High-Resolution 3D Reconstruction of the Surface of Live Early-Stage Toad Embryo

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SIGNATURE
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ABSTRACT

To show the three-dimensional (3D) shape of a live toad embryo is helpful to understand the toad embryo development. It is also desired to make more details visible by using high magnification objective lens. However, limited depth-of-field and field-of-view make it challenging to use high magnification objective lens in imaging the embryo development. This paper shows that 3D reconstruction of live toad embryo can be accomplished by collecting optical section grids in the xy-plane, acquiring each optical section along the z axis using a motor-controlled microscope, making each optical section a single in-focus image with the deconvolution algorithm, combining the image grids into one big image, and then reconstructing to a 3D view. In this study 3D reconstructions were made from both fixed and live early-stage toad embryos.

INTRODUCTION

Although there continues to be great interests in the mechanics of early-stage embryo development, most kinematic data about morphogenetic processes are qualitative, and limited to two dimensions [1]. The challenges are due to the small size of embryos (2 mm diameter or smaller), their high sensitivity to irradiation [2], and their extreme mechanical fragility. Optical methods are excellent for studying embryo development because they can obtain morphometric data without physical contact with the object. Optical methods can also provide sufficient spatial resolutions for studying embryo morphogenesis. These features make optical methods preferable to various scanning and tomography methods suitable for 3D studies of embryo [3].

Limited depth-of-field is a common problem with conventional light microscopy. As shown in figure 1, the specimen’s profile often is more than the depth-of-field of the objective lens. The region of the specimen outside the depth-of-field appears out-of-focus in the acquired image. Moreover, this situation becomes worse when using higher magnification objective lens because the numerical aperture (NA) increases, thus the depth-of-field d becomes smaller:

\[ M = \text{const}.NA \]  \hspace{1cm} (1)

\[ NA = n \sin(\alpha) \]  \hspace{1cm} (2)

\[ d = \frac{\lambda}{(n \sin^2(\alpha))} \]  \hspace{1cm} (3)

where \( \lambda \) is the wavelength of the illumination, \( n \) is the refractive index of the medium in front of the lens, and \( \alpha \) is the angular semi-aperture on the objective side [4]. Consequently, images show certain parts of the specimen in and out of focus, respectively. In this study, one approach was used to image the whole specimen by taking multiple optical sections corresponding to different...
object planes along z axis. Deconvolution techniques can then be used to select from each slice the focused area to reconstruct an image that is in focus anywhere. In this way, it is possible to extend the apparent depth-of-field avoiding the physical limitation of objective lens.

Another limitation of objective lens is the limited field of view (FOV). Only a small portion of the specimen can be seen with high magnification objective lens. Generally, one has to make a tradeoff between the details and the FOV. In practice, objective lens of 4X magnification was typically used for obtaining the complete view of the whole embryo. In this study, a simple approach was proposed to overcome this limitation. Since only a small portion of the specimen can be seen at one time, larger area can be obtained by collecting multiple portions one by one in a pattern of grids in xy-plane, and then combining those portions into a big image. In this way, the FOV of the objective lens can also be extended apparently regardless of the physical limitation.

The purpose of this study was to investigate the feasibility of using appropriate microscopy techniques and software algorithms to overcome the depth-of-field and FOV limitations of the objective lens with relative high magnification. An approach was proposed to obtain more details of the embryo surface using high magnification while still preserving large FOV. Images were recorded from late gastrulation to right before neurulation of live toad embryo for better understanding the toad embryo development. Finally 3D view was reconstructed to show the surface shape of the embryo.

**MATERIALS & METHODS**

**Toad embryo preparation**

Toad embryos were prepared according to the procedures in reference [5]. Embryos were obtained by standard methods [6] and staged according to reference [7]. Whole embryos were fixed in 2 to 5% TCA in phosphate buffered saline (PBS). Live samples were rinsed in PBS with 0.1% Triton-X 100 (PBST), blocked for 1 hr in PBST with 10% heat-inactivated goat serum (PBST+GS), and incubated overnight at 4ºC with 1:500 monoclonal antibody 4H2.

**Microscopy**

In this study, Olympus IX70 light microscopy system was used. The objective lens used was UPlan Apo 10X dry lens with the numerical aperture 0.4. A Hamamatsu Orca CCD camera was used as the detector, which was the original Orca now known as the Orca-1. Because the embryo was not transparent to light, epi-illumination by the fiber optics from both sides of the stage was used.

The optical section grids were acquired by the computer-controlled motor system. An optical section grid of 2 by 2 was obtained in xy-plane, and each grid panel was a stack of 100 optical planes. In xy-plane, the vertical and horizontal step sizes were 50 µm and 70 µm, respectively; and redundant areas near borders of the neighboring grid panels were reserved for the convenience of later combination algorithm. The step along the z axis was 5.6 µm.

**Deconvolution for each stack**

Previous research has proposed two main approaches for deconvolution.

The first one is the point-based deconvolution method, such as stack focuser algorithm [8]. In stack focuser algorithm, a 3x3 median filter is applied on each slice in a stack to reduce noise and then Sobel filter is applied to find edges. The maximum value in the neighborhood of the specified size is taken to determine how far from a focused edge the image is still focused. For every pixel (x, y), based on the choice of the maximum edge value among different slices in the map stack, the pixel value is copied from appropriate original image to new all-in-focus image.
The second method is the multiresolution-based method, for instance, extended depth of field algorithm [9]. The guiding principle for this approach is the assumption that the focused parts contain more details than the out-of-focus areas and thus have more high-frequency components. Extended depth of field algorithm uses discrete wavelet transform to do the image frequency analysis and then determine which area in each slice is in focus.

In this study, stack focuser algorithm was used for deconvolution, because extended depth of field algorithm was relatively complicated and computationally time-consuming. Stack focuser algorithm provided similar results to those of extended depth of field algorithm while it was much faster. The deconvolution was processed by ImageJ (Wayne Rasband, NIMH) plugin of stack focuser [8].

**Image grid combination**

After deconvolution for each grid panel, all-in-focus images were obtained as well as the topology maps providing the height information for each corresponding pixel. A self-written Matlab (Mathworks Inc., Natick, MA) program was then used to combine all of the images into one big image. The redundant areas were first removed by overlapping the neighboring grid panels with the redundant area size estimated by the step size and panel size. Template matching technique was used to aid registration. The topology maps were also processed in the same way.

**3D reconstruction**

The 3D reconstruction was also performed by the self-written Matlab program. The complete all-in-focus image and the corresponding topology map were combined to reconstruct the 3D surface of the embryo. Multiple views from different angles were also reconstructed.

**RESULTS**

The optical section grids for a fixed toad embryo acquired by the motor-controlled light microscopy system were shown in figure 2. Note the out-of-focus areas in the images. And also notice the redundant areas reserved near the borders of the neighboring grid panels.

Figure 3 shows example deconvolution result of for panel 3 stack in figure 2. Stack focuser algorithm was used here.

After deconvolution for each grid panel, all of the grid panel images were combined into one big image. Figure 4 illustrated this procedure by an example of image. The topology maps were processed using the same method, although not shown in figure 4.

The 3D views of the fixed embryo surface are shown in figure 5. The shape of the surface
can be observed clearly in the 3D views.

![Image](image1.png)

Figure 3  The example deconvolution result for grid panel 3. A-E: 5 sample optical planes out of 100 optical planes in the optical section stack; F: deconvolved in-focus image; G: topology map (height map). This is the result of stack focuser algorithm.

![Image](image2.png)

Figure 4  Combination of the deconvolved grid panels. A: the mosaic image, note that redundant areas exist near the border of neighboring grid panels; B: the final combined image, where the redundant areas have been registered correctly.

Figure 6 shows the time series of live toad embryo surface. For publish purpose, only four time points were picked out for demonstrating the morphogenetic processes during the early-stage embryo development.

**CONCLUSION**

In this paper, deconvolution and mosaic combination methods were presented to extend the depth-of-field and field-of-view for the high magnification objective lens of light microscopy system. A new approach acquiring optical section grids then processed by deconvolution and
mosaic combination was proposed to obtain large FOV to contain the complete whole embryo with fine resolution using high magnification objective lens.

The time series of live early-stage toad embryo 3D view showed in this study demonstrated that kinematic data about morphogenetic processes could be obtained using this approach. High resolution 3D stereoscopy can be obtained by automatically controlled image acquisition and real-time processing of deconvolution, combination, and 3D reconstruction, showing 3D information of the toad embryo developing in fine details.
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REFERENCE