

which monocular depth cues were used to compare 2-D and 3-D metrics. For example, the optimal interval between frames is greater for objects when they appear to lie at different depths (13), and minimum frame duration for equal angular rotations in the frontal and depth planes is similar (14). It therefore seems likely that both monocular and disparity cues can be used to compute 3-D proximity.

Our results suggest that correspondence matching makes use of a 3-D spatial representation and that depth, or at least disparity, must be determined before motion matching is performed. This conclusion seems to hold for computer as well as biological vision. Correspondence-matching algorithms can also be improved by using disparity to assign depth (15).

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8. The disks all appeared the same size. There was no obvious effect of size constancy.
9. One pair of disks was always in front of the fixation plane and the other behind. Since fixation was at zero disparity, one pair was in crossed disparity while the other was uncrossed. Similar effects could be obtained when both pairs were at different crossed or uncrossed disparities. Relative rather than absolute disparities seemed to be important.
10. Observers differed somewhat in their sensitivity to frame duration. The 167-msec duration produced

the best motion for J.V.O. and the poorest for K.C. At this speed, K.C., who had never seen stereograms before, found that the disks sometimes dissolved into the background. We believe that J.V.O., being experienced in viewing stereograms, was better at maintaining fusion. When extra fusion cues were provided by darkening the disks, K.C.'s performance was 93 to 100% at all frame rates.

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16. Dedicated to the memory of Paul Kolars. Supported by NIH grant EY05849 to M.G. We thank S. Anstis and H. Ono for helpful comments.

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## Technical Comments

### Nucleosome Structure

Harauz and Ottensmeyer (1) present a structural model for the nucleosome core produced from electron energy loss (EEL) imaging and a new technique for three-dimensional (3-D) reconstruction. Their work can be criticized on two grounds. First, the EEL imaging required doses of electron irradiation that are known to destroy the high-resolution structure that was being imaged. Second, their novel reconstruction technique appears to depend largely on subjective judgments for the fit and selection of images. There are no objective criteria for determining the validity of the images or the reconstruction.

The authors' apparent assumption that the fine details in the EEL images reflect the high-resolution structure of the native nucleosome seems unwarranted in view of the very large electron dose required to obtain them—1000 electrons per square angstrom. Loss of high-resolution order has been demonstrated most precisely for crystalline specimens, where doses of one to ten electrons per square angstrom cause fading and loss of the diffraction pattern (2). This may only show loss of long-range order, but higher resolution has not been convincingly demonstrated for single protein molecules (3).

Perhaps the greatest effect of radiation damage is the loss of 50% of the mass of biological macromolecules, which occurs at a dose of 100 electrons per square angstrom. This has been demonstrated for a variety of model systems (4) and would mean that 50% of the organic matter

(and an undetermined amount of the phosphorus) in the nucleosomes had been blasted away before the image recording could be completed. It is not clear how the residue from such an incineration could reorganize into a skeleton that retains the high-resolution structure of the protein and DNA.

If the images are artifacts, how could a 3-D model be reconstructed? I suggest that their reconstruction system should be easily capable of fitting noise into a plausible model. The only data presented are in a single, tiny image area. One can assume that other images are similar: three to six grainy splotches or streaks within the boundary presumed for the particle. With this limited detail, it is not surprising that most images could be rotated to fit at least one helix projection. The authors state that fully half the "images were rejected if the nucleosomes appeared distorted or severely altered by the electron bombardment." This selection, with no objective criteria for determining the goodness of fit or the validity of each image, raises doubts about the reconstruction.

It seems that the authors increased the pitch of the DNA superhelix from 2.8 to 4.0 nm to obtain a reasonable fit. Clearly this implies that the images are not just random noise, because they constrain the model. I suggest, however, that the only nonrandom feature is the spacing of the streaks and splotches. They are about 4 nm apart, which may reflect the transfer function and optical resolution of their image (like the granularity of the carbon film in conventional microscopy). Obviously one could only get a good

fit if the model had spacings close to those in the image, so the pitch would have to be increased to 4 nm.

Finally, other work on EEL imaging suggests that some fraction, perhaps large, of the detail in Ottensmeyer's images may be amplitude contrast (5). One must be concerned, therefore, that the contrast reflects primarily the mass density and granularity of the specimen and carbon film, with phosphorus making only a small contribution.

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*Response:* Erickson's comments on high-dose imaging are historical theoretical concerns that have been addressed and an-