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Computer-assisted Reconstruction of Vertebrate Embryos from Serial Histological Sections^{1,2}

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Abstract. "HVEM-3D" software has been used to reconstruct the three-dimensional appearance of vertebrate embryos from thick histological sections. Owing to the value of such reconstructions in embryology teaching laboratories and to the growing popularity of computer-assisted instruction in the life sciences generally, we have endeavored to assemble a reliable and versatile system, but one with austere requirements for hardware and software. Output may be viewed on an EGA-compatible color monitor, as 35-mm color transparencies, or as black-and-white hardcopy generated by laser printer.

Serial histological sections of vertebrate embryos give beginning students a limited impression of embryonic anatomy. Students must learn to merge the two-dimensional information on germ layers and organ rudiments into a cogent, three-dimensional image of the organism. Mental reconstruction is a daunting challenge to many individuals. To alleviate their initial frustration and to promote understanding, we have adapted "HVEM-3D" software to the reconstruction of frog, chicken, and pig embryos, the standard laboratory specimens in descriptive embryology courses. Current versions of the software are adequate for reconstructing the younger, structurally simpler embryos, and forthcoming versions should accommodate the older, more complex specimens.

We have found that informative images may be generated by taking simple

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² Abbreviations used in this report include: ASCII, American Standard Code for Information Interchange; CGA, Color Graphics Adapter; EGA, Enhanced Graphics Adapter; MDA, Monochrome Display Adapter; MS-DOS, Microsoft Disk Operating System; RAM, random access memory; TSR, terminate-and-stay-resident; VGA, Video Graphics Array.

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precautions when photographing sections, selecting fiducial (alignment) points, assigning colored contours to embryonic components, and making hardware adjustments to stay within the limited file size imposed by the software. This report summarizes our recommendations for handling large numbers of histological sections and, recognizing the constraints on most teaching budgets, for assembling a reliable and versatile system with austere requirements for hardware and software. Portions of this report have been presented in abstract form (Cavey et al., 1991).

MATERIALS AND METHODS

Serial transverse sections of a 33-h chicken embryo were photographed with a Nikon Optiphot compound microscope equipped with planachromatic objective lenses and a Microflex HFX-II photomicrographic attachment. Exposures were made on Kodak Panatomic-X[®] film. The film was overexposed and underdeveloped, yielding negatives with high informational content and extremely fine grain. These 35-mm negatives were printed full-frame after enlargement to fit standard (8 × 10-inch) sheets of photographic paper.

Version 1.386EGA of the "HVEM-3D" software⁴ (Kinnamon et al., 1986; Royer, 1988; Young et al., 1987) was configured to operate on an IBM XT-compatible computer. (A separate version of "HVEM-3D" is available for IBM AT-compatible systems based on the 32-bit 80386 processor.) Our base system was a Zenith Z-157 personal computer with 640 kilobytes of RAM, a 4.77-MHz 8088 processor, and a 8087 numeric coprocessor operating under "MS-DOS" Version 3.3 Plus (Zenith Data Systems Corporation, St. Joseph, Missouri, U.S.A.). Storage devices included a 5.25-inch, 360-kilobyte floppy drive and a 20-megabyte hard drive. The dual monitor system, dictated by the software, consisted of a Zenith ZCM-1490 Flat Technology color monitor served by a Zenith Z-549 VGA video card with 256 kilobytes of memory and a Roland DataTrain V272A monochrome monitor linked to a V-Tech MDA/CGA video card. These monitors were used to display graphics and text, respectively. A Hitachi HDG-1111C digitizing tablet (emulating a SummaGraphics SummaSketch[®] MM1201 tablet) was used to trace the 10- μ m-thick sections.

The text editor in the TSR program "Sidekick" Version 1.56B (Borland International, Toronto, Ontario, Canada) was used to create procedure files in ASCII text format. Procedure files contain the parameters for reconstruction, and these files may be created or edited without leaving "HVEM-3D" through use of a text editor. Reconstructed images were captured with the FRIEZE utility from "PC Paintbrush IV Plus" Version 1.0 (ZSoft Corporation, Marietta, Georgia, U.S.A.). Reconstructed images were saved to diskette for later retrieval and printing in "PC Paintbrush IV Plus" configured for either a 16-color EGA display (640 × 350 pixels) or a 256-color VGA display (640 × 480 pixels) and a Hewlett-Packard LaserJet[®] series II printer.

A Minolta SRT-200 single-lens reflex camera, fitted with a MC Rokkor 50-mm macro lens and mounted on a sturdy tripod, was used to produce 35-mm color slides. With the front element of the lens positioned about 43 cm from the screen of the graphics monitor, photography of the reconstructed embryos

was accomplished easily in a darkened room. Transparencies were produced with Kodak Ektachrome® 100 film or Kodak Ektachrome® 100 Plus Professional film using 1-sec exposures and f-stops in the range of 5.6 to 8. The latter film, with its color-saturating characteristics, is especially well suited for this application.

RESULTS

Photomicrography and Data Input

The unelevated blastoderm of the sectioned embryo was oriented parallel to the long axis of the film frame. Consistent framing of the sections was accomplished by selecting prominent embryological structures as future fiducial points (Fig. 1A–E). Sections were centered horizontally on the notochord or notochordal process and aligned so that the point where epidermal ectoderm on the right side of the body leaves the frame was centered vertically. In anterior sections through the head fold and in posterior sections through the primitive streak, a notochord is absent. At these levels, the embryo was centered horizontally on the midline of the brain or the midline of the primitive streak. When printing negatives, maintenance of uniform magnification, consistent orientation, and proper alignment of the images were necessary.

On the photomicrographs, structures destined for the reconstructed image were chosen carefully, and their contours were marked directly on the photographic prints with fine-tipped indelible pens, leaving distinct sets of lines for subsequent tracing at the digitizing tablet. Fiducial points also were noted on the prints. Color-coding of primary germ layers (blue for ectoderm, red for mesoderm, green for notochordal mesoderm, and yellow for endoderm) and fiducial points simplified data input at the digitizing tablet and improved the informational content of the final reconstruction. These preliminary steps, although rather time-consuming, standardized the process of digitization and ensured that individual sections could be reproduced precisely if necessary.

Owing to the large number of tracings necessary to depict an embryo faithfully, the BCNF option in “HVEM-3D” was used. BCNF consists of a data-entry program (BIGED) and a reconstruction program (BIGREC). The main menu of BIGED allows: (1) the entry of object contours and fiducial points with the TRACE option; (2) the examination and printing of file information about contours with the DIRECTORY option; (3) the VIEWing of contours and fiducial points; (4) the ability to DELETE and UNDELETE objects and planes; (5) the opportunity to make CHANGES in the file description, magnification, or section thickness; and (6) the option to ALIGN planes by the fiducial points and sort in ascending order. Once in BIGED, the section magnification (in thousands) and section thickness (in micrometers) must be entered for new files. The magnification of our photomicrographs was 175 $\times$, and the section thickness was 10 μ m. By entering these values, however, the entire reconstructed embryo could be displayed only on the graphics monitor at certain angles and at reduced display magnifications (Fig. 2A–E).

A type number for each primary germ layer or organ rudiment is entered

FIG. 1. A 33-h chicken embryo. A. Whole mount, dorsal view, photographed with a Wild M5 stereomicroscope. The embryo (if, intersomitic furrow; ip, anterior intestinal portal; np, neural plate; op, optic vesicle) develops in the inner zone, or area pellucida, of the blastoderm. A large circumferential blood vessel, the sinus terminalis (arrowhead), arises at the junction of the area pellucida and area opaca. Levels of the histological sections in Fig. 1B-E are indicated. B. Transverse section through the mesencephalon (me). The head fold of the embryo (aa, aortic arch I; no, notochord; pl, pharyngeal plate) projects over the proamnion (pa) of the area pellucida. C. Transverse section through the primitive ventricle of the heart (h). The heart is suspended in the coelom

into the procedure file (Table I). The type number defines the drawing parameters for an object and, if the numbers are unique, different combinations of germ layers and rudiments may be selected and viewed in the reconstructions (Fig. 3A–E). Further, if each object has its own TYPE NUMBER, the LINE COLOR, LINE WIDTH, and NO FILL/FILL options may be modified in the procedure file to optimize the appearance of the object in the reconstruction.

Each photomicrograph was placed in turn on the digitizing tablet, loosely aligned by the corners with framing guides, and secured with masking tape. In “HVEM-3D,” each object has a minimum record size of 100 points. Up to 2,000 points may be recorded for an object. With all objects consisting of the minimum 100 points, BCNF can handle a maximum of 3,800 contours. Before digitization begins in earnest, a representative plane should be entered at the default tracing speed to ascertain the number of 100-point records that will be needed. By multiplying the total number of sections by the number of 100-point objects in a typical plane, one can estimate if all sections will fit into the maximal file size allowed by ‘HVEM-3D.’

To ensure that a reasonable facsimile of the embryo is generated, sufficient sections must be entered without exceeding the maximum allowable file size. We elected to trace every second section of the embryo at a reduced plotting speed, thus lowering the number of data coordinates gathered per second and, hence, the amount of memory occupied by the reconstruction data. A total of 292 sections were photographed originally and numbered, but of these only the odd-numbered sections were digitized. Actual section numbers were maintained so that intervening sections could be added later if sufficient memory remained. Data files for even-numbered sections were left empty, even though BIGED has a facility to log blank planes for proper spacing. The blank planes logged by BIGED, unfortunately, must be assigned fiducial points, a requirement that can waste valuable memory when dealing with large numbers of sections.

In the anteromost and posteromost sections, where a notochord is absent, the points where ectoderm leaves the field were used as fiducial points. The notochord, when present, also was used as a fiducial point, but the specification of three points resulted in malalignment of median structures of the embryo. By specifying just two points (the right edge of the embryo and the notochord), sections were aligned acceptably in the reconstruction. After entering or editing data, the sections are ALIGNed from the main menu of BIGED. Speed of alignment is, of course, dependent upon the number of sections on file.

←

beneath the pharynx (ph). D. Transverse section illustrating the open gut. Other prominent features of the body include a pair of dorsal aortae (da), segmental somites (s), and lateral plate mesoderm (lp). E. Transverse section through the primitive node (pn) of the primitive streak. The space between the open gut and uncleaved yolk is the subgerminal cavity (sgc). Scale bars represent 1 mm (A) and 100 μ m (B–E).

FIG. 2. Reconstruction of a 33-h chicken embryo (cf. Fig. 1A-E). A. Reconstruction of an embryo at actual magnification and section thickness reveals little structural detail. B. Digitized plane through the mesencephalon shows how the neural tube has been represented as two quasi-concentric cylinders. A subcephalic pocket (scp) appears between the proamnion and the head fold (da, dorsal aorta; ep, prospective epidermis; hm, head mesenchymal cell; va, ventral aortae). C. The primitive ventricle of the heart, like the rhombencephalon (re), is digitized with outer and inner contours. The coelom (c) appears between the somatic mesoderm (som) and splanchnic mesoderm (spl) of the lateral plate. D. Somites and intermediate mesoderm (im) are closed, filled contours, but somatic and splanchnic mesoderms are represented by open contours. og, open gut; sp, spinal cord. E. The notochordal process and mesoderm beneath the primitive streak (pp, primitive pit) are portrayed as closed, filled contours.

TABLE I
Coding of embryonic structures in the “HVEM-3D” procedure file

Contour or object	Software setting						
	TYPE NUMBER ^a	LINE WIDTH ^b	LINE COLOR ^c	INTERIOR COLOR 1 ^c	INTERIOR COLOR 2 ^c	CLOSED/ OPEN ^d	NOFILL/ FILL ^e
Mesoderm, notochordal	1	0	10	10	10	1	1
Ectoderm, neural	2	1	9	—	—	0	0
Mesoderm, paraxial	3	0	4	4	4	1	1
Mesoderm, intermediate	4	0	12	12	12	1	1
Mesoderm, lateral plate	5	1	12	—	—	0	0
Ectoderm, epidermal	6	1	11	—	—	0	0
Endoderm	7	1	14	—	—	0	0
Mesoderm: blood vessels and heart	8	0	4	12	4	1	1
Mesoderm, prechordal	9	2	4	—	—	0	0
Mesoderm in the primitive ridges and groove; unsegmented mesoderm in the blastocoel	10	0	12	12	12	1	1
Mesoderm in the epiblast	11	0	4	—	—	0	0
Intersomitic furrows	12	0	4	—	—	0	0

^a Program offers 256 choices for TYPE NUMBER: 0 to 255.
^b Program offers five choices for LINE WIDTH: 0 (thinnest) to 4 (thickest).
^c EGA limitation to 16 solid colors: 0 (black), 1 (royal blue), 2 (kelly green), 3 (dark blue-green), 4 (dark red), 5 (purple), 6 (orange), 7 (gray), 8 (dark brown-gray), 9 (medium blue), 10 (lime green), 11 (sky blue), 12 (bright red), 13 (lavender), 14 (yellow), and 15 (white).
^d Program offers two choices: 0 (open) and 1 (closed).
^e Program offers four choices: 0 (no fill), 1 (filled: solid color which covers previously drawn elements and which, in turn, may be covered), 2 (autofill: solid color which covers only its own outline, fill color(s), and blank portions of the screen), and 3 (extended autofill: option to make an enveloping structure “transparent” relative to an enclosed structure).

Reconstruction of the Embryo

BIGREC reconstructs a three-dimensional image of the serial sections entered with BIGED. Main menu selections in BIGREC include: (1) the X ANGLE, Y ANGLE, and Z ANGLE; (2) the VIEWING DISTANCE; (3) the FILTER; (4) the DISPLAY MAGNIFICATION; (5) the Z SCALE (section thickness); and (6) the FILL/NOFILL mode. There also are options on the main screen of BIGREC to GET or MAKE a new procedure file, to VIEW RECONSTRUCTION, to MAKE PICTURE FILE, to GRAB RECONSTRUCTION, and to PRINT RECONSTRUCTION.

Our procedure file (Table I) was kept as simple as possible, thus allowing the embryo to be rotated about the x-axis (left-right axis of the embryo), the y-axis (dorsal-ventral axis of the embryo), and the z-axis (anterior-posterior axis of the embryo) and ensuring that the same (or similar) file could be used for other embryos. Within the procedure file, appropriate colors are matched to the primary germ layers and organ rudiments. For the viewing distance and magnification used, the narrower lines were best suited to photography and

laser printing; the narrowest line was sometimes too thin for laser-printer output. The intermediate and wider lines frequently concealed underlying structures. Few structures were filled, again because of a tendency to obscure deeper structures, even when the transparent-fill option was used.

Reconstructions were manipulated by changes to the VIEWING MODES (NOFILL or FILL), the X, Y, and Z ANGLES (0° to 360°), and the FILTER. Default values for DISPLAY MAGNIFICATION ($1.0\times$) and VIEWING DISTANCE (10,000) were used. The section magnification was unchanged from the BIGED entries ($175\times$), but because only every second plane was entered, the Z SCALE was changed to $20\ \mu\text{m}$. Under these conditions, the reconstructed embryos were proportioned correctly, but they could not be viewed in their entirety. Reducing the DISPLAY MAGNIFICATION to $0.4\times$ made possible fitting the entire embryo on the monitor screen at most angles of rotation; however, reducing the VIEWING DISTANCE tended to distort the perspective. To alleviate such shortcomings, we opted instead to alter the values for section magnification and section thickness. After setting the magnification to $100,000\times$ and the thickness to $0.01\ \mu\text{m}$, default values for DISPLAY MAGNIFICATION and VIEWING DISTANCE could be used at any angle of rotation (Fig. 4A–C). The resulting reconstruction is, of course, compressed in the anterior-posterior axis by the reduced spacing between sections.

Reconstruction of an embryo from every second section in the set of 146 images, did not accentuate the three-dimensional effect. Epidermal ectoderm, for example, appeared as a continuous sheet of color rather than as discrete contours, concealing intraembryonic structures when viewed from various angles. In the procedure file, the reconstruction was modified to display every

FIG. 3. Systemic views of a 33-h chicken embryo. A. Epidermal ectoderm. hf, head fold; scp, subcephalic pocket. B. Neural ectoderm. me, mesencephalon; pe, prosencephalon; re, rhombencephalon; sp, spinal cord. C. Mesoderms. hm, head mesenchymal cell; im, intermediate mesoderm; lp, lateral plate mesoderm; no, notochord; s, somite. Notochordal mesoderm internalizes via the primitive pit (arrowhead) of the primitive streak. D. Heart (bc, bulbus cordis; sa, sinoatrial region; v, primitive ventricle) and blood vessels (da, dorsal aortae). Posterolateral branches of the dorsal aortae are the future vitelline arteries (arrowheads). E. Endodermal derivatives (og, open gut; ph, pharynx). ip, anterior intestinal portal.

FIG. 4. Unique views of a reconstructed 33-h chicken embryo. A. Using adjusted magnification and section thickness, the embryo appears compressed, but structural details such as the heart may be seen between the open gut (og) and the pharynx. op, optic vesicles of the prosencephalon. B. A posteroventral view of the embryo shows the heart (h) and anterior intestinal portal (ip). lp, lateral plate mesoderm; ps, primitive streak. C. Magnified view of the anterior end of the embryo reveals surface contours and features such as the subcephalic pocket and pericardial cavity. ep, prospective epidermis; pa, proamnion of the area pellucida; pe, prosencephalon; sa, sinoatrial region; v, primitive ventricle.

FIG. 5. Rotational sequence of four frames. A. This perspective is from 45° above the blastoderm. At the rear, the primitive node is evident. B. A 60° counter-clockwise rotation on the y-axis reveals the pericardial cavity and the subcephalic pocket beneath the head fold. C. At 120° of rotation about the y-axis, surface contours are shown clearly. D. A 180° rotation completes this sequence, providing a view from the posterior end of the primitive streak toward the head fold.

0°

5A

60°

5B

120°

5C

180°

5D

fourth section. This modification reduced the number of sections used to 73, introducing greater separation between planes and revealing deeper structures with reasonable clarity. Reconstruction time, which is influenced by the number of sections on file, the number of contours within each section, and the plotting speed of the digitizing tablet, seems relatively unaffected by the FILTER option, because the minimum and maximum settings had negligible effects on the delivery time for a completed reconstruction.

The MAKE PICTURE FILE option in BIGREC was used in conjunction with the SHOW animation program to depict rotation of an embryo about the y-axis (Fig. 5A–D). Our 4.77-MHz computer required approximately 8 h and 1.5 megabytes of hard-disk space to create a picture file that rotated the embryo a full 360° in increments of 10°. The resulting 36 frames were viewed in sequence using the SHOW program. The number of frames in a full rotation must not be reduced too far, or the animation will become disjointed.

BIGREC can capture the graphics screen with its GRAB option. Although several TSR screen-capture utilities are available, we chose the FRIEZE utility, owing to its ability to frame the image on the screen and to save in the PCX file format. The PCX format was important in allowing the manipulation of images and their high-resolution reproduction on a laser printer. After creating a reconstruction, selection of the GRAB option pauses the program after reversing the background color of the graphics monitor from black to white. FRIEZE, then, is activated and the image is framed and either saved to diskette or printed. Only images reconstructed in BIGREC can be captured. Individual frames of a SHOW program picture file must be recreated and captured individually to obtain a rotational sequence for laser-printer output. After using FRIEZE, before another reconstruction can be viewed, the GRAB option must be selected again, before pressing the ESCape key to exit. These keystrokes reset the video memory so that the new image does not overlap the old one. (This idiosyncrasy may be unique to the hardware configuration of our particular system.)

Images saved in the PCX format may be loaded into "PC Paintbrush IV Plus." If a VGA card capable of displaying 256 colors is used, the first 16 colors of the palette must be modified to recreate the colors used by the image in BIGREC. An alternate method is to install the "PC Paintbrush IV Plus" program with the 16-color EGA option and to enable the SOLID COLORS ONLY option. Within "PC Paintbrush IV Plus," the PRINT option is used to generate laser-printer output. Settings within the PRINT option allow the image to be reproduced at any size within the memory constraints of the computer. Before printing, HALFTONE SETTINGS should be modified. With mode set to FAT-TING and BRIGHTNESS set to 176 and 150 lines per inch, the laser-printer outputs have good clarity, and the printed pages make excellent masters for photocopying.

DISCUSSION

Tracing contours and marking fiducial points on the photomicrographs serve to standardize the task of digitization and to moderate the complexity of the

final reconstruction. If too much detail is entered into "HVEM-3D," the reconstructed image has a cluttered appearance, and there is always a risk of exceeding the maximum allowable file size. Use of actual values for section magnification and section thickness in the reconstructions will produce images with proper proportions but also will reduce magnification significantly and thus obscure structural details. Experimentation with DISPLAY MAGNIFICATION is mandatory to ensure maximum enlargement of those specimens intended for rotation.

Increasing the section magnification and decreasing the section-spacing were preferable to changing the DISPLAY MAGNIFICATION and VIEWING DISTANCE. By reducing the spacing to the minimum value acceptable to "HVEM-3D," individual planes were moved closer together, and the entire embryo still appeared on the monitor screen at any angle of rotation. By setting up the data files in this manner, reconstructions were simplified somewhat by use of default settings in several data fields.

"HVEM-3D" is able to create three-dimensional images from thick histological sections, but compromises are necessary to handle large numbers of sections. A future release of the program would ideally include capabilities for handling the larger histological specimens. The ability to magnify areas of the reconstruction for closer inspection would, for example, be a valuable addition to this excellent software package.

Data storage in a single file can sometimes complicate the editing of individual images, especially when trying to delete groups of sections to highlight specific areas of the reconstructions. The deletion of sections is often necessary when centering a reconstruction on the graphics monitor for photography. BIGREC always assumes that every plane is present, even if the procedure file specifies a subset of sections. This unfortunate assumption will invariably offset images of anterior or posterior regions of the embryo to the sides of the monitor screen. Facilities for block-deletion of sections in BIGED or incorporated algorithms allowing BIGREC to center images based on user-selected subsets of planes would be desirable additions to future releases of the program.

The dual monitors for graphics and text provide some flexibility, but they do add to the expense of setting up a computer system. Finding two video cards that do not conflict when installed in the same computer is another potential difficulty. A single-monitor system, slated for a forthcoming release of "HVEM-3D" (J. C. Kinnamon, personal communication), will be more practical and should reduce hardware costs. With the growing popularity of VGA cards, we also look forward to the improved resolution that should devolve to "HVEM-3D" reconstructions. A 256-color palette would be particularly welcome when dealing with embryological reconstructions, where different shades of colors (for the primary germ layers) could be used to represent not only their identities but their contributions to various organ rudiments as well. Color-coding with the EGA version of the software is limited to the 16 available solid colors. The FILL option, for closed contours, can increase the number of available colors to 136 through the use of two-color combinations, but this option usually complicates the final appearance of a reconstructed embryo.

The VGA version of the software, which is now being engineered (J. C. Kinnamon, personal communication), in addition to higher resolution and extended color options, would likely produce a better “transparent” effect with overlapping contours.

For the undergraduate teaching laboratory, the images generated with “HVEM-3D” may be exported to computer-assisted instructional modules that help students to visualize a three-dimensional embryo from two-dimensional sections. With the current availability of multimedia authoring software for IBM PC-compatible computers, instructional modules are increasingly easy to create and customize. Such modules might, for example, include facilities to view an embryo from numerous angles, to rotate the embryo about x-, y-, and z-axes, to isolate different combinations of germ layers and organ rudiments, and to pinpoint sections along the length of the embryo to study their two-dimensional compositions. “Pulling” individual sections from a three-dimensional image would be possible, but the design task would be simplified considerably if “HVEM-3D” stored images in individual files rather than a communal file. BIGREC does have the option, in VIEW RECONSTRUCTION, to pause a reconstruction during assembly, but the superimposition of a two-dimensional plane on those already drawn is less informative than viewing a plane in isolation from the others.

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