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THE USE OF RADIAL DENSITY PLOTS TO CALIBRATE IMAGES OF FROZEN-HYDRATED SPECIMENS

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Accurate magnification calibration for transmission electron microscopy is best achieved with appropriate external or internal standards. The use of the icosahedral polyoma virus as a standard in frozen-hydrated preparations was previously described.[1,2] This method used the known diameter of polyoma (from x-ray measurements[3]) as a reference to calibrate the diameters of other viruses. Measurements were made from circular averages computed from individual particle images or from an average image of many individual particle images. The measured diameters of several different spherical viruses are in good agreement with measurements of the same viruses made with x-ray crystallographic and solution scattering techniques.[2]

We found, however, that it is very difficult to accurately measure particle diameters in images taken from frozen-hydrated specimens. This difficulty is attributable to several factors including *i)* uncertainty about the solvent density level, *ii)* superposition of density features that occur in two-dimensional projections of three-dimensional objects, *iii)* uneven outer surfaces of virus particles, *iv)* characteristically high noise and low contrast levels in micrographs, and *v)* contrast transfer function (CTF) effects. We have developed a more objective method, which calibrates invariant features (not particle edges) identified in radial density plots of either three- or two-dimensional data.

Invariant features in plots of standard and "unknown" particles are compared with the use of a general-purpose, least-squares fitting routine to optimize (with respect to scale factors) the fit between the two data sets in the invariant region. For polyoma, we identified the density in the protein capsid region between radii of ~18.5-25 nm to be invariant. A sample of bovine papilloma virus type 1 (BPV-1) was mixed with polyoma and vitrified, images of the frozen-hydrated specimen were recorded (Fig. 1), and two three-dimensional image reconstructions were computed with established procedures.[2,4-6] We used a radial density plot computed from a polyoma x-ray map[7] to calibrate the polyoma reconstruction (Fig. 2), and hence the micrograph magnification. We then used our BPV-1 reconstruction to calibrate a previously computed reconstruction of BPV-1.[8]

A much better fit between the reconstructed density and the x-ray model was obtained (Fig. 2,3) if we produced a "defocused" x-ray map by applying, to the model, a CTF[9] that simulated the microscope conditions of Fig. 1. The scaling procedure used was insensitive to the choice of defocus level for the x-ray data, within the range 0.4 to 2.0 μm underfocus. In addition, large numbers of individual particle images were not needed for accurate analysis. Once a reconstruction is calibrated, future calibrations (using the same particles as standards) can be made by comparing radial density plots computed from circularly-averaged images, thereby eliminating the need to produce another three-dimensional reconstruction.[10]

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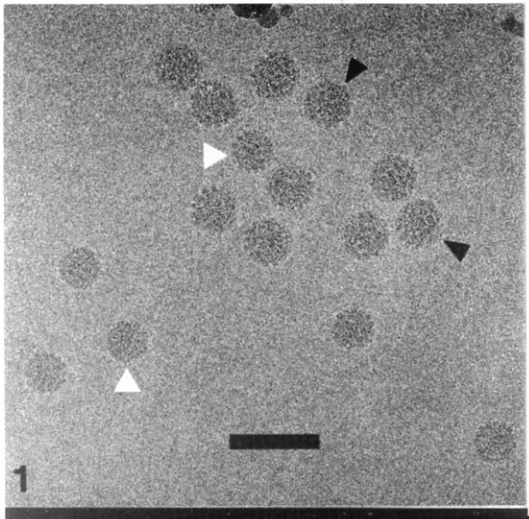
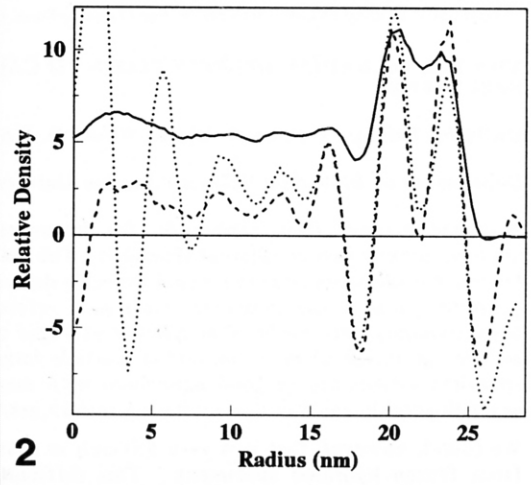
10. We thank W. Murakami, C. Olson, W. Newcomb, and J. Brown for providing virus samples; I. Rayment for the polyoma x-ray virion map; and K. Dryden, N. Dilley, and H. Cheng for programs. Research was supported by NIH grant GM33050 (T.S.B.), the Lucille P. Markey Trust for Structural Biology, and a Frederick N. Andrews Fellowship (D.M.B).





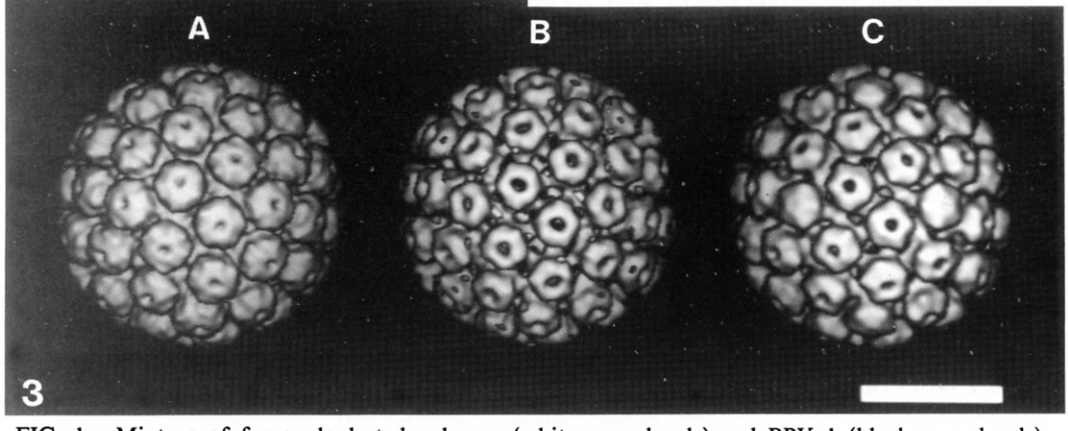


FIG. 1.--Mixture of frozen-hydrated polyoma (white arrowheads) and BPV-1 (black arrowheads). Polyoma is distinguished from BPV-1 by its smaller size (~50 vs. 60 nm diameter). Bar = 100 nm.
FIG. 2.--Radial density plots of spherically-averaged polyoma structures. Solid, dashed, and dotted curves correspond, respectively, to unmodified x-ray model, x-ray model with applied CTF ("defocused"), and EM reconstruction.
FIG. 3.--Surface-shaded views (along two-fold symmetry axis) of polyoma structures: A. Unaltered x-ray structure. B. "Defocused" x-ray structure. C. EM reconstruction computed from 21 polyoma images. Bar = 25 nm.