DIRECT STRUCTURE DETERMINATION OF MULTILAYERED MEMBRANE-TYPE SYSTEMS WHICH CONTAIN FLUID LAYERS

C. R. WORTHINGTON, G. I. KING, *and* T. J. McINTOSH

From the Departments of Biological Sciences and Physics, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

ABSTRACT The theory of direct methods of structure analysis in the case of multilayered membrane-type systems which contain fluid layers is described. Diffraction formulas for this kind of analysis are derived. Deconvolution methods are used when the centrosymmetrical unit cells contain wide fluid layers. When the membrane systems contain narrow fluid layers, other direct methods are used. These direct methods involve computing either the Fourier series representations or the sampling theorem expressions.

INTRODUCTION

The study of cell membranes by X-ray diffraction can in principle provide information on the way the molecular components are assembled in the membrane. If the multilayered specimen contains a number of unit cells with a well-defined repeating unit, the diffraction pattern will show a series of discrete reflections. These X-ray reflections are usually recorded on X-ray film so that a set of integrated intensities $I(h)$ is then obtained. In order to interpret the recorded low-angle X-ray diffraction intensities, knowledge of the Fourier transform of the unit cell is required.

In this paper the notation of an earlier paper (Worthington, 1969) is followed whenever possible. Thus we write $J(h) = |T(h)|^2$, where $|T(h)|$ is the modulus of the Fourier transform value and $J(h)$ is said to be on an absolute scale. The relation between $I(h)$ and $J(h)$ has been given by Blaurock and Worthington (1966). Hence, after suitable processing of the X-ray data, a set of observed intensities $J_{\text{obs}}(h)$ on a relative scale is obtained. A structural analysis of the observed intensities $J_{\text{obs}}(h)$ is the next logical step.

Until recently, it has been customary to use either of two well-known methods of structure analysis, namely, the Fourier method and the method using electron density models. In the Fourier method the phases of the Fourier transform values have to be found in order to compute a Fourier series representation of the unit cell. In this paper the unit cell is assumed to have a center of symmetry. Thus $T(h) =$

$\{\pm\}|T(h)|$ and a Fourier synthesis can be completed on finding the correct set of phases $\{\pm\}$. There are, however, a total of 2^h possible Fourier syntheses and in general there is no reliable procedure for choosing which Fourier is, in fact, the correct one. In the model approach a model is proposed and its correctness is tested by comparing the $J_{obs}(h)$ values with the calculated ones. In practice a large number of possible models are examined and the model which gives the best agreement with the low-angle X-ray data is considered to be the best choice. This favored model then has a certain set of phases which may or may not be the correct set. The question of uniqueness is not easily answered for there is a definite difficulty in trying to prove that one has, in fact, obtained the correct set of phases.

It is clear that, apart from the uniqueness problem, the model approach does provide a structural interpretation in terms of a certain well-defined model. The use of electron density strip models in interpreting the low-angle X-ray data from membrane-type structures has been described (Worthington, 1969). Since that time this method of structure analysis has been used in the study of nerve myelin (for instance, see Worthington, 1972), retinal photoreceptors (Gras and Worthington, 1969), and model membrane systems (Wilkins et al., 1971). In the 1969 theoretical study the relationship between model parameters and the Patterson function was described. Furthermore, it was noted that the relationship was particularly straightforward when the unit cells of the membrane-type specimens contain one or more fluid layers. The membrane assembly is considered to be in a swollen state when the fluid layers are relatively wide. It was recognized in 1969 that the Patterson function of a swollen membrane specimen is identifiable with the autocorrelation function $A(x)$ of the single unit cell. The procedure was to find the model which gave best agreement with $A(x)$. At that time the uniqueness of the favored model remained open to question. It was later realized, however, that knowledge of $A(x)$ was sufficient to solve directly the phase problem in the centrosymmetrical case and thus a unique electron density distribution can be obtained.

The original theory of this direct method was developed by Hosemann and Bagchi (1962). A centrosymmetrical electron density distribution was obtained by a deconvolution of $A(x)$ using either the recursion method or the more involved relaxation method. The original idea of Hosemann and Bagchi was to obtain $A(x)$ from a diffraction study of a specimen containing only a few unit cells. But this direct method did not attract attention until the recent work of Lesslauer and Blasie (1972). Lesslauer and Blasie demonstrated the feasibility of this deconvolution method; they used a small number of layers of barium stearate and derived a centrosymmetrical electron density distribution using the recursion method.

This deconvolution method of structure analysis can also apply to swollen membrane specimens for the autocorrelation function $A(x)$ of a single unit cell can often be obtained via the Patterson function. According to theory the autocorrelation function $A(x)$ has a one-to-one correspondence with a centrosymmetrical n-strip electron density model. There are two ways of interpreting $A(x)$: by building a model to match the shape of $A(x)$ or by deconvolution of $A(x)$. Thus, it can be argued that the favored model proposed for a membrane assembly in the swollen state has the correct set of phases and, in this sense, it is unique. On the other hand, deconcolution of $A(x)$ gives the correct electron density distribution directly and this procedure is clearly preferred. The electron density distribution of certain membrane systems which contain relatively wide fluid layers has been directly determined using this deconvolution method. These systems include retinal photoreceptors (Worthington and Gras, 1972), nerve myelin (King and Worthington, 1972; footnote 1), and air-dried mitochondria.[1]

The purpose of this paper is to describe the theory of direct methods of structure analysis in the case of multilayered membrane-type systems which contain fluid layers. Diffraction formulas needed for this kind of analysis are derived.

REVIEW OF DIFFRACTION FORMULAS

Let $t(x)$ represent the electron density distribution of a single unit cell of width d along an axis at right angles to the planar surface. The unit cell is centrosymmetrical, that is, $t(x) = t(-x)$. $t(x)$ and $T(X)$ are a pair of Fourier transforms, denote $t(x) \rightleftharpoons T(X)$ where

$$T(X) = \int_0^d t(x) \cos 2\pi Xx \, dx, \tag{1}$$

$$t(x) = \int_{-\infty}^{\infty} T(X) \cos 2\pi Xx \, dX, \tag{2}$$

and where x, X are direct and reciprocal space coordinates. Let $g(x)$ denote the electron density of the multilayered specimen which has N unit cells of width d:

$$g(x) = t(x) * \phi(x), \tag{3}$$

where $\phi(x)$ is the lattice generating function and $*$ is the convolution symbol. The Fourier transform of the assembly is $G(X)$ and

$$G(X) = T(X)\Phi(X), \tag{4}$$

where $g(x) \rightleftharpoons G(X)$, $\phi(x) \rightleftharpoons \Phi(X)$ and $\Phi(X)$ is the interference function. If N is relatively large, then the interference function has comparatively discrete maxima which occur at $X = h/d$, h an integer. Integrated intensities $I(h)$ can then be measured and a set of observed intensities $J_{\text{obs}}(h)$ is obtained. The normalization constant K converts $J_{\text{obs}}(h)$ to an absolute scale: $KJ_{\text{obs}}(h) = J(h)$ but the $J_{\text{obs}}(h)$ data set does not include the $h = 0$ value.

In the case of N large, we can write $\Phi^2(X) = 1$ for lattice points $X = h/d$ and

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[1] Worthington, C. R., and T. J. McIntosh. Manuscript in preparation.

$\Phi^2(X) = 0$ for nonlattice points, and it follows that $g(x)$ can then be expressed in series form. It is usual to write the series in terms of the single unit cell electron density $t(x)$. There are three Fourier series representations $t(x)$, $t'(x)$, and $t''(x)$:

$$t(x) = (1/d)\sum_{-\infty}^{\infty} T(h) \cos 2\pi hx/d, \tag{5}$$

$$t'(x) = (1/d)\sum_{-h}^{h} T(h) \cos 2\pi hx/d, \tag{6}$$

$$t''(x) = (2/d)\sum_{1}^{h} \{\pm\}[J_{\mathrm{obs}}(h)]^{1/2} \cos 2\pi hx/d, \tag{7}$$

where $\{\pm\}$ is the phase information. A window function $W(X)$ is defined (Worthington, 1969) such that $W(X)$ has value unity within the observed diffraction range and $w(x) \rightleftharpoons W(X)$. Thus the three Fourier syntheses are related:

$$t'(x) = t(x) * w(x) = (1/d)T(0) + [K]^{1/2}t''(x). \tag{8}$$

The Patterson function can be evaluated without any phase information and this function usually has a central role in direct methods of structure analysis. The Patterson function $P(x)$ of the multilayered assembly is defined:

$$P(x) = g(x) * g(-x), \tag{9}$$

and $P(x) \rightleftharpoons |G(X)|^2$. The multilayered assembly has width Nd and thus $P(x)$ has width $2Nd$. That is, $P(x)$ has values inside the interval $-Nd \leq x \leq Nd$ and $P(x) = 0$ outside this interval. Again, in case of N large, we can write $\Phi^2(X) = 1$ for lattice points and zero for nonlattice points, and then $P(x)$ can be expressed in series form. There are three Patterson functions $P(x)$, $P'(x)$, and $P''(x)$:

$$P(x) = (1/d)\sum_{-\infty}^{\infty} J(h) \cos 2\pi hx/d, \tag{10}$$

$$P'(x) = (1/d)\sum_{-h}^{h} J(h) \cos 2\pi hx/d, \tag{11}$$

$$P''(x) = (2/d)\sum_{1}^{h} J_{\mathrm{obs}}(h) \cos 2\pi hx/d. \tag{12}$$

The Patterson functions are periodic functions and have period d. The three Patterson functions are related as follows:

$$P'(x) = P(x) * w(x) = (1/d)J(0) + P''(x). \tag{13}$$

The sampling theorem of communication theory provides a series-form expression for $T(X)$ in reciprocal space and for $t(x)$ in real space. The expression for $T(X)$ can be formally derived by writing down the Fourier transformation of the Fourier series $t(x)$ in Eq. 5. Thus, an expression for $T(X)$ is obtained:

$$T(X) = \sum_{-\infty}^{\infty} T(h) \operatorname{sinc} (\pi dX - \pi h), \tag{14}$$

where $\operatorname{sinc} \theta = \sin \theta/\theta$. In practice, h is finite so that only the continuous Fourier transform $T(X)W(X)$ can be evaluated:

$$T(X)W(X) \approx \sum_{-h}^{h} T(h) \operatorname{sinc} (\pi dX - \pi h). \tag{15}$$

Note that in Eq. 15 the approximation is valid if all the strong orders of diffraction are included in the summation, that is, it is understood that the cutoff refers to the very weak reflections which are not observed experimentally.

RELATION BETWEEN THE PATTERSON FUNCTION $P(x)$ AND THE AUTOCORRELATION FUNCTION $A(x)$

The autocorrelation function $A(x)$ of a single cell electron density $t(x)$ is defined:

$$A(x) = t(x) * t(-x) \tag{16}$$

and $A(x) \rightleftharpoons J(X)$. The unit cell has width d and thus $A(x)$ has width $2d$. This is shown in Fig. 1. Note that in general $A(x)$ is not easily expressed in series form as $A(x)$ is the Fourier transform of a continuous function $J(X)$. From Eqs. 3, 9, and

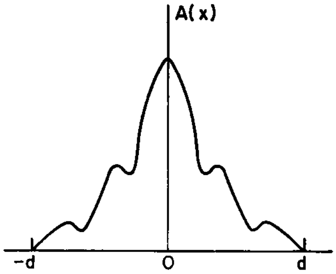

FIGURE 1

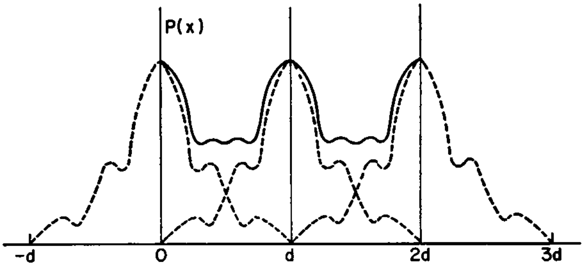

FIGURE 2

FIGURE 1 Autocorrelation function $A(x)$ of a single unit cell of electron density $t(x)$ has width $2d$ whereas the unit cell has width d.

FIGURE 2 The Patterson function $P(x)$ is the summation of autocorrelation functions $A(x)$ centered at intervals of d. In the diagram $A(x)$ is centered at $x = 0$, $x = d$, and $x = 2d$. The $A(x)$ curves are shown by dotted lines and the Patterson function $P(x)$ is shown by a continuous line. In the first positive interval $0 \leq x \leq d$, $P(x)$ contains the positive part of $A(x)$ centered at $x = 0$ and the negative part of $A(x)$ centered at $x = d$. $P(x)$ is a periodic function with period d and has a center of symmetry at half-integral values of d.

16 $P(x)$ and $A(x)$ are related:

$$P(x) = A(x) * \phi(x) * \phi(-x). \tag{17}$$

The Patterson function is therefore composed of a succession of autocorrelation functions centered at intervals of d. This is shown in Fig. 2. The Patterson function in the interval $0 \leq x \leq d$ contains two overlapping autocorrelation functions as $A(x)$ has width $2d$. Consider the case of N large such that the weighting factors $[\phi(x) * \phi(-x)]$ in Eq. 17 can be ignored. In the first positive interval $0 \leq x \leq d$, $P(x) = A(x) + A(d - x)$. In the second positive interval $d \leq x \leq 2d$, $P(x) = A\,(d - x) + A\,(2d - x)$. In the jth positive interval $(j - 1)\, d \leq x \leq jd$, $P(x) = A[(j - 1)\, d - x] + A[jd - x]$ and finally, the Nth positive interval $(N - 1)\, d \leq x \leq Nd$, $P(x) = A[(N - 1)\, d - x]$. Therefore, $P(x)$ in the Nth positive interval is equal to $A(x)$ in the interval $0 \leq x \leq d$. Thus, if N is small, $A(x)$ can be extracted from $P(x)$ as first pointed out by Hosemann and Bagchi (1962).

In order to interpret $J_{\mathrm{obs}}(h)$, it would be convenient if $t(x)$ could be directly obtained from the Patterson function $P''(x)$. However, even if $P(x)$ was available, a rigorous interpretation of $P(x)$ is seldom possible. On the other hand, from Eq. 16 it follows that $t(x)$ can be directly obtained from a deconvolution of $A(x)$ provided that appropriate methods can be found. Thus, it is desirable to separate $A(x)$ from $P(x)$. This is possible when the multilayered specimen contains only a few unit cells, that is, N is small. Another possibility exists; if the unit cell contains a well-defined fluid layer, then the two overlapping autocorrelation functions in $P(x)$ tend to separate. In order to present these ideas, it is convenient to consider an electron density model for the membrane specimen.

DIFFRACTION FORMULAS FOR THE MINUS FLUID MODEL

The model for the electron density distribution $t(x)$ has a membrane unit of electron density $m(x)$ and a fluid layer of electron density F. This model is shown in Fig. 3. The membrane unit has average electron density M and width v whereas the fluid layer has width $d - v$. The origin of the unit cell is at the center of $m(x)$ as shown in Fig. 3.

It is, however, convenient to study a variation of this model denoted $\Delta t(x)$ where $\Delta t(x) = t(x) - F$. This model is called the minus fluid model and is shown in Fig. 4. The membrane unit in this model has electron density $m(x) - F$ within v and a region of zero electron density within the space $d - v$.

The Fourier transform of $\Delta t(x)$ is $\Delta T(X)$, $\Delta t(x) \rightleftharpoons \Delta T(X)$ where

$$\Delta T(X) = \int_0^v \Delta t(x) \cos 2\pi Xx \, dx, \tag{18}$$

$$\Delta t(x) = \int_{-\infty}^{\infty} \Delta T(X) \cos 2\pi Xx \, dX. \tag{19}$$

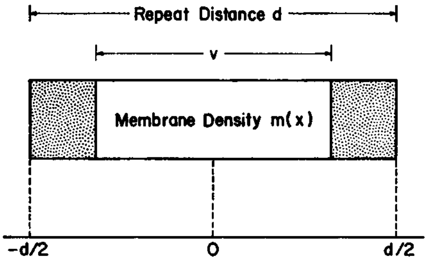

FIGURE 3

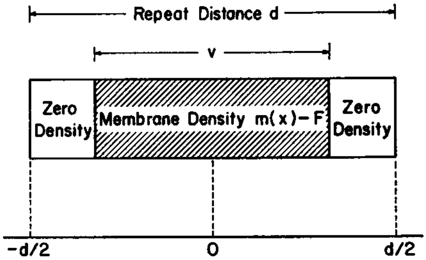

FIGURE 4

FIGURE 3 The electron density model $t(x)$ has a membrane unit of electron density $m(x)$ and a fluid layer of electron density F. The fluid layer is shown as a dotted region. The membrane unit has width v and the fluid layer separating the adjacent membrane units has width $d - v$. The model $t(x)$ has width d and repeat distance d.

FIGURE 4 The minus fluid electron density model $\Delta t(x)$ has a membrane unit of electron density $m(x) - F$ and zero electron density between adjacent membrane units. The minus fluid model $\Delta t(x)$ has width v and repeat distance d.

The Fourier transform of the minus fluid model $\Delta T(X)$ is also given by

$$\Delta T(X) = T(X) - Fd \operatorname{sinc} \pi dX. \tag{20}$$

Now $\Delta T(0) = (M - F)v$ and $T(0) = (M - F)v + Fd$ and therefore $\Delta T(0) \neq T(0)$, but $\Delta T(h) = T(h)$, h a nonzero integer. Hence the observed X-ray data $\Delta J_{\text{obs}}(h) \equiv J_{\text{obs}}(h)$ as the origin value $h = 0$ is not observed. Hence it follows that models $\Delta t(x)$ and $t(x)$ cannot be distinguished on the basis of the observed X-ray data. The Fourier series representations $\Delta t''(x)$ and $t''(x)$ are identical and the Patterson functions $\Delta P''(x)$ and $P''(x)$ are also identical for the same reason. The notation $J_{\text{obs}}(h)$ is used whenever possible, but when the continuous intensity profile is considered, the notation $\Delta J_{\text{obs}}(X)$ and $J_{\text{obs}}(X)$ is retained because $\Delta J_{\text{obs}}(0) \neq J_{\text{obs}}(0)$. Hence it follows that the Fourier series representations $\Delta t'(x)$ and $t'(x)$ are different and, similarly, the Patterson functions $\Delta P'(x)$ and $P'(x)$ are different.

The sampling theorem-type expression for the continuous transform $\Delta T(X)$ is directly obtained from Eq. 14:

$$\Delta T(X) = \sum_{-\infty}^{\infty} \Delta T(h) \operatorname{sinc} (\pi dX - \pi h). \tag{21}$$

Another expression, however, $\Delta T(X)$, can also be formally derived using the Fourier transform pair relations defined by Eqs. 18 and 19. Thus an expression for $\Delta T(X)$ is obtained:

$$\Delta T(X) = (v/d) \sum_{-\infty}^{\infty} \Delta T(h) \operatorname{sinc} [\pi vX - \pi hv/d]. \tag{22}$$

The expressions in Eqs. 21 and 22 are both correct but the expression in Eq. 22 contains the actual value of v and this form may be a convenient one in special situations.

Autocorrelation Function of a Swollen Specimen

The autocorrelation function $\Delta A(x)$ of the minus fluid model is

$$\Delta A(x) = \Delta t(x) * \Delta t(-x), \tag{23}$$

and as $\Delta t(x)$ has width v then $\Delta A(x)$ has width $2v$. From Eq. 17 it follows that, if $d \geq 2v$, then the Patterson function $\Delta P(x)$ in the interval $0 \leq x \leq d/2$ is identical with $\Delta A(x)$ in the interval $0 \leq x \leq v$. If $d \geq 2v$ the autocorrelation functions centered at $x = 0$ and $x = d$ in $P(x)$ do not overlap.

The Patterson functions $\Delta P''(x)$ and $P''(x)$ are both given by Eq. 12 and are directly computed from the X-ray data. The Patterson function $\Delta P'(x)$ is given by

$$\Delta P'(x) = (1/d)\Delta J(0) + KP''(x). \tag{24}$$

Now, if $d \geq 2v$, then $\Delta A'(x) = \Delta P'(x)$ in the interval $0 \leq x \leq v$. It is assumed that the value of v is known. Clearly $\Delta A(v) = 0$ and hence $\Delta A'(v) \approx 0$ and from Eq. 24

$$KP''(v) = (-1/d)\Delta J(0). \tag{25}$$

Hence $\Delta A'(x)$ is given by

$$\Delta A'(x) = K[P''(x) - P''(v)]. \tag{26}$$

Thus, apart from the normalization constant K, the autocorrelation function $\Delta A'(x) = \Delta A(x) * w(x)$ is directly obtained from the low-angle X-ray data of swollen membrane systems. Deconvolution of $\Delta A'(x)$ then provides the electron density distribution $\Delta t'(x) = \Delta t(x) * w(x)$.

Fourier Transform of the Autocorrelation Function

The Fourier transform of the autocorrelation function $\Delta A'(x)$ gives the continuous intensity profile. Since $\Delta A(x) \rightleftharpoons \Delta J(X)$ it follows that $\Delta A'(x) \rightleftharpoons \Delta J(X)W(X)$. Hence the continuous intensity profile on a relative scale $\Delta J_{\text{obs}}(X)W(X)$ is given by

$$\Delta J_{\text{obs}}(X)W(X) = 2\int_0^v [P''(x) - P''(v)] \cos 2\pi Xx \, dx. \tag{27}$$

If this continuous intensity profile has values at $X = h/d$ which are the same as the original $J_{\text{obs}}(h)$ values, then the choice of v is probably correct. Actually the choice of v larger than the correct value will still give the correct intensity profile whereas a choice of v smaller than the correct value will give an incorrect intensity profile. The Fourier transformation of $\Delta A'(x)$ then indicates whether or not the choice of v is correct.

An important consequence of determining the continuous intensity profile

$\Delta J_{\text{obs}}(X)W(X)$ is that the zeros of the intensity profile are obtained. This immediately simplifies the phase problem in that it reduces the number of possible sets of phases. The shape of the intensity regions between successive zeros will often indicate whether a phase change between successive regions actually occurs. Thus, by assuming a $\{+ \text{ or } -\}$ phase for any one region and, by assuming that a phase change occurs between certain regions, two possible sets of phases are obtained. This method provides a check on the final phase solution but the deconvolution method is more direct and is preferred.

Another consequence of obtaining the continuous intensity profile $\Delta J_{\text{obs}}(X)W(X)$ from Eq. 27 is that a value for $\Delta J_{\text{obs}}(0)$ is obtained. Note that neither $\Delta J_{\text{obs}}(0)$ nor $J_{\text{obs}}(0)$ is recorded experimentally. The zero-order intensity can be evaluated from the parameters of the minus fluid model, where

$$\Delta J(0) = (M - F)^2 v^2, \tag{28}$$

and $K\Delta J_{\text{obs}}(0) = \Delta J(0)$. Thus an estimate of M, the average electron density of the membrane unit, can be obtained from knowledge of $\Delta J_{\text{obs}}(0)$ provided that K, v, and F are known. Reliable values for v and F are readily obtained and methods of determining the normalization constant K have been described (Worthington, 1969). Possible pitfalls in this method of obtaining an estimate of M, however, have not yet been studied, for at this time this method has received little attention.

A sampling theorem-type expression for $\Delta J(X)$ can be derived provided that the fluid layer within the unit cell is sufficiently wide. The autocorrelation function $\Delta A(x)$ is identical to the Patterson function $\Delta P(x)$ in the interval $0 \leq x \leq v$ provided that $d \geq 2v$. Thus $\Delta A(x)$ is given by

$$\Delta A(x) = (1/d)\sum_{-\infty}^{\infty} \Delta J(h) \cos 2\pi hx/d. \tag{29}$$

Eq. 29, after Fourier transformation using the appropriate Fourier relations, gives an expression for $\Delta J(X)$:

$$\Delta J(X) = (2v/d)\sum_{-\infty}^{\infty} \Delta J(h) \operatorname{sinc} [2\pi vX - 2\pi hv/d]. \tag{30}$$

In the special case of $d = 2v$, $\Delta J(X)$ is given by

$$\Delta J(X) = \sum_{-\infty}^{\infty} \Delta J(h) \operatorname{sinc} (\pi dX - \pi h). \tag{31}$$

Thus, by sampling points at $X = h/2v$, the continuous intensity transform $\Delta J(X)$ is obtained. But this means that $\Delta t(x)$ of width v is essentially sampled at half-integral points $(h/2)$; this possibility was first noted by Sayre (1952).

In practice, h is finite and an expression for the continuous intensity profile on a relative scale $\Delta J_{\text{obs}}(X)W(X)$ is required. Using the same procedure leading to Eq. 15, $\Delta J_{\text{obs}}(X)W(X)$ can be written

$$\Delta J_{\text{obs}}(X)W(X) \approx (2v/d)\sum_{-h}^{h} \Delta J_{\text{obs}}(h) \operatorname{sinc}\,[2\pi v X - 2\pi h v/d]. \tag{32}$$

Eq. 32 describes a sampling theorem-type expression for the continuous intensity profile but knowledge of $\Delta J_{\text{obs}}(0)$ is required.

Deconvolution of the Autocorrelation Function

The autocorrelation function $\Delta A'(x)$ which is obtained from the low-angle X-ray data in case of $d \geq 2v$ is given by

$$\Delta A'(x) = \Delta t'(x) * \Delta t'(-x). \tag{33}$$

Two methods of deconvolution for obtaining the electron density distribution $\Delta t'(x)$ have been suggested by Hosemann and Bagchi (1962). From diffraction theory (Hosemann and Bagchi, 1962) Eq. 33 yields a unique structure $\Delta t'(x)$ although either the positive structure $+\Delta t'(x)$ or the negative structure $-\Delta t'(x)$ are both valid solutions. Thus, a n-strip centrosymmetrical electron density model has a one-to-one correspondence with the autocorrelation function.

The model of width v is divided into $2m$ equal strips of width $\delta = v(2m)^{-1}$. The electron density of the strips are denoted $[t_m, t_{m-1}, \cdots t_j \cdots t_2, t_1, t_1, t_2 \cdots t_j \cdots t_m]$ where t_1 is the strip closest to the origin and t_m is the strip facing the vacuum space. Note that t_j can be either $\{+ \text{ or } -\}$ as the t_j model refers to the $\Delta t(x)$ model. It is instructive to write down an expression for $\Delta A(x)$ derived from this centrosymmetrical $2m$ strip model in successive steps of δ in the interval $0 \leq x \leq v - \delta$. The values of $\Delta A(x)$ are conveniently expressed in matrix form:

$$\begin{bmatrix} \Delta A(0) \\ \Delta A(\delta) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \Delta A\{(m-1)\delta\} \\ \Delta A(v/2) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \Delta A(v-2\delta) \\ \Delta A(v-\delta) \end{bmatrix} = \delta \begin{bmatrix} t_m\ t_{m-1} \cdot\cdot\ t_j \cdot\cdot\ t_1\ \ t_1 \cdot\cdot\ t_j \cdot\cdot\ t_{m-1}\ t_m \\ t_{m-1} \cdots\cdots\cdots\cdots\cdots t_m \quad 0 \\ \cdots\cdots\cdots\cdots\cdots\cdots 0 \quad 0 \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ t_j \cdots\cdots\cdots\cdots t_m\, 0 \cdots\cdots \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ t_1 \cdots\cdots\cdots t_m\ 0 \cdots\cdots\cdots 0 \\ t_1 \cdots\cdots\cdots t_m\ 0 \cdots\cdots\cdots 0 \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ t_j \cdots t_m\, 0 \cdots\cdots\cdots\cdots\cdots 0 \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ \cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ t_{m-1}\ t_m\ 0 \cdots\cdots\cdots\cdots\cdots \\ t_m \quad 0 \cdots\cdots\cdots\cdots\cdots\cdots 0 \end{bmatrix} \begin{bmatrix} t_m \\ t_{m-1} \\ \cdot \\ \cdot \\ t_j \\ \cdot \\ \cdot \\ t_1 \\ t_1 \\ \cdot \\ \cdot \\ t_j \\ \cdot \\ \cdot \\ t_{m-1} \\ t_m \end{bmatrix}. \tag{34}$$

Eq. 34 contains a $2m$ by $2m$ matrix. The column vector for $\Delta A(x)$ stops at $x = \nu - \delta$ for $\Delta A(\nu) = 0$. The two deconvolution methods, however, aim at solving an m by m matrix in each case instead of the above larger matrix. We first consider the recursion method.

Recursion Method. Because the model for the membrane unit is centrosymmetrical with an origin at $x = 0$, it is convenient to consider only the positive half model $[t_1 t_2 \cdots t_j \cdots t_m]$ defined in the interval $0 \leq x \leq \nu/2$. It is instructive to write down the matrix equation for the column vector $[\Delta A(\nu - \delta), \Delta A(\nu - 2\delta), \cdots\cdots \Delta A(\nu/2)]$:

$$\begin{bmatrix} \Delta A(\nu - \delta) \\ \Delta A(\nu - 2\delta) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \Delta A(\nu/2) \end{bmatrix} = \delta \begin{bmatrix} t_m & 0 & \cdot & \cdot & \cdot & \cdot & \cdot & 0 \\ t_{m-1} & t_m & 0 & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & t_m & 0 & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & t_m & 0 & \cdot & \cdot & \cdot \\ t_j & \cdot & \cdot & \cdot & t_m & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & 0 & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & t_m & 0 & 0 \\ t_2 & \cdot & \cdot & \cdot & \cdot & \cdot & t_m & 0 \\ t_1 & t_2 & \cdot & \cdot & t_j & \cdot & t_{m-1} & t_m \end{bmatrix} \begin{bmatrix} t_m \\ t_{m-1} \\ \cdot \\ \cdot \\ t_j \\ \cdot \\ \cdot \\ t_2 \\ t_1 \end{bmatrix}. \qquad (35)$$

From the above m by m matrix equation the relation for $x = \nu - \delta$ is $\Delta A(\nu - \delta) = \delta t_m^2$ and hence t_m has two values, $t_m = \pm[\delta^{-1}\Delta A(\nu - \delta)]^{1/2}$. The + value leads to the positive structure and the − value leads to the negative structure. The relation for $x = \nu - 2\delta$ is $\Delta A(\nu - 2\delta) = 2\delta t_{m-1} t_m$ and hence a value for t_{m-1} is obtained. Similarly all other values of t_j are obtained. This method of deconvolution is called the recursion method.

In practice the recursion method described by Eq. 35 does not always succeed. The main reason is error propagation. If any errors are introduced into the end values t_m, t_{m-1}, etc. via $\Delta A(\nu - \delta)$, $\Delta A(\nu - 2\delta)$, etc., then these errors are magnified and propagated into the successive values of t_j. This is usually the case unless the values of $m(x)$ are large compared with F at the boundary of the membrane unit where $x \approx \nu/2$. In order to test whether the model t_j values derived from the experimental $\Delta A'(x)$ values in the interval $\nu/2 \leq x \leq \nu$ is the correct solution for $\Delta t'(x)$, the values for $[\Delta A(0), \Delta A(\delta) \cdots\cdot \Delta A\{(m - 1)\delta\}]$ are calculated from the t_j model and compared with the experimental $\Delta A'(x)$ values in the interval $0 \leq x \leq \nu/2$. If the calculated values differ markedly from the experimental $\Delta A'(x)$ values, then the recursion method has not succeeded.

The recursion method has succeeded in the study of retinal photoreceptors (Worthington and Gras, 1972). This deconvolution method could also have been used in some early X-ray studies, for instance, the method when applied to the X-ray data of Rand and Luzzati (1968) from a lamellar phase of mixed lipids also succeeds (Worthington, unpublished). The method, however, does not succeed in the case of nerve myelin even though a sufficiently wide fluid layer to satisfy the condition $d \geq 2v$ has been experimentally recorded. On the other hand, the relaxation method has been used to study the electron density distribution of nerve myelin. The relaxation method avoids the build-up of errors inherent in the recursion method in that a solution is sought for a whole set of equations considered as a unit.

Relaxation Method. In the relaxation method the t_j values are obtained from the $\Delta A'(x)$ values in the interval $0 \leq x \leq v/2$. It is convenient to refer to matrix Eq. 34 but with a smaller column vector, namely $[\Delta A(0), \Delta A(\delta) \cdots \cdot \Delta A\{(m-1)\delta\}]$. That is, half of Eq. 34 is used and it describes a $2m$ by m matrix. This matrix, however, can be rearranged into an m by m matrix and the matrix equation then reduces to m nonlinear equations in m unknown which are the t_j values.

The procedure is to guess at an initial solution following Southwell (1940). A column vector $[\Delta A(0), \Delta A(\delta) \cdots \cdot \Delta A\{(m-1)\delta\}]$ is then calculated from the initial t_j values. These calculated values of $\Delta A(x)$ are then compared with the observed $\Delta A'(x)$ values. A residual function $R_i = \Delta A(i\delta) - \Delta A'(i\delta)$ is computed where $i = 0, 1 \cdots \cdot m - 1$. The t_j values are systematically varied until the R_i values are minimized and are all approximately zero. The set of t_j values which provides a calculated autocorrelation function in closest agreement with the observed autocorrelation is the correct model and the model phases are the correct set of phases.

However, in order to ensure that a correct solution has, in fact, been obtained it is necessary that all possible models be examined systematically. Thus each phase solution is studied in turn and the R_i values for the best t_j model for that particular phase choice is determined. The t_j model which gives the minimum R_i values is the correct model and has the correct set of phases.

An important property of the relaxation method is that $\Delta A'(x)$ only has to be resolved in the interval $0 \leq x \leq v/2$. The necessary condition is $d \geq 3/2v$ whereas the condition that $\Delta A'(x)$ be resolved in the interval $0 \leq x \leq v$ is $d \geq 2v$. This means that the fluid layer can be smaller by a factor of two over that required by the recursion method. For example, if $v = 150$ Å, in the recursion method $d - v \geq 150$ Å with $d \geq 300$ Å is required whereas in the relaxation method only $d - v \geq 75$ Å with $d \geq 225$ Å is required.

MEMBRANE SYSTEMS WITH ONLY SMALL FLUID LAYERS

It is assumed that the unit cell of the multilayered specimen has a membrane unit and a well-defined fluid layer similar to the model shown in Fig. 3. As before it is convenient to study the minus fluid model $\Delta t(x)$ shown in Fig. 4. In a swelling experiment it may happen that the width of the fluid layer only shows small increases and, if $d < 3/2v$, then the deconvolution methods which have been described cannot be used. The kind of swelling considered here assumes that only small or moderate increases occur in the width of the fluid layer and the membrane unit remains unchanged. Different swelling experiments using the same fluid medium invariably lead to different repeat periods, a result first noted by Worthington and Blaurock (1969 *a*) in a study of the swelling behavior of nerve myelin. Thus two different swelling experiments using the same fluid F provide sampling points $X = h/d$, h an integer and $X = H/D$, H an integer on the same Fourier transform $\Delta T(X)$. For the present purposes it is assumed that K, the normalization constant, and $\Delta T(0)$ are known. Because the two sets of X-ray data are derived from the same model but with different periods, then $|\Delta T(h)|$ and $|\Delta T(H)|$ lie on the same transform $|\Delta T(X)|$ and hence it follows the correct set of phases can be found. The correct set of phases can be found by computing either the Fourier series representations or by computing the continuous Fourier transforms. Both methods are equivalent as Fourier series representations and the sampling theorem expressions are related to each other via a Fourier transformation.

In the Fourier method, the Fourier series representations $\Delta t'(x)$ are computed where

$$\begin{aligned}\Delta t'(x) &= (1/d)\sum_{-h}^{h} \{\pm\}\,|\Delta T(h)|\cos 2\pi hx/d,\\ &= (1/D)\sum_{-H}^{H} \{\pm\}\,|\Delta T(\mathrm{H})|\cos 2\pi Hx/D.\end{aligned} \tag{36}$$

The correct set of phases for both sets of data yield the same Fourier $\Delta t'(x)$ whereas an incorrect set of phases will give two different Fouriers.

In the sampling theorem method, the sampling theorem expression for the continuous Fourier transform $\Delta T(X)W(X)$ is computed where

$$\begin{aligned}\Delta T(X)W(X) &\approx \sum_{-h}^{h} \{\pm\}\,|\Delta T(h)|\,\mathrm{sinc}\,(\pi dX - \pi h),\\ &\approx \sum_{-H}^{H} \{\pm\}\,|\Delta T(H)|\,\mathrm{sinc}\,(\pi DX - \pi H).\end{aligned} \tag{37}$$

The correct set of phases for both sets of data yield the same continuous Fourier transform $\Delta T(X)W(X)$ whereas an incorrect set of phases will give two different Fourier transforms.

In actual experiments the two sets of X-ray data $\Delta J_{\mathrm{obs}}(h)$ and $\Delta J_{\mathrm{obs}}(H)$ can be obtained on the same relative scale (Worthington and Blaurock, 1969 *b*) but, again, $\Delta J_{\mathrm{obs}}(0)$ is not recorded. The phases can be found either by computing the Fourier series representations $\Delta t''(x)$ or by computing the continuous amplitude transform $[K^{-1/2}\Delta T(X)W(X)]$. In the Fourier method two sets of phases are obtained, the positive set and the negative set, which give the positive and negative structures $\pm\Delta t''(x)$. Resolution between $\pm\Delta t''(x)$ can sometimes be made if the actual value of $\Delta T(0)$ is known. Fortunately, in nearly every membrane system studied so far, an estimate of $\Delta T(0)$ can be made as reliable values for M, v, and K can be obtained. However, if $M \approx F$, then $\Delta T(0) \approx 0$ and in this case a choice between $\pm\Delta t''(x)$ cannot be made. In the sampling theorem method the phases can be found by computing the continuous amplitude transform $[K^{-1/2}\Delta T(X) W(X)]$. Only one set of phases is obtained using the sampling theorem expression as the origin value $h = 0$ has to be included in the summation. However, in the special case of $\Delta T(0) \approx 0$, two sets of phases are obtained and a choice cannot be made between the $\pm\Delta t''(x)$ structures.

To summarize, phases can be found by computing either the Fourier series representations or the sampling theorem expressions for both sets of X-ray data. This direct method of phase determination is, however, based upon the assumption that both sets of data lie on the same transform. A proof of correctness for the phases can be obtained if this assumption can be verified. Fortunately, this assumption can be verified by obtaining other sets of data which also lie on the same transform. Further swelling experiments are therefore required in order to obtain other sampling points at intervals of $1/g$ where g is a repeat period which differs from d and D. These additional sampling points will tend to trace out a continuous intensity curve $\Delta J_{obs}(X) W(X)$ if the only effect of the swelling is to change the width of the fluid layer. If these additional points also lie on the calculated continuous amplitude transform $[K^{-1/2}\Delta T(X) W(X)]$, then it follows that all the data lie on the same transform. This same conclusion can also be obtained by computing the Fourier series representations using the additional sampling points based upon the period g.

Thus phases can be found for two sets of X-ray data which lie on the same transform. The basic assumption that the data do, in fact, lie on the same transform can be verified by carrying out further swelling experiments. The distinction between the positive and negative set of phases and hence the positive and negative structures can often be made if a reliable estimate of $\Delta T(0)$ is available. A further verification of the choice of phases can be made by changing the electron density of the fluid in a known fashion and conducting a new series of swelling experiments.

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