

10-22-1988

A Rapid Method for Observing the Internal Morphology of Amphibian Embryos

S. C. Smith
University of Ottawa

J. B. Armstrong
University of Ottawa

D. C. Hoppe
University of Ottawa

Follow this and additional works at: <https://digitalcommons.usu.edu/microscopy>



Part of the [Life Sciences Commons](#)

Recommended Citation

Smith, S. C.; Armstrong, J. B.; and Hoppe, D. C. (1988) "A Rapid Method for Observing the Internal Morphology of Amphibian Embryos," *Scanning Microscopy*. Vol. 2 : No. 4 , Article 23.

Available at: <https://digitalcommons.usu.edu/microscopy/vol2/iss4/23>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



A RAPID METHOD FOR OBSERVING THE INTERNAL
MORPHOLOGY OF AMPHIBIAN EMBRYOS

S.C. Smith, J.B. Armstrong*, and D.C. Hoppe

Department of Biology
University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

(Received for publication March 25, 1987, and in revised form October 22, 1988)

Abstract

A simple, rapid method for visualizing the internal morphology of amphibian embryos is described. Fixed embryos of *Ambystoma mexicanum* are surrounded with commercial embedding material and are frozen. Internal structure is revealed by cutting the embryos with a cooled scalpel or razor blade, removing the frozen embedment with an aqueous buffer, and processing the halved embryos for scanning electron microscopy (SEM). This technique allows the internal anatomy of the embryo to be viewed but is much simpler and more rapid than previously described techniques. It should therefore prove useful for understanding and teaching the three-dimensional relationship between tissues in developing embryos. In addition, we believe that this technique could be used, with minor changes, as a rapid method for viewing the internal morphology of a variety of specimens.

KEY WORDS: Internal anatomy, Frozen sectioning, Processing for scanning electron microscopy, Embryo, Amphibian, Mexican axolotl, *Ambystoma mexicanum*, Scanning electron microscopy.

* Address for correspondence:
Department of Biology, University of Ottawa, 30
Marie Curie, Ottawa, Ontario, Canada K1N 6N5.
Phone No. (613)564-4283.

Introduction

The most common means of observing the interior of embryos (and other tissues) is through the use of sectioned material. This provides, at best, a limited two-dimensional view that is often difficult to interpret. As noted by Oppenheimer and Chao (1984) in their atlas, sectioned material cannot provide any of the depth or texture present in living embryos.

To overcome these limitations, early embryologists (and others) resorted to serial sectioning, constructing replicas of the sections, and arranging these to form three-dimensional images. This process, which may now include the use of computer-assisted graphics to reconstruct the image, is still extremely tedious. Further, the reconstructed images still suffer from some loss of detail.

Scanning electron microscopy (SEM) can provide depth, but is limited in that only surfaces can be observed. Since many of the most interesting phenomena in embryos occur internally, ordinary SEM views are of limited use.

Previous efforts to view the internal morphology of embryos have included dissecting or fracturing them either before or after drying (see Waterman, 1980, for review). While this may be satisfactory for many purposes, the fragility of the specimens and the uneven breakage this causes makes consistent results difficult to obtain. Dissecting embryos after drying may also cause a great deal of debris to be deposited on the specimens. As well, dissection is often unsatisfactory for exposing deeper tissue layers.

More successful approaches have involved supporting embryos with a substrate prior to cutting them (e.g., Ukeshima, 1976). More support can be provided by actually embedding the embryos in paraffin (Armstrong, 1971; Armstrong and Parenti, 1973), polyethylene glycol (Nagele et al., 1984) or epoxy resins (Steffens, 1977) and sectioning them to the desired level. However, embedding the embryos subjects them to all of the drawbacks of the method chosen and necessitates that they be soaked in appropriate solvents to remove the embedments. As well, these processes are lengthy and tedious. Similarly, the

fixation/quick-freezing procedure of Carri and Suburo (1979) is lengthy and produces brittle specimens which are difficult to handle.

Perhaps the simplest method for opening embryonic body cavities for SEM is the so-called ethanolic cryofracture technique of Humphreys et al. (1974). This technique involves dehydrating embryos to absolute ethanol, which is used as the embedding medium. The embryos are then quick frozen in liquid nitrogen, and cut with a razor blade struck with a hammer.

In this paper we describe an alternative method for processing amphibian embryos for examination of internal morphology by SEM which is both rapid and simple. Despite the simplicity of the technique, the results resemble those produced by the sectioning and ethanolic cryofracture techniques.

Materials and Methods

Axolotl (*Ambystoma mexicanum*) embryos were obtained from spawnings between animals raised in our laboratory. All embryos were kept in 25% modified Holtfreter's solution (865 mg NaCl, 12.5 mg KCl, 25 mg CaCl₂, 50 mg MgSO₄, 50 mg NaHCO₃ per liter) supplemented with 100 mg/L each penicillin and streptomycin (see Asashima et al., 1988).

Embryos of various stages (Bordzilovskaya and Dettlaff, 1979; Bordzilovskaya et al., 1988) were fixed overnight in 2.5% glutaraldehyde in PIPES buffer containing 5mM CaCl₂ (pH 7.4). Following a rinse with buffer, the embryos were transferred into embedding molds filled with buffer. The buffer was removed, and the embryos were covered with Fisher Histo-Prep frozen embedding medium and immediately placed at -80° C and allowed to freeze.

Once frozen, the blocks were maintained at -20° C until cut. The blocks were manipulated with cooled metal forceps. The blocks were removed from the embedding molds, were held with the forceps, and were cut using a pre-cooled scalpel or single edged razor blade. The blocks were cut so as to give two sagittal halves from each embryo. The block halves were immediately placed into ice-cold fixative and allowed to warm up to room temperature. The use of additional fixative ensured that all internal surfaces were adequately fixed.

The embedding medium was removed by rinsing with several changes of buffer. The sectioned embryos were then post-fixed in 1% OsO₄ in PIPES buffer, dehydrated in a graded ethanol series, and critical point dried from CO₂. They were then mounted on stubs with silver paste, sputter-coated with a 60:40 Au:Pd mixture, and observed with a Philips model 505 scanning electron microscope. Micrographs were taken with Polaroid type 55 film.

Results and Discussion

We have found that the method described above allows us to observe the internal anatomy of amphibian embryos. The technique is both

simple and rapid, and produces cleaved embryos such as those shown in Figure 1.

Several things should be noted when using this technique. First, axolotl embryos have been successfully cut with a scalpel or razor blade, as described. However, if finer cuts or carefully controlled sectional planes are desired, or if this technique is to be adapted to smaller embryos or other specimens, it would be preferable to cut sections to the desired depth with a freezing microtome. Second, under no circumstances should the embryos be allowed to sit in the embedding medium before being frozen, as the body cavities will collapse. (The frozen tissue medium is not intended to act as a true embedding medium and merely surrounds the tissue to make it easier to manipulate without being damaged). However, rapid freezing is not required; an ordinary freezer chest is quite adequate.

Finally, it should be noted that the embryos, once cut, must be thoroughly rinsed with an appropriate aqueous buffer so as to remove all traces of the embedding compound before dehydration. The ethanol used to dehydrate the embryos causes Histo-Prep medium to congeal into a poorly soluble rubbery mass. Remaining traces of the medium may be the cause of the mesh-like precipitate we have noticed on some surfaces (see Figs. 1a and b).

Since the embryos are simply surrounded with commercial frozen embedding medium (Ames OCT compound or Fisher Histo-Prep), the technique is much quicker than previously published techniques. A further advantage is that the frozen sectioning medium is easier to remove than paraffin, polyethylene glycol, or epoxy and does not require that the tissues be soaked in any solvent other than an aqueous buffer (with or without additional fixative). Neither does this method require the use of liquid N₂ or any other rapid freezing agent. Despite the rapidity of the method, we have found that it yields results comparable to those of other workers when used on amphibian embryos. We believe that this technique may also lend itself to use by workers desiring a rapid method for studying the internal morphology of other small and/or delicate tissues.

The ability to see and understand the three-dimensional relationships of the tissues within embryos can aid in understanding the processes and movements which occur during development. Therefore, we think that this technique may prove useful in the study of some developmental processes and particularly, in demonstrating the fundamental processes of embryology to students.

Acknowledgements

This work was funded by an operating grant from the Natural Sciences and Engineering Research Council of Canada to JBA. SCS was supported by a Research Traineeship from the Canadian Heart Foundation. We thank Lise Tassé for help in preparing the manuscript.

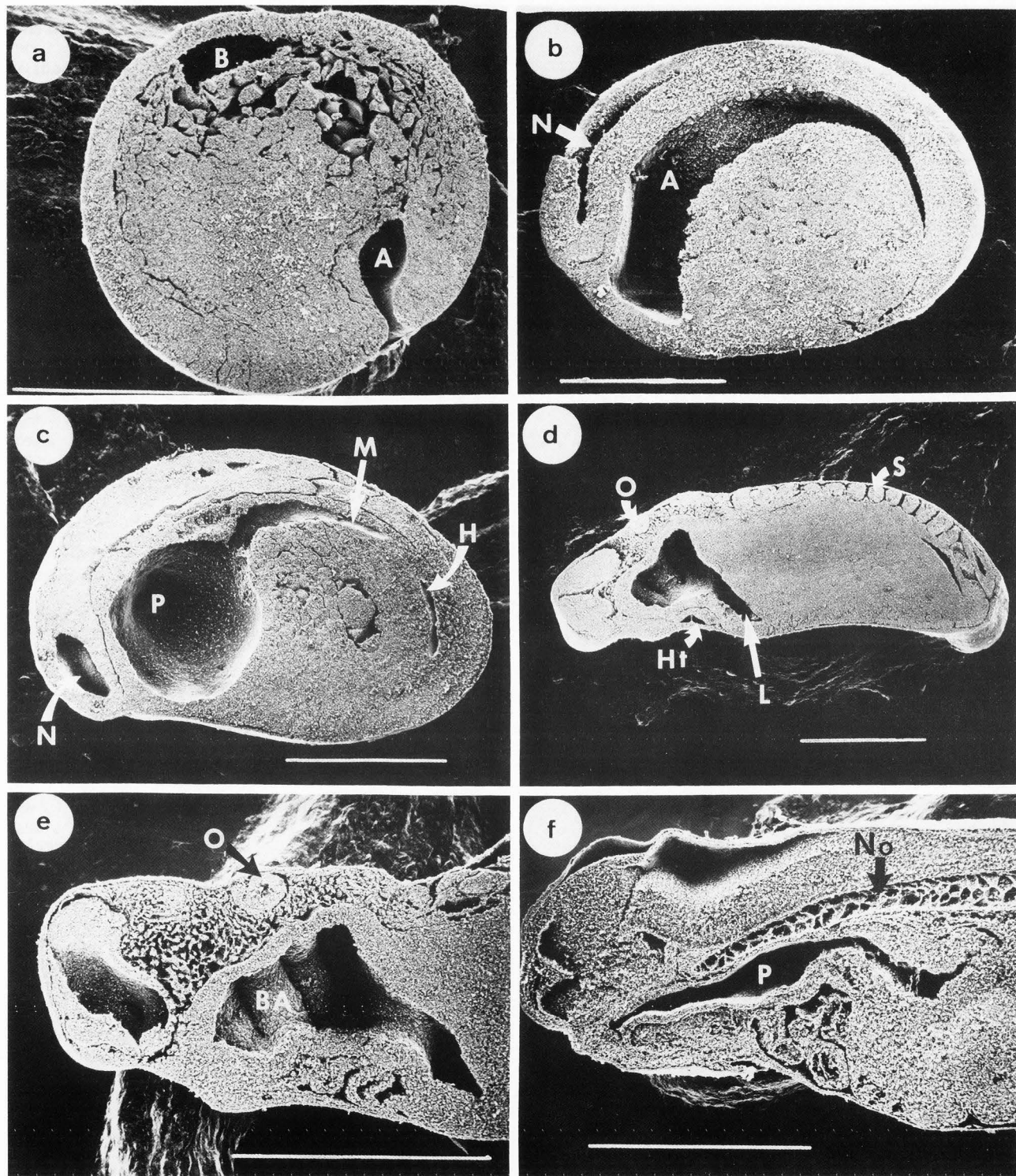


Figure 1: Axolotl embryos which have been cut in half sagittally. In all cases the anterior end of the embryo is to the left. Stages: a) 10½ (gastrula), b) 18 (neurula), c) 24, d) 30, e) 34, and f) 40. A variety of structures are shown. The nature of the fibrillar material present on the cut surfaces in a) and b) is unclear. It may be either residual embedding material or unfixed extracellular matrix which was deposited during dehydration and/or critical point drying. Abbreviation: A, archenteron; B, blastocoel; N, neurocoel; P, pharyngeal cavity; M, midgut; H, hindgut; O, otic vesicle; S, somite; Ht, heart; L, liver diverticulum; BA, branchial arch; No, notochord. Bars = 1mm.

References

Armstrong PB (1971) A scanning electron microscope technique for study of the internal microanatomy of embryos. *Microscope* 19: 281-284.

Armstrong PB, Parenti D (1973) Scanning electron microscopy of the chick embryo. *Dev. Biol.* 33: 457-462.

Asashima M, Malacinski GM, Smith SC (1988) Surgical manipulation of embryos. In: Armstrong JB, Malacinski GM (eds). *Developmental Biology of the Axolotl*. Oxford University Press, Oxford. In press.

Bordzilovskaya NP, Dettlaff TA (1979) Table of stages of the normal development of axolotl embryos and the prognostication of timing of successive developmental stages at various temperatures. *Axolotl Newsletter* 7: 2-22.

Bordzilovskaya NP, Dettlaff TA, Duhon ST, Malacinski GM (1988) Developmental stage series of axolotl embryos. In: Armstrong JB, Malacinski GM (eds). *Developmental Biology of the Axolotl*. Oxford University Press, Oxford. In press.

Carri N, Suburo A (1979) Scanning electron microscopy of frozen sections: A method for studying selected planes in small specimens. *Anat. Rec.* 193: 857-862.

Humphreys WJ, Spurlock BO, Johnson JS (1974) Critical point drying of ethanol-infiltrated cryofractured biological specimens for scanning electron microscopy. *Scanning Electron Microsc.* 1974: 275-282.

Nagele RG, Doane KJ, Lee H, Wilson FJ, Roisen FJ (1984) A method for exposing the internal anatomy of small and delicate tissues for correlated SEM/TEM studies using polyethylene glycol embedding. *J. Microsc.* 133: 177-183.

Oppenheimer SB, Chao RLC (1984) *Atlas of Embryonic Development*. Allyn and Bacon, Inc., Boston, Mass., USA, p. vii.

Steffens WL (1977) A method for the removal of epoxy resins from tissue in preparation for scanning electron microscopy. *J. Microsc.* 113: 95-99.

Ukeshima A (1976) A technique for making the cut surface of the avian embryo for scanning electron microscopy. *J. Electron Microsc.* 25: 49-50.

Waterman RE (1980) Preparation of embryonic tissues for SEM. *Scanning Electron Microsc.* 1980;II: 21-44.

Discussion with Reviewers

M.A. England: Is there any apparent tissue damage from the low temperatures used in this technique?

Y. Shimada: The authors present no higher magnification pictures. How well are the structures of the cell membranes and internal organelles preserved by this procedure?

Authors: We have seen no apparent tissue damage from the temperatures used. It should be noted, however, that we were only interested in observing the gross morphology of the

embryonic body cavities and the morphogenetic processes responsible for forming them. Therefore, as the reviewers have noted, we included no higher magnification micrographs. The method used greatly resembles specimen preparation for light-level frozen sectioning. Therefore, we believe that tissue damage would be comparable to that observed during such preparation. If damage does become apparent at higher magnifications, we would suggest using shorter freezing times (i.e. lower temperatures) and/or infiltrating the specimen with a cryoprotectant such as DMSO prior to freezing.

Y. Shimada: I find that ethanolic cryofracture is very simple, since the fracture can be done during dehydration. Further, the three dimensional structures are rather well preserved by the ethanolic procedure. In the present method, embedding in and removal of the embedding medium is necessary. This seems to be rather tedious in comparison to the ethanolic method. What is the advantage of this method in terms of simplicity and structural preservation?

Authors: Ethanolic cryofracture is, indeed, a useful technique. The major advantage of our method is that we do not require the use of liquid nitrogen. In this way, the hazard and expense of liquid nitrogen is avoided. Furthermore, the worker is not obliged to position the specimen rapidly and strike a blade with a hammer to make the cut. If our method were performed in a cold chamber or, indeed, on a glass plate cooled with wet or dry ice, the worker could take his time in positioning the specimen and the blade to obtain the desired cut. It should even be possible (using the cooled glass plate) to observe the specimen with a dissection microscope while cutting. As well, if a freezing microtome is used (as suggested in the text) much more closely controlled sectional planes can be obtained than with ethanolic cryofracture.

Finally, as we said in the text, the frozen "embedding" medium, despite its name, is NOT a true embedding medium in the sense that the tissue is infiltrated and the medium permeates all the cells. As with light-level frozen sectioning, the medium (at low temperatures) merely provides an external support to prevent damaging delicate surfaces during handling and to allow the tissue to be cut without tearing and collapsing. Under no circumstances should one attempt to infiltrate the tissue with this medium, as the internal cavities will collapse! As such, it is only necessary to rinse the excess medium off the surface with a few quick rinses. The medium is not being removed from infiltrated tissues and therefore extended soaking in solvent (which is aqueous in this technique) is not necessary.