Journal of
Applied Crystallography
ISSN 0021-8898

Received 27 January 2001
Accepted 12 June 2001

Simulation of electron density maps for two-dimensional crystal structures using *Mathematica*

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The simulations presented here are based on the program *Mathematica* as a tool to present electron density maps of two-dimensional crystal structures. The models give further insights into the relationship between the thermal displacement parameters and the quality of the electron density maps. Furthermore, users can readily test the effects of several crystallographic parameters on the electron density maps, such as, the number of reflections, the thermal displacement parameters and the unit-cell dimensions.

1. Introduction

The aim of this paper is to describe an elementary computational tool that serves as auxiliary material for courses in crystallography for postgraduate students in biophysics, biochemistry and molecular biology. This material has been used by the authors for crystallography courses at the State University of São Paulo (Brazil) for four years.

Teaching the interpretation of electron density maps to future biocrystallographers has been shown to be quite a difficult task. To overcome this difficulty, at least partially, we have developed a simple simulation to help in teaching the interpretation of electron density maps. This simulation, based on the program *Mathematica* (Wolfram, 1999), allows the user to visualize the electron density maps of two-dimensional crystals. Furthermore, it allows visualization of the effect of thermal vibration on the resolution of electron density maps.

Analysis of the electron density map is a critical step in the crystallographic refinement of any molecular structure determined by X-ray diffraction techniques (Blundell & Johnson, 1976; Delatorre, Fadel & Azevedo, 2001; Delatorre, Olivieri *et al.*, 2001; Azevedo *et al.*, 1996, 1997; Kim *et al.*, 1996) and teaching the interpretation of these maps is frequently quite laborious. There are many sophisticated programs used to visualize protein structures and the electron density maps (McRee, 1993). However, in these programs most of the characteristics of the electron density are omitted, which can, for the beginner in biocrystallography, make the visualization of the relations between thermal vibration and electron density function an arduous task.

The use of the program *Mathematica* to simulate the electron density map for a hypothetical two-dimensional crystal structure is described here. In addition, the effect of thermal vibration on the resolution is simulated.

2. Methods

2.1. Structure factors and electron density function computations

The atomic scattering factor (f) of an atom depends on the electron density and can be found in the *International Tables for X-ray Crystallography* (1974, Vol. III). The thermal motion of the atoms affects this factor. In the simple case in which the components of vibration are the same in all directions, the vibration is called isotropic and the atomic scattering factor is

$$f = f_o \exp[-B(\sin^2 \theta_{hk})/\lambda^2], \quad (1)$$

where θ_{hk} is the scattering angle, f_o is the scattering power of a given atom for a given reflection, λ is the wavelength, and B is related to the mean square amplitude ($\overline{u^2}$) of atomic vibration by

$$B_j = 8\pi^2 \overline{u_j^2}. \quad (2)$$

The structure-factor [$F(hk)$] calculation for centrosymmetric two-dimensional crystals, used in this paper, was performed using

$$F(hk) = 2 \sum_j f_j \cos[2\pi(hx_j + ky_j)], \quad (3)$$

where f_j is the atomic scattering factor for each atom in the unit cell, h and k are reflection indexes, and x_j and y_j are fractional coordinates for each atom in the asymmetric unit. The summation is over atoms not related by the centre of symmetry (Stout & Jensen, 1989).

The Fourier expansion of the electron density function for a centrosymmetric two-dimensional crystal is

$$\rho(xy) = 2 \sum_h \sum_k F(hk) \cos[2\pi(hx + ky)], \quad (4)$$

where h and k are integers over which the series is summed.

Table 1
Atomic and fractional coordinates for the model used in the simulations.

Atom	X (Å)	Y (Å)	x	y
C_1	3.0419	2.855	0.178935	0.167941
C_2	1.8338	3.5525	0.107871	0.208971
C_3	1.8338	4.9475	0.107871	0.291029
C_4	3.0419	5.645	0.178935	0.332059
C_5	4.25	4.9475	1/4	0.291029
C_6	4.25	3.5525	1/4	0.208971

2.2. Model building

To build a hypothetical crystal structure, we used a benzene molecule inserted into a two-dimensional unit cell. The unit cell is centrosymmetric to facilitate the computation of the atomic scattering factor, structure factors and electron density function. Fractional coordinates for the atoms in the model are shown in Table 1. A square unit cell was used with $a = b = 17$ Å. There are two benzene molecules in the unit cell, related to each other by a centre of inversion at $x = 1/2$, $y = 1/2$. The wavelength used was 1.5418 Å and the maximum value of the reflections h and k was 22 for the two-dimensional unit cell. This value is obtained from Bragg's law,

$$2\sin\theta/\lambda = 1/d_{hk}, \tag{5}$$

where $1/d$ is

$$1/d_{hk} = h2^{1/2}/a \tag{6}$$

for h equals k. The maximum value for the reflections is that for which $\sin\theta = 1$:

$$h_{\max} = 2a/\lambda 2^{1/2}. \tag{7}$$

The use of smaller values for h and k can illustrate the effect of resolution on electron density maps.

3. Results and discussion

A simulation of the electron density function for the two-dimensional crystal is shown in Fig. 1. In this simulation, all B factors were taken as zero. It can be seen that all electron density peaks are sharp and the molecular geometry can easily be identified. The program *Mathematica* allows rotation of the model, which facilitates the identification of individual atomic positions. Figs. 2 and 3 are simulations in which all B factors were taken as 2 Å^2 and 4 Å^2, respectively. When thermal vibration is introduced into the model, peak broadening is observed in the electron density map. In Fig. 3, where $B = 4$ Å^2 for all atoms, it is practically impossible to identify individual atomic positions. The B factor is a consequence of the dynamic disorder in the crystal caused by the temperature-dependent vibration of the atoms in the structure (Drenth, 1994) and the overall effect of increasing the B factor is the broadening of all peaks in the electron density map.

In addition to the effect of the B factors on the electron density maps, the effect of the resolution has been analysed. For Figs. 1 to 3, the resolution attainable from all the available data is 0.8 Å, which is more than needed to resolve the atoms.

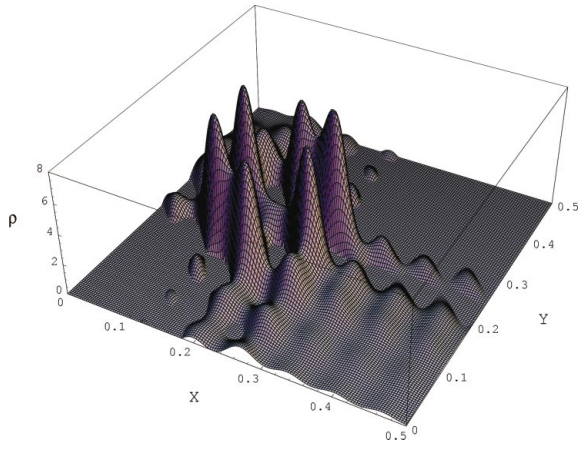

Figure 1
Electron density map for a two-dimensional crystal structure at 0.8 Å resolution ($h_{\max} = k_{\max} = 15$). All atoms have $B = 0$ Å^2.

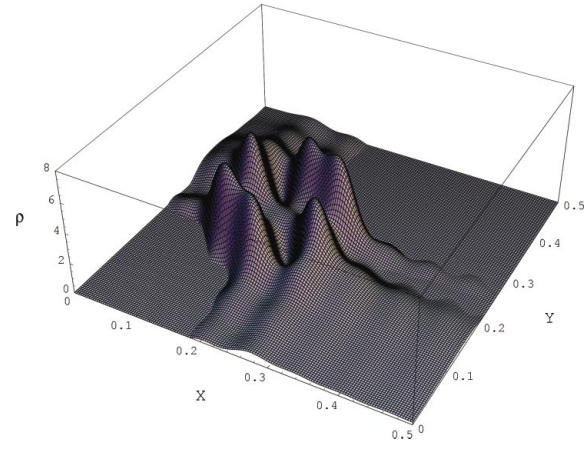

Figure 2
Electron density map for a two-dimensional crystal structure at 0.8 Å resolution ($h_{\max} = k_{\max} = 15$). All atoms have $B = 2$ Å^2.

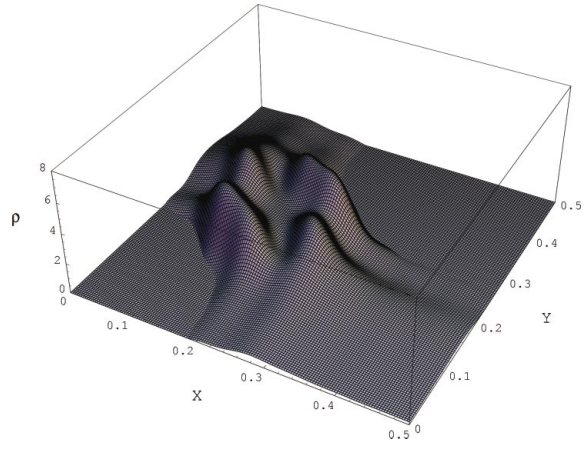

Figure 3
Electron density map for a two-dimensional crystal structure at 0.8 Å resolution ($h_{\max} = k_{\max} = 15$). All atoms have $B = 4$ Å^2.

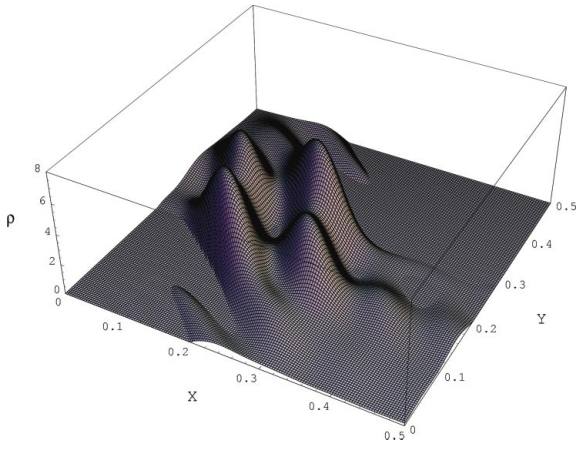

Figure 4
Electron density map for a two-dimensional crystal structure at 1.2 Å resolution ($h_{max} = k_{max} = 10$). All atoms have $B = 0$ Å^2.

A simulation of the electron density function at 1.2 Å resolution is shown in Fig. 4. In this simulation, all B factors were taken as zero and the h_{max} and k_{max} were reduced to 10. Comparison of Figs. 1 and 4 makes clear the degenerating effect of the reduced resolution on the electron density peaks, since we can barely resolve adjacent atoms at 1.2 Å resolution.

The input scripts used in the simulations presented here can be found at http://www.biocristalografia.df.ibilce.unesp.br/Xtal/simul/index.html.

We are grateful to Professor Åke Oskarsson (Lund University) for valuable suggestions about this paper and to Ms Gláucia R. Peglow Borges (State University of São Paulo) for her help with the program *Mathematica*. This work was supported by grants from FAPESP, CNPq, CAPES, FUNDUNESP and LNLS (Brazil). WFA is a researcher for the Brazilian Council for Scientific and Technological Development (CNPq, 300851/98-7).

References

Azevedo, W. F. Jr de, Leclerc, S., Meijer, L., Havlicek, L., Strnad, M. & Kim, S.-H. (1997). *Eur. J. Biochem.* **243**, 518–526.
Azevedo, W. F. Jr de, Mueller-Dieckmann, H.-J., Schulze-Gahmen, U., Worland, P. J., Sausville, E. & Kim, S.-H. (1996). *Proc. Natl Acad. Sci. USA*, **93**, 2735–2740.
Blundell, T. L. & Johnson, L. N. (1976). *Protein Crystallography*. London: Academic Press.
Delatorre, P., Fadel, V. & de Azevedo, W. F. Jr (2001). *Rev. Bras. Ens. Fis.* **23**, 63–73.
Delatorre, P., Olivieri, J. R., Ruggiero Neto, J., Lorenzi, C. C. B., Canduri, F., Fadel, V., Konno, K., Palma, M. S., Yamane, T. & de Azevedo, W. F. Jr (2001). *Biochim. Biophys. Acta*, **1545**, 372–376.
Drenth, J. (1994). *Principles of Protein X-ray Crystallography*. New York: Springer-Verlag.
Kim, S.-H., Schulze-Gahmen, U., Brandsen, J. & de Azevedo, W. F. Jr (1996). *Progress in Cell Cycle Research*, Vol. 2, edited by L. Meijer, S. Guidet & L. Vogel, pp. 137–145. New York: Plenum Press.
McRee, D. E. (1993). *Practical Protein Crystallography*. San Diego: Academic Press.
Stout, G. H. & Jensen, L. H. (1989). *X-ray Structure Determination. A Practical Guide*, 2nd ed. New York: John Wiley.
Wolfram, S. (1999). *The Mathematica Book*, 4th ed. Champaign: Wolfram Media/Cambridge University Press.