical solution.

- Since we are keen to deliver a working usable solution we have decided to move to our back up solution... a numerical method.
- A numerical solution can be as accurate as we wish, the only thing that places constraints on us is how accurate our input data is and how much memory and computer power we have.

A Numerical solution.

- We begin by discretising the transmission integral equation....

$$\tilde{T} = \frac{1}{n} \sum_{i=1}^n \prod_{j=1}^m e^{-\mu_j x_j^i} = \frac{1}{n} \sum_{i=1}^n \exp \left[\sum_{j=1}^m -\mu_j x_j^i \right]$$



A Numerical solution.

- Doing this allows us to solve the transmission integral equation (and hence find an absorption correction) numerically.
- This approach allows us to take into account crystal, mother liquor and loop (or sample holder) accurately.



What's needed?

To compute an accurate absorption correction that isn't dependent on the data we've collected we need the following things...

- An accurate description of the shape of the experimental sample.



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To compute an accurate absorption correction that isn't dependent on the data we've collected we need the following things...

- We feel the easiest way to gain the accuracy needed is to use tomographic imaging.
- This will give us an accurate description of the sample that is already discretised on a lattice.



What's needed?

To compute an accurate absorption correction that isn't dependent on the data we've collected we need the following things...

- A computer with enough power to calculate an accurate absorption correction.



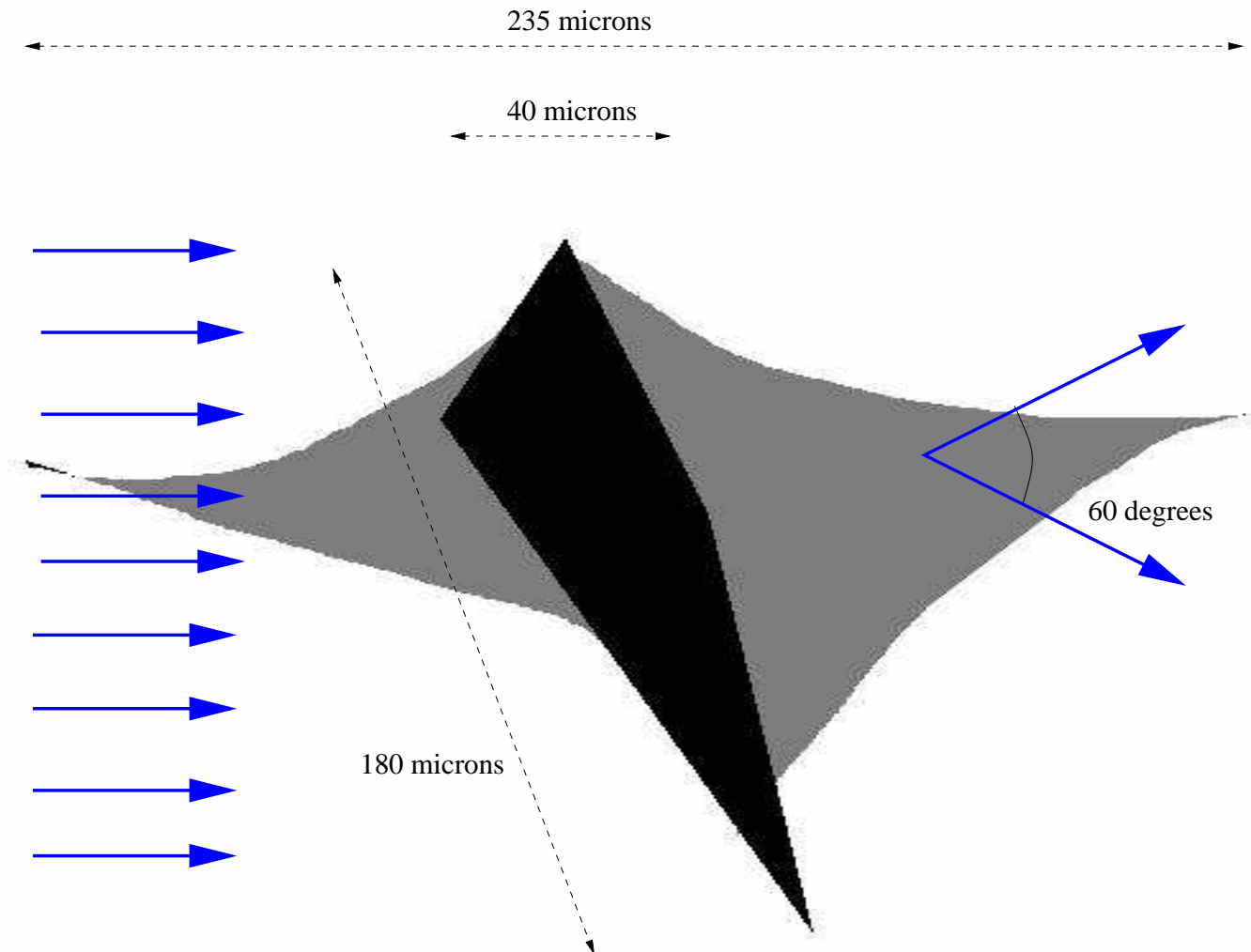
What's needed?

To compute an accurate absorption correction that isn't dependent on the data we've collected we need the following things...

- Although it seems a standard desktop computer could produce a relatively accurate absorption correction in a reasonable time we are looking onto using Tesla computers on the beamline so that the correction could be done within minutes of the experiment finishing.

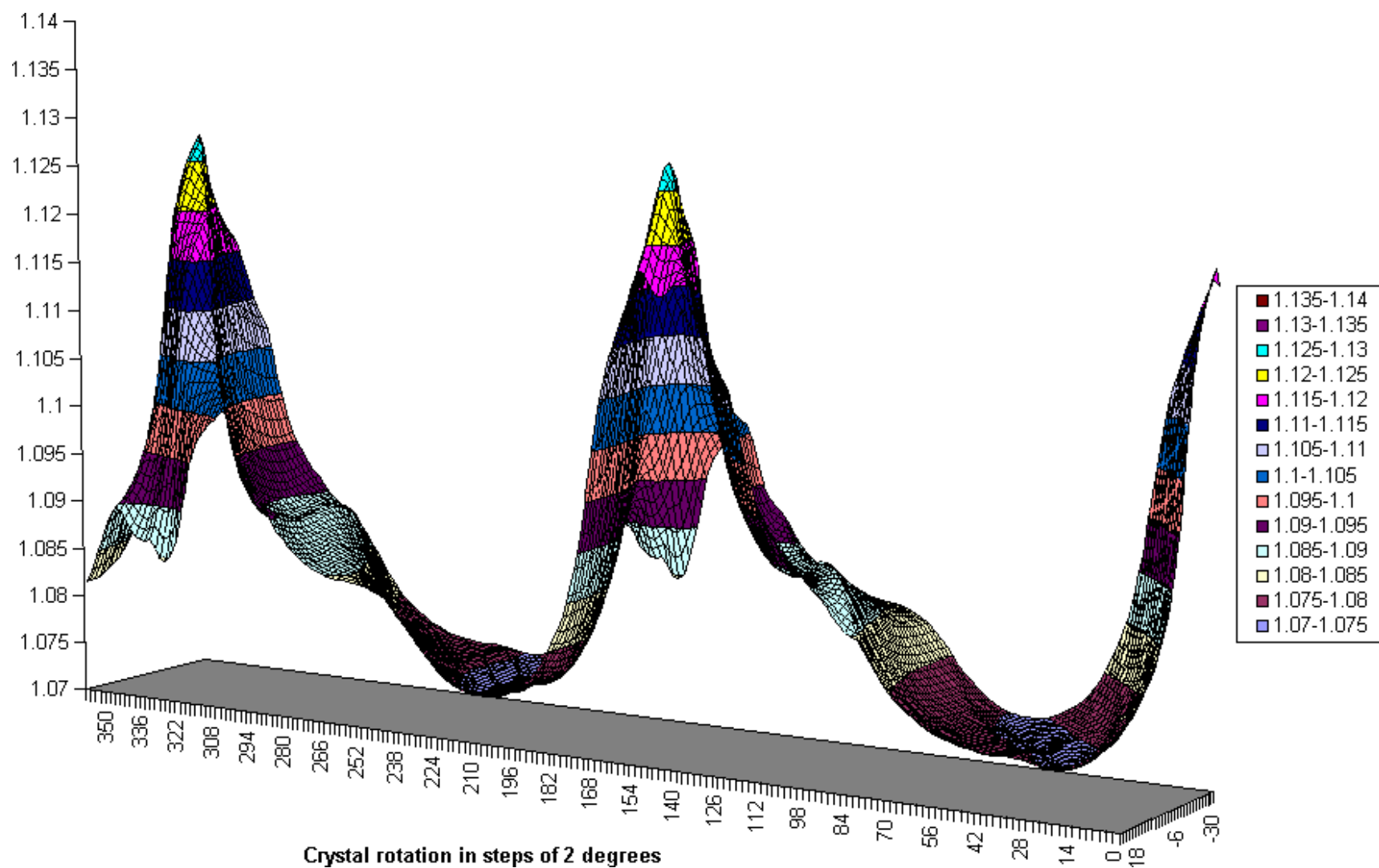
2D

Consider the following 2D crystal and mother liquor...



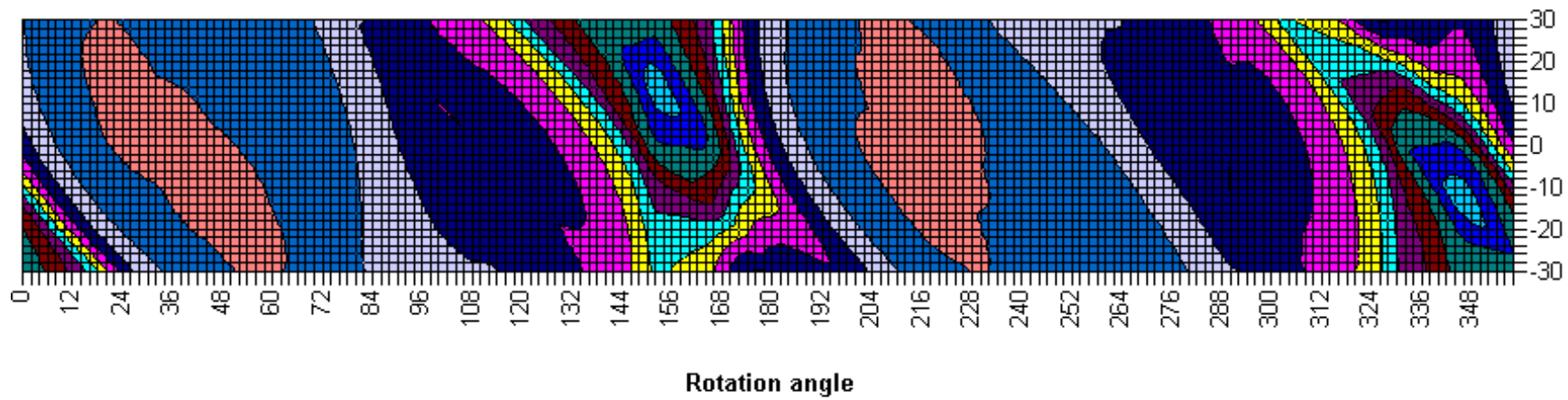
2D

Results of 360° rotation...



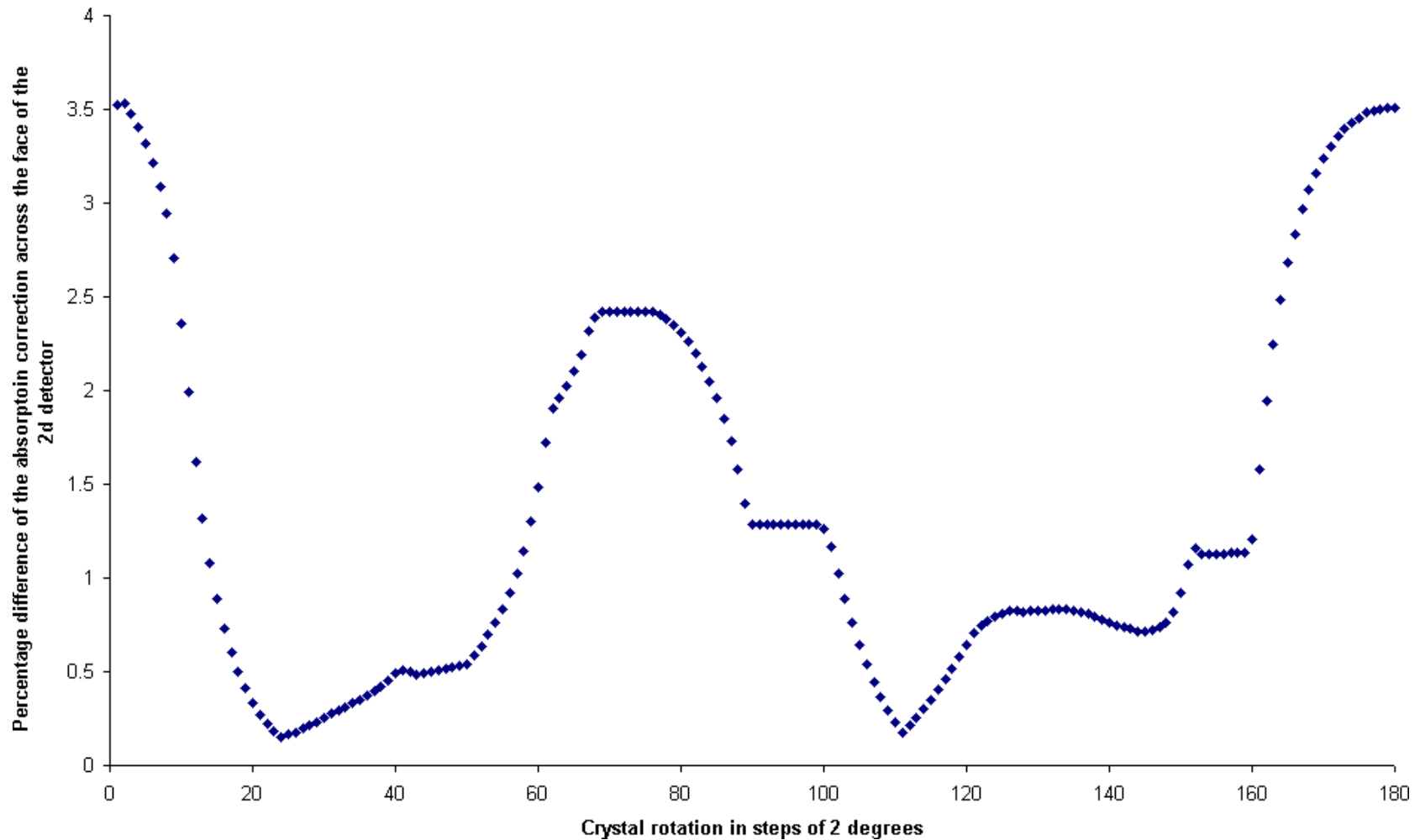
2D

As a contour plot...



2D

As the percentage difference at a given angle across the face of the detector.



2D

What have we learned...

- For a needle (40 microns x 180 microns) at 1.5Å we see a moderate absorption correction ranging from around 7% to 13%.
- We note that the variation in this, not only as the crystal rotates but also across the face of the detector is around 4%.
- Simulating at 2° intervals over 360° of rotation and a scattering angle of -30° to 30° takes about 2 minutes on a standard pc.



3D

A spherical crystal in a spherical droplet...

A z-slice projection of the volume data

The associated absorption correction...



3D

Consider the following plate in an ellipsoid droplet

The associated absorption correction for 120° of data collection

Summery.

What has been achieved?

- Working 3D code that produces absorption corrections for a crystal fully bathed in beam.
- The code is split up such that all of the physically dependent and computationally intensive tasks are done at the beamline. The application of the absorption correction to data can be done on a desktop pc.
- Analytical methods that will allow us to vastly reduce the number voxels that need to be considered.

Summery.

Future work for the next 6 months(ish)...

- Partially illuminated volumes. This will allow for the treatment of crystals that are not fully bathed in beam. This will also give an exact volume for each part of the crystal that has been illuminated (i.e. no need to scale for V)
- Incorporation of analytical methods to reduce memory and cpu requirements.
- Future spin-off project would be to expand the code to calculate the absorbed dose for each illuminated volume.