

Generation of the Dimensional Embryology Application (App) for Visualization of Early Chick and Frog Embryonic Development

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Abstract: The study of embryonic development of multiple organisms, including model organisms such as frogs and chicks, is included in many undergraduate biology programs, as well as in a variety of graduate programs. As our knowledge of biological systems increases and the amount of material to be taught expands, the time spent instructing students about embryology is becoming more abbreviated. In addition, limitations in budget and resources make the laboratory components of embryology courses more difficult to support. We have generated a free mobile tablet application (App) called Dimensional Embryology (<http://itunes.apple.com/app/id985989230>) that can be used to quickly and easily teach students about the organization, shape and position of embryonic structures in both early chick and frog embryos. For each organism, we have highlighted different time points in early development, and have two-dimensional and three-dimensional images of the embryos. Using color enhanced images and images with the major structures isolated away from the background, students can make correlations between observations of serial sections and the three-dimensional shape and position of each internal structure. Students that used the Dimensional Embryology App found it to be very informative, easy and convenient to use.

Key words: Embryology, development, chick, frog, three-dimensional, computer-assisted learning

INTRODUCTION

The development of an embryo begins with a fertilized egg, which undergoes cleavage, gastrulation and organogenesis/ histogenesis. Knowledge of fundamental embryology is critical for understanding general organismal development, for comparing the ontogeny of different organisms, and for helping elucidate the establishment of different developmental disorders and diseases. However, exclusive embryology courses are no longer part of many curricula, due to a lack of time in which to teach the material, along with a lack of financial support for supplies, equipment, and instructional faculty (Scott et al., 2013). Portions of embryology are now incorporated into multiple courses, as students and faculty alike understand the importance of establishing a foundational knowledge of embryonic development (Burk et al., 2013; Hamilton and Carachi, 2014; Scott et al., 2013). Introductory biology, developmental biology, anatomy and physiology, histology, and vertebrate comparative anatomy courses, in pre-medical biology undergraduate programs, medical schools, veterinary and dental schools often teach about the anatomical structures in a range of organisms and how those tissues and organs develop in the embryo (Beale et al., 2014; Burk et al., 2013).

Many of the courses that include embryology use a traditional lecture style paired with a laboratory

component and are required within major degree programs, or are part of a self-directed learning program. Within lectures on embryology in these courses, two-dimensional images of different times in development are often drawn or displayed on a screen. In the laboratory, both live and fixed fertilized embryos are observed. For early embryonic development, vertebrate model organisms such as frog and chick embryos are used to identify anatomical structures, to observe when structures develop in the embryo by looking at different time points, and to evaluate the differences and similarities in embryonic development among different organisms. Live frog and chick embryos allow students the ability to directly observe changes over time, however the live embryos present some difficulties. Students can observe gross, external anatomical changes but cannot visualize any internal characteristics. Furthermore, due to the cost of purchasing live organisms, the need for approval to use live vertebrate organisms for research and the necessity for available space for housing live organisms, laboratory experiences with live developing embryos are limited. Historically, preserved whole mount embryos and serial cross-sections of embryos that have been fixed at different times during development have been used for evaluation. While the prepared slides are useful to visualize some of the structures, this approach also

has limitations. The whole mounts do not allow for the observation of internal structures. Using traditional histological serial sections, the students do not appreciate what the structures actually look like and cannot distinguish the different parts of the embryo. Students have difficulty making the association between what is observed in serial sections and the three-dimensional shape and location of structures in the whole mounts. Students lack the ability to visualize the complexity of the structures and the relationships between the structures in the embryo. In addition, slide observation needs to be done in the lab where microscopes are available and an instructor is present to help guide the students as they visualize the sections of the embryo and compare them with the Atlas of Descriptive Embryology (Shchoenwolf, 2008) and websites such as Developmental Biology Online (Scadding, 1998) and DevBio: Vade Mecum³ (Tyler and Kozlowski, 2010).

To address the problems with embryology, we have generated a mobile tablet application, an iPad App, called Dimensional Embryology that can be used to visualize chick and frog embryo serial sections, along with three-dimensional models of the embryos at different times in development. The App can be used as supplemental information, in web-based or self-study programs, and in programs that have limited funding and equipment. Furthermore, unlike many labs where the students cannot revisit the specimens or repeat the observations, by using a mobile tablet application the students can review the material at any time, allowing for more self-study time. To our knowledge, there is no resource that clearly demonstrates the three-dimensionality of the structures within chick and frog embryos. This is a critical component of student knowledge of embryonic development that is lacking. Websites and applications similar to ours have been created to visualize mouse, such as **EMAP eMouse Atlas Project** (<http://www.emouseatlas.org>) (Richardson et al., 2014) and human embryo serial sections and three-dimensional structures (Ecker et al., 2003; Museum of Health and Medicine, 2012; O'Loughlin, 2008; Sulik and Beam Jr., 2015) but not for other organisms. The Dimensional Embryology App uses different colors to label the structures in every serial section and uses the serial sections to create a three-dimensional, color enhanced model of the embryo that can be rotated on both the X and Y axes.

The Dimensional Embryology App can be downloaded for free to an iPad through the iTunes store, <http://itunes.apple.com/app/id985989230>. It is easy to use and provides a quick, convenient resource for the study of embryology.

METHODS

Image preparation

Standard sequential serial cross-section slides of 24 hour (hr), 48hr, and 72hr chick embryos, and hatched (4mm) and 10mm frog embryos were obtained from Carolina Biological Supply Company, (catalog #311532, 311604, 311652 for chick and special order for frog). To generate a library of images for each embryo at each developmental time point, the embryonic sections were imaged using a Leica microscope at 10X, with a digital camera. The images were transferred to a Dell computer with Leica Application Suite. For larger sections that were too large to be imaged in one frame, multiple images were photomerged using Adobe Photoshop. Background inconsistencies and excess embedding wax remaining from sectioning procedure was manually removed from the image in Photoshop (Fig 1A). The three-dimensional model was generated from the sequence of images in ImageJ, freeware from the National Institutes of Health (Fig 1B) (<http://imagej.nih.gov/ij/index.html>).

The major tissues and organs in the developing organisms were color enhanced in Adobe Photoshop, using the color replacement tool to pseudo-color the structures, to allow for easier identification within the embryo. The two-dimensional enhanced series of images and the subsequent three-dimensional model of the embryo were generated in ImageJ (Fig1C, 1D). To generate the isolated images, the enhanced, pseudocolored organs were further processed by removing all of the unlabeled tissue from the images of the embryo. Again the sequence of images was used to generate a three-dimensional model (Fig 1E, 1F). The scale bar is 200µm.

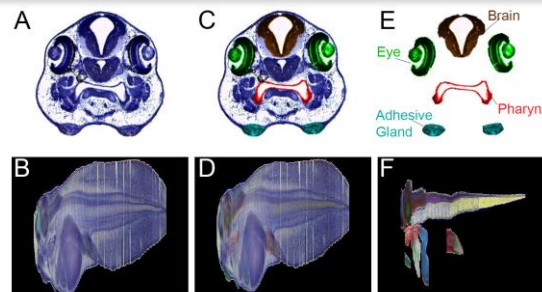


Fig. 1. 10mm Frog embryo with nonenhanced, enhanced and isolated two- and three-dimensional images. (A) nonenhanced serial section (B) single view of a nonenhanced three-dimensional model (C) enhanced serial section (D) single view of an enhanced three-dimensional model (E) isolated serial section showing the major structures with unlabeled, excess tissue removed (F) single view of an isolated three-dimensional model

iPad App Creation

The app was created for the iPad because of its high performance and its popularity in academics. The core challenge in writing the application was to enable the user to smoothly iterate through thousands of images using a scroll bar. The use of web technology was considered but would require a user to download thousands of images ultimately resulting in slow wait times and/or slow performance. The iPad App bundles all of the images with the App when it is originally downloaded and installed. The app works by allowing a user to select an animal, development time, and enhancement choice. The image selector slider position, along with the user choices, dictates the image that will be shown (Fig2A). The image files were named such the file name also represents the animal, development time, enhancement choice and slide number. An example file name was chick48hisol0257.jpg, indicating that the animal was chick; development time was 48 hours; enhancement choice was isolated, and slide number was 257.

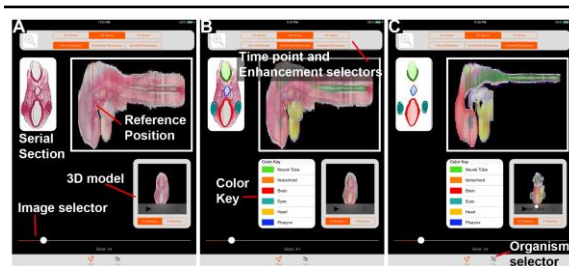


Fig. 2. Dimensional Embryology App screen with highlighted features. (A) Non-enhanced images for 48hr Chick (B) Enhanced images for 48hr Chick (C) Isolated images for 48hr chick

Assessment

Thirteen students in an undergraduate developmental biology course used the Dimensional Embryology App to first look at the non-enhanced two-dimensional serial sections and the generated three-dimensional model to see if they could identify structures in the embryo and describe the three-dimensional shape of the embryo. Then they used the enhanced images and the three-dimensional models, to begin to determine the position of the major structures in the embryo. Finally, using the isolated images and the three-dimensional model, students evaluated the relative positions and shapes of all major internal structures. During the use of the App, students were observed and were asked to qualitatively assess the usefulness and clarity of the App and to state their overall impressions in a survey.

RESULTS

The students evaluated the App for ease of use, clarity and ability to identify the three-dimensional structures within the embryos contained in the image

library, including chick embryos that are 24hrs old, 48hrs old and 72hrs old, as well as hatched (4mm) and 10mm frog embryos. By showing the traditional, two-dimensional serial sections (Fig 1A), the students first saw the general histological sections with dark and light staining patterns, indicative of the tissue diversity. When they began with the non-enhanced serial sections, and the accompanying three-dimensional model that rotates in both X and Y to visualize the entire embryo (Fig 1A,B), they had difficulty determining where structures like the heart and eyes were, and found it near impossible to describe the three-dimensional shape of those structures. They were able to understand the relationship between the slide number (section) and its position in the embryo using the reference position on the App (Fig 2A).

The enhanced images with the color-coded structures allowed the students to visualize the anatomical positions and organization of the tissues and organs in relationship to the other structures in the embryo (Figs 1C, 1D, 2B), while the isolated images displayed some of the organs and tissues in the embryo without the presence of the connective tissue and other less emphasized structures in the embryo. By looking at the enhanced and isolated structures in both two- and three- dimensions (Fig 1E, 1F), students detect the shape, structure, size and detail of the embryonic components. When using the three-dimensional models of the isolated structures, the students quickly identified and described the three-dimensional nature of the structures (Figs 1F, 2C). For example, the lens and ocular cup of the eye were clear to the students in the enhanced and isolated images and the entire structure of the eye was clear in the three-dimensional model. In addition, the students found it very easy to see the looped heart of the chick in the three-dimensional model (Fig 2C), while in the serial sections the students only saw two separate circular structures and could not determine the final shape of the heart.

The Dimensional Embryology App also provided the students with an easy tool for evaluating how the embryonic structures change through development using the time point and enhancement selectors and differences between organisms using the organism selector (Figs 2B, 2C, 3). When they began to compare the neural tube across the chick developmental time points they were able to see the change in structure of the forming neural tube. By switching to the frog embryo, the comparable neural tube was apparent. Similarly, the progression of eye development and the commonality of development among species (Fig 3) were clarified by simply switching from one screen of the App to another. The students preferred to use the App and not the original images for making these types of comparisons.

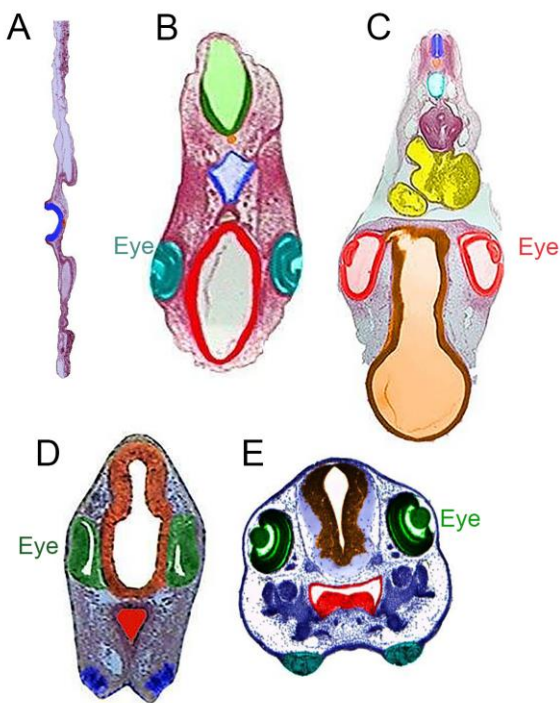


Fig. 3. Enhanced two-dimensional serial sections of Chick and Frog embryos at different developmental time points. (A) 24hr Chick (B) 48hr Chick (C) 72hr Chick (D) Hatched (4mm) Frog (E) 10mm Frog

DISCUSSION

Research has shown that the use of computer-assisted learning (CAL) has aided in an increase in student understanding and consequently has resulted in improved test scores (Burk et al., 2013; Khalil et al., 2005; Khalil et al., 2010). Additionally, CAL decreases the costs associated with teaching expensive anatomy, embryology and vertebrate biology laboratories, by replacing the need for purchasing live or fix embryos with virtual labs, websites, and applications (Beale et al., 2014; Nieder and Borges, 2012; Petersson et al., 2009). Furthermore, the use of CAL can decrease the amount of class or laboratory time spent on teaching a topic, as students can use the tools individually, spending time outside of the classroom to master the material. Over eighty percent of dental schools use CAL for embryology and anatomy (Burk et al., 2013). As embryology ceases to exist as a standalone course, the time spent teaching embryonic development is limited. There are a number of CAL resources currently available to support the study of embryonic development, including extensive support of understanding human development (Ecker et al., 2003; Hill, 2015; Museum of Health and Medicine, 2012; O'Loughlin, 2008; Sulik and Beam Jr., 2015). Model organisms, such as chicks and frogs, are often used to study development, however there are less resources available to evaluate their development (Muneoka, 2000; Scadding, 1998). The resources that

are available do not clearly demonstrate the relationship between two-dimensional serial sections and three-dimensional embryonic structures for frogs or chicks, while indicating the different tissues in the images.

The library of images contained in the Dimensional Embryology App spans multiple times in development for chick and frog embryos (Fig 2, 3). The App uses a series of two- and three-dimensional images of embryos to help students gain a better understanding of embryonic structures and their relative positions in the embryo. By providing the students with a series of images for different time points in both chick and frog development, they can begin to compare the organisms and the timing of development of different structures in the embryos. The comparative anatomy of the developing eye in frog and chick embryos for example, demonstrates the developmental similarities between species and the progression of eye development within one organism over time (Fig 3).

When student learning of embryology and development of multiple species was qualitatively assessed, the students that used the enhanced or isolated images on the App found it easier to understand the structure and organization of the chick and frog embryos as compared to using the serial section slides. They commented that the App was easy to use, clear to understand and convenient. Our qualitative assessment suggests that the students support the use of the App and are more likely to learn about embryology quickly and easily. Further testing is needed to quantitatively assess student learning when using the App.

Future directions include adding additional time points for chick and frog embryos, other organisms such as pig embryos, and specific mouse organs, to allow a user to experience more exposure to embryology. Additional learning tools could be added to the Dimensional Embryology App, such as textual explanations, videos of specific slides and self-quizzes. We anticipate and encourage constructive feedback from users suggesting changes and new features.

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REFERENCES

- BEALE, E.G., TARWATER, P.M., AND LEE, V.H. 2014. A retrospective look at replacing face-to-face embryology instruction with online lectures in a human anatomy course. *Anatomical sciences education* 7: 234-241.
- BURK, D.T., LEE, L.M., AND LAMBERT, H.W. 2013. Embryology and histology education in North American dental schools: the Basic Science Survey Series. *Journal of dental education* 77: 744-756.
- ECKER, P., ECKER, G., BUTLER, C.D., MATHERS, L.H., WILSON, D., et al. 2003. Simbryo - Animated Embryology. Accessed from <http://simbryo.stanford.edu/index2.html>. on 15 March 2015.
- HAMILTON, J., AND CARACHI, R. 2014. Clinical embryology: is there still a place in medical schools today? *Scottish medical journal* 59: 188-192.
- HILL, M.A. 2015. UNSW Embryology. (University of New South Wales). Accessed from https://embryology.med.unsw.edu.au/embryology/index.php?title=Embryo_Serial_Sections. on 1 December 2014.
- KHALIL, M.K., LAMAR, C.H., AND JOHNSON, T.E. 2005. Using computer-based interactive imagery strategies for designing instructional anatomy programs. *Clinical anatomy* 18: 68-76.
- KHALIL, M.K., MANSOUR, M.M., AND WILHITE, D.R. 2010. Evaluation of cognitive loads imposed by traditional paper-based and innovative computer-based instructional strategies. *Journal of veterinary medical education* 37: 353-357.
- MUNEOKA, K. 2000. Embryology - Online Developmental Atlas. (Tulane University). Accessed from <http://www.tulane.edu/~embryo/index.htm>. on 14 May 2014.
- MUSEUM OF HEALTH AND MEDICINE, N. 2012. Developmental Anatomy - Human Development Anatomy Center. (Silver Spring, MD). Accessed from <http://www.medicalmuseum.mil/index.cfm?p=collections.hdac.anatomy.index>. on 1 March 2015.
- NIEDER, G.L., AND BORGES, N.J. 2012. An eight-year study of online lecture use in a medical gross anatomy and embryology course. *Anatomical sciences education* 5: 311-320.
- O'LOUGHLIN, V. 2008. Human Embryology Animations. (Indiana University). Accessed from http://www.indiana.edu/~anat550/embryo_main/. on 13 February 2015.
- PETERSSON, H., SINKVIST, D., WANG, C., AND SMEDBY, O. 2009. Web-based interactive 3D visualization as a tool for improved anatomy learning. *Anatomical sciences education* 2: 61-68.
- RICHARDSON, L., VENKATARAMAN, S., STEVENSON, P., YANG, Y., MOSS, J., et al. 2014. EMAGE mouse embryo spatial gene expression database: 2014 update. *Nucleic acids research* 42: D835-844.
- SCADDING, S. 1998. Developmental Biology Online. (University of Guelph, Guelph, Ontario, CA). Accessed from <http://nte-serveur.univ-lyon1.fr/nte/embryon/www.uoguelph.ca/zoology/devobio/dbindex.htm>. on 27 August 2012.
- SCOTT, K.M., CHARLES, A.R., AND HOLLAND, A.J. 2013. Clinical embryology teaching: is it relevant anymore? *ANZ journal of surgery* 83: 709-712.
- SCHOENWOLF, G.C. 2008. Chapter 6: Amphibian Development and Chapter 7: Avian Development. In *Atlas of Descriptive Embryology*, 7 ed. Pearson Benjamin Cummings, San Francisco, CA.
- SULIK, K.K., AND BEAM JR., P.R. 2015. Embryo Images Normal and Abnormal Mammalian Development. (Greenwood Genetic Center, Greenwood, SC and University of North Carolina at Chapel Hill, Chapel Hill, NC). Accessed from https://syllabus.med.unc.edu/courseware/embryo_images/. on 15 February 2015.
- TYLER, M.S., AND KOZLOWSKI, R.N. 2010. *DevBio Laboratory: Vade Mecum3*. Sinauer Associates, Inc., Sunderland, MA.