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7. The length of the standard genomes (measured by sizing of restriction fragments) varied by up to 450 bp, as described by L. D. Densmore, J. W. Wright, W. M. Brown, *Genetics* **110**, 689 (1985). The region containing continuous length variation (CV) was duplicated in L and L* (Fig. 2). The novel "3.2"-kb Sst II fragment in L and L* contains a CV region and thus varies from 3.2 to 3.4 kb.
8. Cleavage site comparisons of widely distributed *C. exsanguis* give sequence divergence estimates from 0.16% to 0.67% (C. Moritz, J. W. Wright, W. M. Brown, unpublished data). Methods for restriction endonuclease analysis are given in Densmore *et al.* (7).
9. ³²P-labeled probes were obtained by nick-translation [P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977)] of (i) the 4.8-kb Pvu II fragment, isolated from a low-melting point agarose gel [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)], (ii) the *C. exsanguis* 17.4-kb mtDNA genome, and (iii) cloned mitochondrial rRNA genes from gorilla [1.4-kb fragment; J. E. Hixson and W. M. Brown, *Mol. Biol. Evol.* **3**, 1 (1986)]. Digested mtDNA was transferred from 1.0% agarose gels to nylon membranes (GeneScreen Plus; DuPont) by the acid-depurination/alkali transfer method of K. C. Reed and D. A. Mann [*Nucleic Acids Res.* **13**, 7207 (1985)]. The hybridization protocol followed by Maniatis *et al.* above. The filter was washed under stringent conditions: 0.1× SSC (saline sodium citrate) (0.75× SSC for the gorilla mtDNA probe) at 60°C. Prior to incubation with a new probe, the previously bound probe was removed by washing in 0.4N NaOH at 37°C until no beta emission was detectable.
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20. We thank J. W. Wright for providing specimens; D. Foran, L. Szura, and T. Dowling for technical help; T. Dowling, D. Foran, T. Gharrett, J. Palmer, and L. Vawter for comments on the manuscript; G. P. Wallis for permission to cite an unpublished manuscript; M. Van Bolt for illustration; and D. Bay for photography. Supported by a Thurnau postdoctoral fellowship to C.M. and grants from NSF (BSR-8516645, BSR-8517830) and NIH (GM30144).

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Correspondence Matching in Apparent Motion: Evidence for Three-Dimensional Spatial Representation

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The path of an object in apparent motion depends on correspondence matching, the decision that images seen at different places and at different times represent the same object. One determinant of correspondence is proximity. Still debated, however, is whether proximity is defined in a two- or three-dimensional spatial representation. Observers judged the motion path taken by an object with two neighbors of different apparent depth. Given similar two-dimensional distances, objects moved toward the neighbor of the same apparent depth. This is evidence that correspondence operates in a three-dimensional spatial representation.

VIEWING A SEQUENCE OF STATIC pictures, or "frames," may produce a compelling experience of apparent motion. This apparent motion requires the matching of images seen at different places and at different times. If each frame contains multiple images, the visual system is confronted with a problem; each image in one frame has several potential matches in the next. How does the visual system decide which images correspond and represent the same object? The solution to this "correspondence problem" lies in the application of two heuristics: (i) match images of similar form and (ii) match images that are nearest neighbors in space. Although it has proven difficult to determine the relevant form properties (1), recent studies have demonstrated preferential matching between objects of similar orientation (2, 3), spatial frequency (2), luminance polarity (4), and color (4).

Several studies (5, 6) have also demonstrated the importance of proximity. Given a choice of several alternatives, objects tend to match their nearest neighbor. A remaining

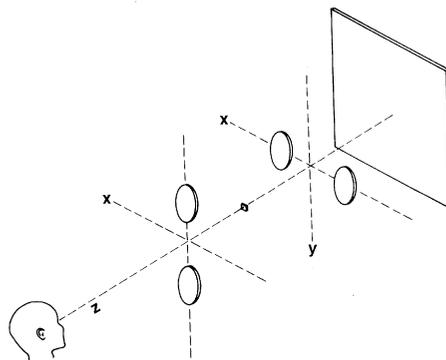


Fig. 1. Schematic representation of the display as seen by the observers. Viewing distance was 100 cm.

question is whether nearest neighbor is defined in two-dimensional (2-D) retinal coordinates or by distance in an internal, three-dimensional (3-D) reconstruction of space. Previous studies (5, 7) suggested that correspondence operates only on 2-D retinal coordinates. We have found, however, that objects preferentially match neighbors of the same retinal disparity—evidence that correspondence uses a 3-D proximity metric.

We controlled apparent depth by varying binocular disparity, the relative position of images on the two retinæ. Each frame in the display was a stereogram consisting of separate left- and right-eye random "dot" matrices made from equal numbers of light (169.0 cd m⁻²) and dark (0.2 cd m⁻²) squares. All frames contained a background matrix, four submatrices, and a red fixation square (Fig. 1). The background was viewed with an uncrossed disparity of 24 arc min, so that it appeared far behind the fixation mark. Using this as a base, we added four disk-shaped submatrices, each having a diameter of 1.3°. The submatrices were presented as pairs of different apparent depth (12 arc min crossed and 12 arc min uncrossed) lying on the circumference of an imaginary circle with the fixation square at the center. The radius from the center of the

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fixation mark to the center of each disk was 1.8° . When the stereograms were fused, the fixation square and pairs of disks appeared to float in front of the background (8).

Observers viewed a series of eight frames in which the disks' positions were rotated by 45° steps. Two successive frames of the display are shown schematically in Fig. 2. If correspondence matching is based only on 2-D proximity, direction of rotation is ambiguous; each object in frame 1 is equidistant from two potential matches in frame 2. If 3-D proximity is used in the distance metric, images should match neighbors in the clockwise direction. When viewing the sequence of frames, observers readily perceived clockwise motion.

We tested an experienced (J.V.O.) and an experimentally naïve (K.C.) observer. Direction of rotation and frame duration were randomized across trials, and disparity (9) was varied between blocks. After each frame sequence, observers made a forced-choice decision about direction of apparent motion. Large disparities produced motion toward the neighbor in the same depth plane (Fig. 3A) (10). Decreasing disparity reduced the preference, and at zero disparity, when there were two equidistant neighbors, direction of motion was totally ambiguous. Similar results were obtained with the disks arrayed on circles with larger (2.5°) and smaller (1.2°) radii.

The generality of our initial experiment was limited in two ways. (i) Unlike most objects in the real world, our test images were cyclopean, that is, invisible when

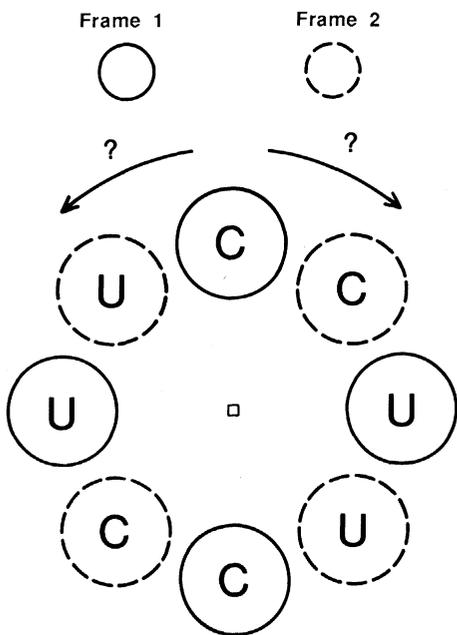


Fig. 2. Visual display in the x - y plane. "U" disks were in uncrossed disparity, and "C" disks were in crossed disparity. The C disks appeared to be nearer and the U disks farther away.

viewed monocularly. (ii) We used only slow rotation rates since the disks would dissolve into the background if frame duration were too brief. To produce monocularly visible images and prevent dissolution, we darkened the light squares in the disks to 10.0 cd m^{-2} . Observers then perceived dark gray disks floating in front of the lighter background. We tested two observers (K.C. and M.G., 60 trials per condition) with a disparity difference of 24 arc min and added an 84-msec condition. Rotational motion in these conditions was even clearer. Motion toward the neighbor of the same depth was seen on 100% of the trials at 84, 167, and 334 msec and on 93% of trials at 668 msec. We supposed that the dark edges provided an additional fusion cue and prevented the disks from dissolving.

A control experiment ensured that observers had not used monocular motion cues to discriminate direction. Braddick (11) has shown that monocular presentation of stereograms may give rise to a "short-range" motion percept. Although they reported judging the motion of solid disks of differing depth, our observers were retested with one eye occluded. Cyclopean disks created a torus of incoherent motion, whereas dark gray disks produced flicker. In neither case were observers able to perform at better than chance levels. This result indicates that the step sizes used in the experiment stimulated only a long-range motion process.

These results suggested that correspondence matching occurs in a spatial representation where proximities are determined tridimensionally. If so, it should be possible to trade distances in the frontal and depth planes. We tested this supposition by repeating our experiment with greater rotation angles. Suppose that images rotate clockwise by slightly more than 45° . Each object will have a single nearest neighbor in the x - y plane. However, this neighbor will be at a different disparity and lie in the "backward" counterclockwise direction. Therefore, 2-D matching predicts that clockwise rotations with angles greater than 45° should produce counterclockwise rotation. If matching occurs in 3-D space, the x - y plane proximities must overcome the additional distance created by the differences in depth. As a result, rotations slightly greater than 45° should still produce "forward" clockwise motion. At a sufficiently large rotation, however, distances between images in different planes become smaller, and "backward" motion should be seen. The angle at which backward motion occurs should depend on the size of the disparity; for larger disparities, there are greater distances in the z direction to overcome. Larger rotation angles should be needed to produce backward motion.

We tested these predictions with an experiment in which both rotation angle and disparity were randomized across trials. The disks were dark gray, the radius of the imaginary circle was 1.4° , and duration was 137 msec. Figure 3B shows that forward motion was still the dominant percept for rotation angles slightly greater than 45° . At sufficiently large rotations, however, backward motion, accompanied by a movement in depth, became more frequent. Our results also confirm the prediction that larger disparities require a greater rotation angle before backward motion is seen.

Disparity is only one of many possible depth cues, and we do not know whether monocular depth cues would also produce matching based on 3-D proximities. Our display probably produced long-range motion, which is thought to occur at a relatively advanced stage of perceptual processing (11, 12). Disparity cues, in contrast, are presumably used at an early level of the visual system. This particular situation may have favored the use of a 3-D spatial representation in matching. However, our results are consistent with data from other studies in

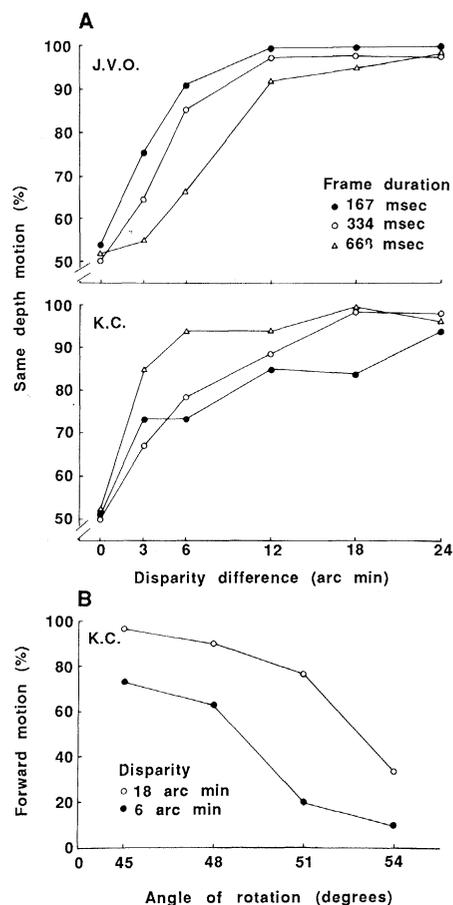


Fig. 3. (A) "Correct" motion reports as a function of disparity. Each data point is based on 60 judgments. (B) "Forward" responses as a function of rotation angle. Each data point was based on 60 judgments of K.C.

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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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-