

# Investigating the Process of Converting Microscopy Data Sets for Use with Additive Manufacturing

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## Abstract

The objective of this research is to explore the use of advanced imaging technology for utilization with additive manufacturing (AM) to produce detailed and accurate three-dimensional biological models at the cellular level for use in biological instruction and laboratory study. At this time, three-dimensional models in a virtual computer environment and two-dimensional analyses of microscopy images are the primary methods employed allowing researchers the capacity to observe specimens being investigated. Instructional aides for specimens on this scale consist of inaccurate renditions of what is actually taking place, making them unusable for research investigations due to the inherent inaccuracies they possess. This work illustrates the development of a methodology to produce more accurate three-dimensional, tactile cellular models allowing for a new way to view the biological structures and processes captured through microscopy. It is anticipated that this new capability will assist researchers in their study of biological specimens and events, as well as help students at all levels to gain a better understanding of structures and events taking place under the microscopes lens. Through this work, a protocol for cellular model construction has begun to be established for the conversion of file types containing data sets from various microscopy platforms to file types commonly found in the AM field. In addition, procedural steps that were followed in the extrapolation of this dataset prior to the build have been detailed in the researcher's log book, and will later be published for the benefit of future study.

**Keywords:** Rapid prototyping, Microscopy, Cellular modeling, Additive manufacturing

## 1. Introduction

This research introduces the concept of using additive manufacturing to produce accurate three-dimensional physical models of cellular level images from data sets generated by advanced microscopy techniques. Current and previous research has focused on the creation of detailed physical models at the molecular level or focused on converting the data sets generated by magnetic resonance imaging (MRI) or computerized tomography (CT) scans for use with AM<sup>1,2</sup>. The goal of this research however is to move beyond previously established protocols that make use of CT and MRI files, and to produce an accurate physical model of a cellular level specimen utilizing data sets originating from select microscopy platforms. The significance of this research lies in its ability to provide research biologists a valuable tool to study cellular models in a three-dimensional, tactile form that may lead to greater understanding of the specimen being studied. While this does not seek to replace methods and procedures currently being utilized, it hopes to augment the resources currently available. Furthermore, students at all levels may benefit from models that can enhance the learning experience by opening up new routes of discovery, allowing both visual, as well as kinesthetic routes for comprehension and innovation<sup>3</sup>.

Current three-dimensional cellular level models tend to be artists' renditions depicting cellular structures and their internal composition. This research was done to create a superior model that acts as a teaching aid for use in both academic and research realms. The reasoning in focusing on microscopy files for modeling is the belief that the preeminent way to accurately depict events taking place at the cellular level is through microscopy. Confocal laser scanning microscopy (LSCM) and multiphoton laser scanning microscopy (MPLSM) were the platforms of choice because of their optical sectioning capabilities that allow for a graphic, non-invasive route to study specimens in infinitesimal detail throughout the complete cellular structure<sup>4</sup>. While LSCM and MPLSM images were the initial focus, other microscopy platforms cannot be discounted as valid routes of producing cellular level models. However, a file that was available from a MPLSM was selected for the initial build because of its ready availability, and the significance of the scientific research being done utilizing this particular species, the *Caenorhabditis elegans*, commonly referred to as a *C. elegans*<sup>5</sup>. The file type generated by this particular microscope dictated the progression of steps that were taken to bring this build to fruition.

To date, no previous published work has been found, after extensive literature searches, that indicate that microscopy files have been converted for use with additive manufacturing, although the possibility of such has been mentioned<sup>6</sup>. This being said, the methodology that was devised for this particular research did not have the benefit of previous protocols for the production of these cellular level biological models. With this in mind, extensive documentation has been undertaken so that the knowledge gained from this project may serve as a guide for future research and production of cellular level models.

## 2. Microscopy

Fluorescence microscopy, specifically LSCM and MPLSM were the focus of this study due to the stacked nature of the data sets produced by the optical sectioning of these platforms. Each layer in the stack represents a separate scan taken at a progressively lower focal plane along the Z axis, allowing for detailed imaging through the entirety of the specimen<sup>7</sup>. In the past, these data sets have been difficult to convert to file formats that allow for their enhancement outside of provided software due to the proprietary nature of software packaged with these imaging systems. To remove this obstacle, steps were taken to convert these image files for their use with various enhancement programs prior to conversion to .stl files commonly used in the AM field. Numerous factors must be considered by microscopists when preparing specimens and adjusting microscope parameters, if the ultimate goal is to produce accurate physical models. These steps become more arduous for microscopists when *in vivo* imaging techniques are to be considered and detailed protocols for this methodology have been established<sup>8</sup>. Although discussing the myriad of specimen preparation variances and microscope settings is beyond the scope of this research, information regarding the settings utilized to produce the image files used for this project has been made available as a base of information for further research<sup>8</sup>. Some basic general considerations related to scan settings and specimen selection will be discussed later in this paper as they relate to this project and are considered a starting point in the development of an imaging protocol for modeling to be expanded upon by further work. The system used to produce the scan used for this research was done with a *Nikon Quantum* inverted microscope. 85 time points consisting of 30 to 60 optical sections spaced 1.0 or 0.5 microns apart were taken over a period of several hours allowing four-dimensional representation of embryogenesis of the *Caenorhabditis elegans* embryo being studied<sup>9</sup>.

## 3. Additive manufacturing

Additive manufacturing, commonly known as rapid prototyping, is a technique used to produce complex three-dimensional objects built one cross-section at a time. In the additive manufacturing process, layers of a selected resin or powder are solidified by various methods in successive layers starting from the bottom with succeeding layers added on top until the complete product is produced. Numerous variations on the process exist but all operate in much the same manner. In the case of stereolithography (SLA), one of the processes used for this project, a build platform is positioned at a preset distance below the surface of a vat of photosensitive liquid polymer. A helium cadmium (HeCd) laser follows a path of preset coordinates, curing the initial layer of resin. The build platform is lowered (for this study .004 inches), a sweeper device skims the surface of the polymer to ensure proper resin uniformity, and the laser scans the next layer, and so on, until the entire part is complete. Post-production processing consists of the removal of support structures, if present, draining excess liquid resin, and post curing with ultraviolet light to complete the process. A process known as 3D or Z printing was also employed for model production in this project. 3D printing utilizes ink jet based technology to apply ink containing a binding agent to

simultaneously color and cure a cross-section of plaster powder. Post processing of these parts generally consists of applying heat to increase strength, removal of excess powder and application of a hardening agent. These parts tend to show exceptional color and detail; however, other forms of AM will create a more robust product. Different strengths and weaknesses are inherent with each of the various AM platforms as well as the selected materials used with each. As such, specific applications and needs dictate which process is best suited for individual applications.

## 4. Methodology

The data set used for this project is a sample data set of *C. elegans* undergoing cell fusion; made freely available to the public, and consists of a sequence of 85 time points, each time point in the data sets consisting of 33 focal planes, for a total of over 2000 scans<sup>10</sup>. Converting the *Bio-Rad* PIC file of the *C. elegans* to TIFF for use with other software was accomplished by using *ImageJ*<sup>11</sup>. This program, an open source Java based imaging analysis program from the National Institute of Health, along with user written plug-ins, was obtained from <http://rsb.info.nih.gov/ij/> for use on this research. After downloading, extracting, and opening the *ImageJ* toolbar, the *Bio-Formats* and *OME* plug-ins were selected and imported into the *ImageJ* plug-in folder. A drag and drop feature enables smooth importation of data into the toolbar. The raw data set was next converted to TIFF file format allowing for the viewing of all 85 separate time points making up the data set. The fourth time point was selected because of the superior definition of the cell membrane through a high number of focal planes. Other deciding factors were that higher numbered time points in the data set were less complete in the upper and lower regions of the embryo, and also had greater variations in the cell membrane between the focal planes. Many features available with the *ImageJ* toolbar for enhancement of the raw data set were explored, but time constraints dictated that more familiar commercially available software be used for extrapolation.

### 4.1. segmentation

*Mimics*, a medical imaging software program by *Materialize*, was used for this investigation to perform segmentation operations on the grayscale two-dimensional image slices imported from *ImageJ* and viewed one at a time. After importing the file, a slice distance of four microns was arbitrarily selected for modeling. Thresholding, the first operation performed with this software, allows for the user to create a brightness histogram of image data, and apply a color mask to an area containing a specified density of pixels. Brightness histograms were created by measuring pixel intensity along axes of a profile line moving from inside of an individual cell, through the embryo wall, and into the medium. Bracketing a specific area of the histogram scale was done next, allowing for isolation of the cuticle of the embryo to be given a selected color mask. This operation was performed numerous times on selected areas of the specimen to further isolate areas of interest. The dynamic region growing function was next utilized. Dynamic region growing allows the user to eliminate random pixels, known as noise, and create an encapsulating color mask using only connected pixels in a specified grayscale range. After the dynamic region growing operation, more thresholding operations were performed prior to morphology operations to further clarify the region of interest. A variety of morphology operations (open, close, dilate, erode) were used to allow for a minimum number of selected pixels, the distance between them specified, to be connected so that features of the cell may be drawn out that would otherwise be difficult to identify without the use of the mathematical modeling of this software. Conversely, selected pixels can also be removed if the density of the pixels falls below a certain value. Through a number of these operations, cellular walls within the embryo were able to be progressively more defined. After enhancement of the cellular walls was completed, a Boolean operation was performed creating two separate segmentation masks, one of the cellular structures and one consisting of the exterior walls of the embryo. The cell structure was then saved and exported to a 3D modeling program. The entire interior portion of the embryo was next filled with a color mask to represent the exterior walls of the embryo using the cavity fill operation. The file was then saved and exported to the 3D modeling program for further work.

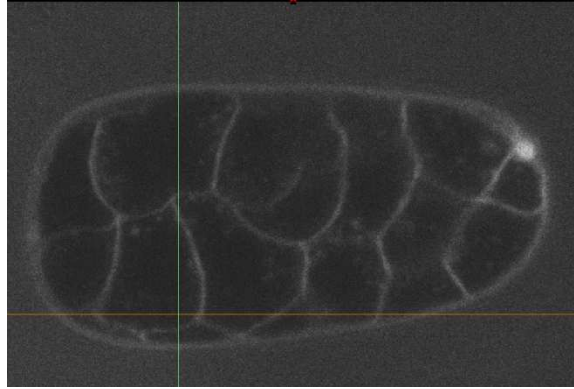


Figure 1 Raw image of time point 5, focal plane 22.

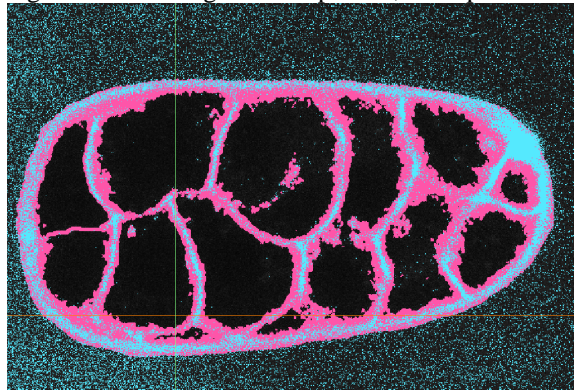


Figure 2. Mimics enhancement process.

## 4.2. modeling

The 3D modeling program, *Freeform® Modeling™*, allows the user to turn the three-dimensional computer images to virtual clay and permits modeling similar to that of an artist sculpting clay in the physical world. The *Phantom® Desktop™* haptic device enables the user to manipulate the clay model on the screen with force feedback, allowing for a physical interaction with the virtual model. A variety of tools are available that add, modify and refine the digital model on the screen. Automatic smoothing was the first operation carried out on the two individual files with this program. This was done to eliminate the stepping effect created by the variance in diameter of consecutive optical sections. A moderate level of smoothing was specified in the hopes of retaining the embryo's true surface texture. Interior cellular spaces were rendered solid in the software with walls removed; insuring pathways traversing individual cells were unobstructed allowing for more distinction of the cellular walls when modeling. The removal of obvious scatter imported with the image file was done prior to saving both files for modeling, demonstrating the effects of minimal extrapolation. It is understood that the lower and uppermost regions of the embryo were missing since the scanning did not cover these planes of the specimen. To demonstrate extrapolation capabilities, the missing upper cellular structures were created by drawing a line connecting multiple points surrounding the missing sections. Next, using the Tug function of the software, the uppermost region of the cellular structures was generated. The exterior portion of the embryo was than fashioned in a similar manner, creating a cap for the embryo. Lastly, consecutive smoothing operations were performed before saving the files and prior to conversion to .stl format for use with the AM machines.



Figure 3. 3D virtual model prior to extrapolation.

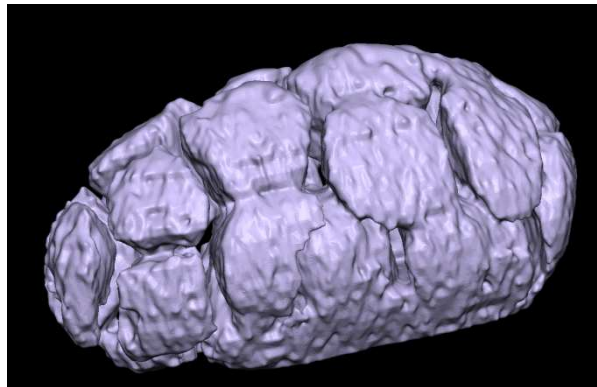


Figure 4. Virtual model after extrapolation.

### 4.3. additive manufacturing

A 3D printer and SLA were scheduled for simultaneous builds. *Z Corporation's Spectrum Z™ 510*, full color system, in conjunction with *zp® 131* plaster powder and *zb®60* acrylate binder was utilized to produce a cut-away model for the first build, consisting of focal planes 12-22. Prior to the build, color was added to the virtual model using *ZCorp™ Zprint* software. In this process, the cell membrane was color coded green, with the embryo's exterior coded gray to provide contrast between these areas. A layer depth of .0035 inches was used to produce a 7.75x4 x2.3 inch model for this build. Following the build, the part remained in the powder bed and a post-curing temperature of 100°F was applied for 70 minutes to allow for proper curing of the binder. Post-processing of the finished structure required utilizing compressed air and a horse hair brush to remove the residual powder prior to infusion of the model with a cyanoscrilate infiltrate that was applied using a dropper to increase the strength and durability of the finished product.

A second and third model were scheduled for simultaneous build using the *3D Systems™ 250 SLA* and *Huntsman® 9300* medical resin. This particular polymer was chosen because of its unique properties that allow selected areas within a clear matrix of the resin to be caste in a reddish hue by multiple passes of the laser to highlight the regions of interest. For the simultaneous build, the second model was to depict a complete, smoothed specimen, showing the capabilities of software extrapolation. The third model was to illustrate the stepping that occurs in the model when the optical sections are stacked and complete smoothing is not performed. The reason for scheduling a build with little enhancement was to show the limitations of building a model when scan settings are not optimized, illustrating the stepped nature of the model this creates. The first attempt to create these models failed, with the minimally enhanced model warping to the point it was caught beneath the sweeper and pushed into the smoothed model, pushing both models off position on the build platform. After clearing the platform, only the smoothed and complete embryo model was slated for a build. For the SLA build, a .02mm cure depth was utilized with the laser programmed for multiple passes in specified locations to add a red hue in these regions enabling the display of cellular structures through a clear embryo shell. Post-processing for this build included applying a coat of *Huntsman RenShape® SL-L-9000* UV lacquer. A third build, also using a *3D Systems™ 250 SLA* along with *DSM Somos' Watershed® XC 11122* resin was performed. The *Watershed®* resin, like the *9300* medical resin,

allows for objects to be viewed through a clear matrix of resin; however, with the *Watershed*® resin the object of interest will be a pale brown color instead of red. The method of creating an entity inside the cube, as done with the *Watershed*® resin, uses a process where the laser speed is decreased and programmed for multiple passes in select areas to highlight the cell membrane. No post processing other than cleaning the excess resin off of the model is needed.

## 5. Results

Prior to discussing the results, it must be understood that little in-depth knowledge of the scan settings or specimen was realized when this research began, therefore no precise scaling of the model was attempted and only subjective results will be addressed. The model produced employing an SLA and *Huntsman 9300* medical resin, representing a complete embryo, displayed poor results. The entire model was nearly completely solid red in color and little cellular membrane was distinguishable from the environment in which it resided. After the consecutive smoothing operations performed in *Freeform® Modeling™* little texture remained on the surface of this model, removing any tactile representation of the embryo's exterior.



Figure 5. SLA model.

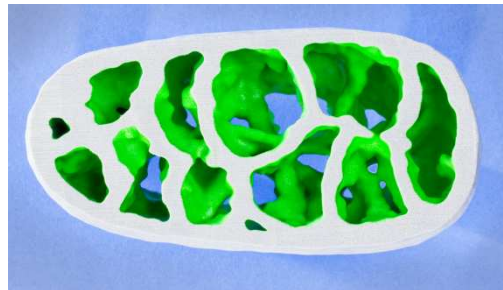


Figure 6. 3D printer model.

As shown in Figure 5, the SLA model constructed using *DSM Somos Watershed®11122* produced superior results with outlines of the cellular membrane easily discernable from the rest of the structure. Transparency of the cube matrix allowed for clear view of the structure within the matrix when a light table illuminated the model. However, what the figure does not demonstrate is that without backlighting cloudiness within the resin reduces the transparency of the structure. Lines from the layered production are clearly visible when viewed from the four sides, but aren't present when viewed from the top or bottom of the cube. No evidence of defined optical sections are noticeable and no discernable difference between extrapolated portions of the structure and those taken directly from the image file were noted.

Figure 6 shows the model produced using 3D inkjet printing, which shows exceptional color clarity and variation. Not seen in the illustration, are lines representing the stacked focal planes which are still prominent on the exterior of the model. No indication of stacking is noticeable on the cellular membrane. A number of the cell walls appear incomplete although cells structures nearer to the poles are the most complete. Muted construction layer lines, with close examination, are evident on cell membranes, however they are more prominent on the exterior of the model; with poor color uniformity on the models exterior as well.

## 6. Discussion

Although successful production of cellular level models has been accomplished through this research, a number of issues should be addressed to help refine the process. To model the complexities of many biological events and structures, a more advanced modeling technique should be investigated by future research. The relatively vast spacing between structural components of many biological entities as well as a low level of connectivity of the structures comprising these specimens will make cellular modeling using current AM technology alone difficult to achieve. Experimentation with elastomeric and polymer modeling techniques in conjunction with AM may help to address this issue until the time that AM techniques alone can produce such structures. Until the development of another methodology, highly connected organisms should first be considered for use with AM on its own. Though the 3D printers have relatively poor material properties compared to other AM platforms, the ability to produce color models with complex geometrical shapes sets it apart from other AM methods and makes 3D printing a clear choice for a large number of applications of modeling cellular structures.

Through this work it became clear that a number of microscopy parameters need to be considered when modeling is the ultimate goal. When *in vivo* imaging is to be considered, a specimen with low mobility should allow for the best modeling, decreasing the variance between optical sections created by the movement of the subject. It seems as though the scan rate for this particular data set was a bit slow, allowing for deviation between the focal planes of the live specimen, making it suboptimal for highly accurate model production. An elevated number of focal planes would be ideal when modeling is to be considered, which theoretically should reduce the stepping effect that was encountered on this investigation. Actions taken to reduce the stepping effect will undoubtedly remove details from the surface of the structure and in doing so; decrease the accuracy of the model. The distance between focal planes is also a consideration worth noting. Since any distance between the optical sections will necessitate interpolation, the closer the optical sections are together, the more representative of the true nature of the subject it will be. No issues were encountered with resolution or magnification on this study; however, these parameters should be considered in future studies. Without the consultation of a biologist or the microscopist responsible for the data set utilized in this study, it is not possible to say with any confidence that the modeling process has provided an accurate representation of the original specimen. With this in mind, if the extrapolation of the dataset will be handled by a party outside of the imaging laboratory, detailed parameter settings should accompany the image file and close collaboration would be advantageous to ensure that the highest level of accuracy is achieved.

Although a microscopy image is but a rendition of the actual event taking place under the microscopes lens, the first key stroke made in extrapolation of the subject has the possibility of greatly altering the realism of the subject. Very rarely, it is surmised, will optical datasets have the capability to be modeled with no enhancement whatsoever. With this knowledge, and the availability of software to radically change the appearance of the image being studied, care must be taken not to overly compromise the accuracy of the specimen. When enhancement is necessary, alterations that will impact model accuracy need to be clearly indicated so that the physical model is in no way thought to be more precisely representative of the specimen than it actually is.

## 7. Conclusion

Through the research done on this project, a proof of concept has been established for the production of cellular level modeling utilizing raw microscopy data sets. Efforts have been made to preserve the integrity of the specimen and the capabilities of extrapolation and interpolation methods were demonstrated. Although only six different file types have been converted during the course of this work, the ability to convert 69 different proprietary microscopy file types for use with AM is believed to be possible. A novel process for creating three-dimensional, tactile models has been created, that with further refinement, will allow greater understanding and discovery of events taking place at the cellular level to be realized by students and research scientists alike. Benefits and limitations of additive manufacturing platforms have been revealed and the groundwork for a standard protocol to be used by microscopists has been established for cellular level biological modeling.

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