22

The Preparation of Stereoscopic 3D Illustrations of Confocal Data Sets for Publications and Slides

Gabriel G. Martins, Alan T. Stonebraker, and Robert G. Summers

1. Introduction

In this chapter we describe a simple method to prepare 3D illustrations from stereo-pairs of images from confocal sections. Methods for preparing both stereo slides and stereo images for publication using Microsoft's PowerPoint® 97 and CorelDraw® 8 are described. The parallel and cross-eyed viewing methods are also described and used to prepare the 3D illustrations. The procedure assumes previous preparation of stereo-pairs from the confocal data sets. Therefore, stereoscopy theory and methods of preparation of stereo-images are not described in this chapter but can be found in the operating manuals for the confocal microscopes and in more detail in ***(1–4)***.

1.1. Considerations Before Starting

Images prepared for a book or for slide projection must be prepared with different values of pixel shift or rotation. In other words, if you try to project slides of stereo-pairs prepared to print in a textbook, they will appear too "deep" and distorted for the audience. This is due to the phenomenon of "distance deformation" that leads to Z-stretched images when viewed from long distances ***(2)***. For slide projection, try to use half the pixel shift (or half the rotation angle) that you would use for publication stereos. Note that this is just a "rule of thumb" and does not necessarily apply to all images and compositions. Often, you will have to try different pixel shifts or rotations, and choose the best. If desired, the formulas used to calculate the rotation or pixel-shift necessary for correct 3D viewing can be found in **ref. *4***.

Make sure both images in a stereo-pair have exactly the same brightness/contrast and sharpness settings, as well as the same magnification.

From: *Methods in Molecular Biology, vol. 122: Confocal Microscopy Methods and Protocols*
Edited by: S. Paddock © Humana Press Inc., Totowa, NJ

1.2. Simple Methods for Viewing Stereoscopic Images

There are basically two approaches for viewing stereo-paired images in 3D. Some methods rely on the use of different colors for both images (e.g., anaglyphs of red-green/blue, producing monochrome 3D images), whereas others rely on the side-by-side display of both images. The latter is a preferable method because it provides a genuine separation of the paired images, allows the display of full-color 3D images, and avoids red/green vision problems.

One way of viewing these 3D images is to use a stereo viewer that causes the eyes to merge the paired stereo-images; unfortunately, these viewers are not always available. Another way is to merge the images yourself using the cross/parallel-eyed methods. It is well worth the time spent learning these viewing methods.

In this chapter, we introduce a simple method for mounting stereoscopic 3D images using Microsoft PowerPoint® 97 for preparation of slides and CorelDraw® 8 for preparation of plates for publication. These methods require the ability to either cross or diverge the eyes to merge the stereo-paired images on screen while working on them. If you are not familiar with these viewing methods read the instructions in the Methods section. Most confocal images can be viewed with either of the methods.

2. Materials

1. A stereo-pair originated from a confocal data-set (or other method)
2. Computer system with:
   - Microsoft Powerpoint® 97 and/or Corel Draw® 8 (or similar drawing/presentation software)
   - Slide printer and/or paper printer
3. 3D viewer for side-by-side prints (stereoscope)
4. Stereo slide projection system ***(3)*** with:
   - Two slide-projectors mounted on an adjustable rig
   - Silver screen
   - Two polarizing filters for projectors
   - Polarized 3D glasses
   - 3D viewers for "twin" slides
5. Accessories for mounting slides:
   - Stereoscope (3D viewer for side-by-side prints)
   - Small fluorescent light box
   - 35-mm Glass slide mounts (e.g., Gepe mounts)
   - Alignment set for mounting 3D film "chips," including alignment grid
   - Ruler

Note: Some of the materials can be acquired through the companies mentioned in the Websites section (page 400).

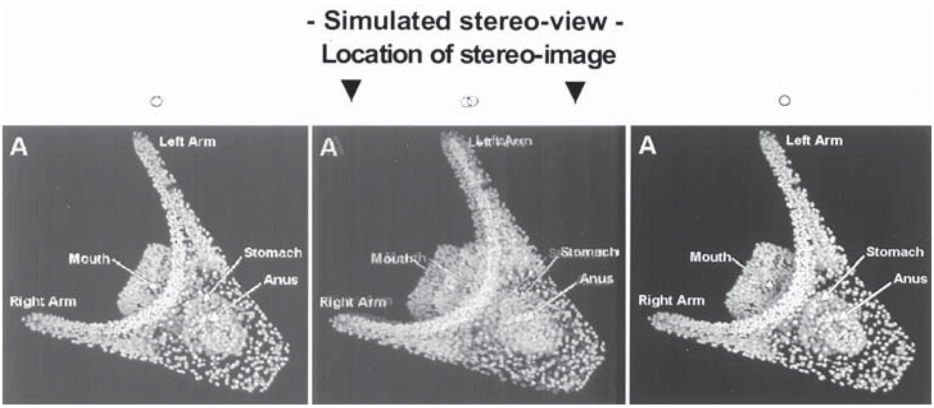


Fig. 1. Representation of the stereo-image resulting from merging the stereo-pair in **Fig. 7A** with the the cross-eyed method. The small circles on top of the image should be used to achieve correct stereo viewing. The goal is to fuse the two circles in the center (location of the stereo-image). Vertical displacement of the circles is corrected by slightly tilting the head and horizontal displacement by changing the degree of convergence of the eyes.

3. Methods

3.1. Viewing Stereo-Images

With paired stereo-images, the trick is to "persuade" each eye to see only its respective image. Once the mind accepts it, the viewer perceives a 3D view. In theory it sounds simple, but accomplishing a comfortable 3D view is difficult and takes practice and patience at first. There are basically two methods (use Fig. 7A as an example).

3.1.1. The Cross-Eyed Method

The idea is to cause the eyes to focus onto a close point (like the tip of the nose!), while looking at a stereo-pair. This way the right eye will focus on the image on the left side, and the left eye vice versa. When both images are focused, they fuse into a central image (*see* **Fig. 1**) that the brain interprets as a 3D image. To practice, try placing your index finger onto Fig. 7A and slowly bring your finger toward your nose. Keep focusing on the tip of your finger but also try to see what happens to the image in the background. It should start to look something like **Fig. 1**. You will perceive three images on the page, the one at the center being somewhat visually confusing.

There is a point where both the finger and the image on the center become less confusing (when your finger is at approx 4 inches from your nose). This middle image is the fusion of the stereo-pair and is gradually perceived as a 3D image. You may have to make small adjustments to your finger, by moving it toward and away from you very slowly.

Also, tilt your head slowly to either side to visually position the two middle circles at the same vertical level. These circles should merge into a single one when correct stereo is achieved. Do not let your eyes uncross, but allow them to focus on the center image (on the location of stereo-image; *see* **Fig. 1**), and remember…RELAX YOUR EYES, WAIT, BE PATIENT AND PERSISTENT! It may take a while, but with practice, your brain will get used to this unusual view and finally perceive the image three-dimensionally.

All the stereo images in this chapter are prepared for cross-eyed viewing, as we find this to be the easiest and most useful method to learn. However, be aware that the conventional display of stereo-pairs for publication is the parallel-eyed method, since it is the method used by most 3D viewers and stereoscopes. We also suggest that everyone obtain stereo-glasses (available from EM and 3D suppliers mentioned in the References section) to view published stereo-images in greater detail.

3.1.2. The Parallel-Eyed Method

Instead of crossing your eyes, the idea is to diverge them as when focusing on a distant object. This method is normally used to view the recently popular random dot stereograms, and is the most common in publications, although it is more difficult and restrictive then cross-eyed viewing. Hold Color Plate IV in front of your eyes and try to focus on a distant point ahead (such as the wall across the room). Then, keeping the eyes diverged, focus on the stereo images on the page. As with the cross-eyed method, you will see three images, the one at the center being somewhat confusing. Again…BE PATIENT...! This method is more difficult to master (see also the "Showing 3D Images" section).

Note: Viewing Fig. 7 (made for cross-eyed viewing) with the parallel-eyed method will produce an image that is inverted with respect to the viewer. This is effective only on a transparent specimen (e.g., a confocal image); otherwise, an unnatural pseudoscopic image is obtained.

3.2. Creating the 3D Illustrations

The procedure described can be easily adapted for any other program with the same capabilities. Be sure to read the section "Techniques and Tricks" (*see* **Notes 1** and **2**), even if you are an experienced computer user.

To prepare slides, start by formatting the page to twice the width of a normal slide (Width-height aspect ratio of 3:1), to accommodate both images of the stereo-pair, side by side. If you are preparing confocal images, the background should be set to black to conform to the unstained background of your confocal image. You should also use a *threshold* function after acquiring the confocal slices (or set the contrast) to create a pure black background on your image. This

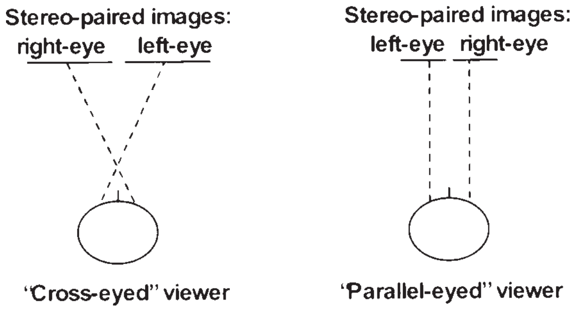


Fig. 2. Diagrammatic representation of the two standard stereo viewing conventions, as seen from above the viewer.

will prevent the appearance of visually confusing spots and blurs on your 3D image. Remember to perform exactly the same transformations on both images of the stereo-pair. To prepare plates for publication using CorelDraw®, create a graphic page of the desired size, according to the instructions for authors. Then, create a black box 5 inches wide to be used as background for the illustration.

To view the stereo image on the computer screen, the cross-eyed method should be used since the parallel-eye method does not work on stereo-images wider than 5 inches (2× the average interpupillary distance—approx 13 cm), while the width of computer screens may vary from 11 inches to 16 inches. With the cross-eyed method the right-eye image is placed on the left side of the stereo-pair and vice versa for the left-eye image (for the parallel-eyed method it is the opposite; *see* **Fig. 2**). Accordingly, insert the right-eye image on the doubled slide (pixel shift to the left or volume rotation to the right) and position it on the left half. Then repeat with the left-eye image and place it in right half. It should look somewhat like **Fig. 3**. After positioning, the images should be cropped to eliminate any areas that do not contain important objects or points of interest. When preparing stereo-pairs for publication, usable area is precious because the image size is constrained to 5 inches, because of the requirements for parallel-eyed configuration. If cropping is done, the images should be resized identically to fill the printable area, and repositioned.

To create a 3D image comfortable for viewing, it is essential to have perfect horizontal alignment of both images of the stereo-pair. To do this, first activate the *guides*. Then move one of the images (using the <ALT> key + mouse) to make homologous points of both images coincident with the horizontal guide (*see* Fig. 5). Notice that, in PowerPoint, up to eight vertical and 8 horizontal guides can be created. Repeat this process for several points from top to bottom on the stereo-pair (*see* Fig. 5). If the images are exactly at the same zoom (magnification) then all points should be simultaneously adjustable at the same

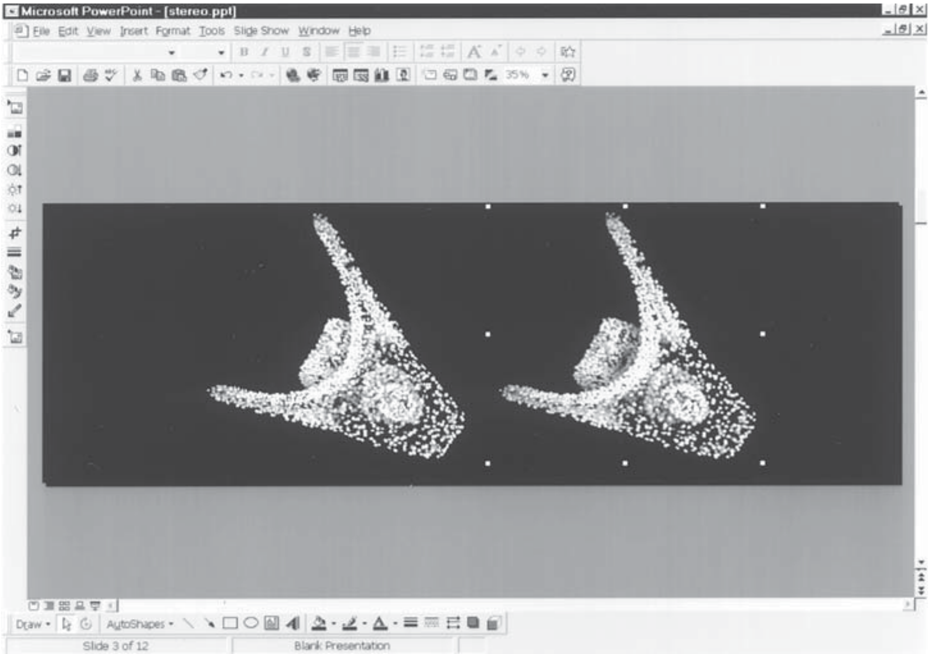


Fig. 3. Appearance of PowerPoint after inserting the stereo-images, and during alignment of the stereo-pair. Note the use of a page with the double width of a normal page formatted for 35-mm slide. The left eye image is placed on the right side and vice versa.

horizontal level; otherwise, one of the images will have to be resized until all the homologous points coincide with the guides.

Now that the stereo-pair is created and aligned, the 3D image should be moved inside the "stereo-window," in order to make it appear more natural and comfortable to the viewer (**Fig. 4**). The "window" refers to the plane in which the image is printed; in this case, the computer's screen. Start by creating vertical guides on the center, on the sides and exactly in the middle of each frame, as shown in **Fig. 5**. This will produce a grid that can be seen in stereo and that is exactly in the plane of the "window." The nearest point in the object (in this case, the left arm of the sea urchin pluteus) should lie directly behind the grid. To relocate the object, move the frames of the stereo-pair closer (towards the center), causing the 3D image to move toward the back of the "window" (inside the screen). Moving them apart causes the 3D image to move toward the viewer, in front of the "window" (outside the screen).

Understanding this concept is not easy and also takes practice, but this method should make it easier. After preparing and aligning the stereo-pair, you can add labels and other objects to the 3D illustration.

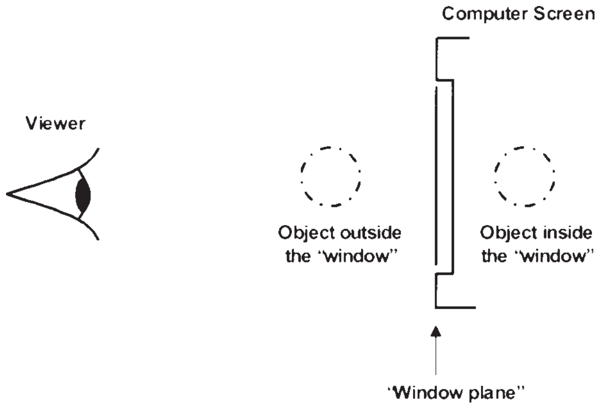


Fig. 4. Diagrammatic representation of the stereo "window." For example, in **Fig. 7B** the embryo is inside the "window" and the inset is outside.

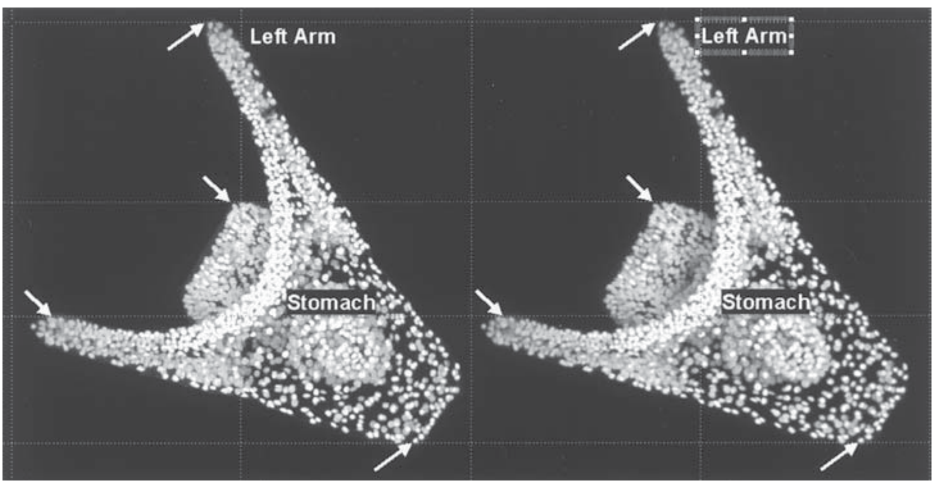


Fig. 5. Alignment of the stereo-pair and placing of labels in the 3D illustration. Note the horizontal guides (*dashed lines*) that are being used to align both images to coincide on the homologous points indicated by *arrows*. Vertical guides are placed exactly in the middle of each frame, creating a 3D grid at the level of the "window" plane. The 3D image of the embryo is placed behind this "window."

3.3. Labeling of Stereoscopic 3D Illustrations

Start by creating a textbox and placing it close to a point of interest on the left image. Then copy that textbox to the right image and place it close to the homologous point. Be sure that the textboxes are in perfect horizontal align-

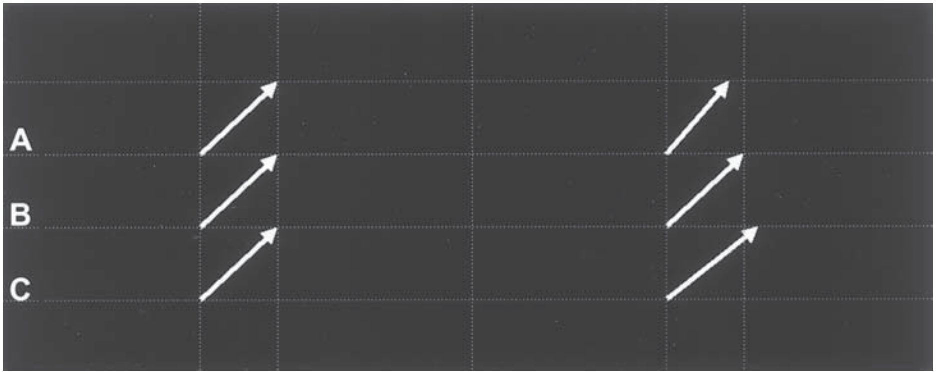


Fig. 6. 3D arrows. These arrows were created in PowerPoint as paired images. Then, the right arrows were resized to create a 3D stereoscopic effect. **(A)** Arrow pointing inside the "window." **(B)** "Flat" arrow. **(C)** Arrow pointing outside the "window." The guides (*dashed lines*) are used to show the relative sizes and positions of the stereo-paired arrows, and the "stereo-window" plane.

ment: if you are not sure, select both and use the *align* function, before viewing in 3D.

To position the label in a precise location in space, select one of the textboxes. While viewing the image in 3D (crossing your eyes), move the selected text-box to the left or right using the cursor keys (i.e., closer or farther apart, resulting in a label that moves towards or away from the viewer, respectively). It is best to start by placing the labels close to their homologous structures. However, there are restrictions to placing labels. For example, the label "stomach" in **Fig. 5** cuts into the image, and we cannot embed the label within the original bitmap (under the embryo's skin). In such a situation, it is best to use an arrow (preferably a 3D arrow) pointing to the structure of interest (*see* Fig. 7A).

To create arrows, start by drawing an arrow on the left frame connecting the label to the structure. Then, copy both the arrow and the label to the right image and position them on the appropriate homologous point. This will produce a flat arrow and label (**Fig. 6B**). To make it 3D, the right arrow will have to be resized to simulate the left eye perspective of an arrow in space. Once again, horizontal alignment should be maintained. Reducing the length of the right arrow will produce a 3D arrow pointing inside the window (**Fig. 6A**), while enlarging it will produce an arrow pointing outside the window (**Fig. 6C**). Care should be taken to minimize this resizing; otherwise, the 3D arrow will be confusing and difficult to perceive comfortably (being too "deep"). Creating 3D images that are too "deep" is a common mistake that leads to uncomfortable viewing.

After creating the right arrow, reposition it to match the location in space of both the arrow and structure. Colors can also be used to make the image more interesting visually (*see* Color Plate IV). Using the same principle, it is possible to create other objects in 3D such as insets, magnification bars, geometric figures, or circles/dots to help viewers achieve correct stereo, like in **Fig. 7A**. Any program that allows drawing 3D objects (such as 3D graphs in PowerPoint®) can be used to create the stereo-paired views of objects (by rotation or horizontal shift) that can then be used in the illustration. It is possible, e.g., to create a stereoscopic 3D graph (*see* Color Plate IV or **Fig. 8**), using these principles.

3.4. Preparing for Printing or Publication

3.4.1. Printing the Stereo-Slides for Projection

After finishing the stereo illustration in PowerPoint®, all the objects should be grouped on two separate frames (left and right) to prevent any accidental displacement of labels or arrows. To do this, select all objects of the left frame and group them, and then repeat the same operation for the objects in the right frame.

To prepare the slides for this projection method it will be necessary to create a new presentation containing one frame per slide (stereo-projection systems use two projectors simultaneously, for the left and right frames). While keeping the presentation with the illustration opened, create a new blank presentation and format the slides for 35-mm film (in Page Setup). Next, define a black background. Go back to the illustration, select the left grouped frame and copy it to the clipboard (Edit > Copy). Then go to the new blank presentation and "paste" that object into one slide. It is also a good idea to add a very small (almost unnoticeable) text label in the corner of the slide, briefly describing the slide and whether it is the left or right frame of the stereo-pair. Then create a new slide and repeat the same operation for the right frame.

Because the two frames are now separated into different slides, the image will have to be repositioned in the "stereo-window" using a slightly different method. Start by identifying the point that will be nearest to the viewer (in the case of **Figs. 5** and **7A** it seems to be the tip of the left arm). Activate the guides and position the left frame on the slide. Place one vertical guide exactly at that "nearest point" on the left frame. Then, change to the right frame slide and position the whole frame so that the same homologous point coincides with that guide, and be sure to maintain the horizontal alignment.

Finally, run a PowerPoint slide-show and by using <PageUp> and <PageDown> repeatedly you should see the objects in the illustration undergoing a slight rotation, and not stepping horizontally or vertically. The nearest point should look like a fixed point. It is also possible to use the slide sorter

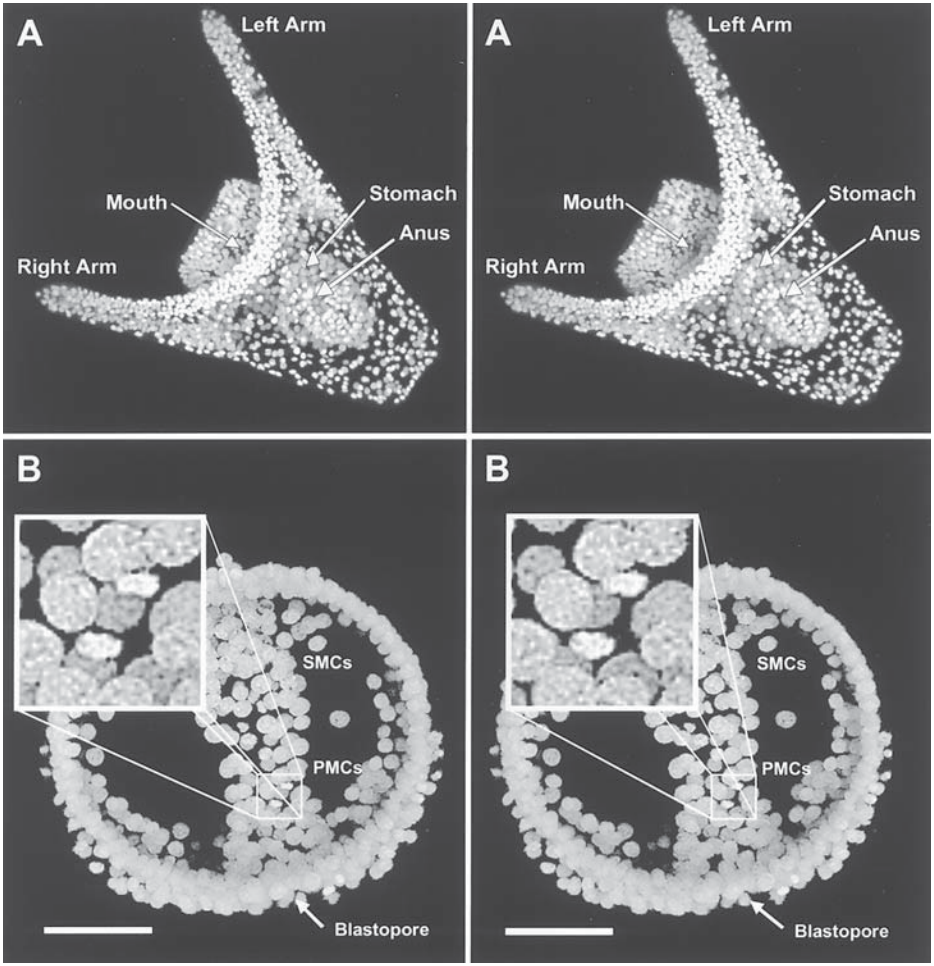


Fig. 7. Examples of 3D illustrations prepared in PowerPoint, from confocal slice-series of sea urchin embryos. The two circles on top of the figure can be used to obtain correct stereo viewing with the cross-eyed method. **(A)** Several anatomical features of the embryo are labeled with 3D arrows and labels. **(B)** The inset is outside the stereo "window," and the embryo is inside. Note the use of magnification bars in 3D. For a color version of these illustrations prepared for the parallel-eyed method, *see* Color Plate IV. Viewing these images with the parallel-eyed method will produce a pseudoscopic effect. Also, try to cross your eyes to see Color Plate IV for the same effect. Note that the pseudoscopic effect of the graph is very confusing.

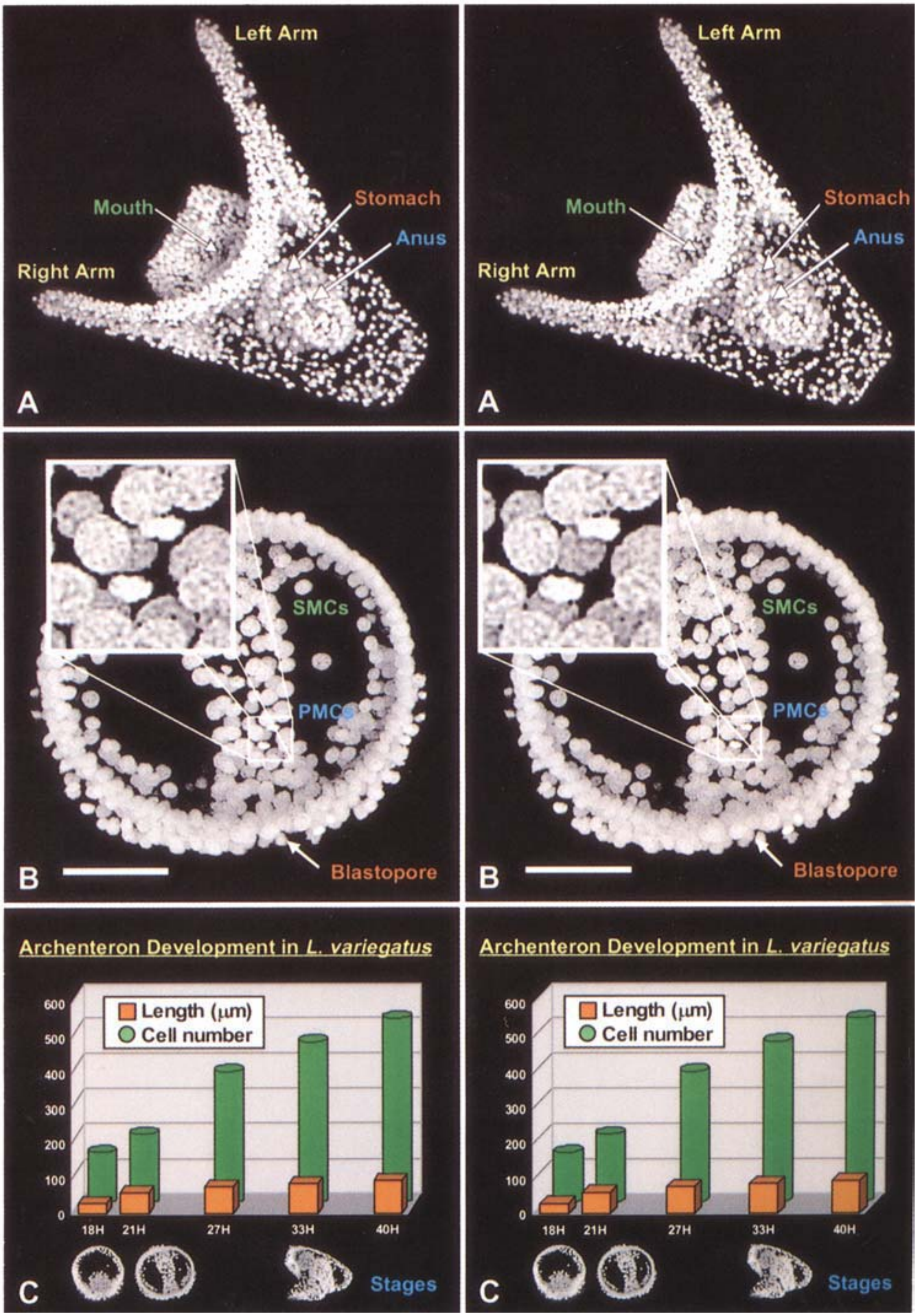
view at 100% zoom to view both slides side by side, and confirm the stereo quality by crossing your eyes. Remember that the right-eye slide should be on the left side and vice versa.

This operation will save you time when trying to align the "film-chips" later, if the company that processes the film is careful enough to mount all the slides accurately (which very rarely occurs). We suggest the manual mounting of the "film-chips" using glass slide mounts (e.g., Gepe mounts available from vendors mentioned in the References section). During manual mounting it will be necessary to go through the process of realigning the chips to maintain the 3D image in the stereo "window" and at perfect horizontal alignment. When aligning the slides on a fluorescent box, keep a separator of 0.5 inch between both mounts. A transparent aligning grid and instructions to mount slides are available through Reel 3D Enterprises (*see* References section).

3.4.2. Printing Plates with Stereo-Pairs for Publication

The same principles apply for the preparation of stereo-plates for publication. However, in this case, stereo-images are printed side-by-side on the plate. Since convention dictates the publication of stereo-pairs for the parallel-eyed viewing method, you must switch the frames that were prepared with the cross-eyed method (*see* **Fig. 2**). Keep in mind that in this case, the stereo-pair width is restricted to 5 inches. Also, a white line separating the two frames should be added. *See* Color Plate IV (or **Fig. 8**) for a version of some of the stereo-images presented on this chapter, as prepared for publication.

To keep the stereo-image inside the "window" use vertical guides exactly in the middle of each frame (–2.5 inches and +2.5 inches from the center). Then move the frames apart to place the image inside the "window" or closer to bring it outside the "window." In this case, when you use the cross-eyed method to view a parallel-eyed stereo-pair, you will see an inverted 3D image—a pseudoscopic image (with confocal images, the pseudoscopic image is also easily perceptible, unlike stereo-images from SEM or the 3D graph in **Fig. 8C** or Color Plate IV C). The object will be inside the "window" if it looks "outside" and vice versa (try viewing **Fig. 5** with both methods)! You may often find situations where there is a compromise between using printable area and keeping the image inside the "window." Reducing the size of the frames may be necessary, for placing the image inside the "window." If detail is important, be aware that images outside the "window" can also be perceived (*see* **Figs. 7B**, **8B**, and Color Plate IV C).



Left Arm
Mouth
Stomach
Anus
Right Arm
A
SMCs
PMCs
Blastopore
B
Archenteron Development in L. variegatus
Length (μm)
Cell number
600
500
400
300
200
100
0
18H
21H
27H
33H
40H
Stages
C

3.5. Showing 3D Images

3.5.1. Projection of Stereo-Slides

More detailed information about how to set up a stereo-slide projection system can be found elsewhere ***(2,3)***. In brief, two slide projectors are necessary to simultaneously project the left and right frames on a silver screen. These two projected images have to be in perfect register (both horizontally and vertically superimposed). The left and right images are then separated with the use of polarized filters in front of the projectors' lenses, set at a 90° angle to one another (45° and 135°). Viewers also have to wear polarized glasses to allow each eye to see only the frame for that eye. These glasses and filters can be ordered through several companies, and information about some of these can be found on "Websites" at the end of this chapter.

3.5.2. Viewing Stereo-Images with Glasses and Stereoscopes

Other stereoscopes for slides include twin 35-mm 3D slide viewers, which can also be purchased in the same way. These are particularly useful for presentations of posters at scientific meetings, where no projection capabilities are readily available. They also constitute an easy way of assessing the final quality of your printed slides before projection, but keep in mind that deformation due to smaller viewing distance will be noticeable (the 3D image will look somewhat flat, i.e., "squeezed," when compared with the 3D image resultant from proper slide projection).

For viewing published stereo-images, unless the viewer is familiar with the cross/parallel-eyed viewing techniques, stereo-glasses have to be used. These

---

Fig. 8. *(previous page)* Example of 3D illustrations prepared for the parallel-eyed viewing method (standard method for publication). This figure is reproduced in Color Plate IV, following page 372. **(A)** 40-h *L. variegatus* sea urchin pluteus larva. The nuclei of cells are visible and several anatomical features are labeled in 3D. **(B)** 21-h *L. variegatus* sea urchin gastrula. The inset represents a magnification of a mitotic figure within the archenteron wall. Some mesenchymal cell types are labeled (SMC = secondary mesenchyme cells; PMC = primary mesenchyme cells). **(C)** Stereoscopic 3D graph representing the continuous increase in cell numbers that accompanies archenteron length increase during development in the sea urchin *L. variegatus*. The stereographs were created in Excel97® by rotation (8° apart) and by adjusting sizes for perfect horizontal and vertical match. For this type of illustration it is preferable to create only the graphs in Excel and then add the legends, titles, and other objects in PowerPoint® or CorelDraw®, using the methods described for preparation of slides and stereo-images for publication. Titles and legends were repositioned to create the 3D stereoscopic effect and stereo-images of embryos were added to depict some of the stages.

can be acquired in the same way as the polarized glasses. Their prices vary with quality and generally, plastic glasses are of poor quality. There are also tricks to "force" people to see stereo-images. A common method is to use a piece of card (letter size) and place it vertically between the two images. Then, the viewer can look down from the top edge of the card (with the nose touching the edge of the card) and allow the eyes to focus only on the respective image. It is a simple method for beginners to learn how to diverge the eyes, for the parallel-eyed method, and it creates a less confusing 3D image. There are also similar card-mounts with lenses available through Reel 3D Enterprises, Inc. and other suppliers.

4. Notes

4.1. PowerPoint® Techniques and Tricks

When pasting or inserting images, use the function "*Paste Special...Picture*." Microsoft® applications handle bitmaps in the *"Picture"* format more effectively.

Use the <CTRL> key to copy objects: Holding the <CTRL> key while moving an object with the mouse will create a copy of that object and place it in the new location. This is most useful for creating the labels and arrows for the second image of the stereo-pair.

Use the <ALT> key for fine adjustments: Holding the <ALT> key while moving an object with the mouse will move the object freely and not within the predefined grid. This function is essential for the fine adjustment necessary for horizontal alignment and comfortable stereo viewing.

Use the <SHIFT> key for multiple selections: It is possible to select two or more objects without having to select an area. Pressing the <SHIFT> key and then clicking on several objects will enable multiple selection. This function is useful for moving paired labels of the stereo-pair at the same time, and ensures that they remain aligned.

Use the <SHIFT> key to move objects in one direction: Objects can be moved horizontally or vertically pressing the key <SHIFT> while moving them with the mouse. This is useful to maintain horizontal alignment when copying labels.

Group objects that are to be handled together: Use the command Draw > Group to group a multiple selection of objects into a single object.

Use *Guides* to align objects: Activate guides with <CTRL> + G, or menu View > Guides. They are most useful to verify on screen, the alignment of objects, and are not printed with the illustration (*see* **Fig. 5**).

Move objects with the arrow keys: After selecting an object (as when clicking on the border of a textbox) that object can be moved with the arrow keys on the keyboard. However, this method does not allow for finer adjustments in PowerPoint.

To align objects:

1. Select the objects you would like to align.
2. Go to the Align menu via DRAW > Align or Distribute, and then align on *top* or *bottom*.

Turn off the automatic spell-checker: The red lines that recent versions of spell-checkers use to underline misspelled words are confusing and often prevent correct stereo viewing.

When resizing objects, be sure to preserve the original aspect ratio by using only the corner handles of an object + <SHIFT> key, and never the side handles.

4.2. CorelDraw® Techniques and Tricks

Use the <CTRL> key to constrain object movement in one direction: Hold down <CTRL> while selecting and moving an object.

Use the standard COPY and PASTE functions to create multiple instances of objects such as labels or arrows. Be sure to constrain movement of the copied object in the horizontal direction if it is to be paired with the original object.

Use the arrow keys to make fine adjustments to an object's position. The default "nudge" setting is too coarse in CorelDraw; to change it, go to the menu TOOLS > Options > Workspace > Edit. Set Nudge to 0.02 inches, or to a comfortable setting. This function is essential for the fine adjustment necessary for horizontal alignment.

Group objects that are to be handled together: Use <Ctrl> + G to group selected objects.

Create guides to check alignment of homologous points: Click and drag a horizontal guide from the horizontal ruler onto the illustration.

To align objects:

1. Select the objects to be aligned.
2. Go to the Align menu via ARRANGE > Align & Distribute, or the "Align…" button located on the upper right portion of the toolbar. <CTRL> + A is the shortcut for this option.
3. Select the appropriate alignment button and press "OK." Align TOP, CENTER, or BOTTOM are the settings used to align stereo elements in the horizontal direction.

Use the <SHIFT> for multiple selections: It is possible to select two or more objects without having to select an area. Pressing the <SHIFT> key and then clicking on several objects will enable multiple selection. This function is useful for moving paired labels of the stereo-pair at the same time, and ensures that they remain horizontally aligned.

When resizing objects, be sure to preserve the original aspect ratio by using only the corner handles of an object and never the side handles.

Note: In CorelDraw®, be aware that text placed on a stereo-pair sometimes appears "jumpy" in stereo, i.e., the characters in a word do not all appear to be within the same plane. This is only a display anomaly, and should not appear in the printed piece.

Acknowledgments

Figures 1, 3, 5, 7A, 8A were adapted from Martins, G. G., Summers, R. G., and Morrill, J. B. (1998) *Dev. Biol.* **198,** 330–342, with permission of Academic Press Inc.

References

1. Chen, H., Swedlow, J., Grote, M., Sedat, J., and Agard, D. (1995) The collection, processing and display of digital three-dimensional images of biological specimens, in *Handbook of Biological Confocal Microscopy*, (Pawley, J., ed.), Plenum Press, New York, pp. 197–209.
2. Ferwerda, J. G. (1987) *The world of 3-D, A Practical Guide to Stereo Photography*, 3D Book Productions, The Netherlands, 300 pp.
3. Wergin, W. P. and Pawley, J. B. (1980) *Recording and Projection of Scanning Electron Micrographs.* SEM/I, SEM Inc., AMF O'Hare, IL 60666, pp. 239-250.
4. White, N. (1995) Visualization systems for multidimensional CLSM images, in *Handbook of Biological Confocal Microscopy*, (Pawley, J., ed.), Plenum Press, New York, pp. 211–254.

Websites

Stereoscopy.com
http://www.stereoscopy.com
A good starting point for general 3D resources. Numerous links.

Reel 3-D Enterprises, Inc.
http://www.stereoscopy.com/reel3d/index.html
Purveyor of 3D instruction books, images, and equipment by mail order.

How to make 3D pictures by computer
http://www.stereoscopy.com/3d-info/index.html
Basic theory about stereoscopy and principles for preparing stereo-images from photographic pictures.

Stereoscopy and Illusions
http://www.lhup.edu/~dsimanek/3d/3dpage.htm
Various links to related sites, documents on 3D, and optical illusions.

3-D Scanning Laser Confocal Microscopy
http://www.cs.ubc.ca/spider/ladic/confocal.html
An extensive site for confocal microscopy resources, from specimen preparation to volume visualization. Includes postings of upcoming courses and meetings related to confocal microscopy and 3D. A must for the confocal microscopist.

Confocal Assistant
ftp://ftp.genetics.bio-rad.com

An anonymous FTP site where Confocal Assistant® V4.02 can be downloaded. This program can be used to create the stereo-pairs from confocal datasets, using both the pixel-shift and rotation methods.

Alan Gordon Enterprises, Inc.

http://www.A-G-E.com

Supplier of a large selection of stereoscopes. The catalog can be requested at this site or at: 1430 Cahuenga Blvd., Hollywood, CA 90029, USA.