Journal of
Applied Crystallography
ISSN 0021-8898

Received 28 April 2004
Accepted 30 June 2004

HELIX: a helical diffraction simulation program

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The program *HELIX* has been devised as a simple aid to understanding the origin and appearance of fibre diffraction patterns from helical structures. Helices are common as preferred conformations in both natural and synthetic macromolecules (*e.g.* DNA, α-helices, polysaccharides, synthetic polymers), and occur frequently in extended macromolecular aggregates (*e.g.* actin filaments, myosin filaments, microtubules, amyloid filaments *etc.*). For this reason, a simple way of visualizing the kinds of diffraction patterns that these structures can give should have educational value and should also be useful as a quick means of testing possible symmetries in structural investigations before embarking on full helical diffraction analysis. Despite its simplicity, there is no other public program that provides these possibilities. The *HELIX* program, running under Microsoft Windows, is freely available from the CCP13 website (http://www.ccp13.ac.uk).

1. Introduction

The technique of fibre diffraction and the theory of helical diffraction were early developments in 20th century molecular structural studies and can be said to have formed a central part in the elucidation of the double-helical structure of DNA, which is probably the single greatest discovery in biology in recent times. The determination of the structures of the α-helix, the β-sheet, the triple helical structure of collagen and the double helix of DNA were all based on or confirmed by fibre diffraction analysis. The theory of helical diffraction was developed in parallel in the early 1950s by Cochran *et al.* (1952) and Stokes (unpublished), and since then these theories have been fully developed and described in numerous reports (Holmes & Blow, 1965; Harford & Squire, 1997; Squire, 2000; Chandrasekaran & Stubbs, 2001). However, understanding the relationship between a polymer structure and the diffraction pattern generated from it is not a trivial task, especially for those who are not diffractionists, and there are many who wish to use fibre diffraction methods as tools in their research (*e.g.* in probing muscle structure) without carrying out complete modelling studies. In addition, there are others who seek to analyse fibre diffraction data and who have structural models in mind, but do not have the programs available for quick tests of their ideas.

In previous publications we have sought to illustrate helical diffraction in a non-mathematical way by means of optical diffraction analogues (Squire, 1981, 2000; Harford & Squire, 1997). For the same kind of reason, we have now developed the *HELIX* program, described here, as a teaching aid to illustrate the nature of helical diffraction patterns and how these patterns change as a function of the different parameters which define the symmetry and size of the structure. However, it is apparent that *HELIX* will also be useful for those wishing to carry out preliminary tests of possible interpretations of their data in terms of alternative molecular models prior to a full analysis. The *HELIX* program is freely available from the CCP13 website (http://www.ccp13.ac.uk), is easy to use, and, we hope, will be highly educational. However, it is not intended to provide a means to determine fully the corrected diffracted intensities for a completely described molecular model. This can be achieved with other more research-oriented diffraction programs [*e.g.* the CCP13 programs *MOVIE* (AL-Khayat *et al.*, 2004) and *LALS* (Arnott & Wonacott, 1966; Okada *et al.*, 2003)].

2. Description of the *HELIX* program

HELIX is a didactic program designed for students and researchers who want to improve their understanding of the relationship between structural models of helical molecules or filaments and their diffraction patterns. It runs under Microsoft Windows 98 or XP and was developed with Microsoft Visual Basic 6.0. *HELIX* allows the construction of simple models through the input of a series of structural parameters *via* a user-friendly graphical user interface and it produces a display of the model so that the structure can be visualized and checked. It then calculates the cylindrically averaged diffraction pattern to a chosen resolution (q range) for visual inspection. It also allows the chosen model parameters to be saved as a parameter file, which can be re-input at a later time if required, it can save the full coordinates of the model structure that is generated, and it can save the calculated simulated diffraction pattern as an image. If desired, the output coordinate data can be used in other CCP13 programs

(*e.g. FibreTrans*; see http://www.ccp13.ac.uk) to generate a more complete description of the diffraction pattern.

In addition to its pedagogic value, the *HELIX* program can be extremely useful when recording experimentally a diffraction pattern from a novel polymer for which relatively little information is available. In particular, the program allows rapid qualitative testing of different possible helical structures for comparison with the recorded diffraction patterns. The information obtained from *HELIX* can itself be refined to mimic more closely the observed data, and the parameter information can then be used as input to other more sophisticated programs to improve and refine the model.

3. Parameters of a helix

The symmetry of a helical structure can be defined in terms of a number of parameters, as illustrated in Fig. 1(*a*). These include the subunit axial translation (h), the pitch of the helix (P), the repeat of the helix (C) if the number of subunits in a pitch is not an integer, and the radius of the helix (r). The number of subunits in one pitch is clearly $N = P/h$, and since the helix turns through 360° around the helix axis in a complete pitch, the amount turned from one subunit to the next is $\varphi = 360°/N$, an angle which we term the azimuthal rotation angle between subunits. As an example, the structure of the B form of DNA has exactly ten sugar-phosphate-base subunits along one strand in a complete pitch length.

This might be written as a 10/1 or 10_1 helix. The subunit axial translation is 3.4 Å, the pitch is 10 × 3.4 Å, and the azimuthal rotation angle between subunits is 360/10 = 36°. Because there is a whole number of subunits in one pitch, in this case the repeat C is the same as the pitch P.

The form of the helical diffraction pattern (Fig. 1*b*) is a series of layers of intensity, so-called layer-lines, perpendicular to the helix axis. The line in the diffraction pattern (by convention taken to be vertical) that is parallel to the helix axis and passes through the middle of the pattern (*i.e.* through the undiffracted beam direction) is termed the meridian and diffraction peaks on it are called meridional reflections. The horizontal line through the pattern centre is called the equator and this has equatorial reflections. The equator is part of the series of horizontal layer-lines. It is not appropriate here to give details of fibre diffraction theory, which is dealt with very well elsewhere (Holmes & Blow, 1965; Harford & Squire, 1997; Squire, 2000). Suffice it to say that, bearing in mind the reciprocal relationship between normal (real) space and diffraction (reciprocal) space, the positions of the layer-lines are related to successive orders of the repeat C of the helix. This means that, starting from the equator, they are at spacings related to $0/C$ (the equator), $1/C$ (the first layer-line), $2/C$ (the second layer-line) and so on. All of these layer-lines have intensity each side of the meridian, but not on the meridian, except for the layer-lines which correspond in spacing to orders of the subunit axial translation (h). These meridional intensities occur at axial positions related to m/h, where m is an integer, positive, negative or zero.

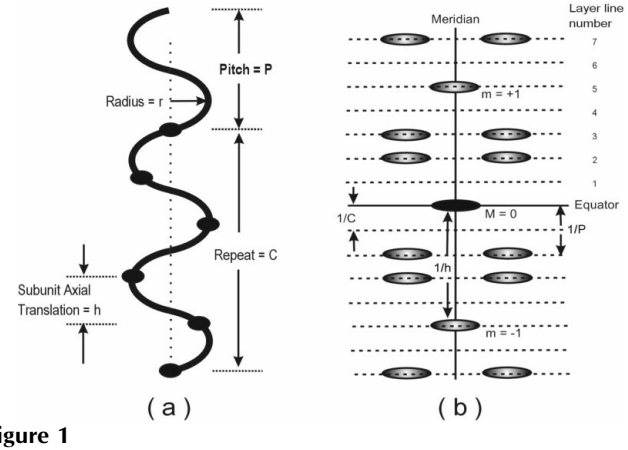

Figure 1
(*a*) Parameters of a helical structure (in this case a 5/2 or 5_2 helix, *i.e.* $S = 5$ and $T = 2$) and (*b*) the sort of distribution of diffracted intensities that is obtained from the helix in (*a*). The first meridional peak ($m = 1$) is on the fifth layer-line (*i.e.* $S = 5$) and the strongest peaks close to the meridian are on the second layer-line (*i.e.* $T = 2$) and related positions (layer-lines at $m \pm 2$).

As a good rule of thumb, if one is dealing with a helix with S subunits in T turns of the helix, then the diffraction pattern can be drawn by counting out to the Sth layer-line at S/C from the equator and placing a meridional reflection there (see Fig. 1*b*), and then counting out to the Tth layer-line at T/C from the equator and putting reflections each side of the meridian there. One can also count T layer-lines up and down from the meridional reflection on the Sth layer-line and put other off-meridional peaks there at the same radial positions as on the layer-line at T/C from the equator.

Finally, another important parameter of a helix is its radius (r). Because features in the diffraction pattern are at distances which are reciprocal to distances in the object, helices with a small radius will give off-meridional peaks at large reciprocal radii (R) along the layer-lines (they will be a long way from the meridian) and helices with a large radius will give peaks close to the meridian. Putting this the other way around, the radial position (R) of the peaks in the diffraction pattern can be used to determine the radius (r) of the helix.

4. Description of the *HELIX* interface

Fig. 2 shows that the *HELIX* graphical user interface has several areas with specific roles. On the left-hand side and along the bottom are boxes where various parameter values can be inserted (input boxes). On the right is a box where the structure generated by these parameters will be displayed (it is one of two picture boxes, namely the Structure Picture Box). At the top left are two command buttons. One is called Display Structure, which when activated will use the parameters that have been input to generate a structure that is displayed in the Structure Picture Box. This image can be adjusted by changing the scale and by changing the position of the image in the window. When the model structure has been checked, the second command button, Calculate Fourier Transform, can be activated and the diffraction pattern of the object structure will be calculated to the chosen resolution and

displayed in the central picture box, namely the Transform Picture Box. Depending on the number of objects included in the chosen structure, the transform calculation may take a few seconds or sometimes much longer. The cursor will display the busy sign while this is happening. Once calculated, the transform will appear in the Transform Picture Box. The intensity range in this transform can be adjusted at will by adjusting the FT Image Greyscale Parameters and then clicking on Refresh Image.

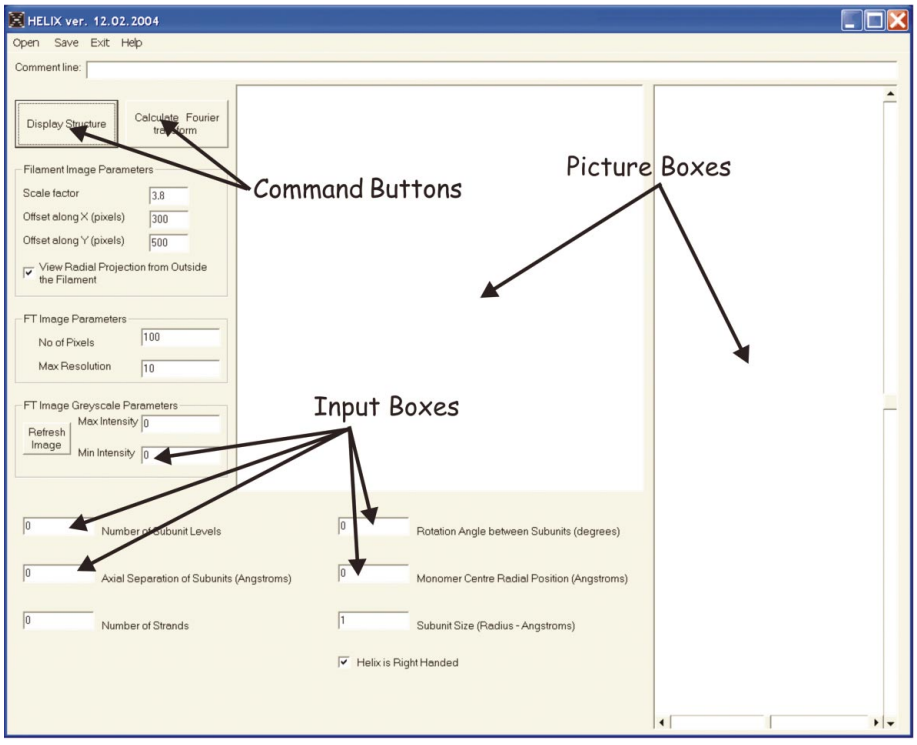

Figure 2
The *HELIX* graphical user interface.

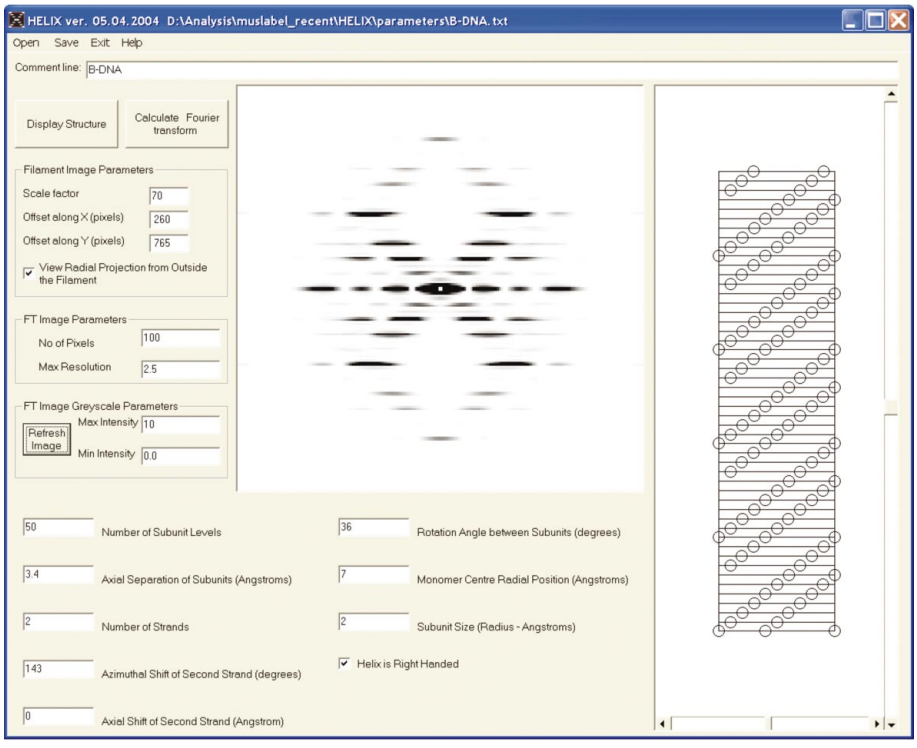

Figure 3
Simulation of the symmetry and diffraction pattern from B-DNA.

Once a useful image and diffraction pattern have been achieved, the parameters, structure image and Fourier transform image can be saved, as mentioned above, using the drop-down menus at the top of the *HELIX* window. There is also a space towards the top of the window for comments to be written. These comments will be saved as part of the parameter file. In addition, the complete *HELIX* window can be saved by using `Alt` + `Print Screen` on the computer keyboard and then pasting the saved window into a graphics program, as in Figs. 2 and 3 here.

5. Setting up the simulated structure and diffraction pattern of B-DNA

Here we use the example of the B-DNA structure to illustrate the application of *HELIX*. *HELIX* can be used to generate multi-stranded helices since many structures (myosin filaments, microtubules, troponin on actin filaments, *etc.*) consist of co-axial helices. The number (n) of strands can be input as a parameter in the *HELIX* interface where it is assumed that the strands are separated azimuthally by equal angles $360°/n$. Thus, an n-start or n-stranded helix will have n-fold rotational symmetry around the helix axis. However, in the case of $n = 2$, a special provision is included to allow the two strands to be non-equivalent. If 2 is put into the Number of Strands input box, then extra parameter boxes appear (Fig. 3), namely the azimuthal offset of the second strand and the axial shift of the second strand. In the case of a structure with two equivalent strands, then the azimuthal offset angle is 180°.

In the case of structures like DNA, the second strand in the double helix is not equivalent to the first. In fact, in reality, the second strand is anti-parallel to the first, but since *HELIX* does not allow the inclusion of

Table 1
Parameter values for some well known helical structures for use in simulations generated by the *HELIX* program.

	B-DNA 10/1	A-DNA 11/1	Collagen 10/3	Three-stranded 9/1 myosin filament	13/6 Actin filament	18/5 Alpha-HELIX
(*A*) Structure parameters						
Axial separation (*h*) of subunits (Å)	3.4	2.54	2.9	143	27.5	1.49
Rotation (φ) between subunits (°)	36	32.7	108	40	166.154	100
Monomer centre radial (*r*) position (Å)	7	9	5	150	25	2.5
Monomer subunit size (Å)	2	1.2	1.5	30	10	0.5
Hand of helix	RH	RH	RH	RH	LH	RH
No. of subunit levels	50	140	140	100	100	140
No. of strands	2	2	1	3	1	1
Azimuthal shift of strand 2 (°)	143	170	N/A	N/A	N/A	N/A
Axial shift of strand 2 (Å)	0	2	N/A	N/A	N/A	N/A
(*b*) Display parameters						
Scale factor	60	100	50	2	100	100
Offset along *X* (pixels)	240	230	255	250	275	255
Offset along *Y* (pixels)	645	685	690	680	695	690
(*c*) Transform parameters						
No. of pixels	100	100	100	100	100	100
Maximum resolution (Å)	2.5	2	2	40	20	1.3
Maximum intensity	25	25	10	15	25	25
Minimum intensity	0	0	0	0	0	0

detailed asymmetric subunit structures, the simulation simply requires a second strand at the same axial position as the first but offset by about 140–145° rather than 180°. The resulting structure, shown in Fig. 3, is very like the basic symmetry of the B-DNA double helix and the computed transform in Fig. 3 simulates quite well the general distribution of intensity on the observed layer-lines in the observed X-ray diffraction pattern of B-DNA, apart from the sampling along the layer-lines (see Fig. 4).

6. Conclusion

It has been shown that the program *HELIX* can simulate the diffraction pattern of a structure like B-DNA. Since it is easy to use and since such parameters as helical radius, the monomer size, the *q* range of the diffraction pattern, and so on, can be altered at will, it is a very useful means of discovering the effects that these parameters have on the observed pattern. A much fuller description of the program is given in the manual available at the CCP13 website. Apart from simulating B-DNA diffraction, *HELIX* is equally good at simulating the observed patterns from many other structures. The parameter data appropriate for a few well known examples are shown in Table 1. Many of these structures are also included as downloadable parameter files on the CCP13 website, where some other simulations are also illustrated.

Figure 4
Comparison of (left) the diffraction pattern of B-DNA simulated using *HELIX* and the parameters discussed above and (right) a recorded X-ray diffraction pattern from a fibre tilted to bring the 3.4 Å meridional reflection ($m = 1$) into the diffraction condition (unpublished pattern recorded by JMS). This tilting explains why the diffraction features at the top of the recorded pattern are so strong. The *HELIX* program, even with a very simple structure, simulates quite well the relative strengths of the different layer-lines. However, it does not show the observed sampling of the layer-lines along vertical row-lines, since lattice sampling effects have not been included. Here we are simulating the diffraction from a single molecule which consists of unsampled layer-lines.

We are indebted to the BBSRC, EPSRC and CLRC for support of CCP13 (#28/B10368 and #28/B15281) and to the Wellcome Trust (#061729) for a grant to JMS in support of CK while *HELIX* was being developed.

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