

Three Dimensional Reconstructions of Spherical Viruses by Fourier Synthesis from Electron Micrographs

by

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Methods are developed for computing three dimensional density maps from transmission electron micrographs using Fourier transforms. The reconstructions objectively combine data from several different views of one or more particles. When applied to negatively stained spherical viruses, the technique gives clear reconstructions of their surface structures.

CONVENTIONAL electron microscopes possess a large depth of focus. Consequently a transmission electron micrograph represents a projection of the scattering density in the specimen onto a plane normal to the direction of the electron beam. Knowledge of three dimensional relationships between the various parts of the specimen must therefore be obtained from one or more of these projected views of the object. A general method involving the use of Fourier transforms has been proposed¹. It has been applied¹⁻³ to biological assemblies with helical symmetry, for which special case a single view of the particle may provide sufficient information to reconstruct the object at least to limited resolution, since the two dimensional transform obtained directly from one image can be used in a Fourier-Bessel inversion. For non-helical particles it is necessary to combine data from several different projected views and this, in turn, requires data reduction and interpolation⁴. We have developed mathematical and computational procedures for implementing the method and have applied these to reconstructions of spherical viruses, where several different views are required.

In such viruses the coat protein molecules are arranged with icosahedral symmetry⁵ to form a protective covering for the nucleic acid. This kind of particle is well preserved in negatively stained preparations, and the high symmetry and possibility of recognizing the orientation of certain special views make it a natural choice for testing the extended system of reconstruction. Above all, the presence of high symmetry means that in principle only a few distinct views are required for their reconstruction. This is effectively because a general view of a particle made up of subunits related by symmetry contains within itself as many different views of the subunit—related in a known way—as there are subunits in the particle.

We now describe briefly how the method combines several different views and then discuss its application to human wart and tomato bushy stunt viruses.

Outline of the Method

The method of reconstruction is based on the projection theorem, which states that the two dimensional Fourier transform of a plane projection of a three dimensional density distribution is identical to the corresponding central section of the three dimensional transform normal to the direction of view. The three dimensional transform can therefore be built up section by section using transforms of different views of the object, and the three dimensional reconstruction then produced by Fourier inversion. The approach is similar to conventional X-ray crystallography, except that the phases of the X-ray diffraction pattern cannot be measured directly, whereas in electron microscopy they can be computed from an image.

The different views may be collected either from a single particle by using a tilting stage in the microscope, or from several particles in different but identifiable

orientations. We have used the first method of data collection for human wart virus and the second for bushy stunt virus. In general, it is desirable to combine data from different particles so that imperfections can be averaged out. When different particles are viewed from different angles their absolute orientation must first be found; we have developed a method for identifying the direction of view, which is based on the symmetry known (or thought) to be possessed by the particle.

The reconstruction method is straightforward in principle. The area of interest on the micrograph is converted to an array of optical density values by means of a computer-controlled film scanner⁶. All further operations required for the reconstruction are performed by a digital computer. The digitized densities are transformed by computation into a set of Fourier amplitudes and phases. Each view of the particle provides a central section of Fourier space and the symmetry of the particle can be used to generate further central sections related to the first by the symmetry. For example, in an icosahedral particle a general section determines fifty-nine other sections, although in the special case of a view down a symmetry axis the number of related sections is fewer. By inserting data from views of the same particle in different orientations, or from different particles in independent orientations, and making use of the symmetry, Fourier space is "filled up" to enable the reconstruction to be carried out to a given degree of resolution. How do we decide when the degree of filling up is sufficient?

Interpolation in the Fourier Transform

For computation of a correct Fourier inversion of the three dimensional data so collected, the values of the transform must be available at regularly spaced points throughout its volume. Otherwise an undistorted representation of the required density will not be obtained

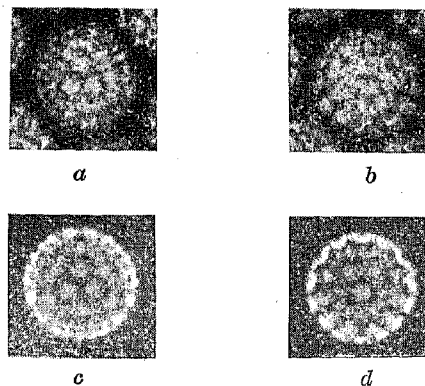


Fig. 1. Two views of the same negatively stained particle of human wart virus from micrographs taken with a tilting stage. *a*, Close to a three-fold axis; *b*, after a tilt of approximately 20° about a vertical axis. *c* and *d*, Corresponding simulated images* used as preliminary confirmation of the orientations.

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Table 1. EQUATIONS RELATING REQUIRED REGULARLY SPACED TRANSFORM VALUES TO IRREGULARLY SPACED VALUES OBTAINED FROM THE DATA

Cartesian coordinates

The Whittaker-Shannon interpolation formula* expresses the transform value F_h at a known non-lattice point $h'(h',k',l')$ in terms of the lattice values F_h :

$$F_{h'} = \sum_h \frac{\sin \pi(l-l')}{\pi(l-l')} \frac{\sin \pi(k-k')}{\pi(k-k')} \frac{\sin \pi(l-l')}{\pi(l-l')} F_h$$

Write this in matrix form as

$$F' = B F$$

Form the normal equations

$$B^\dagger F' = B^\dagger B F$$

which give a least squares solution for the required F_h as:

$$F = (B^\dagger B)^{-1} B^\dagger F'$$

Reconstruction achieved by normal Fourier inversion of F_h :

$$g(x,y,z) = \sum_{hkl} F_h \exp(-2\pi i(hx + ky + lz))$$

* Ref. 13, with a crystallographic implication in ref. 14.

(formally, this happens because the result of a Fourier inversion of a sampled transform is the convolution of the required density with the transform of the sampling function). In general, however, the planes of collected data will contain relatively few of the regularly spaced transform points. To use them efficiently, the values of the transform at points where they happen to be available must somehow be interpolated to convert them to the values at the regularly spaced points required for the Fourier inversion.

We have developed several procedures, described in detail elsewhere⁴, for carrying out this interpolation. Two types of grid system commend themselves. If normal Fourier inversion in cartesian coordinates is used, the transform values must be available at the points of a three dimensional lattice. For Fourier-Bessel inversion in cylindrical polar coordinates the transform values are required on the grid formed by the intersection of the r , ϕ and z surfaces. According to the symmetry of the object and the way in which the data have been collected, one or other of these representations may be particularly appropriate. In either case, the grid spacings are determined by the limited extent of the original object.

Both methods of interpolation lead to sets of linear equations of the form shown in Table 1. The equations

Cylindrical coordinates

Expand the required density in cylinder functions:

$$g(r,\phi,z) = \sum_n \int g_n(r,Z) \exp(in\phi) \exp(2\pi izZ) dZ \quad (1)$$

Fourier transform is then expressible in the form¹⁵:

$$F(R,\phi_j',Z) = \sum_n G_n(R,Z) \exp(in(\phi_j' + \pi/2))$$

where the available transform planes cut an annulus of radius R on section Z at known points ϕ_j' . On each annulus write as:

$$F' = B G$$

Form the normal equations

$$B^\dagger F' = B^\dagger B G$$

which give a least squares solution for the required G_n on each annulus as

$$G = (B^\dagger B)^{-1} B^\dagger F'$$

Reconstruction achieved by Fourier-Bessel inversion of G_n to give g_n :

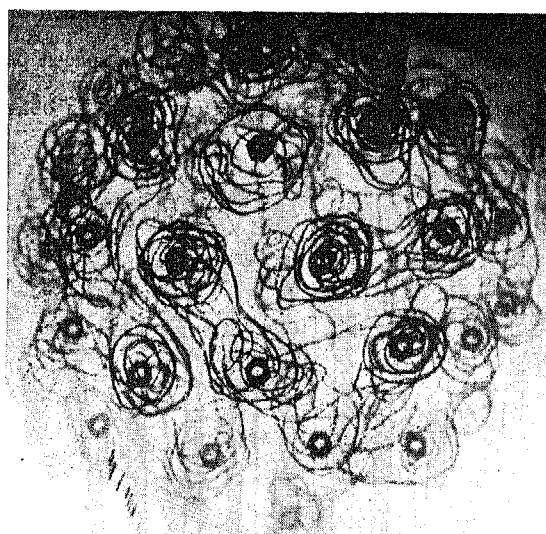
$$g_n(r,Z) = \int G_n(R,Z) J_n(2\pi Rr) 2\pi R dR$$

The g_n are then combined using (1) to give $g(r,\phi,z)$.

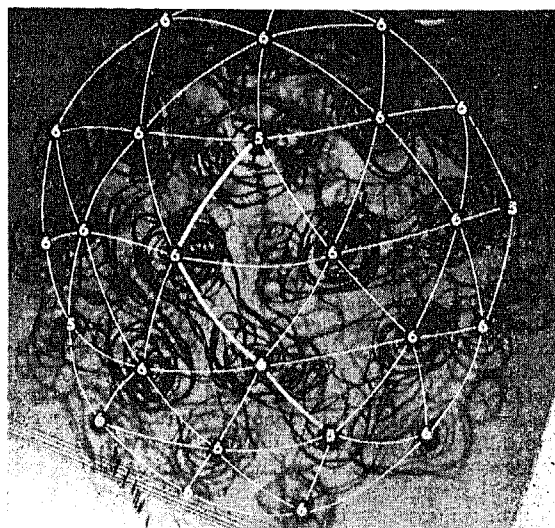
relate the required regularly spaced transform values to the irregularly spaced values obtained from the data. A necessary condition for the equations to be soluble is that there should be as many measured values as unknowns. This is not a sufficient condition, however, because the data points may be unfavourably spaced so that some of the required values of the transform are only poorly determined. In practice extra views of the object must be introduced so that there are rather more data points than unknowns in each interpolation problem. We then solve these extended sets of equations by the method of least squares.

The requirement that all the sets of least squares equations be soluble ensures that a satisfactory three dimensional reconstruction is possible to the degree of resolution aimed for. If too few views of the original object are included, the equations will not be soluble. More views must be included until they are. Formally we can investigate this by computing the eigenvalue spectrum of each normal matrix. None of the eigenvalues should be zero or close to zero; in practice there is a lower limit for the minimum eigenvalue below which one cannot usefully invert the normal matrix, but this limit depends on the errors in the original data.

In the reconstructions described here, we have used a



a



b

Fig. 2. a, Contour map of the reconstruction of human wart virus produced from the two views shown in Fig. 1. Contours represent the absence of stain. The morphological units are indicated by nuts inserted between the Perspex sheets on which the map is plotted. b, The same map with part of the $P=7$ surface lattice⁶ superimposed. The morphological units lie on the five-fold and local six-fold axes of the lattice. The relationship between two neighbouring pentamers is like a right-handed knight's move in chess⁶, as indicated by the bold lines.

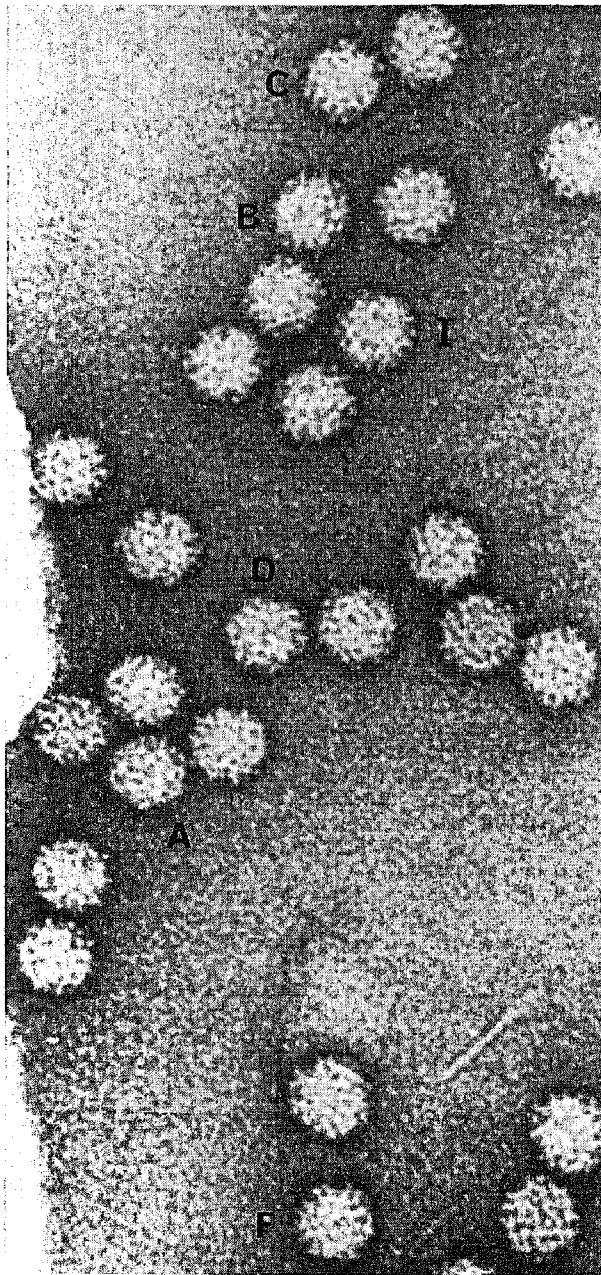


Fig. 3. A field of particles of tomato bushy stunt virus embedded in negative stain over a hole in the substrate. Some of the particles used in the reconstructions are indicated.

cylindrical grid together with Fourier-Bessel inversion, because the normal matrices are then of such a size as to be conveniently handled. For independent reconstructions to give consistent results in problems of the scale studied here, this method of interpolation requires three or four views of the particle. Using fast computers with very large stores, the more powerful Whittaker-Shannon interpolation, for which the normal matrices are at present inconveniently large, may ultimately turn out to be the method of choice, except where the nature of the symmetry or the method of data collection (for example, tilts about a single axis) lends itself to the use of cylindrical symmetry.

Recognition and Refinement of Particle Orientation

To use the symmetry of the spherical viruses and also to relate different particles, we must know the orienta-

tion relative to the symmetry axes of any view we propose to include in the reconstruction. This can be determined by a method which depends on the existence of a set of pairs of "common lines" in the two dimensional transform of any view of a symmetrical particle. These arise as follows. An observed section of the transform intersects an identical symmetry-related section in a line, along which the transform must have the same value in both sections. The common line lies in the original section. But, regarded as lying in the symmetry related section, it must have been generated by the symmetry operation from some other line in the original section. We have therefore a pair of lines in the original transform plane along which the transform must have identical values. A similar pair of lines will be generated by each possible choice of pairs of symmetry operations. The angular positions of all these lines depend on the orientation of the particle.

Because the disposition of the various pairs of common lines in the transform of a particle cannot be recognized directly, it is necessary to search for them by computation. We compare the differences in the observed values of the transform along the set of common lines corresponding to a particular choice of orientation parameters. The minimum value of the sum of these differences will occur when the angular parameters correspond to the true orientation of the particle. The orientation of an unknown view of a particle can be determined by searching a complete asymmetric unit of rotation space. The search can be restricted to a small range if a preliminary estimate of the orientation of the view is available (from, say, comparison of the image with a trial model), in which case the method serves for refinement.

The sum of differences at the point with minimum value gives a measure of the degree of preservation of the icosahedral symmetry in the particular particle the image of which is being investigated. Because we can compute this residual to various limits in Fourier space, it is possible to decide to what degree of resolution the details in a particle image are related by icosahedral symmetry. Clearly, data should be included only if they show some degree of icosahedral correlation: beyond this limit any transform data are contributing only noise to the final reconstruction.

Thus the method of determining orientation gives at the same time a measure of the quality of the image. This is analogous to the procedure that has been used for helical particles where the optical diffraction pattern, or its computer-generated equivalent, provides an objective measure of the degree to which the helical symmetry is preserved¹.

Human Wart Virus

Human wart virus (HWV) has a diameter of 560 Å. Its coat protein comprises 420 subunits arranged on a $T=7$ icosahedral surface lattice and clustered into pentamers and hexamers to form seventy-two morphological units⁷. The surface lattice can exist in right and left-handed forms and HWV has been shown to be right-handed^{7,8}.

We have produced reconstructions including data out to a cut-off of 60 Å, for which problem two views of the particle are sufficient. We have obtained these two views from a pair of micrographs taken with a tilting stage, using an experimental procedure described in ref. 8. Fig. 1 shows two views of a typical negatively stained particle together with the corresponding simulated images taken from a gallery of such images based on a trial model. Fig. 2 shows the reconstruction obtained from the pair of views. The morphological units are well resolved from one another, as indicated by the markers. Two pentamer units are shown and bear the correct "knight's move" relationship to one another.

Other reconstructions from pairs of views of different

particles show the same general structure. We have included data only to 60 Å, although for good particles the icosahedral correlation on common lines extends to at least 40 Å. It will therefore be possible to produce reconstructions at higher resolutions by combining data from different particles. Preliminary studies at higher resolution show that the morphological units appear as ring-like structures.

Tomato Bushy Stunt Virus

Tomato bushy stunt virus (TBSV) has a maximum diameter of 330 Å and a spherically averaged diameter of 310 Å (review in ref. 9). The major component of the coat protein is arranged on a $T=3$ icosahedral surface lattice, the 180 quasi-equivalent structural units being clustered in dimers to form ninety morphological units¹⁰. Of these ninety morphological units, thirty lie on the strict two-fold axes of the surface lattice, and sixty on the local two-fold axes. These sixty are arranged in twelve rings of five, the centres of which partially exclude the stain indicating the presence of protein.

Until recently, no simple correlation could be made between the morphology and the published physico-chemical data for this virus or for the very similar turnip crinkle virus^{10,11}. Recent work by Dr J. Butler of this laboratory on both viruses has established the molecular weight of the major protein subunit as 38,000*, consistent with a total of 180 in the particle. Each of the ninety morphological units would therefore correspond to a pair of protein subunits, in agreement with the expected symmetry. Butler's work has also revealed a minor protein component in a suitable amount to account for the matter observed at the five-fold positions of the surface lattice.

For the reconstructions we chose a particularly favourable micrograph, where particles are embedded in negative stain over a hole in the carbon substrate, as shown in Fig. 3. Experimental details of virus preparation and electron microscopy are given in ref. 10. The particles in this field have shrunk by about 10 per cent from their normal diameter in solution, but this shrinkage is in many cases isometric and preserves the icosahedral symmetry, as judged by the common lines technique. Icosahedral

* A similar value has been independently found for bushy stunt virus by Drs S. C. Harrison and K. Weber (private communication).

correlation appears to extend to at least 25 Å for good particles, showing that detailed symmetry is preserved almost to the limiting resolution of the negative staining technique.

Six of the best particles indicated in Fig. 3 were combined three at a time to produce two independent reconstructions. Data were included out to a cut-off of 28 Å. The result of one such reconstruction from particles B, F and I is shown in Fig. 4. The reconstruction from particles A, C and D gave an almost identical result. Fig. 5 shows a stereo-pair of a density plot of part of the reconstruction B, F and I. Fig. 6 shows a comparison between particle B, which is very close to a two-fold view, and a projection down a two-fold axis of the reconstructed density. The agreement between the two is remarkably good, particularly as the reconstructed particle has not been "embedded in negative stain" before projecting, so that the relative weights of the various parts of the projection may not be quite correct.

As expected, the main concentrations of density in the reconstruction are on the strict and local two-fold axes of the surface lattice so that the morphological units are presumably dimers. These morphological units are arranged in rings of six and five, and there is also density at the centres of the rings of five, giving them the appearance of five-pointed stars. We cannot yet tell whether this density arises from the shape of the principal structure unit itself or from the minor protein component mentioned above. Density also appears in the rings of six, but at a much smaller radius, and probably represents a coming together of the inner parts of the structure units, possibly in association with the nucleic acid.

The reconstruction also shows that the dimers on the local two-fold axes (that is, those dimers forming rings of five) lie at a greater radius than those dimers on strict two-fold axes, so that the five-pointed "stars" stand out from the surface. This effect also shows itself as a puckering of the rings of six, which is visible in Fig. 5. We estimate the difference in radius to be about 10 Å. This observation confirms the results of X-ray crystallographic studies of TBSV¹¹.

Finally, there is a marked absence of density at the local three-fold positions, that is approximately between the points of the "star", indicating deep penetration of stain into these places. This suggests that the nucleic

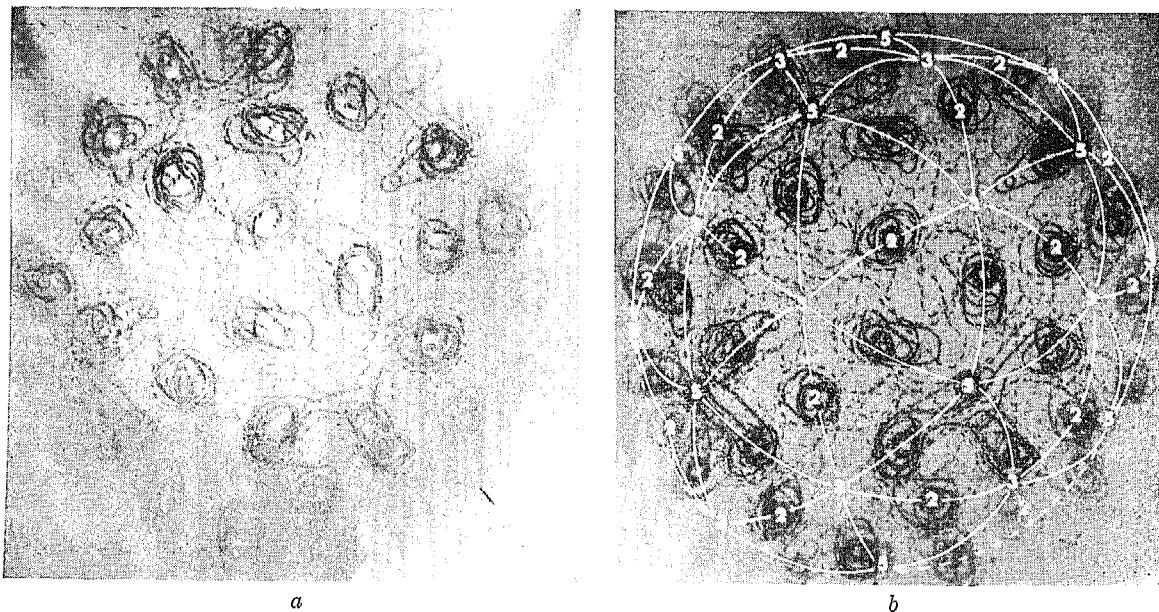


Fig. 4. *a*, Contour map of a reconstruction of tomato bushy stunt virus; *b*, the same map (photographed using different illumination) with the $T=3$ surface lattice superimposed. Contours indicate the absence of stain. The principal morphological units lying on the local two-fold axes of the lattice are indicated by nuts while the subsidiary morphological units at the five-fold positions are marked by washers.

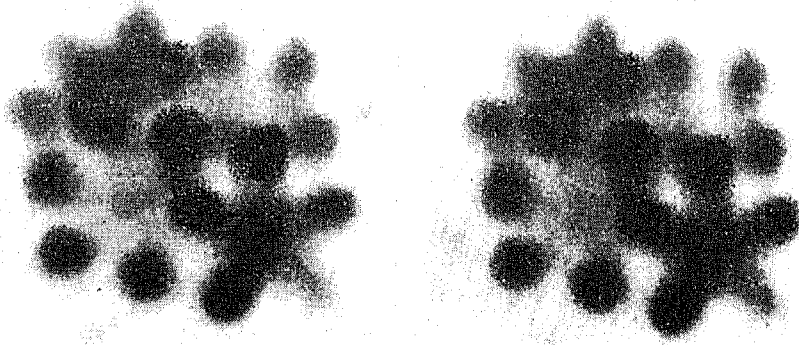


Fig. 5. A stereo-pair of part of a density plot of the TBSY reconstruction, in which high density indicates the absence of stain. The view is close to a two-fold axis, which relates respectively two relatively empty rings of six units and two five-pointed "stars". Notice the way in which the "star" at the top left, seen almost edge-on, projects the rings of six a puckered appearance. For full effect this diagram must be viewed with a stereoscopic viewer.

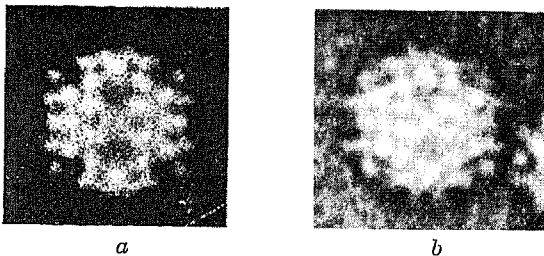


Fig. 6. Comparison of (a) the projection of the reconstructed density down a two-fold axis and (b) particle B from Fig. 3, which is very close to a two-fold view. The good agreement between the two pictures extends to fine detail, even though the relative weights of some features are not quite correct since the reconstruction was not "embedded in negative stain" before computing its projection.

acid is folded within the particle in such a way as to avoid these regions.

Future Applications

The reconstructions described here demonstrate that it is in practice possible to combine data from several different views of a given particle or from views of different particles. Using fast Fourier transform methods¹² for computing the two dimensional transforms, the total time needed to compute a reconstruction from three views, starting from raw densitometer data and ending with a computer display suitable for contouring, is about 1.5 h on an IBM 360/44 computer. Although in the case of spherical viruses with their high symmetry the number

of views needed is small, there is in principle no reason why the method should not be extended to systems with lower symmetry for which many more views must be combined to achieve the same degree of resolution. For successful implementation it will be necessary to improve methods of specimen preservation during a tilting series in the electron microscope and to use larger and faster computers to process the large volume of data that will be needed.

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Contemporary Depositional Environments of the Omo Delta

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Geomorphological and sedimentological studies of depositional environments of the modern Omo River delta and floodplain are essential to an understanding of the Pliocene to Pleistocene Mursi, Nkalabong and Kibish Formations of the Lower Omo Basin (south-western Ethiopia).

THE first field season of the Omo Research Expedition, in 1967 (ref. 1), made it very clear that, if the palaeoecology of the Pliocene and Pleistocene Omo Basin was to be understood, considerable work had to be expended on contemporary depositional environments and their ecology. I therefore carried out geomorphological studies of the modern Omo Delta and, to a lesser extent, of the basin

peripheries, in 1967 to 1969. This work consisted of mapping, air photo interpretation and sediment analyses. Miss Claudia Carr of the University of Chicago also investigated the plant ecology of the area.

Many facets of the investigation were necessarily only exploratory, but none the less it is felt that the six or seven modern sediment and soil samples analysed in detail are