International Tables for X-ray Crystallography (1974). Vol. IV. Birmingham: Kynoch Press.
LOFTHUS, A. (1959). *Mol. Phys.* **2**, 367–371.
MAIN, P., LESSINGER, L., WOOLFSON, M. M., GERMAIN, G. & DECLERCQ, J.-P. (1977). *MULTAN 77. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.* Univs. of York, England, and Louvain, Belgium.
MULLEN, D. & HELLNER, E. (1978). *Acta Cryst.* B**34**, 1624–1627.
OTTERSEN, T. (1975). *Acta Chem. Scand. Ser. A*, **29**, 934–944.
RAE, I. D. & DYALL, L. K. (1966). *Aust. J. Chem.* **19**, 835–839.
ROLLETT, J. S. (1969). *Crystallographic Computing*, edited by F. R. AHMED, pp. 169–172. Copenhagen: Munksgaard.
SMITH, H. W., CAMERMAN, A. & CAMERMAN, N. (1978). *J. Med. Chem.* **21**, 468–471.
TALBERG, H. J. (1979). *Structural Aspects of Nitrosobenzenes*, Department of Chemistry, Oslo Univ., Oslo.
TEMPLETON, L. K., TEMPLETON, D. H. & ZALKIN, A. (1973). *Acta Cryst.* B**29**, 50–54.
WASER, J. (1963). *Acta Cryst.* **16**, 1091–1095.

Acta Cryst. (1982). B**38**, 2181–2184

A Photographic Method for Reconstructing Three-Dimensional Information

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(*Received* 23 *December* 1981; *accepted* 18 *February* 1982)

Abstract

A photographic method for obtaining the three-dimensional coordinates of atoms constituting a Kendrew-type molecular model is described. The method is based on the fact that a photograph is a perspective projection. The method needs two photographs taken from arbitrary directions. The two-dimensional coordinates of any atom are measured on these photographs with the aid of computer graphics. The coordinates of several standard points are measured by another method, say a plumb bob and thread, and used to obtain the transformation matrix from the two-dimensional coordinates on the photographs to the three-dimensional coordinates. An accuracy of 0·3 Å is easily achieved, and the method was successfully applied to the structure analysis of cytochrome c_3.

At the final stage of an X-ray structure analysis of a protein crystal, model-building is usually carried out, fitting the polypeptide models of standard bond lengths and angles into the electron density map. Computer graphics systems are increasingly used for such a purpose and it is not necessary to build a structure model. Nevertheless, a protein crystallographer tends to build a model, perhaps because such a model represents the secondary and tertiary structures better, and it is easy to explain the biochemical importance of his work. In other words, a structure model better visualizes the significance of his results.

It then becomes an important process to read out the atomic coordinates from a structure model. Some devices which do this have been described in the literature (Richards, 1968; Salemme & Frehr, 1972; Mitsui, 1979), but there may well be other methods used by protein crystallographers. It can be said that each laboratory has its own method for measuring the atomic coordinates, and any such method must be rapid as well as precise.

Here we wish to describe a photographic method to be used for such a purpose. This method uses the following procedure. Two photographs of a model are taken from any direction; it is desirable that these directions are perpendicular to each other, but information about the spatial relationship between them is not necessary. The optical density of every unit area on the photographs is converted to a digital value using a high-speed rotating drum scanner. Several standard points or atoms in the model (at least six) are read out from the model by any method. The transformation matrix can then be calculated. The two-dimensional coordinates from a set of photographs are then combined to reconstruct the three-dimensional coordinates. The method is quite general and is also applicable to other purposes. A remarkable feature of this method is the need for information on only a few points; this number is far less than the number of points to be measured.

0567-7408/82/082181-04$01.00

Rossmann & Argos (1980) have proposed a method for reconstructing the three-dimensional image from a stereo pair of plotted figures of a protein. The photographic method somewhat resembles this method, but an important difference is the fact that our method is not limited to a stereo pair.

A new computer algorithm for reconstructing a scene from two projections has been reported recently (Longuet-Higgins, 1981). This method differs from ours in their necessity for the knowledge of the spatial relationship between the two projections.

Algorithm

The mathematical background of the method has been described by Rogers & Adams (1976). Here we summarize useful equations as briefly as possible.

Let (x,y,z) be the three-dimensional coordinates of any point in an object, and (X,Y,Z) be its transformed ones. The general perspective transformation can be represented as a 4 × 4 matrix, **T**.

$$[x\,y\,z\,1]\mathbf{T}' = [X\,Y\,Z\,H] \tag{1}$$

$$\mathbf{T}' = \begin{pmatrix} T'_{11} & T'_{12} & T'_{13} & T'_{14} \\ T'_{21} & T'_{22} & T'_{23} & T'_{24} \\ T'_{31} & T'_{32} & T'_{33} & T'_{34} \\ T'_{41} & T'_{42} & T'_{43} & T'_{44} \end{pmatrix} \tag{2}$$

H represents a scale factor. A photograph is a perspective projection. We consider that the results can be projected onto a two-dimensional plane, say $z = 0$, by using

$$\mathbf{T}'' = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}. \tag{3}$$

Multiplication of the two matrices gives

$$\mathbf{T} = \mathbf{T}'\,\mathbf{T}'' = \begin{pmatrix} T_{11} & T_{12} & 0 & T_{14} \\ T_{21} & T_{22} & 0 & T_{24} \\ T_{31} & T_{32} & 0 & T_{34} \\ T_{41} & T_{42} & 0 & T_{44} \end{pmatrix}. \tag{4}$$

Therefore, the overall transformation can be written as

$$\begin{aligned}[x\,y\,z\,1]\mathbf{T} &= [X\,Y\,0\,H] \\ &= H[x^*\,y^*\,0\,1].\end{aligned} \tag{5}$$

Here x^* and y^* are the coordinates in the perspective projection onto the plane $z = 0$; that is, the coordinates on the photograph. Writing out (5) yields

$$T_{11}x + T_{21}y + T_{31}z + T_{41} = Hx^* \tag{6}$$

$$T_{12}x + T_{22}y + T_{32}z + T_{42} = Hy^* \tag{7}$$

$$T_{14}x + T_{24}y + T_{34}z + T_{44} = H. \tag{8}$$

Substituting (8) into (6) and (7):

$$\left.\begin{aligned}&(T_{11} - T_{14}x^*)x + (T_{21} - T_{24}x^*)y \\ &\quad + (T_{31} - T_{34}x^*)z + (T_{41} - T_{44}x^*) = 0 \\ &(T_{12} - T_{14}y^*)x + (T_{22} - T_{24}y^*)y \\ &\quad + (T_{32} - T_{34}y^*)z + (T_{42} - T_{44}y^*) = 0.\end{aligned}\right\} \tag{9}$$

If two perspective projections (two photographs) are available, two sets of equations like (9) can be written attaching superscripts (1 to 2) to T and x^*, y^*. If one wishes to determine T, then one has to know x,y,z and x^{*1}, y^{*1}, x^{*2}, y^{*2} for several standard points. Equation (9) includes twelve unknown T_{ij} elements and, therefore, the knowledge of the coordinates of at least six standard points is necessary. Equation (9) is homogeneous for T_{ij}, and we can solve it by supposing that T_{44} is unity with the known method of least squares; one can then derive the three-dimensional coordinates of any point from a set of x^*, y^* for two photographs using equation (9). There are four equations for three unknowns, and then (x,y,z) are determined by the method of least squares.

Method

The outline of the procedure is presented schematically in Fig. 1.

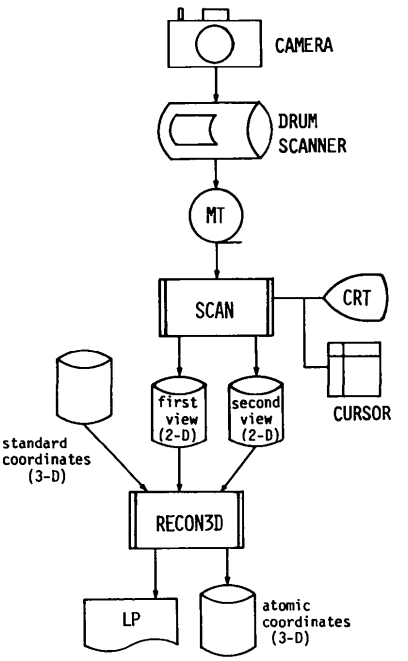

Fig. 1. A schematical representation of the photographic-method procedure for reconstructing the three-dimensional information.

Photographs

A pair of photographs are taken from two arbitrary directions. An ordinary 35 mm film camera with a standard lens is adequate. It is desirable to take the second photograph from a direction perpendicular to that of the first. The positional error of the measurement with the photograph is believed to be not greater than 1% (Watanabe, 1976).

Standard points

The standard points must be distributed uniformly over every part of the model. The measurement of the three-dimensional coordinates of several standard points may be carried out by any method. We use a plumb bob and thread. A piece of graph paper is placed at the bottom of the model. A plumb bob with thread is hung from a measuring point. Two-dimensional coordinates of the point can be read out on the graph paper and the last coordinate is given by the length of the thread from the point to the top of the plumb bob.

Measurement of the two-dimensional coordinates

The optical density of the whole area on the photographs is digitized by an Optronics P-1000 drum scanner. A slit aperture 25 × 25 μm is used. The density is converted to an eight-bit numeral, the values 0 to 255 corresponding to 0 to 2 D in optical density. The digitized data are transferred to a computer. The picture is displayed on a raster-scan display. An N-6940 color data scope (640 × 512 pixels, eight colors) connected to an ACOS-700 computer system is used. The user indicates the atomic position with a cross-hair cursor and types in the identification code of the atom (Fig. 2). The two-dimensional coordinates of the atom on the photograph are then stored in the computer. The second photograph is then processed in the same way. This stage is performed by the program *SCAN*.

Fig. 2. A photograph of the frame displayed on a graphics display terminal representing part of the molecular model.

Reconstruction of the three-dimensional information

At first the transformation matrices shown in equation (4) are computed from the data of the standard points using equation (9). The program *RECON3D* does this by the least-squares method. The residual is expected to be very small if the choice of standard points is adequate and the measurement of the coordinates is free from error. Therefore, one can judge whether the transformation matrices are well determined from the value of the residual. The three-dimensional coordinates of atoms are then easily obtained using equation (9). There are four equations for three variables, so the coordinates are computed by the method of least squares. Here the residual again gives an estimate as to whether the coordinates have been well determined. *RECON3D* lists warning messages when the residual is unusually high, implying some mistakes in the measurement of the two-dimensional coordinates.

Limits and scope of the method

This method has been successfully applied to the structure analysis of cytochrome c_3. The Kendrew-type model was constructed by comparing the electron density map with the polypeptide models of standard bond lengths and angles with variable dihedral angles. The scale was 1 Å = 2 cm. A set of coordinates of the atoms in the main chain and side groups thus obtained could be used as input parameters for the crystallographic refinement of the structure. A stereo pair of the structure at this stage is shown in Fig. 3.

Here we wish to give some results of calculations using the coordinates of α-carbon atoms. A stereoscopic view of the connected α-carbon atoms with

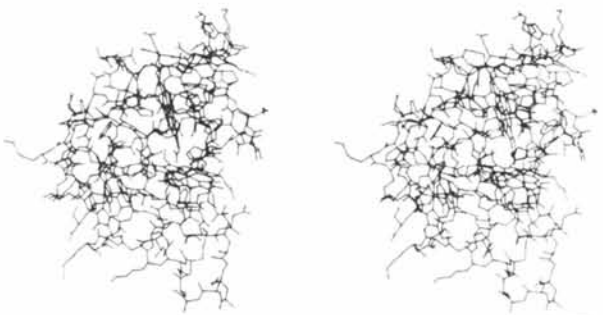

Fig. 3. A stereoscopic drawing of the cytochrome c_3 molecule. The coordinates of these atoms have been obtained by the procedure described in the text.

Table 1. *Dependence of the root-mean-square errors on the choice of standard points*

R_1 denotes the root-mean-square error for all atoms, while R_2 is the corresponding value excluding the standard points.

Case	Number of standard points	Root-mean-square error R_1	R_2	Comment
(1)	6	0·42 Å	0·43 Å	Every part of the molecular surface
(2)	8	0·29	0·30	
(3)	10	0·27	0·29	
(4)	12	0·27	0·28	
(5)	6	1·13	1·16	Randomly chosen
(6)	8	0·29	0·31	
(7)	10	0·31	0·32	
(8)	107	0·27	–	All atoms

heme groups is shown in a previous communication (Higuchi, Bando, Kusunoki, Matsuura, Yasuoka, Kakudo, Yamanaka, Yagi & Inokuchi, 1981). The coordinates of α-carbon atoms have already been obtained by a plumb bob and thread. The remaining atoms in the peptide backbone and in the side groups have been obtained by the photographic method described above. The coordinates of α-carbon atoms were treated as standard points. To demonstrate the accuracy of the present method, some calculations using these known coordinates have been carried out. Some of the α-carbon atoms were treated as standard points to calculate the transformation matrix and the coordinates of the remaining atoms were derived from a set of two-dimensional coordinates measured on photographs and the transformation matrix. These coordinates were then compared with those obtained by the plumb bob and thread method. The results are summarized in Table 1. The R value is the root-mean-square deviation, defined as $R = [\sum_N(\mathbf{r}_b - \mathbf{r}_p)^2/N]^{1/2}$, where $\mathbf{r}_b$ and $\mathbf{r}_p$ designate the positional vectors obtained by the plumb bob and thread method, and the photographic method, respectively.

The results for cases (1)–(4) in Table 1 help us to estimate how many standard points must be used to obtain sufficient precision. In these cases we had chosen the standard points carefully from every part of the molecular surface. If we select ten standard points, it is sufficient to reproduce the atomic coordinates within 0·3 Å. Case (8) uses all the α-carbon atoms as standard points, and is considered to give the available precision of the present method. Cases (5)–(7) demonstrate that the selection of the standard points is important to obtain accurate coordinates. In these cases we selected them randomly; that is, in case (5) for example, we selected every fifteenth α-carbon atom along the protein sequence, and so on. Such a random choice gave a rather high R value compared to the case where standard points have been selected to cover every part of the molecular surface.

Although the method is very simple and applicable to many cases, some atoms happen to overlap on a photograph and it is not possible to determine their exact two-dimensional coordinates. If a given protein has as many as 500 residues, overlap of atoms may often be observed. In this case a third photograph will be added to the procedure described above. We believe that the present method is one of the simplest for obtaining three-dimensional coordinates from a molecular model.

References

HIGUCHI, Y., BANDO, S., KUSUNOKI, M., MATSUURA, Y., YASUOKA, N., KAKUDO, M., YAMANAKA, T., YAGI, T. & INOKUCHI, H. (1981). *J. Biochem.* **89**, 1659–1662.

LONGUET-HIGGINS, H. C. (1981). *Nature (London)*, **293**, 133–135.

MITSUI, Y. (1979). *J. Appl. Cryst.* **12**, 135.

RICHARDS, F. M. (1968). *J. Mol. Biol.* **37**, 225–230.

ROGERS, D. F. & ADAMS, J. A. (1976). *Mathematical Elements for Computer Graphics*, pp. 78–83. New York: McGraw-Hill.

ROSSMANN, M. G. & ARGOS, P. (1980). *Acta Cryst.* B**36**, 819–823.

SALEMME, F. R. & FREHR, D. G. (1972). *J. Mol. Biol.* **70**, 697–700.

WATANABE, K. (1976). *Shashin Keisoku*, p. 11 (in Japanese). Tokyo: Kyoritsu Shuppan.