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Imaging Early Stage Axolotl Salamander Embryos Inside and Out

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ABSTRACT

Preliminary imaging all sides of amphibian embryos using time-lapse microscopy during early stage development has been accomplished. Also, Optical Coherence tomography was successfully used to image the interior of a blastocoel stage embryo. Due to the yolk in amphibian eggs they cannot be seen using confocal microscopic techniques. Using The images from the exterior of the embryo and images of the interior of the eggs will be correlated to further the study of the forces that cells experience during early development including neural plate and early brain development. This will further the study of chordate and human brain development.

INTRODUCTION

Imaging Inside the embryo

The inside of the early stage live salamander egg/embryo was imaged using Optical Coherence Tomography (OCT). The OCT apparatus used was a quadrature OCT using a swept source frequency operating at 1310 ±55 nm. A main advantage of using a quadrature OCT system over a standard swept-source OCT system, is that the complex conjugate artifact in the OCT image is suppressed, allowing for a greater imaging depth to be achieved [1].

The egg/embryo was at stage 9 when the blastocoel and neural plate develop [2]. The main goal of the study is to follow the blastocoel development in relation to the neural plate. This will give insights into how the blastocoel helps form the neural plate and the brain. An image and approximate orientation of an OCT sagittal slice is shown in Figure 1. This type of imaging is used in medical practice to view the interior of the eye and is a less invasive method of viewing inside a delicate developing embryo [3]. Salamander eggs have highly reflective yolk so microscopes that require visual light transparent tissue cannot be used.

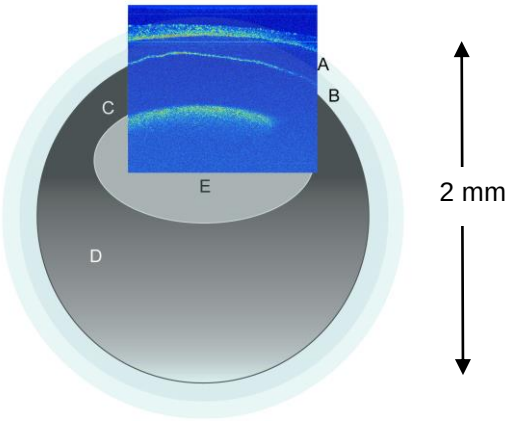

Figure 1: The salamander egg is 2 mm in diameter. The blue portion is the OCT image taken of the developing egg (0.7 mm) – blastocoel albino Axolotl egg at stage 8 – 9. **A** outer jelly, **B** Inner jelly, **C** Animal half of the egg/embryo, **D** Vegetal half of the egg/embryo, **E** Blastocoel.

This partial image of the developing blastocoel shows that the developing blastocoel can be imaged using Optical Coherence Tomography. This will enable the study of how blastocoel development interacts with neural plate development in early stage axolotl embryos.

Imaging the Outside of the Embryo

As most of early development occurs on the outside, such an approach can be quite fruitful. In a new technique developed by Dr.

Richard Gordon [4], the eggs are rotated rapidly and then slowly rotate back to an upright position because they are bottom heavy. In a nontoxic, water based viscous fluid (containing methylcellulose), the time for up-righting can be stretched to many seconds. The rotation of the eggs can be accomplished using a small microcontroller with specific timing so that all sides of the eggs/embryos can be viewed and images can be taken as the embryo uprights itself. The images will then be montaged to produce a 4D time lapse microscopy display of the whole surface as the embryo develops.

The outside images are collected using a microscope stage that flips a salamander egg so that its heavy yolky bottom end is on top has been successfully developed. As the egg rolls back, a microscope movie of the surface is made, from which the positions, shape changes, differentiation waves, and mixing of the cells can be ascertained [5,6]. This will give us unprecedented data on how a vertebrate embryo forms itself.

The instrument uses Arduino microcontrollers and servo motors to control a stage that rotates and then flips the egg/embryo. The rotation guarantees that the embryo rolls back a different way each time, so that the whole surface is imaged. Thus the rest of the apparatus consists of a stereo microscope with an attached digital SLR camera that is triggered repeatedly by an additional Arduino microcontroller. Lighting was accomplished using an LED ring light and side LED lights so that the spherical egg is evenly lighted on all sides.

This project was undertaken to view all sides of the surface of the egg while it is developing. This is necessary to view the movement of cells as they change from epithelial tissue into neural tissue, forming the brain and spinal cord.

Development of the Flipping Microscope Stage

The concept of the stage is to turn the axolotl egg/embryo upside down and then photograph it rapidly as it uprights itself. This happens within seconds because the eggs are bottom heavy with more yolk in the bottom vegetal portion of the egg than in the top animal portion.

The requirements of the stage are that it be fast enough in flipping motion to turn the egg upside down and allow it to right itself, while the camera takes several photographs of the surface. Then the stage needs to turn the embryo so that the next flipping motion will cover a different portion of the egg. A close-up photo of the finished stage in Figure 2.

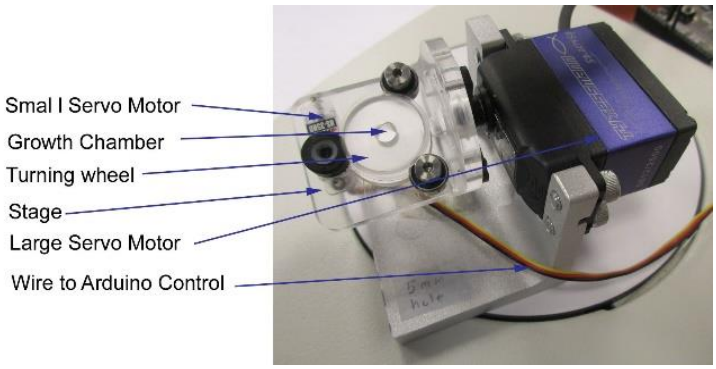

Figure 2: Flipping Microscope Stage. A salamander egg/embryo is place in the growth chamber and the Arduino microcontroller moves the large servo motor 180° within a half a second. This flips the egg upside down and then the egg uprights itself while the camera takes 9 or more pictures. The small servo then turns the small wheel which changes the direction of the egg so that all sides can be imaged. The concept drawing was designed by James R. Young and Drafting and final product Leon Bauman of General Metal, Winkler.

The large servo moto is a SAVOX SB-227-SC and the small servo motor is a HiTec HS-35HD. Both servo motors turn 360° and so can continuously turn if needed. The small wheel driving motor has a rubber wheel attached to it and the holding wheels are steel ball bearings. The 5 mm hole is the egg chamber and allows for an egg with part of its jelly removed to be placed in the chamber. Further design of the chamber needs to be done so that the egg does not dry out but can still breath.

The whole microscope setup

The whole microscope consists of a stereo microscope (light microscope, LM) which is the lens for a digital SLR camera, and a stand. The stand positions the lens in focus on the object below – in this case the egg. Only parts of the egg are in focus so the top portion of the egg was chosen as the focus point. The LM scope does have a good depth of field. The field of view is approximately 3 mm giving ample room for the 2 mm egg. The camera used is a Nikon D7200 and has good low light capabilities so that a moving object can be photographed using the LM Scope lenses. The camera is set to 6 Megapixels as this corresponds to the resolution of the LM scope. The ring light is from Titan Tool Supply Inc. and the LEDs are at an angle so that even lighting is achieved at the level of the stage.

CONCLUSION

The Flipping stage microscope take about 15 Gigabytes of data an hour which is approximately 5,000 pictures at 6 Mega Pixel size images in jpeg mode. The development of the salamander neural plate and then neural tube and brain closure called Neural Tube Closure takes about 48 to 52 hours. NTC is when the brain and spinal column have become a closed tube and begin internal development. The amount of storage needed per egg will be about 750 Gigabytes for jpeg images or 7500 Gigabytes uncompressed RAW images. The system will show cell behavior as the surface cells develop into neural tissue, namely brain and spinal cord.

ACKNOWLEDGEMENTS

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