Deconvolution Analysis in Membrane Diffraction Using the Modulus of the Continuous Fourier Transform

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(Received 15 January 1981; accepted 14 April 1981)

Abstract

A method of deconvolution using the modulus of the continuous Fourier transform of the unit cell is described. This method differs from previous deconvolution methods in membrane diffraction in that calculations are carried out in reciprocal space. The modulus profile is obtained from the continuous intensity transform which is itself the Fourier transform of the autocorrelation function. Sampling theorem methods are used to reconstruct the continuous Fourier transform of the unit cell. The various phase choices are examined and compared. In membrane diffraction, the autocorrelation function is derived in two distinct situations: when the interference function is broad as in the case of a few unit cells and when the membrane systems contain wide regions of constant electron density. It is concluded that, in the first situation, the derived autocorrelation function contains missing information and is incorrect. On the other hand, in the second situation, the derived autocorrelation function is a good representation of the true autocorrelation function.

Introduction

Membranes either occur naturally or can be artificially prepared in certain well-defined configurations. The application of direct methods of structure analysis to the X-ray data recorded from certain membrane configurations provides a solution to the phase problem. In this paper the direct method using deconvolution is considered. The first step in the deconvolution method is to obtain the autocorrelation function of a single centrosymmetrical unit cell of the membrane assembly. Deconvolution of the autocorrelation function provides an electron density solution \( s(x) \), with the well-known sign ambiguity. The original theory of the deconvolution method was developed by Hosemann & Bagchi (1962), while the theory of deconvolution methods as applied to membrane-type systems has been presented (Worthington, King & McIntosh, 1973; Worthington, 1973).

The particular case when \( h \) diffraction orders are recorded from a multilayered membrane assembly with repeat distance \( d \) is treated. The unit cell is centrosymmetrical so that the Fourier transform values have phases of \(+1\) or \(-1\). Thus, there are \( 2^h \) possible phase choices. Deconvolution of the autocorrelation function of the unit cell has previously been carried out using the recursion or the relaxation method (Hosemann & Bagchi, 1962; Worthington et al., 1973). The recursion method has the advantage that the deconvolution calculation is rapid and uncomplicated but, in practice, it is an approximate procedure because of error propagation inherent in the recursion process. Two other methods using either a Fourier series (Pape, 1974) or a power-series expansion (Moody, 1974) have been proposed as an alternative to the recursion method. The recursion and these two later methods provide only one solution apart from the sign ambiguity. Once a solution has been obtained we still need to know whether there are other solutions which might also account for the X-ray data. In other words, a statistical measure is required in order to verify that the deconvolution process has, in fact, provided the best solution.

The relaxation method can be set up so as to examine each possible solution but, so far, it has only been used in membrane diffraction on one occasion (McIntosh & Worthington, 1974) although the method was once used in an earlier study of line broadening (Paterson, 1950). The relaxation method is not straightforward to apply as the solution \( s(x) \) has to be systematically varied until the difference between the calculated and observed autocorrelation functions is minimized. The relaxation method is seldom used for the reason that the minimization procedures complicate the method. In this paper an alternative to the relaxation method is proposed. This new method uses the modulus of the continuous Fourier transform of the unit cell and enables each solution to be examined and compared.

The question of whether one solution is superior to the next solution often arises. Even in the ideal case, when the autocorrelation function is known exactly, this question cannot be readily answered as it is not easy to apply error analysis to any deconvolution method. In practice, the experimentally derived autocorrelation function is an approximation to the true autocorrelation function owing to experimental errors in the intensity measurement as well as possible errors in data processing. The autocorrelation function is obtained in two separate situations. The first situation is when the interference function is known, for instance when the
multilayered specimen contains only a few unit cells (Hosemann & Bagchi, 1962; Lesslauer & Blasie, 1972), while the second situation is when the unit cell contains sufficiently wide regions of constant electron density either fluid as in swollen membrane systems (Worthington et al., 1973) or lipid as in lipid bilayers (Worthington & Khare, 1978). We find that, in the first situation, only a poor representation of the autocorrelation function is obtained because the X-ray information at very small angles of diffraction is missing. On the other hand, in the second situation, a good representation of the autocorrelation function is obtained for the reason that X-ray information at very small angles of diffraction is included.

Diffraction theory

The diffraction theory of the deconvolution method as applied to membrane systems has been described (Worthington, 1969; Worthington et al., 1973) and only a brief account is given here.

Let \( t(x) \) represent the electron density distribution in a direction at right angles to the membrane surface and let \( T(X) \) represent its Fourier transform. Denote \( t(x) \rightleftharpoons T(X) \), where \( t(x) \) and \( T(X) \) are a Fourier transform pair and where \( x, X \) are real- and reciprocal-space coordinates. The diffracted intensity, after data processing, is denoted \( \mathcal{I}(X) \) and

\[
\mathcal{I}(X) = J(X) \Phi^2(X),
\]

where \( J(X) = |T(X)|^2 \) is the continuous intensity transform and \( \Phi^2(X) \) is the interference function. In (1), the range of \( X \) is from \(-\infty \) to \( +\infty \). In practice, only a finite range of \( X \) is recorded, namely, \( X_{\text{min}} \leq X \leq X_{\text{max}} \) where \( X_{\text{min}} \) is the smallest value of \( X \) and \( X_{\text{max}} \) is the cut-off value of \( X \). Window functions \( E(X) \) and \( W(X) \) are defined such that they have a value of unity for \( 0 \leq X \leq X_{\text{max}} \) and \( 0 \leq X \leq X_{\text{max}} \) respectively and are zero outside this range. Denote \( e(x) \rightleftharpoons E(X) \) and \( w(x) \rightleftharpoons W(X) \) respectively. The diffracted intensity in the observed range is denoted \( \mathcal{I}_{\text{obs}}(X) \) and

\[
\mathcal{I}_{\text{obs}}(X) = \mathcal{I}(X) \left[ W(X) - E(X) \right].
\]

In the first situation, when the multilayers have either only a few unit cells or else have large lattice disorder, then the interference function \( \Phi^2(X) \) is relatively broad so that a continuous intensity profile \( \mathcal{I}_{\text{obs}}(X) \) is obtained. In the second situation, when the multilayers contain a large number of unit cells, the interference function \( \Phi^2(X) \) is sharply defined and discrete X-ray reflections are recorded at \( X = h/d \), \( h \) is the diffraction order and \( d \) is the cell dimension. Integrated intensities are measured and after data processing a set of corrected intensities \( J_{\text{obs}}(h) \) is obtained. The notation \( KJ_{\text{obs}}(h) = J(h) = |T(h)|^2 \), where \( K \) is the normalization constant is retained. The set of corrected intensities contain diffraction orders \( h = 1 \) to \( h_{\text{max}} \), the zero-order reflection is not included.

In the second situation, when the membrane systems contain regions of constant electron density, it is convenient to study either the minus fluid model (Worthington, 1969) or the minus L model (Worthington & Khare, 1978). The electron density of the minus fluid model is defined as

\[
\Delta t(x) = t(x) - F,
\]

where \( F \) is the electron density of the fluid. Note that the \( \Delta \) symbol is used to specify the minus fluid or the minus \( L \) models. The Fourier transform of \( \Delta t(x) \) is \( \Delta T(X) \) where \( \Delta t(x) \rightleftharpoons \Delta T(X) \) and the notation \( \Delta J(h) = |\Delta T(X)|^2 \) is retained. The relationship between \( T(X) \) and \( \Delta T(X) \) of the minus fluid model is

\[
\Delta T(X) = T(X) - F d \text{sinc} \pi d X,
\]

where sinc \( \theta = \sin \theta /\theta \). Hence, \( \Delta T(h) = T(h) \) and \( \Delta J(h) = J(h) \) provided that \( h \) is a non-zero integer. This means that there is no way to distinguish between \( t(x) \) and \( \Delta t(x) \) on the basis of the X-ray data unless the \( h = 0 \) reflection is recorded. We note that, in the minus \( L \) model, \( L, \) the electron density of the hydrocarbon chains, replaces \( F \) in (3) and (4).

The notation of single and double primes is adopted (Worthington, 1969) so as to specify the extent of the X-ray data which is used to define the appropriate function. A double prime refers to the data set \( J_{\text{obs}}(h) \) where \( h = 1 \) to \( h_{\text{max}} \). Thus, the Patterson function for both models is denoted \( P''(x) \) and

\[
P''(x) = (2/d) \sum_1^h J_{\text{obs}}(h) \cos 2\pi hx/d.
\]

The Fourier series representation for both models, which are assumed to be centro-symmetrical, is denoted \( t''(x) \) and

\[
t''(x) = (2/d) \sum_1^h \{ \pm \} |J_{\text{obs}}(h)| \cos 2\pi hx/d,
\]

where \( \{ \pm \} \) is the phase information. A single prime refers to the data set \( J_{\text{obs}}(h) \) but also includes the \( h = 0 \) value. Thus, the Fourier series expression for the minus fluid model is denoted \( t'(x) \) and

\[
t'(x) = (1/d) \sum_{-h}^h \{ \pm \} |T(h)| \cos 2\pi hx/d.
\]

Now, \( t'(x) \rightleftharpoons T(X) W(X) \) and, hence, \( t'(x) = t(x) \ast \ast w(x) \), where \( \ast \) is the convolution symbol.

The autocorrelation function \( A(x) \) of a single unit cell of electron density \( t(x) \) is defined as

\[A(x) = t(x) \ast t(-x),\]

and \( A(x) \rightleftharpoons J(X) \). The autocorrelation function \( \Delta A(x) \) of the minus fluid model \( \Delta t(x) \) is of importance as it can be experimentally determined in membrane diffraction (Worthington et al., 1973; McIntosh & Worthington, 1974). From (8), the autocorrelation function \( \Delta A(x) \) is given by

\[\Delta A'(x) = \Delta t'(x) \ast \Delta t'(-x),\]

where \( A'(x) = \Delta J(X) W(X) \).
Determination of the autocorrelation function

First situation

The first situation refers to the case when the interference function $\Phi^2(x)$ is broad and a continuous corrected intensity profile $\mathcal{I}_{\text{obs}}(x)$ is measured. The intensity profile is defined by (1) and (2). In previous X-ray work (Lesslauer & Blasie, 1972; Schwartz, Cain, Dratz & Blasie, 1975; Herbette, Marquardt, Scarpa & Blasie, 1977), an autocorrelation function has been derived from $\mathcal{I}_{\text{obs}}(x)$ either by taking the Fourier transform of $\mathcal{I}_{\text{obs}}(x)$ and using the function at its end points or by assuming knowledge of $\Phi^2(x)$. From (1) and (2), $\mathcal{I}_{\text{obs}}(x)$ after effective division by $\Phi^2(x)$ is given by

$$\mathcal{I}_{\text{obs}}(x) \left[ \Phi^2(x) \right]^{-1} = J(x) W(x) - J(x) E(x).$$

An autocorrelation function $\mathcal{A}(x)$ is obtained by Fourier transformation of (10) and $\mathcal{A}(x)$ is given by

$$\mathcal{A}(x) = A'(x) - A(x) \ast e(x).$$

The derived autocorrelation $\mathcal{A}(x)$ is a poor representation of the true autocorrelation function $A'(x)$ as it contains a missing term which has variation over the whole range of $x$. Moreover, deconvolution of $\mathcal{A}(x)$ does not provide $t'(x)$ or $\Delta t'(x)$ but provides an incorrect electron density solution. The previous determinations of phases with $\mathcal{A}(x)$ (Schwartz et al., 1975; Herbette et al., 1977) are, therefore, suspect to errors.

It is evident that the effect of the missing $J(x) E(x)$ term can be reduced or eliminated by the inclusion of the zero-order term $A J(0)$. The zero-order term can be estimated with the procedure developed in sampling theorem methods by King & Worthington (1971).

Second situation

The second situation refers to the case when the membrane systems contain regions of constant electron density fluid or lipid. The derivation of the autocorrelation function $A A'(x)$ of the electron density of the minus fluid or minus L models has been described (Worthington et al., 1973; McIntosh & Worthington, 1974; Worthington & Khare, 1978) and only a brief account is given here. The Patterson function $P''(x)$, as defined by (5), is computed. The autocorrelation function $A A'(x)$ is obtained from the Patterson function by shifting the base line so that $A A'(v) = 0$. The necessary condition for the derivation of $A A'(x)$ is that $d \geq 2v$, where $v$ is either the width of the membrane region in the minus fluid model or the width of the head-group region in the minus L model.

The autocorrelation function $A A'(x)$ is on a relative scale as

$$A A'(x) = K [P''(x) - P''(v)],$$

where $K$ is the normalization constant and $A A'(x) \equiv J(x) W(x)$. The origin value $A J(0)$ is given by

$$A J(0) = -K d P''(v).$$

The derived autocorrelation function $A A'(x)$ is a good representation of the true autocorrelation function $A A'(x)$ in that the derived function contains information on the zero-order diffraction. This information was obtained as a result of noting that $A A'(x) = 0$ for $x \geq v$.

A Fourier transformation of the derived autocorrelation function $A A'(x)$ provides the continuous intensity transform $J J(x) W(x)$. The modulus of the continuous Fourier transform generally shows $n$ regions, where $n$ is less than $h_{\text{max}}$ (McIntosh & Worthington, 1974). When $n$ is less than $h_{\text{max}}$ two or more diffraction orders are in the same region and have the same phase. As each region can be $\{\pm\}$, there are a total of $2^n$ possible phase choices to consider. A reconstruction of the Fourier transform of a single unit cell is denoted $\Delta T_i(x)$ and $\Delta T_i(x)$ is defined using the sampling theory of communication theory as

$$\Delta T_i(x) = \sum_{-k}^k \{ \pm \} |\Delta T(ih)| \sin(\pi dX - \pi h),$$

where a finite number of orders are used in the summation, including the $\Delta T(0)$ term which is obtained from (13). $N$ values of $\Delta T_i(x)$ are computed at successive intervals ($i$) of width $Y$, where $(N-1) Y = X_{\text{max}}$. Typically, a value of $N = 100$ might be used. In order to compare the matching of the calculated $\Delta T_i(x)$ values with the derived Fourier transform values $|\Delta T(X)|$, it is convenient to define the R equivalent (RE) as

$$\text{RE} = \frac{\sum_j |\Delta T_j(Y)| - |\Delta T(jY)|}{\sum_j |\Delta T(jY)|},$$

where the summation is from $j = 0$ to $j = N - 1$. The phase choice with the lowest RE value is the favored solution. The question of whether it is the correct solution or only a possible solution is dependent on the magnitude of the RE values of the other phase choices.

Discussion

In the deconvolution method, the autocorrelation function of a single unit cell is derived in two separate experimental situations. We find that in the first situation, when the interference function is broad, the autocorrelation function as derived previously contains missing information which means that an incorrect electron density solution is obtained. In future work, it will be possible to include the missing information by using sampling theorem methods (King & Worthington, 1971). On the other hand, the derived autocorrelation function in the second situation is a good representation of the true autocorrelation function as it contains no missing information.

The deconvolution method described in this paper uses the Fourier transform of the autocorrelation function $A A'(x)$ which is $A J(x) W(x)$, the continuous
intensity transform expressed in reciprocal-space coordinates. The modulus of the continuous Fourier transform of a single unit cell of the minus fluid or minus \( L \) models \( |\Delta T(X)|W(X) \) is obtained from \( \Delta J(X)W(X) \). According to diffraction theory, the phase choice which generates a calculated Fourier transform identical to the experimentally derived transform is the correct choice. A statistical measure, namely the RE value, is used to examine all possible choices. We note that the deconvolution method described here is similar in certain respects to the reconstruction method using sampling theorem methods (Worthington et al., 1973).

The present deconvolution method operates in reciprocal space and differs from the recursion, the relaxation and other methods which are carried out in real space. The autocorrelation function is found once the continuous intensity transform has been experimentally defined. The physical interpretation of the deconvolution method when expressed in reciprocal space is as follows: There is only one phase choice (with the sign ambiguity) that will generate an intensity profile which is identical to the experimentally derived intensity transform.

This work was aided by a grant from the US Public Health Service.

References