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North A C T, Phillips D C & Mathews F S. A semi-empirical method of absorption correction. *Acta Crystallogr. A—Found. Crys.* 24:351-9, 1968.
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The paper describes an experimental method of correcting for the absorption of different X-ray reflections from crystals; the corrections are easily measured and applied, and the method has been widely used by protein crystallographers. [The *SCI*® indicates that this paper has been cited in more than 2,545 publications.)

Easily Applied Absorption Corrections for Protein Crystals

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In 1955,1 joined the protein crystallography group of Sir Lawrence Bragg at the Royal Institution in London. Other members of the group included David Phillips and David Green (who, with Max Perutz and Vernon Ingram, had just used isomorphous replacement to find the phases of X-ray reflections from protein crystals, opening the way to determining their structures).

Photographic film was the normal recording medium for diffraction from protein crystals, but Perutz, concerned about nonlinearity in the films, asked me to measure the strongest reflections from his hemoglobin crystals on our manually operated diffractometer.

Protein crystals contain water, filling the gaps between irregularly shaped protein molecules. Consequently, they are soft and must be Kept moist during the experiment, normally by sealing them with some other liquor in a thin glass tube.

A meticulous experimentalist, Perutz personally mounted all the crystals. His assistant brought them by train from Cambridge, and I put each on the diffractometer—with some trepidation, lest I broke the capillary.

Each X-ray intensity needed to be corrected for its absorption, which could be done theoretically, knowing the paths of the incident and diffracted rays through the crystal. With proteins, the glass capillary also absorbs X-rays and the crystal is mounted off-axis against the capillary wall, with mother liquor between crystal and capillary. Although M. Wells extended his absorption correction computer program to take into account capillary and liquid,[1] the problem was to measure accurately the crystal dimensions, its position within the capillary, and the space filled by liquid—all extremely difficult because of the distorting effects of the capillary glass.

This led Phillips and me to develop an experimental method, proposed by T.C. Furnas,[2] to derive approximate corrections. When a crystal axis is parallel to the spindle of the goniometer on which it is mounted, the reflecting planes of an axial reflection are perpendicular to that axis and remain in position for all rotational angles about it. The variation in intensity with azimuthal angle gives a first-order correction, assuming that crystal absorption depends only upon orientation about the goniometer axis. Our improvement was to average the values corresponding to the directions of the incident and diffracted rays. We also measured absorption curves for several axial reflections, choosing the one with an index nearest to the reflection to be corrected.

We found that the method greatly improved agreement among intensities of symmetry-equivalent reflections.[3] A further check, with the help of Scott Mathews, showed that our method compared favorably with accurate measurement of a crystal and computation of its absorption.

The next paper to ours in *Acta Crystallographica,* by R. Huber and colleagues,[4] described a method of similar aims. By making many more measurements than we, they evaluated an "absorption surface" defining absorption in all possible directions through the crystal. While Huber's method is more accurate, many additional measurements are required. This is time-consuming, but, more importantly, the limited life of protein crystals means that as more time is spent on evaluating the absorption correction, less is available for collecting intensity data.

1. **Wells M.** Computation of absorption corrections on EDSACII. *Acta Crystallogr.* 13:722-6, 1960.
2. **Furnas T C.** *Single crystal orienter instruction manual.* Milwaukee: General Electric Company, 1957. (Cited 330 times.)
3. **Blake C C F, Mair G A, North ACT, Phillips D C & Sarma V R.** On the conformation of the hen egg-white lysozyme molecule. *Proc. Roy. Soc. B* 167:365-77, 1967. (Cited 525 times.)
4. **Kopfmann G & Huber R.** A method of absorption correction by X-ray intensity measurements. *Acta Crystallogr. A—Found. Crys.* 24:348-51, 1968. (Cited 140 times.)

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