

Algebraic Reconstruction Techniques (ART) for Three-dimensional Electron Microscopy and X-ray Photography

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(Received 12 August 1970)

We give a new method for direct reconstruction of three-dimensional objects from a few electron micrographs taken at angles which need not exceed a range of 60 degrees. The method works for totally asymmetric objects, and requires little computer time or storage. It is also applicable to X-ray photography, and may greatly reduce the exposure compared to current methods of body-section radiography.

There are a number of biological structures which have not yet been crystallized, and whose structure therefore cannot be determined by X-ray crystallography. The most outstanding example is the ribosome, whose mechanochemical functioning in the translation of messenger RNA into protein is a basic unsolved problem. Other examples are chromosomes and numerous uncrystallizable enzymes. DeRosier & Klug (1968) have given a Fourier method for the reconstruction of three-dimensional objects from electron micrographs. Unfortunately, there are limitations on their method, which make it practical only for highly symmetrical objects. They estimate that in order to obtain a 30 Å reconstruction of a 250 Å ribosome, electron micrographs would have to be taken at approximately 30 different angles, on a stage capable of tilting $\pm 90^\circ$. This number of pictures, if taken by ordinary electron microscopy, would destroy the ribosome and cover it with a thick layer of dirt from the microscope chamber.

We will present an entirely new, direct method, an Algebraic Reconstruction Technique (ART), which has the following advantages over the Fourier method:

- (1) the ART method works readily for completely asymmetric objects;
- (2) it produces considerable detail of such objects with only 5 to 10 views;
- (3) ordinary tilting stages may be used, since the views may be taken over a relatively small range of angles ($\pm 30^\circ$);
- (4) computing time is approximately 30 seconds per section on a Control Data 6400;
- (5) small computers may be used, since little storage is required;
- (6) ART is directly applicable to macroscopic X-ray photography, and should require considerably less radiation than present methods of body-section radiography (Kane, 1953).

The computing requirements are emphasized, since Crowther, Amos, Finch, DeRosier & Klug (1970), studying highly symmetrical spherical viruses by the Fourier method, needed 1.5 hours on an IBM 360/44 computer, and apparently most of its core storage.

Using a tilt stage, we may rotate the object around a single axis. Then each plane through the object, perpendicular to the axis, projects into a line on the electron micrograph. We may reconstruct each plane in turn from the densities of its corresponding lines in the different views, and stack the planes to get the three-dimensional reconstruction. (The separation of the planes should be no greater than the resolution of the microscope.) Thus the problem is reduced from three dimensions to two.

Consider a square around the object in a given plane, and suppose that we are satisfied with reconstructing this plane at $n \times n$ points within the square. Thus we must find the optical density ρ_{ij} at each point (i, j) , $i = 1, \dots, n$; $j = 1, \dots, n$.

We define a *ray* of a projection or view at angle θ as a band of width w across the plane at that angle. (w ought to be less than or equal to the resolution of the microscope.) This ray will intersect a subset of the discrete points defined above. Thus the total optical density of the ray across a plane of the real object somehow has to be distributed amongst these discrete points in the reconstruction.

Let $R_{k\theta}$ be the known total optical density of ray k of the projection at angle θ , as measured from the appropriate photograph. Each ray yields a linear equation

$$R_{k\theta} = \sum_{(i,j) \in \text{ray}(k,\theta)} \rho_{ij} \quad k = 1, \dots, r_\theta. \quad (1)$$

For instance, if $\theta = 0^\circ$, then $k = j$ and

$$R_{j,0^\circ} = \sum_{i=1}^n \rho_{ij} \quad j = 1, \dots, n \quad (2)$$

provided $w =$ one unit of the grid. The number of rays in a given projection at angle θ , r_θ , is of the order of n/w (see Appendix A). If we take p photo-

graphs, we have approximately pn linear equations of type (1) in the n^2 unknowns ρ_{ij} .

Suppose we are using an electron microscope with 5 Å resolution to ascertain the internal structure of a 250 Å wide ribosome. In order to use the microscope to maximum advantage, we ought to choose $n \gtrsim 50$ points across. If we take five photographs of the ribosome at different angles, we will have only, say, 250 equations in 2500 unknowns. The number of solutions is infinite. Yet we would like to believe that we have obtained a certain amount of information about the three-dimensional structure. The problem is how to make this visible.

The real, but unknown structure of the ribosome is, of course, one of the numerous solutions to our undetermined equations. If we had some reason to choose one solution over another, we might get reasonably close to the real structure.

We have previously presented three Monte Carlo algorithms (Gordon & Herman, 1970) for finding solutions to equations (1). In these, the optical densities ρ_{ij} were quantized, and single density bits were added or subtracted to an originally blank array ($\rho_{ij} = 0$), until the equations were satisfied. In order to test the algorithms, we used a digitized picture of a little girl, Judy, to represent one plane of the three-dimensional object (Plate I). The results of the Monte Carlo algorithms were reconstructions which looked something like the original, but were highly "peppered". Thus it is apparent that not all solutions are "smooth".

One way we could get a smooth reconstruction was to average a number of individual peppered reconstructions. This procedure is valid, because the average of two or more solutions to equations (1) is also a solution. Thus it is clear that smooth solutions exist. This is presumably a desirable feature of reconstructions of real objects. Moreover, the average of a number of peppered reconstructions was closer to the original picture $\{\rho'_{ij}\}$ in terms of the Euclidean "distance"

$$\delta = \left[\frac{1}{n^2} \sum_{i=1}^n \sum_{j=1}^n (\rho_{ij} - \rho'_{ij})^2 \right]^{1/2} \quad (3)$$

We also found that the "entropy"

$$S = \frac{-1}{\ln n^2} \sum_{i=1}^n \sum_{j=1}^n \left(\frac{\rho_{ij}}{T} \right) \ln \left(\frac{\rho_{ij}}{T} \right) \quad (4)$$

was nearly maximized by averaging, which bolstered our intuition that the best solution would be the one which maximized entropy, or alternatively, gives us no more "information" than we put in; i.e. the solution maximizing equation (4) would seem to be the "least biased". (T is the total density of the picture.) These notions open up some unanswered questions about the

