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SHORT NOTE

# ENHANCING THE POWER OF DENSITY MODIFICATION

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"Density modification" is a technique for the refinement, resolution extension, and/or partial structure completion of images obtained by X-ray crystallography, and may be useful as well for some applications in electron microscopy. The method consists of the following: One begins with a density function, $\rho_0$, which is either given experimentally or calculated from an approximate or incomplete set of amplitudes and phases $[F_0, \phi_0]$. The density function is then modified by the imposition of known constraints, and new amplitudes and phases are obtained by an inverse transform. From the initial and calculated transforms, one then generates a set of amplitudes and phases for the synthesis of a new image for the next cycle. The iterative procedure, schematically written as follows *

$$\rho^j \xrightarrow{M} \rho_m^j \xrightarrow{T^{-1}} [F_c, \phi_c]^j \xrightarrow{S} [F_S, \phi_S]^j \xrightarrow{T} \rho^{j+1}$$

is thus a mixed real and reciprocal space method, which is conceptually transparent – it consists of a modification step, M, and a synthesis step, S. Using the fast Fourier algorithm for the forward and reverse transforms, it is also very rapid. While in principle any operation on the image can be done either on $\rho$ or its transform, it is often the case that it is simpler and cheaper to implement it in either real or reciprocal space. Thus, for example, the squaring of $\rho$ which is implicit in the convolution expression of the Tangent Formula, is usually best performed in real space [1]. The truncation of negative values, the prevention of density accumulation in single peaks, the elimination of noise outside the boundary of the molecule (e.g. solvent fluctuations in a crystal), are all often simplest to perform directly on $\rho$. Recently [2] it has been shown that the use of local symmetry elements, i.e., redundancy of structural information in the crystallographic asymmetric unit, is also most simply performed in real space. Other procedures are more appropriately done in reciprocal space, such as weighting of the Fourier synthesis, which very often has a powerful effect on the quality of the map, integration of information from initial data and/or past cycles with the calculated quantities of the current cycle (e.g., combining phase probability curves), and monitoring the progress of the refinement.

Such a procedure readily lends itself to the combination of many different tactics for improving the density function, and the present note reports on some explorations of the limits of the power of this approach. The main result of these studies so far is the following: About half of a 31 atom structure is assumed known: 16 delta functions of equal height are placed at grid points closest to the true atomic positions. (Grid point intervals in $x$, $y$, $z$ were 0.34, 0.54, and 0.42 Å.) Then noise peaks (delta functions of the same height) are added at random locations. Using the correct (calculated) amplitudes for the entire structure to 2 Å, it is possible to solve a starting map consisting of as many as 256 random peaks added to the 16 true ones in the asymmetric unit. That is, the density-modification procedure automatically, in 15 cycles, eliminates all the noise peaks and brings up to full height the missing 15 true peaks (and no new incorrect peaks), lowering the (amplitude-weighted) average phase error from 83° to 25°. This is quite a dramatic result.

The work was motivated by the hope that if the cycling procedure were made sufficiently powerful, the

* Notation: $[F_S, \phi_S]$ are "synthesis" amplitudes and phases used to calculate the next map; $[F_c, \phi_c]$ are "calculated" values from previous modified $\rho_m$; $F_0$'s are "observed" amplitudes which, in these model studies, are calculated from atomic coordinates.

procedure might possibly converge on the correct solution (or a partially correct one, which, by summing different solutions, might yield the correct result) starting from completely random atoms. This has not been achieved, although we have not exhausted all our ideas on improvements in modification and synthesis. It is interesting to note that with 512 randomly placed peaks, where the method presently fails, the signal to noise of the starting map approximately matches that fraction of the grid points of the correct map which have appreciable density values; i.e., which lie at or sufficiently near atomic positions.

Details of the procedure used and results obtained follow. We employ a method similar to that of Collins et al., [3] who based their approach partially on the earlier work of Hoppe, Huber and Gassman [4] and Barrett and Zwick [1]. Collins introduced the innovative 3–2 modification:

$$\rho_m = \begin{cases} 3\rho^2 - 2\rho^3, & \rho \geqslant 0, \\ 0, & \rho < 0, \end{cases}$$

where $\rho$ is normalized to a maximum of 1. This modification first of all truncates non-physical negative density. The polynomial expression suppresses peaks less than 1/2, and augments those greater than 1/2; i.e., it enhances contrast. Or one might regard this modification as first squaring the density and then using a smoothed upper level truncation to prevent peaks from getting too large. (For structures having heavy atoms, the modification expression is altered to allow for higher values.)

We have here used this modification, and their $(2F_0 - F_c)$-synthesis method, with a small alteration:

$$F_S^j = \max (2F_0 - F_c^j, \lambda_1 F_0)$$

where the $F_0$ are calculated amplitudes from the complete 32-atom structure. The $2F_0 - F_c$ expression [4] accentuates peaks which would be present in a $[F_0, \phi_c]$ synthesis, but which are absent in the $[F_c, \phi_c]$ synthesis, $\rho_m$, and suppresses peaks which would be absent in the former but are present in the latter. Taking the maximum of this quantity and some fraction of the observed amplitude is an alteration in the direction of conservatism. Low values of the multiplier $\lambda_1$ reflect the desire not to allow negative amplitudes, which would cause the phase to flip by $\pi$. Larger $\lambda_1$ select values near to $F_0$, when $2F_0 - F_c$ is small.

We add to the synthesis step a weighting term in $\tanh (\lambda_2 \cdot E_0 \cdot E_c)$ where the $E$'s are normalized structure factors. This has the effect of strengthening high amplitude reflections [5]. Other weighting terms, exponentials in amplitude or vector differences, were also tried. These were generally better than no weighting, but inferior to the tanh function. We introduce also accelerated phase changes

$$\phi_S^j = \phi_S^{j-1} + \lambda_3 (\phi_c^j - \phi_s^{j-1}),$$

since it is our experience that convergence can often be successfully speeded up. In addition, calculated and synthesis amplitudes ($F_c$ and $F_s$) are rescaled in shells of resolution to $F_0$. Finally, a weak temperature factor of the form $\exp(-\lambda_4/d^2)$ where $d$ is resolution in Å. is applied to the $F_s$ after rescaling to $F_0$, to weight down slightly the higher resolution reflections. It is likely that the high frequency phases are the less reliably known, since a high resolution reflection, $h$, has less than the full set of possible vector pairs ($k$, $h-k$), and higher order terms to contribute to its proper determination. In real space terms, we thus de-emphasize the sharpening effects of the modification.

To sum up the procedure for generating the synthesis amplitudes: $F_c$ is normalized, the maximum of $(2F_0 - F_c, \lambda_1 F_0)$ taken, and then multiplied by the tanh term. The result is rescaled to $F_0$ and the final temperature factor applied. In all calculations, the correct $F(0,0,0)$ term was included. The principal result mentioned earlier came from a run with $\lambda_1 = 1$, $\lambda_2 = 0.25$, $\lambda_3 = 2$, $\lambda_4 = 0.5$. The model system was the alkaloid, panamine, which was crystallized in P2 with $a = 10.91$ Å, $b = 8.57$ Å, $c = 13.58$ Å, and $\beta = 112.7°$ and solved by symbolic additions [6]. (The structure has two chlorine atoms per molecule, which are removed in calculating the "observed" amplitudes and correct phases, leaving only light atoms.)

The starting map, which contains 16 true plus 256 random peaks in the asymmetric unit, had an average phase error of 85°, a phase error weighted by $F_0$ of 83°, an R-factor $(\Sigma|F_0 - F_c|/\Sigma F_0)$ of 0.61, and a $\psi_0$ of 17.7 on an arbitrary scale ($\psi_0$ is $\Sigma F_c$ for reflections for which $F_0$ is small, and should therefore be small as well [7]). In 15 cycles of refinement, these values steadly dropped to 32°, 25°, 0.33, and 7.3, respectively. The average phase change in the last two cycles was 6° and 4°; its largest value was 29° (cycle 4). The final map had peaks only at the 31 true positions, with

typical values of 6, compared with a noise level of about 1.

It should be obvious that we did not simply start out these tests of the power of density modification by adding 256 noise peaks to half of the structure. Earlier versions of this procedure which did not have all of the modification and synthesis features, or had different options altogether, were unable to solve starting cases with many fewer random atoms. For example, a simple $2F_0 - F_c$ synthesis with a modification of negative density truncation, squaring, and upper level truncation (to prevent peaks from becoming too large) could not even complete the half-structure with no noise peaks added. The refinement is not too sensitive to values in some of the parameter constants, although the convergence rate will vary. The procedure was tested also in the remaining 15 atoms of the structure, and a different set of 256 noise peaks, with virtually identical results.

A number of simpler problems were also tried. For example, as part of a study of the usefulness of density-modification for heavy atom refinement, amplitudes were calculated for a two-atom structure in $P6_222$ with relative peak heights of 12 and 10. Starting phases were obtained from an artificially broadened peak centered at the location of the weaker site, with an average weighted phase error of 89°. This refined to 7° in 13 cycles, with the R-factor dropping from 0.53 to 0.23 and $\psi_0$ from 11.2 to 2.8. The final map had the two peaks at the correct relative height.

Calculations were done with a system built around the CHAFF program [8] and on a 370-195 computer; refinement of the 16/256 case took about 10 minutes.

A manuscript describing in greater detail these and other experiments with density modification is in preparation.

In conclusion, by simple combination of rational procedures for density modification and construction of synthesis coefficients, and, admittedly, some considerable empirical testing, we have arrived at a procedure which seems remarkably powerful. It is hoped this will encourage greater experimentation and use of this method, although results with real data can hardly be expected to be as dramatic. Nonetheless, a solution to the phase problems along these lines, which is not restricted to small structure (where a small number of reflections may dominate), is not inconceivable.

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## References

[1] A.N. Barrett and M. Zwick, Acta Cryst. 27 (1971) 6.
[2] G. Bricogne, Acta Cryst. A30 (1974) 395.
[3] D.M. Collins, F.A. Cotton, E.E. Hazen, Jr., E.F. Meyer, Jr., and C.N. Morimoto, Science 190 (1975) 1047.
[4] W. Hoppe, R. Huber and J. Gassman, Acta Cryst. 16 (1963) A4.
[5] G. Germain, P. Main and M. Woolfson, Acta Cryst. A27 (1971) 368.
[6] I.L. Karle and J. Karle, Acta Cryst. 21 (1966) 860.
[7] W. Cochran and R.M. Douglas, Proc. Roy. Soc. A227 (1957) 486.
[8] D. Bantz and M. Zwick, Acta Cryst. A10 (1974) 257.