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Computer-generated Fourier transforms of helical particles†

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Abstract. An alternative method has been found to display the information contained in the Fourier transform of a helical particle. This allows a strong selection rule to be defined for the transform on the layer lines and consequently the discrimination between signal and noise contributions to the data can be improved.

1. Introduction

When proteins aggregate to form a solid, cylindrical structure they usually do so in a manner such that the cylinder can be described as consisting of identical axially repeating units each geometrically related to the one below it by a screw rotation. This type of structure is called a discrete helix (Cochran *et al* 1952) and its mass distribution, λ, satisfies the following two conditions.

For all z

$$\lambda(r, \theta, z) = \lambda(r, \theta+\zeta, z+p), \qquad p > 0 \tag{1.1}$$

and

$$\lambda(r, \theta, z) = 0, \qquad r > a; \tag{1.2}$$

$\lambda(r, \theta, z)$ is the mass-density distribution function and (r, θ, z) are cylindrical polar coordinates in real space: (R, Θ, Z) will be used to denote the corresponding Fourier space variables. The axis of the helix is the line $r = 0$; p is the smallest nonzero distance such that (1.1) is satisfied (ie the depth of the axially repeating unit) and ζ is the smallest corresponding screw rotation. For simplicity we choose $a = 1$.

Electron micrographs of helical particles deposited on the grid so that their axes are perpendicular to the incident electron beam can often be used to obtain structural information about the particles. The general technique for obtaining the three-dimensional mass distribution in such particles using micrograph data is now well established (De Rosier and Klug 1968, De Rosier and Moore 1972, Mellema and Klug 1972). The method relies on the fact that the optical density of the electron micrograph can be used to obtain approximate estimates of the projected mass density of the helix along the direction of the electron beam. In general such projection data are contaminated by noise.

This can be written as

$$f^{\mathrm{D}}(x, z, \phi) = f(x, z, \phi) + f^{\mathrm{N}}(x, z, \phi) \tag{1.3}$$

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where

$$f(x, z, \phi) = \int_{-\infty}^{\infty} \lambda(\sqrt{x^2+y^2}, \phi + \tan^{-1}(y/x), z)\, dy \tag{1.4}$$

is the projected density function of the helix, f^N is the noise contribution and f^D are the measured data. x is the coordinate perpendicular to z in the projection plane. ϕ is the absolute view angle of the helix with respect to an angular origin whose choice is arbitrary. Consequently we may, without loss of generality, write $f(x, z)$ for $f(x, z, 0)$; $\phi = 0$ being understood.

Given image information in the form of projection data the central section theorem states that the two-dimensional Fourier transform of the projection, $f(x, z)$, is an axial central section of the three-dimensional Fourier transform of the helix (cf equation (2.6) in Smith *et al* 1973 to be referred to as I). This can be stated generally as:

$$\Lambda(R, \Theta, Z) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} dz\, dx \exp[-2\pi i(Zz + Rx)] f(x, z, \Theta) \tag{1.5}$$

where Λ is the three-dimensional Fourier transform of λ.

Such axial central sections have a simple structure (cf Cochran *et al* 1952, Klug *et al* 1958) consisting of so-called layer lines (which are planes in the three-dimensional transform) on which the transform is nonzero. The intensity distribution of the transform on each line depends on those angular spatial frequencies of the helical mass distribution which are sampled on that particular layer line. This distribution is such that the first significant contribution to the intensity distribution can be no closer to the line $R = 0$ than the position of the first maximum of the Bessel function $J_L(2\pi R)$ where L is the lowest angular order sampled on that layer line. Noise contributions on the other hand are distributed over the whole Fourier plane.

In a practical case where a structural analysis is to be attempted the distinctive structure of the axial central section of the Fourier transform can be used:

(i) To find the screw rotation angle of the helix, ζ, its axial rotational symmetry and the length, p, of the axially repeating unit consistent with the positions of the intensity maxima on the layer lines.

(ii) To provide an opportunity to reduce noise contributions to the data by filtering the transform so as to eliminate contributions where it is constrained either to be zero or to be very small.

The first of these two tasks is usually done using the optical diffraction pattern of the micrograph as the principal tool, with the computer-generated transform† providing additional phase information. Once the helical parameters mentioned above are established and an appropriate sampling has been chosen, the computer transform can then be used to filter the data.

In the presence of a high noise level such filtrations are often hard to do, mainly because the 'selection rule', based on the expected intensity distribution along a layer line, does not allow *a priori* a completely unambiguous discrimination between those spatial frequencies which can, in principle, contribute structurally significant information about the helix and those which cannot. A further complication is often the rather coarse sampling of the transform data. This sampling can be improved by numerically transforming a larger matrix, obtained from the data array by adding columns and rows of

† It is assumed that computer transforms will be done using the fast Fourier transform algorithm (FFT). See for example Ten Eyke (1973).

zeros at its edges. Although this improved sampling is easier to work with, the practical advantages, as regards discrimination between signal and noise contributions, are not very great.

In the following sections we describe an alternative method for presenting the information in the transform which does allow a clear-cut distinction to be made between those spatial frequencies which can and those which cannot contribute structural information. This in turn permits a better-defined filter to be constructed for the projection data in the computer than is allowed by the Fourier transform itself.

2. Theory

An alternative interpretation of the numbers generated by the FFT program is to consider them as coefficients of a series expansion fit to the continuous Fourier transform using the so-called two-dimensional sinc functions† (Goodman 1968, pp 21–5). Our solution to the 'filtration problem' is simply to make use of a different series expansion for the transform data whose coefficients possess a more convenient selection rule than the coefficients of the two-dimensional sinc functions.

The set of functions which seems to be most convenient in this respect is a generalization to three-dimensional cylindrical polar coordinates of the functions suggested, in various forms, by Cormack (1964), Smith *et al* (1973) and Zeitler (1974) as being useful for performing two-dimensional reconstructions. One advantage of this particular set of functions is that the basis functions used to describe the real space density distribution are transformed into other simple, computable transcendental functions under the operations of projection (eg equation (1.4)) and three-dimensional Fourier transformation.

Using equations (4.16) and (4.17) of I and substituting for the hypergeometric functions using equations (22.5.42) and (22.4.1) of Abramowitz and Stegun (1968) the following expansion can be found for a general cylindrical mass-density distribution $\lambda(r, \theta, z)$:

$$\lambda(r,\theta,z) = \frac{1}{2}\Bigg(\sum_{l=-\infty}^{\infty} \sum_{n \geqslant |l|}^{\infty} a_{nl}(z)(2n+1)(-1)^n r^{2l} P_{n-l}^{(0,2l)}(2r^2-1)\, e^{i2l\theta} + \sum_{l=-\infty}^{\infty} \sum_{n>|l|}^{\infty} b_{nl}(z) 2n(-1)^{n+1} r^{2l+1} P_{n-l-1}^{(0,2l+1)}(2r^2-1)\, e^{i(2l+1)\theta} \Bigg) \tag{2.1}$$

where the $P_{\nu}^{(\eta,\xi)}(Y)$ are Jacobi polynomials‡. These are closely related to the Zernicke polynomials used by Cormack (1964) and Zeitler (1974). Equation (2.1) can be Fourier transformed to obtain an expression for $\Lambda(R, \Theta, Z)$:

$$\Lambda(R,\Theta,Z) = \frac{1}{2}\Bigg(\sum_{l=-\infty}^{\infty} \sum_{n \geqslant l}^{\infty} A_{nl}(Z)(2n+1) \frac{J_{2n+1}(2\pi R)}{R} e^{i2l\Theta} + \sum_{l=-\infty}^{\infty} \sum_{n>|l|}^{\infty} B_{nl}(Z) 2n \frac{J_{2n}(2\pi R)}{R} e^{i(2l+1)\Theta} \Bigg). \tag{2.2}$$

† A two-dimensional sinc function has the form:

$$\operatorname{sinc}(x)\operatorname{sinc}(y) = \frac{\sin(\pi x)}{\pi x} \frac{\sin(\pi y)}{\pi y}.$$

‡ A simple recurrence relation given in Abramowitz and Stegun (1968, equation (22.7.1)), allows the Jacobi polynomials to be calculated easily without undue loss of precision through rounding errors.

In this expression $J_\nu(Y)$ is a Bessel function of order ν and argument (Y) and $A_{nl}(Z)$ and $B_{nl}(Z)$ are the Fourier transforms with respect to z of $a_{nl}(z)$ and $b_{nl}(z)$ respectively.

If $\lambda(r, \theta, z)$ is the mass-density distribution of a discrete helix the A's and B's have important properties as can be seen from the following calculation:

$$A_{nl}(Z) = \int_{-\infty}^{+\infty} a_{nl}(z)\, e^{-2\pi i z Z}\, dz \tag{2.3}$$

from (1.1) however we have that

$$a_{nl}(z+p) = a_{nl}(z)\, e^{-i2l\zeta}. \tag{2.4}$$

Consequently $A_{nl}(Z)$ can be factorized to obtain

$$A_{nl}(Z) = A'_{nl}(Z) \sum_{m=-\infty}^{\infty} \delta\left(Z - \frac{m}{p} - \frac{2l\zeta}{2\pi p}\right) \tag{2.5}$$

and similarly

$$B_{nl}(Z) = B'_{nl}(Z) \sum_{m=-\infty}^{\infty} \delta\left(Z - \frac{m}{p} - \frac{(2l+1)\zeta}{2\pi p}\right) \tag{2.6}$$

where

$$A'_{nl}(Z) = \int_0^p a_{nl}(z)\, e^{-2\pi i z Z}\, dz \tag{2.7}$$

and

$$B'_{nl}(Z) = \int_0^p b_{nl}(z)\, e^{-2\pi i z Z}\, dz. \tag{2.8}$$

From equations (2.5) and (2.6) we see that $A_{nl}(Z)$ and $B_{nl}(Z)$ are, for a perfect helix, only nonzero on layer planes.

We now wish to see whether the expression (2.2) together with conditions (2.5) and (2.6), which the A's and B's must satisfy, can be used to obtain the necessary selection rule. As pointed out previously our data, f^D, consist of noisy measurements of the projected density of our presumed helix. Following I we expand $f^D(x, z)$ as a series of Chebychev polynomials:

$$f^D(x, z) = \sum_{n=0}^{\infty} \{s_n^D(z) \cos[(2n+1) \sin^{-1} x] + t_n^D(z) \sin(2n \sin^{-1} x)\} \tag{2.9}$$

where $s_n^D(z)$ and $t_n^D(z)$ are the coefficients of the expansions of the even and odd parts of f^D with respect to reflection in the line $x = 0$.

Using equation (1.5) above we can now obtain a noisy estimate of the axial central section of the Fourier transform of our helix for $\Theta = 0$, by Fourier transforming equation (2.9):

$$\Lambda^D(R, \Theta, Z)_{\Theta=0} = \frac{1}{2} \sum_{n=0}^{\infty} \left(S_n^D(Z)(2n+1) \frac{J_{2n+1}(2\pi R)}{R} + T_n^D(Z) 2n \frac{J_{2n}(2\pi R)}{R} \right) \tag{2.10}$$

where $S_n^D(Z)$ and $T_n^D(Z)$ are the Fourier transforms with respect to z of $s_n^D(z)$ and $t_n^D(z)$.

We now re-arrange the summations in equation (2.2) and evaluate $\Lambda(R, \Theta, Z)$ at $\Theta = 0$:

$$\Lambda(R, \Theta, Z)_{\Theta=0} = \frac{1}{2}\sum_{n=0}^{\infty}\left[\left(\sum_{l=-n}^{n} A_{nl}(Z)\right)(2n+1)\frac{J_{2n+1}(2\pi R)}{R} + \left(\sum_{l=-n+1}^{n-1} B_{nl}(Z)\right)2n\frac{J_{2n}(2\pi R)}{R}\right]. \tag{2.11}$$

From a comparison of (2.10) and (2.11) we can see that the contribution, f, to the data which come from the helix can be related to sums of the A's and B's as follows:

$$S_n(Z) = \sum_{l=-n}^{n} A_{nl}(Z) \tag{2.12}$$

$$T_n(Z) = \sum_{l=-n+1}^{n-1} B_{nl}(Z). \tag{2.13}$$

S_n and T_n consequently satisfy the conditions (2.5) and (2.6) through (2.12) and (2.13).

We now define

$$D(Z, k) = S^{D}_{k/2}(Z) \qquad \text{for } k \text{ even} \tag{2.14a}$$

and

$$D(Z, k) = T^{D}_{(k+1)/2}(Z) \qquad \text{for } k \text{ odd.} \tag{2.14b}$$

This new quantity D possesses the two strong selection rules which we need because when the noise contribution is zero:

(i*a*) $D(Z, k) = 0$ except where an integer m can be found such that

$$Z = \frac{m}{p} + \frac{L\zeta}{2\pi p} \tag{2.15}$$

where L is the angular harmonic being sampled; and

(ii*a*) $D(Z, k) = 0$ except for $k \geqslant L$. These two selection rules can be recast in a simpler form if $(\zeta/2\pi)$ is a rational number (t/u). In this case:

(i*b*) $D(Z, k) = 0$ for all $Z \neq N/up$ where N is an integer; and

(ii*b*) $D(Z, k) = 0$ except for those values of N and k for which an integer m can be found such that

$$k \geqslant \left|\frac{N - mu}{t}\right|. \tag{2.16}$$

The two selection rules arise due to the combination of the consistency condition, discussed in I (§ 5) and reflected in the summation limits in (2.1), with the helical selection rule (Klug *et al* 1958) which, using our notation, is

$$N = tL + mu. \tag{2.17}$$

The helical selection rule, (2.17), allows one to determine the number of the layer line N on which a given axial angular harmonic L is sampled (see equations (2.5) and (2.6)): selection rule (ii*b*) then says that for perfect helical projection data, $D(Z, k)$ will be zero on a layer line for values of k less than the smallest angular harmonic sampled on that layer line.

It is clear that a noise contribution to the data, which is constrained neither by the consistency condition nor by helical symmetry, can lead to nonzero values for the $D(Z, k)$ where helical information is excluded. The two selection rules allow contributions which are inconsistent with the two assumed constraints on the data to be unambiguously eliminated.

3. Computation

The coefficients $D(Z, k)$ can be obtained from real projection data† by means of the following general procedure.

The data should consist of samples of the projection of the helical density distribution taken over a rectangular region bounded by the two edges of the particle (taken to be $x = -1$ and $x = 1$ respectively) and including an integral number of helical repeats. The coefficients for the expansion (2.9) are obtained by transferring the data by means of an interpolation scheme to a new sampling grid which is uniform in $\sin^{-1}(x)$, separating it into its even and odd parts with respect to reflection symmetry in the line $x = 0$ and then finding the cosine and sine series fits to the two parts of the data. A series of one-dimensional Fourier transforms in the z direction completes the calculation. Both the Fourier transforms and the sine and cosine series fitting steps can be performed using the FFT routines described by Ten Eyke (1973).

The inverse transform can be obtained by reversing the order of the steps outlined in the above procedure.

The calculation of the $D(Z, k)$ coefficients is, of course, slower than the calculation of the Fourier coefficients; although it need not be very much slower. The actual speed of the calculation depends on the details of the program and in particular on the method used for interpolating the data. At present we make use of four-point Lagrange interpolation which is rather slow, but it has been suggested to us that a simple linear interpolation would not only be faster but would also be less susceptible to interpolation artefacts when noise levels were high. Alternatively interpolation could be avoided altogether and the coefficients for the expansion (2.9) be obtained directly using the orthogonality relations (see Zeitler 1974). A study of the effects of noise on the computation and a comparison of various interpolation schemes have not yet been done.

4. Discussion and conclusions

The array of coefficients $D(Z, k)$ obtained from the computer, which we shall call a $D(Z, k)$ plot, looks similar to the array of Fourier coefficients one would obtain from a simple two-dimensional Fourier transform of the data, but its simpler structure makes it easier to interpret.

The principal advantage of the $D(Z, k)$ plot is that a computer generated filter mask can be constructed for it only on the basis of the axial rotational symmetry and the helical selection rule (Klug *et al* 1958) which defines the screw angle ζ. The simplicity of this filter makes it possible to transform an array, filter it and then reconstruct a filtered image in three simple operations. If this filtration procedure results in the loss of a large fraction of the image power and a severe degradation of the structure seen in

† It is assumed that the data have been pre-processed as described by De Rosier and Moore (1972) prior to this step.

the original image it indicates either that an error was made in choosing the selection rule and symmetry or that the original image structure was largely artefactual.

At present we make use of both the two-dimensional Fourier transform and of the $D(Z, k)$ plot in preparing micrograph data for three-dimensional reconstruction. The Fourier transform is used in the first instance to obtain a general low-pass filtered image where high spatial frequency noise has been removed. In subsequent steps it is used to locate the axis of the particle and to scale it correctly in the axial direction. The $D(Z, k)$ plot is used in the final stage of the processing as an overall check on the helical parameters and to obtain a filtered image suitable for the three-dimensional reconstruction proper.

Our intention is, therefore, to make use of both the Fourier and the $D(Z, k)$ transformations so as to utilize their respective qualities in a simple and complementary fashion. The $D(Z, k)$ plot is, we feel, a theoretically satisfying and expedient computational tool to use in the analysis of micrograph data from helical particles. A further communication is planned concerning the implementation of these results in practical examples of three-dimensional reconstructions of helical biological structures.

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