# Helical Diffraction. II. The Inclusion of a Subunit of Finite Size

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## Abstract

In paper I of helical diffraction [Worthington & Elliott (1989). *Acta Cryst.* A45, 645-654] an expression for the autocorrelation function (a.c.f.) for a helix was obtained. The Fourier transform of this a.c.f. gave a new expression for the diffracted intensity. In paper II the theory is extended to real helices containing subunits of finite size. There are two main approaches to consider. The first approach centers on the calculation of the a.c.f. of the helical array of subunits. In the special case when the subunit contains an assembly of discrete atoms, the expression for the intensity is shown to be equivalent to the classical formula of Franklin & Klug [*Acta Cryst.* (1955), 8, 777-780]. The second approach bypasses the a.c.f. calculation and expresses the intensity in terms of the Fourier transform of the subunit. The second approach may prove useful in molecular modeling studies of biological helical structures.

## Introduction

In paper I of this series (Worthington & Elliott, 1989) it was shown that the Fourier transform of the autocorrelation function (a.c.f.) for a helix led to new expressions for the diffracted intensity. These expressions allow an examination of the effects of helical disorders. The formulation in paper I was elementary to establish the theoretical basis of helical diffraction. Specifically, the treatment referred to a helical array of atoms or to a helical array of point scattering centers.

In paper II I extend the theory to real helices and hence derive the diffracted intensity for a helical array containing a three-dimensional subunit of arbitrary size. The diffraction formulas have similar properties to the corresponding expressions in paper I and this will allow the inclusion of helical disorders (Worthington & Elliott, 1989).

There are two different approaches to evaluate the diffraction intensity for a helix containing subunits of finite size. The first approach is to calculate the a.c.f. contribution directly. The diffracted intensity is then obtained by cylindrically averaging the Fourier transform of the a.c.f. of the helix. The present treatment applies to subunits which have a three-dimensional distribution of electron density. In the special case when the subunit contains an assembly of discrete atoms it will be shown that the cylindrically averaged intensity formulas obtained using the first approach are equivalent to the classical formula for helical diffraction (Franklin & Klug, 1955). The second approach bypasses the calculation of the a.c.f. contribution and expresses the intensity in terms of the Fourier transform of the subunit. The intensity profiles are finally obtained by computer. This second approach appears to be the more useful method for the X-ray analysis of biological structures and for the inclusion of helical disorders.

## Statement of problem

I have used the same notation as in paper I. The reader will find that paper II assumes a fair knowledge of paper I. The point atoms in the elementary helix in paper I are now replaced with three-dimensional subunits of electron density $t(\mathbf{r})$. The helix contains $S$ subunits. There are $M$ subunits in $N$ turns with radius $r_0$, subunit repeat $h$ and pitch $p$. The helix repeat is $c$ and $c = Mh = Np$. A list of symbols used in this presentation is summarized in Table 1. The lattice generating function $\psi(\mathbf{r})$ for this helical array of subunits is

$$\psi(\mathbf{r}) = \sum_{j=0}^{j=S-1} \delta(\mathbf{r}-\mathbf{r}_j) * t(\mathbf{r}), \qquad (1)$$

where $\mathbf{r}_j$ has cylindrical coordinates $r_0, j\varphi_0, jh$ and $*$ is the convolution symbol. The Fourier transform of $t(\mathbf{r})$ is $T(\mathbf{R})$ where $t(\mathbf{r}) \Leftrightarrow T(\mathbf{R})$. The a.c.f. of the subunit is $j(\mathbf{r}) = t(\mathbf{r}) * t(-\mathbf{r})$ and the Fourier transform of $j(\mathbf{r})$ is $J(\mathbf{R}) = |T(\mathbf{R})|^2$. The a.c.f. for this general helix follows from (I.18) [equation (18) of paper I] and is given by

$$l(\mathbf{r}) = Sj(\mathbf{r}) + \sum_{m=1}^{m=S-1} (S-m)u_m(\pm \mathbf{r}) * \delta(z \pm mh), \quad (2)$$

where $u_m(\pm \mathbf{r})$ is the a.c.f. contribution at levels $z = \pm mh$ in the a.c.f. $l(\mathbf{r})$ of the general helix. The contribution $u_m(\mathbf{r})$ occurs at level $z = mh$ whereas the contribution $u_m(-\mathbf{r})$ occurs at level $z = -mh$. The function $u_m(\mathbf{r})$ is given by

$$u_m(\mathbf{r}) = \delta(\boldsymbol{\rho}-\boldsymbol{\rho}_m) * \mathscr{I}_m(\mathbf{r}), \qquad (3)$$

where $\boldsymbol{\rho}_m$ has cylindrical coordinates $\Delta_m, \varphi_m$: the radius $\Delta_m$ is given by (I.19) and $\varphi_m = \pi/2 + m\varphi_0/2$.

## Table 1. *List of symbols*

| | |
|---|---|
| $c$ | Helix repeat |
| $h$ | Subunit repeat of helix |
| $M$ | Subunits in $N$ turns |
| $p$ | Pitch of helix |
| $\varphi_0$ | Axial rotation angle of helix |
| $S$ | Total number of subunits in helix |
| $x, y, z$ | Cartesian coordinates in real space |
| $\rho, \varphi, z$ | Cylindrical coordinates in real space |
| $X, Y, Z$ | Cartesian coordinates in reciprocal space |
| $\xi, \Phi, Z$ | Cylindrical coordinates in reciprocal space |
| $I(\xi, l)$ | Cylindrically averaged intensity on layer line $l$ |
| $\psi(\mathbf{r})$ | Lattice generating function of helix |
| $l(\mathbf{r})$ | Autocorrelation function (a.c.f.) of helix |
| $\Delta_m$ | Radius of ring at levels $z = \pm mh$ in a.c.f. $l(\mathbf{r})$ of primitive helix |
| $\varphi_m$ | $\varphi$ coordinate of the first delta function on the above ring |
| $t(\mathbf{r})$ | Electron density of subunit |
| $T(\mathbf{R})$ | Fourier transform of $t(\mathbf{r})$ |
| $t(\mathbf{r}')$ | Electron density of rotated (in $x, y$ plane) subunit |
| $T(\mathbf{R}')$ | Fourier transform of $t(\mathbf{r}')$ |
| $j(\mathbf{r})$ | A.c.f. of subunit $t(\mathbf{r})$ |
| $J(\mathbf{R})$ | Fourier transform of a.c.f. $j(\mathbf{r})$ |
| $\mathscr{S}_m(\mathbf{r})$ | Cross-correlation function (c.c.f.) between $t(\mathbf{r}')$ and $t(\mathbf{r})$ |
| $\mathbb{J}_m(\mathbf{R})$ | Fourier transform of c.c.f. $\mathscr{S}_m(\mathbf{r})$ |
| $u_m(\mathbf{r})$ | C.c.f. at level $z = mh$ of a.c.f. $l(\mathbf{r})$ |
| $U_m(\mathbf{R})$ | Fourier transform of c.c.f. $u_m(\mathbf{r})$ |
| $\mathbb{U}_m(\xi, l)$ | Cylindrically averaged Fourier transform of $U_m(\mathbf{R})$ |
| $\sigma_m$ | Vector in c.c.f. $\mathscr{S}_m(\mathbf{r})$ |
| $\Sigma_m$ | Vector in c.c.f. $\mathscr{S}_m(\mathbf{r})$ |
| $\psi_m$ | Angle defining the orientation of vectors $\mathbf{p}_m$ and $\mathbf{q}_m$ |
| $l_m$ | Length of vectors $\mathbf{p}_m$ and $\mathbf{q}_m$ |

The function $\mathscr{S}_m(\mathbf{r})$ in (3) is defined as

$$\mathscr{S}_m(\mathbf{r}) = t(\mathbf{r}') * t(-\mathbf{r}), \qquad (4)$$

where the $\mathbf{r}'$ axes are rotated an angle $\varphi = m\varphi_0$ relative to the $\mathbf{r}$ axes in the $xy$ plane. Thus, there are $m\mathscr{S}_m(\mathbf{r})$ functions as the angle between axes is a linear function of $m$. When $m = 0$ the $\mathscr{S}_0(\mathbf{r})$ term is simply the a.c.f. of the subunit itself and, consequently, the $m = 0$ term in (2) is $Sj(\mathbf{r})$. From (4), $\mathscr{S}_m(\mathbf{r})$ is a cross-correlation function (c.c.f.) between $t(\mathbf{r}')$ and $t(\mathbf{r})$. The term c.c.f. is normally used when the two functions are different whereas the term a.c.f. is normally used when the two functions are the same (Champeney, 1973).

Since $\mathscr{S}_m(\mathbf{r})$ is a c.c.f. it follows that $u_m(\mathbf{r})$ is also a c.c.f. There are two c.c.f.s to consider: $u_m(\mathbf{r})$ at level $z = mh$ and $u_m(-\mathbf{r})$ at level $z = -mh$. The c.c.f. $u_m(-\mathbf{r})$ at level $z = -mh$ is given by

$$u_m(-\mathbf{r}) = \delta(\mathbf{\rho} + \mathbf{\rho}_m) * \mathscr{S}_m(-\mathbf{r}), \qquad (5)$$

where $\mathscr{S}_m(-\mathbf{r})$ is the c.c.f. between $t(\mathbf{r})$ and $t(\mathbf{r}')$. The relationship between $u_m(\mathbf{r})$ and $u_m(-\mathbf{r})$ simply denotes that there is a center of symmetry at the origin of the a.c.f. $l(\mathbf{r})$ of the helix.

The Fourier transform of $\mathscr{S}_m(\mathbf{r})$ is $\mathbb{J}_m(\mathbf{R})$, where

$$\mathbb{J}_m(\mathbf{R}) = T(\mathbf{R}')T(-\mathbf{R}). \qquad (6)$$

The $\mathbf{R}'$ axes are rotated an angle $\Phi = m\varphi_0$ relative to the $\mathbf{R}$ axes in the $XY$ plane. This rotation is expressed as follows: define a particular point in $T(-\mathbf{R})$ in cylindrical coordinates as $\xi, 0, Z$ then the corresponding point in $T(\mathbf{R}')$ is at $\xi, \Phi, Z$ and the $\Phi$ coordinate $\Phi = \pi + m\varphi_0 = 2\varphi_m$.

The Fourier transform of the c.c.f. $u_m(\mathbf{r})$ is $U_m(\mathbf{R})$ and, when expressed in cylindrical coordinates, the Fourier transform $U_m(\xi, \Phi, Z)$ is

$$U_m(\xi, \Phi, Z) = \exp\left[i2\pi\xi\Delta_m \cos(\Phi - \varphi_m)\right]$$
$$\times \mathbb{J}_m(\xi, \Phi, Z), \qquad (7)$$

where $\mathbb{J}_m(\xi, \Phi, Z)$ is defined in (6). In the general case when the electron density $t(\mathbf{r})$ is not centrosymmetric, the Fourier transforms $T(\mathbf{R}')$ and $T(-\mathbf{R})$ are both complex. Moreover, their product $\mathbb{J}_m(\mathbf{R})$ and the Fourier transform $U_m(\mathbf{R})$ as defined in (7) are also complex. However, in the cylindrical averaging process only the real part of $U_m(\mathbf{R})$ is used, that is, $\mathrm{Re}[U_m(\mathbf{R})]$. In paper I the first step in the derivation of the diffracted intensity from the elementary helix with point atoms was to write down the Fourier transform contribution due to each delta function on the ring of radius $\Delta_m$. In the case of a general subunit of electron density $t(\mathbf{r})$ each c.c.f. term at level $z = mh$ is denoted $u_m(\mathbf{r})$ and is defined by (3). The Fourier transform of $u_m(\mathbf{r})$ is $U_m(\mathbf{R})$ and is defined by (7). The cylindrically averaged contribution is denoted $\mathbb{U}_m(\xi, Z)$ and, in integral form, is given by

$$\mathbb{U}_m(\xi, Z) = (1/2\pi)\int_0^{2\pi} \mathrm{Re}[U_m(\xi, \Phi, Z)]\, d\Phi. \qquad (8)$$

Note that the c.c.f.s $u_m(-\mathbf{r})$ which occur at levels $z = -mh$ have Fourier transforms $U_m(-\mathbf{R})$. Although the Fourier transforms $U_m(\mathbf{R})$ and $U_m(-\mathbf{R})$ are different the real part of the two transforms are, however, the same. Thus, $\mathrm{Re}[U_m(\mathbf{R})] = \mathrm{Re}[U_m(-\mathbf{R})]$. While there is a choice of levels within the a.c.f. $l(\mathbf{r})$ to study, the same cylindrically averaged contribution $\mathbb{U}_m(\xi, Z)$ is consequently obtained in both descriptions.

The Fourier transform of the a.c.f. $l(\mathbf{r})$ for the general helix is $L(\mathbf{R})$ and, as $L(\mathbf{R})$ contains the electron density information, $L(\mathbf{R}) = I(\mathbf{R})$, where $I(\mathbf{R})$ is the diffraction intensity. The cylindrically averaged diffraction intensity $I(\xi, l)$ on layer line $l$ is given by

$$I(\xi, l) = SJ(\xi, l) + 2\sum_{m=1}^{m=S-1}(S-m)\mathbb{U}_m(\xi, l)$$
$$\times \cos 2\pi ml/M, \qquad (9)$$

where $Z = l/Mh$, $l$ an integer. Note that (9) has the same form as the corresponding equation (I.20). The difference is that $\mathbb{U}_m(\xi, l)$ in (9) replaces the $J_0(2\pi\xi\Delta_m)$ term in (I.20).

In order to appreciate the content of (1) to (9), it is necessary to consider fully the properties of the c.c.f.s $\mathscr{S}_m(\mathbf{r})$ and $u_m(\mathbf{r})$ and their Fourier transforms $\mathbb{J}_m(\mathbf{R})$ and $U_m(\mathbf{R})$. It is instructive to consider in detail an idealized subunit which contains two discrete scattering centers.

## Simple subunit model

I treat the case when $t(\mathbf{r})$ contains two scattering centers with weights $w_1$ and $w_2$ and with coordinates $z = 0$ and $\varphi = 0$. The electron density $t(\mathbf{r})$ of the subunit in Cartesian coordinates is $t(x, y)$ as the $z$ dimension can be omitted. Thus, $t(x, y)$ is given by

$$t(x, y) = w_1\delta(x + a, y - 0) + w_2\delta(x - a, y - 0). \quad (10)$$

The subunit $t(\mathbf{r})$ is not centrosymmetric. The $\mathbf{r}'$ axes are rotated an angle $m\varphi_0$ relative to the $\mathbf{r}$ axes of subunit $t(\mathbf{r})$. A drawing of the two subunits $t(\mathbf{r}')$ and $t(\mathbf{r})$ is shown in Fig. 1. The total rotation of the $-\mathbf{r}$ and $\mathbf{r}'$ axes is $\pi + m\varphi_0 = 2\varphi_m$. The c.c.f. $\mathscr{I}_m(x, y)$ contains four vectors: two have length $\sigma_m = 2a \sin m\varphi_0/2$ while the other two have length $\Sigma_m = 2a \cos m\varphi_0/2$. A drawing of the c.c.f. $\mathscr{I}_m(x, y)$ is shown in Fig. 2. Note that both the subunit $t(\mathbf{r})$ and the c.c.f. $\mathscr{I}_m(\mathbf{r})$ are defined relative to the origin at the center of the subunit.

The c.c.f. $\mathscr{I}_m(x, y)$ for the model is given by

$$\mathscr{I}_m(x, y) = w_2^2\delta(x - \sigma_m \cos \varphi_m, y - \sigma_m \sin \varphi_m)$$
$$+ w_1^2\delta(x + \sigma_m \cos \varphi_m, y + \sigma_m \sin \varphi_m)$$
$$+ w_1 w_2[\delta(x - \Sigma_m \cos m\varphi_0/2, y$$
$$- \Sigma_m \sin m\varphi_0/2)$$
$$+ \delta(x + \Sigma_m \cos m\varphi_0/2,$$
$$y - \Sigma_m \sin m\varphi_0/2)]. \quad (11)$$

Because of the cylindrical averaging process as defined in (7), the c.c.f. $\mathscr{I}_m(x, y)$ is more conveniently expressed in cylindrical coordinates and $\mathscr{I}_m(\xi, \varphi)$

then becomes

$$\mathscr{I}_m(\rho, \varphi) = w_2^2\delta(\rho - \sigma_m, \varphi - \varphi_m)$$
$$+ w_1^2\delta(\rho - \sigma_m, \varphi - \pi - \varphi_m)$$
$$+ w_1 w_2[\delta(\rho - \Sigma_m, \varphi - m\varphi_0/2)$$
$$+ \delta(\rho - \Sigma_m, \varphi - \pi - m\varphi_0/2)]. \quad (12)$$

The Fourier transform $\mathbb{J}_m(\xi, \Phi)$ for this idealized subunit model is

$$\mathbb{J}_m(\xi, \Phi) = w_2^2 \exp[i2\pi\xi\sigma_m \cos(\Phi - \varphi_m)]$$
$$+ w_1^2 \exp[-i2\pi\xi\sigma_m \cos(\Phi - \varphi_m)]$$
$$+ 2w_1 w_2 \cos[2\pi\xi\Sigma_m \cos(\Phi - m\varphi_0/2)].$$
$$(13)$$

Note that as $w_1$ and $w_2$ are unequal the Fourier transform $\mathbb{J}_m(\xi, \Phi)$ is complex.

### 1. The first approach

The initial step in the first approach is to obtain an analytic expression for the c.c.f. $u_m(\mathbf{r})$. The c.c.f. $u_m(\mathbf{r})$ consists of two terms: $\delta(\mathbf{\rho} - \mathbf{\rho}_m)$ and $\mathscr{I}_m(\mathbf{r}_m)$. The first term has been treated in paper I while the second term $\mathscr{I}_m(x, y)$ is shown in Fig. 2. The c.c.f. $u_m(\mathbf{r})$ generates the c.c.f. $\mathscr{I}_m(\mathbf{r})$ at the end of $\mathbf{\rho}_m$. A drawing of the c.c.f. $u_m(x, y)$ for the helical array of the idealized subunits is shown in Fig. 3. The c.c.f. $u_m(x, y)$ contains four vectors $\mathbf{\Delta}_m \pm \mathbf{\sigma}_m$, $\mathbf{p}_m = \mathbf{\Delta}_m + \mathbf{\Sigma}_m$ and $\mathbf{q}_m = \mathbf{\Delta}_m - \mathbf{\Sigma}_m$. The vectors $\mathbf{p}_m$ and $\mathbf{q}_m$ have the same length $l_m$ since $l_m^2 = \Sigma_m^2 + \Delta_m^2$. The angle $\psi_m$ is defined by $\tan \psi_m = \Sigma_m/\Delta_m$. Since vector $\mathbf{\rho}_m$ has
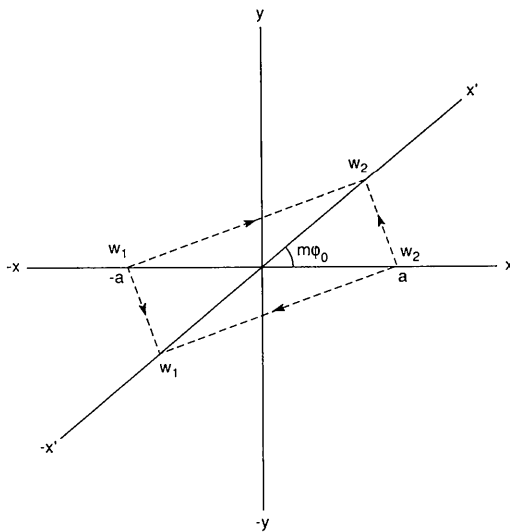
Fig. 1. A diagram of the subunits $t(x')$ and $t(x)$. The $x'$ axis is rotated an angle $m\varphi_0$ relative to the $x$ axis. The dotted lines with arrows indicate the four vectors of $\mathscr{I}_m(x, y)$ which is redrawn in Fig. 2.
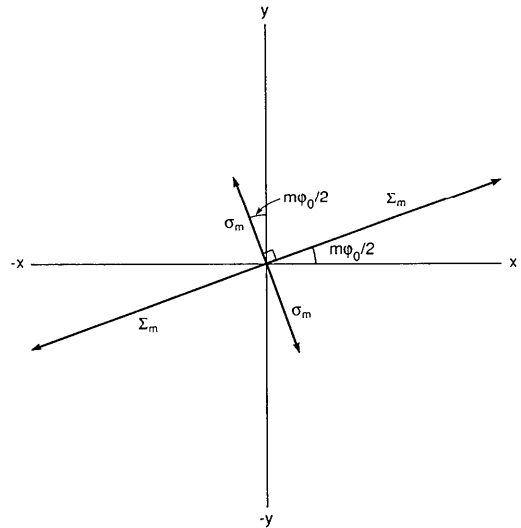
Fig. 2. The c.c.f. $\mathscr{I}_m(x, y)$ for the simple subunit model. The c.c.f. $\mathscr{I}_m(x, y)$ contains four vectors $\pm\sigma_m$ and $\pm\Sigma_m$ with lengths $\sigma_m = 2a \sin m\varphi_0/2$ and $\Sigma_m = 2a \cos m\varphi_0/2$, respectively. Note that the subunits $t(x')$ and $t(x)$ in Fig. 1 and the c.c.f. $\mathscr{I}_m(x, y)$ have been drawn to the same scale.

cylindrical coordinates $\Delta_m$, $\varphi_m$, the origin of $\mathcal{I}_m(x, y)$ is at $\Delta_m$, $\varphi_m$ in the $\rho$, $\varphi$ plane.

The c.c.f. $u_m(\mathbf{r})$ can be expressed in Cartesian coordinates using Fig. 3, similar to $\mathcal{I}_m(x, y)$ in (11). I omit this step and write $u_m(\mathbf{r})$ using cylindrical coordinates and the diagram in Fig. 3. The c.c.f. $u_m(\rho, \varphi)$ is

$$
\begin{aligned}
u_m(\rho, \varphi) = w_2^2\delta[\rho - (\Delta_m + \sigma_m), \varphi - \varphi_m] \\
+ w_1^2\delta[\rho - (\Delta_m - \sigma_m), \varphi - \pi - \varphi_m] \\
+ w_1 w_2\{\delta[\rho - l_m, \varphi - (\varphi_m - \psi_m)] \\
+ \delta[\rho - l_m, \varphi - (\varphi_m + \psi_m)]\}.
\end{aligned} \quad (14)
$$

The Fourier transform of $u_m(\rho, \varphi)$ is $U_m(\xi, \Phi)$ and $U_m(\xi, \Phi)$ is given by

$$
\begin{aligned}
U_m(\xi, \Phi) = w_2^2 \exp[i2\pi\xi(\Delta_m + \sigma_m) \cos(\Phi - \varphi_m)] \\
+ w_1^2 \exp[-i2\pi\xi(\Delta_m - \sigma_m) \cos(\Phi - \varphi_m)] \\
+ w_1 w_2(\exp\{i2\pi\xi l_m \cos[\Phi - (\varphi_m - \psi_m)]\} \\
+ \exp\{i2\pi\xi l_m \cos[\Phi - (\varphi_m + \psi_m)]\}).
\end{aligned} \quad (15)
$$

From (8) and (15) the cylindrically averaged transform $\mathbb{U}_m(\xi)$ for the helical array of idealized subunits is

$$
\begin{aligned}
\mathbb{U}_m(\xi) = w_2^2 J_0[2\pi\xi(\Delta_m + \sigma_m)] \\
+ w_1^2 J_0[2\pi\xi(\Delta_m - \sigma_m)] \\
+ 2w_1 w_2 J_0(2\pi\xi l_m).
\end{aligned} \quad (16)
$$

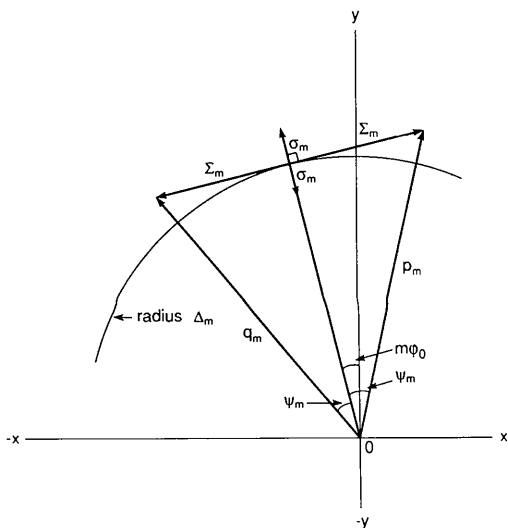The cylindrically averaged diffraction intensity $I(\xi, l)$ is then obtained from (9).

## 2. Comparison with the classical formula

I next consider the classical formula for intensity $I(\xi, l)$ for an assembly of discrete atoms with scattering factors $f_j$ and coordinates $r_j$, $\varphi_j$, $z_j$. The classical formula for $I(\xi, l)$ was first derived by Franklin & Klug (1955) and describes the cylindrically averaged intensity along layer line $l$. The intensity $I(\xi, l)$ is given by

$$
I(\xi, l) = \sum_n \sum_{i,j} f_i f_j J_n(2\pi\xi r_i) J_n(2\pi\xi r_j) T_{ij},
$$

where

$$
T_{ij} = \cos[2\pi(z_i - z_j)l/c - n(\varphi_i - \varphi_j)], \quad (17)
$$

and where $n$ is an integer determined by the selection rule for helical diffraction. Note that the origin of coordinates in (17) is at the center of the helix whereas the scattering centers in our subunits are defined relative to the origin of the subunit. In the case of the simple subunit model, $T_{ij} = 1$; $w$ replaces $f$; $r_1 = r_0 - a$ and $r_2 = r_0 + a$ and, from (17), $I(\xi, l)$ becomes

$$
\begin{aligned}
I(\xi, l) = \sum_n w_1^2 J_n^2[2\pi\xi(r_0 - a)] + w_2^2 J_n^2[2\pi\xi(r_0 + a)] \\
+ 2w_1 w_2 J_n[2\pi\xi(r_0 - a)] J_n[2\pi\xi(r_0 + a)]. \quad (18)
\end{aligned}
$$

In order to compare the classical formula (18) for the simple subunit model with our formulas (9) and (16), it is necessary to re-examine the four vectors of $u_m(x, y)$ at level $z = mh$. A drawing of these four vectors is shown in Fig. 4. In Fig. 4, the two scattering centers in the plane $x, y, 0$ have been moved to the plane $x, y, mh$. The four vectors are $\Delta_m + \sigma_m$, $\Delta_m - \sigma_m$, $\mathbf{p}_m$, $\mathbf{q}_m$ and they all subtend the angle $m\varphi_0$ at the origin of the circle of radius $r_0$. The expansion (Jeffreys & Jeffreys, 1962) for $J_0(2\pi\xi V_m)$, where $V_m$ refers to the length of each of the four vectors,



Fig. 3. The c.c.f. $u_m(x, y)$ for the simple subunit model. The c.c.f. $u_m(x, y)$ contains four vectors $\Delta_m \pm \sigma_m$, $\mathbf{p}_m$ and $\mathbf{q}_m$. Vectors $\mathbf{p}_m$ and $\mathbf{q}_m$ have the same length $l_m$, where $l_m^2 = \Delta_m^2 + \Sigma_m^2$. The angle $\psi_m$ is given by tan $\psi_m = \Sigma_m/\Delta_m$.
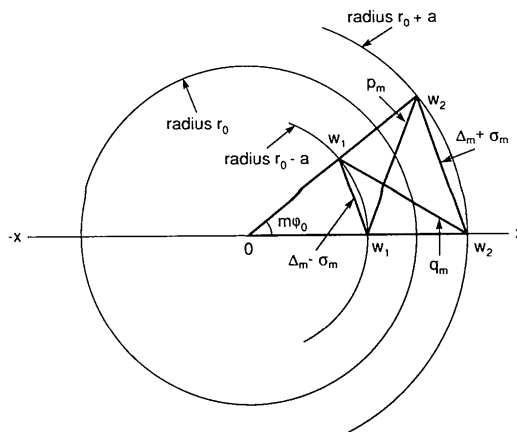


Fig. 4. The c.c.f. $u_m(x, y)$ for the simple subunit model with $z = mh$. The c.c.f. $u_m(x, y)$ in the plane $(x, y, mh)$ contains four vectors $\Delta_m \pm \sigma_m$, $\mathbf{p}_m$ and $\mathbf{q}_m$. All four vectors subtend angle $m\varphi_0$.

therefore applies and is

$$J_0[2\pi\xi(\Delta_m \pm \sigma_m)] = \sum_{n=-\infty}^{n=\infty} J_n^2[2\pi\xi(r_0 \pm a)] \cos nm\varphi_0,$$

whereas

$$J_0(2\pi\xi l_m) = \sum_{n=-\infty}^{n=\infty} J_n[2\pi\xi(r_0 - a)]$$

$$\times J_n[2\pi\xi(r_0 + a)] \cos nm\varphi_0. \quad (19)$$

In paper I a formula for $I(\xi, l)$, (I.20), was obtained. This equation is similar in form to (9) but it differs in that the $U_m(\xi, l)$ factor in (9) is replaced by the $J_0(2\pi\xi\Delta_m)$ term. Moreover, it was shown in paper I [see (I.20)-(I.25)] that, after invoking the expansion for the $J_0(\ )$ function (Jeffreys & Jeffreys, 1962), the $I(\xi, l)$ formula was in agreement with classical theory (Cochran, Crick & Vand, 1952). The formula for $U_m(\xi)$ for the subunit model in (16) contains four $J_0(\ )$ functions and, from (19), it therefore follows that the formula for $I(\xi, l)$, described by (9) and (16), is in agreement with the classical formula of Franklin & Klug (1955).

Although it was to be expected that the two methods for deriving the intensity formula of helical diffraction were equivalent, the proof was not easily obtained. Consider the case of a single subunit of electron density $t(\mathbf{r})$ by itself. The diffracted intensity $I(\mathbf{R})$ from the subunit can be obtained in two ways. The standard crystallographic method is to write $I(\mathbf{R}) = J(\mathbf{R}) = |T(\mathbf{R})|^2$, where $T(\mathbf{R})$ is the Fourier transform of $t(\mathbf{r})$. Another way (used here) is to obtain the a.c.f. $j(\mathbf{r}) = t(\mathbf{r}) * t(-\mathbf{r})$ and hence obtain the intensity $I(\mathbf{R}) = J(\mathbf{R})$, where $J(\mathbf{R})$ is the Fourier transform of $j(\mathbf{r})$. The equivalence of the two methods follows from the convolution theorem. It has been shown that in the case of helical diffraction these two methods are also equivalent and the equivalence again follows from the convolution theorem.

## 3. The second approach

The second approach follows the first approach but bypasses the calculation of the c.c.f. $u_m(\mathbf{r})$. The Fourier transform of $u_m(\mathbf{r})$ is $U_m(\mathbf{R})$ and, after cylindrical averaging, it is the $U_m(\xi, l)$ term that is required in order to evaluate $I(\xi, l)$ from (9). The Fourier transform $U_m(\mathbf{R})$ is given by

$$U_m(\mathbf{R}) = \exp(i2\pi\boldsymbol{\rho}_m \cdot \boldsymbol{\xi}) \mathbb{J}_m(\mathbf{R}), \quad (20)$$

where $\boldsymbol{\rho}_m$ has coordinates $\Delta_m \cos \varphi_m$, $\Delta_m \sin \varphi_m$ and $\boldsymbol{\xi}$ has reciprocal-space coordinates $X, Y$ and where $\mathbb{J}_m(\mathbf{R})$ is given by (6). Thus, in principle, the only unknown in (20) is $T(\mathbf{R})$, the Fourier transform of $t(\mathbf{r})$.

The electron density $t(x, y)$ of the simple subunit model is defined in (10). The Fourier transform of

$t(-x, -y)$ is $T(-X, -Y)$ and this transform is given by

$$T(-X, -Y) = w_1 \exp(i2\pi aX) + w_2 \exp(-i2\pi aX). \quad (21)$$

The Fourier transform of $t(x', y')$ is $T(X', Y')$ and this transform is given by

$$T(X', Y') = w_1 \exp(-i2\pi aX') + w_2 \exp(i2\pi aX'), \quad (22)$$

and, as the axes are rotated an angle $m\varphi_0$, $X' = X \cos m\varphi_0 + Y \sin m\varphi_0$ and, on substituting $X'$ in (22), $T(X', Y')$ becomes

$$T(X', Y') = w_1 \exp[-i2\pi a(\cos m\varphi_0 X + \sin m\varphi_0 Y)]$$

$$+ w_2 \exp[i2\pi a(\cos m\varphi_0 X + \sin m\varphi_0 Y)]. \quad (23)$$

The Fourier transform $\mathbb{J}_m(X, Y)$ from (6) is obtained by multiplying (21) and (23) so that $\mathbb{J}_m(X, Y)$ becomes

$$\mathbb{J}_m(X, Y) = w_2^2 \exp\{-i2\pi a[(1 - \cos m\varphi_0)X$$

$$- \sin m\varphi_0 Y]\}$$

$$+ w_1^2 \exp\{i2\pi a[(1 - \cos m\varphi_0)X$$

$$- \sin m\varphi_0 Y]\} + 2w_1w_2 \cos 2\pi a$$

$$\times [(1 + \cos m\varphi)X + \sin m\varphi_0 Y]. \quad (24)$$

The Fourier transform $U_m(X, Y)$, from (20), can be written as

$$U_m(X, Y) = \exp[i2\pi(\Delta_m \cos \varphi_m X + \Delta_m \sin \varphi_m Y)]\mathbb{J}_m(X, Y), \quad (25)$$

where $\mathbb{J}_m(X, Y)$ is given by (24). The next step is to convert the Cartesian coordinates $X, Y$ to cylindrical coordinates $\xi, \Phi$ so as to obtain $u_m(\xi, \Phi)$. The cylindrically averaged transform $U_m(\xi)$ is obtained using (7) and $U_m(\xi)$ then becomes

$$U_m(\xi) = (1/2\pi) \int_0^{2\pi} \text{Re}\{\exp[i2\pi\xi\Delta_m$$

$$\times \cos(\Phi - \varphi_m)]\mathbb{J}_m(\xi, \Phi)\} \, d\Phi. \quad (26)$$

In general, $\mathbb{J}_m(\xi, \Phi)$ is not known explicitly so that (26) is expressed in series form similar to (I.32). The diffraction intensity $I(\xi, l)$ for the subunit model is then obtained by substituting $U_m(\xi)$ from (26) into (9).

## 4. Comparison between the two approaches

The cylindrically averaged diffraction intensity $I(\xi, l)$ for the subunit model will be the same for the two approaches provided that the averaged transform $U_m(\xi)$ is the same. In the first approach an analytic

expression was obtained for the $U_m(\xi, \Phi)$ transform, see (15) and, consequently, this led to an explicit expression for $\mathbb{U}_m(\xi)$, see (16). In the second approach the $U_m(\xi, \Phi)$ transform for the subunit model is given by (24) and (25). Since the expression for $U_m(\xi, \Phi)$ is seemingly complicated, the averaged transform $\mathbb{U}_m(\xi)$ is obtained by computer after expressing (26) in series form.

Since diagrams of the c.c.f.s $\mathscr{I}_m(x, y)$ and $u_m(x, y)$ for the subunit model are shown in Figs. 1, 2 and 3, it is possible to simplify the $U_m(X, Y)$ transform of (25). The trigonometric conversions are

$$a(1 - \cos m\varphi_0) = -\sigma_m \cos \varphi_m,$$

$$a \sin m\varphi_0 = \sigma_m \sin \varphi_m,$$

$$a(1 + \cos m\varphi_0) = \Sigma_m \cos m\varphi_0/2,$$

$$a \sin m\varphi_0 = \Sigma_m \sin m\varphi_0/2.$$

The $x$ and $y$ coordinates of vectors $\mathbf{p}_m$ and $\mathbf{q}_m$ are denoted $p_{m,x}, p_{m,y}$ and $q_{m,x}, q_{m,y}$ respectively. From Fig. 3, it follows that

$$p_{m,x} = \Delta_m \cos \varphi_m + \Sigma_m \cos m\varphi_0/2,$$

$$p_{m,y} = (\Delta_m + \sigma_m) \sin \varphi_m,$$

$$q_{m,x} = \Delta_m \cos \varphi_m - \Sigma_m \cos m\varphi_0/2,$$

$$q_{m,y} = (\Delta_m - \sigma_m) \sin \varphi_m,$$

where the length $l_m$ of the two vectors $\mathbf{p}_m$ and $\mathbf{q}_m$ is given by

$$l_m^2 = p_{m,x}^2 + p_{m,y}^2 = q_{m,x}^2 + q_{m,y}^2.$$

On substituting the above conversions into (24) and (25) an explicit expression for $U_m(X, Y)$ is obtained and, after converting the Cartesian coordinates $X, Y$ to cylindrical coordinates, it is evident that the $U_m(\xi, \Phi)$ transform is identical to the $U_m(\xi, \Phi)$ transform of (15) which was derived using the first approach. Thus, the same averaged transform $\mathbb{U}_m(\xi)$ is obtained in the two approaches and the two approaches therefore yield the same diffraction intensity $I(\xi, l)$.

## General subunit model

So far, the subunit model with only two scattering centers has been treated in detail. The search for an explicit expression for the transform $U_m(\xi, \Phi)$ was straightforward but non-trivial as it involved some new concepts. The reader might like to extend the model by increasing the number of parameters, that is, by adding extra scattering centers and by including $z$ and $\varphi$ coordinates. I will omit this treatment (which is inferred) and next consider the case of a general subunit model with numerous parameters.

I finally consider the case of a three-dimensional subunit of electron density $t(\mathbf{r})$. The first approach

does not seem to be a good choice as the evaluation of $U_m(\xi, \Phi, Z)$ involves two steps: the calculation of the c.c.f. $u_m(\rho, \varphi, z)$ followed by the computation of the Fourier transform $U_m(\xi, \Phi, Z)$. The second approach appears to be a better choice even though the evaluation of $U_m(\xi, \Phi, Z)$ again involves two steps: the calculation of the Fourier transform $T(\xi, \Phi, Z)$ followed by the formation of $\mathbb{J}_m(\xi, \Phi, Z)$ using (6) and, finally, $U_m(\xi, \Phi, Z)$ using (7).

In both approaches, only knowledge of the Fourier transform $U_m(\xi, \Phi, Z)$ in rings of constant $\xi$ and constant $Z = l/c$ are required. Moreover, only discrete evenly spaced $\Phi$ values on each ring in reciprocal space are needed in order to obtain $\mathbb{U}_m(\xi, l)$ using the series form of (8). The author anticipates that the second approach will prove the more useful but this is a practical matter of programming and it is too early for a final verdict.

### Two special cases

Previous calculations of helical structures have used subunits of discrete atoms (Franklin & Klug, 1955) or, in the case of muscle, spherical subunits of uniform electron density (Worthington, 1961) or spherical subunits with Gaussian electron density (Haselgrove, 1980). I briefly consider the methods for dealing with the cases when the subunit is either a cylinder or a pillar with uniform electron density. Note that the Fourier transforms of these two subunits are well known (Champeney, 1973). The objective here is to illustrate how the Fourier transform $\mathbb{U}_m(\xi, l)$ is obtained in these two cases.

A. *Cylinder.* The three-dimensional subunit $t(\mathbf{r})$ has uniform electron density $\tau$ and is a cylinder with area $a = \pi\sigma^2$ and length $\lambda$. The long axis with length $\lambda$ is along the $x$ axis. The Fourier transform of $t(\mathbf{r})$ is $T(\mathbf{R})$ and, using Cartesian coordinates, $T(X, Y, Z)$ is given by

$$T(X, Y, Z) = \tau a \lambda W(Y, Z) \operatorname{sinc} \pi \lambda X, \quad (27)$$

where $W(Y, Z) = 2J_1(m)/m$, $m = [2\pi\sigma(Y^2 + Z^2)^{-1/2}]$ and where $\operatorname{sinc} \theta = \sin \theta/\theta$. The Fourier transform $T(\xi, \Phi, l)$, $Z = l/c$ then becomes

$$T(\xi, \Phi, l) = \tau a \lambda W(\xi, \Phi, l) \operatorname{sinc} \pi \lambda \xi \cos \Phi, \quad (28)$$

where $Y = \xi \sin \Phi$. The Fourier transform can then be evaluated at discrete values of $\Phi$ for a series of $\xi$ values and for a constant layer line $l$. The averaged transform $\mathbb{U}_m(\xi, l)$ for the cylinder of uniform density can be computed using (28) and the series form of (8).

B. *Pillar.* The three-dimensional subunit $t(\mathbf{r})$ has uniform electron density $\tau$ and is a rectangular pillar with dimensions $y_0$ and $z_0$ and length $\lambda$ along the $x$ axis. The pillar has cross-sectional area $y_0 z_0$. The

Fourier transform of $t(\mathbf{r})$ is $T(\mathbf{R})$ and, using Cartesian coordinates, $T(X, Y, Z) = T(X, Y)T(Z)$. This is a simplification in the computation of the diffraction intensity $I(\xi, l)$. One can write, after omitting the weight factors, the Fourier transform $T(X, Y)$ as

$$T(X, Y) = \text{sinc } \pi\lambda X \text{ sinc } \pi y_0 Y, \qquad (29)$$

and $T(l), Z = l/c$, is given by

$$T(l) = \text{sinc } \pi z_0 l / c. \qquad (30)$$

The Fourier transform $T(X, Y)$ in cylindrical coordinates $\xi, \Phi$ is

$$T(\xi, \Phi) = \text{sinc } \pi\lambda\xi \cos \Phi \text{ sinc } \pi y_0\xi \sin \Phi. \qquad (31)$$

The averaged transform $\mathbb{U}_m(\xi)$ for the pillar of uniform density can be computed using (31) and (26).

## Discussion

A new formula for the diffracted intensity $I(\xi, l)$ for a helical array of subunits has been derived. This formula is exact. It may have computational advantages in fiber diffraction but further study is required. The present formulation allows the examination of the effects of helical disorders. The treatment of dis-

order in the case of the elementary helix was described in paper I.

The electron density of each subunit is defined relative to its own origin independent of the helical parameters. This straightforward application of the Fourier transform of the subunit may prove to be useful in molecular model-building studies of biological helical structures and in a variety of disorder problems relating to helical structure. A long-term aim of this work is eventually to study the dynamics of biological structures.

### References

CHAMPENEY, D. (1973). In *Fourier Transforms and their Physical Applications*. London, New York: Academic Press.
COCHRAN, W., CRICK, F. H. C. & VAND, V. (1952). *Acta Cryst.* **5**, 581–586.
FRANKLIN, R. E. & KLUG, A. (1955). *Acta Cryst.* **8**, 777–780.
HASELGROVE, J. C. (1980). *J. Muscle Res. Cell Motil.* **1**, 177–191.
JEFFREYS, H. & JEFFREYS, B. (1962). In *Methods of Mathematical Physics*. Cambridge Univ. Press.
WORTHINGTON, C. R. (1961). *J. Mol. Biol.* **3**, 618–633.
WORTHINGTON, C. R. & ELLIOTT, G. F. (1989). *Acta Cryst.* **A45**, 645–654.

# The Locked Rotation Function

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## Abstract

It frequently occurs that a biological assembly in a crystallographic asymmetric unit has more than one noncrystallographic symmetry operator. For instance, a tetramer might have the point group 222 or a spherical virus will have the point group 532. A self-rotation function searches for the direction and angle of rotation of the individual noncrystallographic symmetry operations, while a cross-rotation function searches for the relationship of a structure in one unit cell with similar structures in another cell. The power of the rotation function can be greatly enhanced by searching for all noncrystallographic symmetry operators simultaneously. The procedure described previously [Rossmann, Ford, Watson & Banaszak (1972). *J. Mol. Biol.* **64**, 237–249] has been generalized. The increased power of this 'locked' rotation function permits a good determination of the orientation of an icosahedral virus in the presence of less than 1% of the possible diffraction data to 7 Å resolution. In addition, the peak-to-noise ratio is substantially improved.

## Introduction

The rotation function (Rossmann & Blow, 1962; Hoppe, 1957) determines the direction and angle of rotation of noncrystallographic symmetry operators in a crystal lattice. The latter is any operator that is valid within a local volume (as opposed to infinite volume) of the crystal lattice. In many cases, biological assemblies contain point groups of fairly high symmetry. When crystals are available, the rotation function can be used to determine the point group