Creation of a Physical 3D Homo sapiens Single-cell Model using Confocal Microscopy Data via Rapid Prototyping

Paul Hausch
Department of Physics
Ripon College
300 Seward Street PO Box 248
Ripon, WI 54971

Rapid Prototyping Center
Milwaukee School of Engineering
1025 N. Broadway
Milwaukee, WI 53202

Faculty Advisor: Dr. Vipin Paliwal

Abstract

The purpose of this research was to further develop a novel process of converting confocal microscopy images to create a physical three-dimensional cell model. This work is a continuation of John Konieczny’s research from 2009 where two-dimensional images of a cell cluster, specifically a Caenorhabditis elegans embryo, were converted to create numerous physical models. Data images of a single Homo sapiens endothelial cell were created through the process of rapid prototyping, which has not been done before at this level of magnification. Confocal Microscopy image data sets of the singular cell were collected as TIFF files. To enhance the images, they were imported into the medical imaging program Mimics for numerous enhancements including thresholding, dynamic region growing, various morphology, and Boolean operations were performed to create two separate segmentation masks. The files were exported into the Freeform® Modeling program, allowing for the creation of a virtual 3D clay model. Moderate smoothing was done using the program Phantom® Desktop, to reduce the stepping effect created by data collection of the 2D images. Next these files were converted into .STL file format, which is compatible with rapid prototyping machines. Physical 3D Models were created using both the ZTM 510 (Z Corporation) 3D printer and 250 SLA (using the DSM Somos Watershed XC 11122). This work increases cellular comprehension in education, research, and clinical applications by allowing for kinesthetic exploration of an exact cell model.

Key words: Rapid Prototyping, Endothelial, Cytokinesis

1. Introduction

This research is expanding off of previous work to create 3D cell models from confocal microscopy data images through the process of additive manufacturing (commonly known as rapid prototyping)\(^1\). The microscopy technique used for data collection was confocal microscopy. This method was used for the high levels of magnification and
detail capabilities. The previous work resulted in the creation of a physical cell model of a nematode *Caenorhabditis elegans* embryo. Expanding off this cell cluster model was the idea to create six cellular models of the phases illustrating mitosis/cytokinesis of a single human endothelial cell, which has never made accomplished before. The model was made to highlight two structures in the cell, the DNA and tubulin. This work was done to move away from artist’s three-dimensional renditions by making exact models of cells. These models will kinesthetically aid understanding for researchers, educators, and clinical applications.

Mitosis and Cytokinesis are the stages in a cell’s life where one cell replicates its own DNA and divides into two separate cells. This process was selected as the model of choice because it is an essential part of every cell’s life. Human endothelial cells form a thin layer along the interior of blood vessels and lymphatic vessels, and together they are called the endothelium. This cell type was chosen because knowledge could be gained about human progression through life. Mammal cells have not been made before so a human cell seemed like the perfect fit for further exploration. An addition to this project’s direction was to create a model of a single cell. Previously a cell cluster was created but by making just one, we might be able to understand the intercellular life of a cell in a more personal way.

2. Methodology

The technique of confocal microscopy was used to collect images of human endothelial cells spaced evenly throughout the cell stage of mitosis/cytokinesis. Six cells of the cell process were imaged including prophase, metaphase, early anaphase, anaphase, telophase, and cytokinesis. A seventh stage depicting a cell in its normal life stage was also imaged to make comparisons. The images were TIFF files collected along the z-axis of each specimen consisting of 35-74 optical sections spaced 0.12 – 0.2 microns apart. Each specimen was triple stained highlighting the nucleus, DNA, and tubulin. The staining consisted of 405 DAPI for the nucleus, alexa 488 for the DNA, and alexa 568 for the tubulin.

2.1. segmentation

The medical imaging software program Mimics developed by Materialize was used to perform segmentation operations on the grayscale two-dimensional TIFF image slices individually. First a thresholding operation was performed to create a brightness histogram of the image pixel density, starting from the center of the cell towards the exterior. The nucleus was eliminated to make the replication and migrating of the DNA more visible in the models. Dynamic region growing function was completed to eliminate noise (random pixels) that were picked up during the image collection phase. Thresholding operations were performed again prior to multiple morphology operations (including open, close, dilate, erode). These morphology operations were done to increasingly define the cell walls by connecting pixels together of a certain density without using mathematical modeling, and delete pixels below a specified density. Then the cell was saved and exported to Magics, a CAD software development rapid prototyping program, to correctly orient and scale the models.

![Figure 1. Raw image, focal plane 30.](image1)

![Figure 2. Mimics enhancement process.](image2)
2.2. modeling

The program Freeform® Modeling™ was used to turn the 2D data images into a virtual 3D clay model allowing for clay-like molding and alterations to be performed. Then using the Phantom® Desktop™ haptic device, force feedback was possible to physically interact with the virtual model. First, automatic smoothing was performed moderately to eliminate the stepping effect between layers, while retaining the cell’s original texture. The stepping effect forms by the difference in diameter of each consecutive optical slice. After the final smoothing steps were performed, the file was converted to .stl file format, which is compatible with rapid prototyping machines.

![Virtual 3D model in Freeform® Modeling™](image)

2.3. rapid prototyping

Rapid prototyping is the process of creating three-dimensional objects built one cross-section at a time. Layers of a specific resin or powder are solidified in successive layers starting at the bottom with succeeding layers added on top until the object is produced. There are numerous types of rapid prototyping processes but for this build, the 3D SystemsTM 250 SLA along with DSM Somos’ Watershed® XC 11122 resin was used and the Z Corporation’s Spectrum Z™ 510 along with the zp® 131 plaster powder and zb®60 acrylate binder. The stereolithography (SLA) machine was chosen for its clear properties and visibility, while the 3D printer was used for its color capabilities.

The SLA machine cured the layers of resin starting at the bottom and moving upward until the whole model was completed. A vat of photosensitive liquid polymer on a build platform was lowered .004 inches each layer and a helium cadmium (HeCd) laser followed the path of preset coordinates to cure the resin. The Watershed® resin created a clear square plate with the highlighted cell structures housed inside it. The process was completed with no post-curing needed.

A second build using the Z Corporation’s Spectrum Z™ 510, full color system was used to produce a 3D cell model sitting on top of a thin square plate for stability. Prior to the build, color was added to the virtual model using ZCorp™ Zprint software. In this process, the cell was color coded red to provide contrast between the actual cell and supporting structure. Labels of each of the phase names were engraved into each of the cell models, but on the back to allow for classroom testing of mitosis/cytokinesis. A layer depth of .004 inches was used to produce a 2.5x2.5x0.25 inch model for this build. After the printing was completed, the part remained in the powder bed for post-curing at a temperature of 100°F for 70 minutes to allow for the binder to harden. Compressed air and a horse hair brush was used to remove extra powder left over. Post-processing was completed by the infusion of the model with a cyanoscrylate infiltrate was applied using a dropper to increase the durability of the finished cell model.
3. Results

Figure 4. SLA model, normal cell state

Figure 5. Z-Corp model, normal cell state

The SLA model constructed using the DSM Somos Watershed® 11122 resin produced satisfactory results with outlines of the DNA and tubulin structures highlighted. The models small scale made it hard to distinguish between the separate structures and even the model as a whole. This thinness of the endothelial cell made the model very hard to see because it forced the laser highlighting to be very faint. Without backlighting the resin seemed cloudier with a reduction of structure visibility. The side view shows a blurred image of the cell from the slices placed successively on top of each other, however when viewed from the top or bottom of the cube the image becomes clear.

The models produced using 3D inkjet printing shows the areas of interest with greater clarity. These color-coded models were easier to see because of the contrast of the red cell against the gray supporting structure. The names of each of the phases etched into the back of each model made it very easy to discern each phase from the next. Overall these models were much more cleaner as a completed set and displayed the stage of mitosis/cytokinesis in a clear and clean way.

4. Discussion

These models have shown that microscopy parameters need to be recorded when modeling. The actual scaling of each model after the various conversion processes is important to know how big the specimen is in reality. Additionally the increase of optical sections of the specimen meant that less smoothing operations were needed for the models. This allowed for the models to be closer to the actual image data collected and how a true to nature cell would look. When enhancement is performed, this will impact the model’s accuracy so this must be stated to insure that the physical model is in no way thought to be a more accurate representation than it actually is. Also consultation of a biologist or microscopist is necessary to say with confidence that the modeling process here has provided an accurate cellular representation. For both models the cell is shown as an extremely thin structure along the z-axis. The cell is analogous to a sunny side up egg where the nucleus is the yolk and the tubulin structure is the egg white thinning at the edges.

The SLA models were not as successful due to the faint laser highlighting that the rapid prototyping machine was only able to achieve. The specimen was extremely thin in this case and lacked three-dimensional volume that was necessary to create a darker outline of the cellular structures. The thinning of the specimen is due in part by the imaging process of a single cell. Since there is only one cell standing alone during the image collection phase there is no support from the surrounding cells to retain its volumetric shape and therefore the single cell flattens. For future models using the SLA machine, cells that have a greater three-dimensionality should be used in order to insure that the laser highlighting process would be more visible to the naked eye. The visibility of these models did however, increase with the use of backlighting in a dark room. The light coming from below illuminated the cell model and showed the start to a 3D model forming but still was somewhat lacking in clarity of individual structures within the cell.

The 3D inkjet printing models are far superior to the SLA models in that they are visible to the naked eye without the need for magnification or backlighting. You can interact with these models in an entirely different way by actually being able to touch them and feel the three-dimensionality. These models are ideal for an educational setting because the bottom of each model is labeled with the name of that cell phase. This underside engraving
would fit well for a self-testing exercise where a student could try to put the models in order and check to see if they are correct.

For future models different methods of adding color to the clear resin models could be explored. For instance a hollow cavity of a cellular structure could be filled with some sort of solid color material, which would bring actual color to a SLA instead of just the shading from the laser burning process. Or creating a SLA cell model in two sections, filling each side individually and fusing them together to create a full model.

5. Conclusion

This research increases cellular comprehension in education, research, and clinical applications. A new way to observe mitosis/cytokinesis has been created by having the ability to hold and feel the structures involved in this cellular process. This form of learning is a superior method for kinesthetic learners who will have a greater understanding through the physical interactions of holding actual objects. This work also further developed the process of creating cell models through the process of rapid prototyping by creating a single human cell model, which has never been done before at this level of magnification. We can also open the door to new discoveries overlooked on 2D models and maybe lead to unknown knowledge about Homo sapiens.

6. Acknowledgments

This project was funded by the National Science Foundation (NSF) and the Milwaukee School of Engineering (MSOE) Rapid Prototyping Center (RPC). The author would like to thank Ann Bloor, Director of Research Administration, Dr. Vipin Paliwal, Betty Albrecht, Eric Beccue, Joe Peroutka, and Dr. Subha Kumpaty. Special thanks to Dr. Padmanabhan Vakeel and Dr. Ramani Ramchandran for collaboration on this project. Additional thanks to all of the faculty advisors and REU participants for a wonderful experience.

This material is based upon work supported by the National Science Foundation under Grant No. EEC-1062621. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

7. References