

Embryonic Mitosis Detection Using Time-Lapsed Images

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Abstract— This paper presents an application using computer vision methods to detect embryonic mitosis. The goal of this project is to reproduce Cicconet’s results, build the cell tracking application using Tkinter, a platform independent graphical user interface package. Time-lapsed images along with Gaussian filters and edge detection methods are used to track the cells as they multiply. The overlapping of embryos in a two-dimensional image present a challenge when tracking in the later stages since the program isn’t able to detect the entire shape of an embryo. The new application created allows users to manually track cells as they divide and saves statistics on the cells.

Keywords: Computer vision, embryo division detection, image analysis, in vitro fertilization, platform independent Graphical User Interface

I. INTRODUCTION

Many parents have described the euphoric feeling of holding their newly born in their hands as being flooded with emotions. Unfortunately, many couples are unable to experience those emotions due to difficulties in conceiving a baby. According to the United States Department of Health and Human Services, common causes of infertility in woman include poor egg quality, ovulation complications, Endometriosis (the growth of uterus lining outside the uterus) and fallopian tubes issues [7]. The Center of Disease Control and Prevention state 12.3% of woman between 15-44 years of age are unable to conceive or carry a baby to full term [6]. Infertility in men can be caused by medical issues like diabetes or enlarged and overheated testicles, toxins in the environment or an unhealthy lifestyle rife with overconsumption of alcohol and/or drug use. Luckily for those couples, In Vitro fertilization is an option commonly used to become parents.

In Vitro fertilization, an assisted reproductive technology (ART), is the process of fertilization by manually combining an egg and sperm in a laboratory dish and then transferred to the uterus. As with any medical procedure, there are risks that are weighed against potential benefits. There is a risk of multiple births associated with IVF, related to transfer of multiple embryos. Multiple births greatly affect the health of a woman and her children. The risk can be mitigated by reducing the number of embryos transferred. The time lapsed recording of the embryos is very important for identifying the best quality embryos to transfer that ultimately improve IVF success rates [1]. Primo Vision allows detailed embryo monitoring to perform the most accurate evaluations and provides an easy

way to analyze, compare and report the development of embryos [12]. On the other hand, embryo scope is the world’s most used time lapse system for observation of embryo development [9].

The embryo scope time lapse system is adopted worldwide for observation of embryo development. This system provides a stable incubation environment that result in a very high quality image of embryos [12]. A software has been used to create time lapse videos of embryo development. The embryos are placed in a dish and a photo of the embryo is taken every 20 minutes. Further, these images are stored and made into a continuous data record [9]. Considering mouse embryos for cell tracking, bio imaging uses frames and algorithms that aim to show reasonable cell growth within the embryos. The image processing continues to revolutionize the way science deals exploits with microscopy. Allowing scientist to analyze fully the state of embryos from origination to ending will be imperative [10].

Embryo detection using phased time elapsed tracking methods can be useful in trying to find out different spatial patterns in cell cycles. Embryonic time/detection methods used provide a better sense of these events [13]. Cellular reproduction requires a nucleus and mitochondria to begin the process of mitosis.

The objective of this project includes porting over the CellTracker application created by Cicconet [3] to a different programming language and graphical user interface in order to be able to run on any operating system, reproducing the results Cicconet achieved and reporting the statistical results. Currently, the Cicconet’s Celltracker application can track up to the 4-cell stage automatically then needs manual intervention to track the later stages. The Opencv-python library was used to track the cells, detect their division and track the radii sizes of the cells.

Section 2 reviews literature on sentiment analysis and the word2vec algorithm along with other effective models for sentiment analysis. Section 3 describes methodology and computer vision techniques used. Section 4 concludes the paper with a review of our results in comparison to previous works. Section 5 discusses the future work to be undertaken. Section 6 details the user manual for the application.

II. LITERATURE REVIEW

Time-Lapse microscopy (TLM) and image analysis is basic clinical embryo development research. TLM is used to figure out the growth of embryo development. Many researchers have used TLM to study embryos. Everything referenced in this paper is to increase the success rate of in vitro fertilization (IVF) with the use of Time-Lapse microscopy. There are advantages as well as challenges in this process when working with IVF. TLM will one day increase accuracy with the use of automation and computers.

In the specific research done by Marcelo Cicconet et al, the research team devises a method through which a database can be created and maintained which records information and techniques specific to the monitoring of cells. This includes the techniques detection of cellular division, problems tracking cells, and the recording thereof using time lapsing video of mammalian embryos. While the paper relies heavily on the research of previous scientists on which to base the foundations of their own research, the major discoveries are advancements in this particular field based on the following contributions: (1) a method for counting embryos in a well, and cropping each individual embryo across frames, to create individual movies for cell tracking; (2) a semi-automated method for cell tracking that works up to the 8-cell stage, along with a software implementation available to the public (this software was used to build the reported database); (3) an algorithm for automatic tracking up to the 4-cell stage, based on histograms of mirror symmetry coefficients captured using wavelets; (4) a cell-tracking database containing 100 annotated examples of mammalian embryos up to the 8-cell stage; (5) statistical analysis of various timing distributions obtained from those examples [3].

One of the advantages of using TLM over the traditional method, Time-Point microscopy (TPM), is the usage of live video clips so that the researcher can observe the changes in the cellular tissue being analyzed rather than relying on images that merely show the developments at a certain stage in the observation without allowing the researcher to see exactly how those developments took place [15]. TLM is more effective than TPM as the time points used in the latter method are often selected for convenience or arbitrary reasons rather than because of biological/scientific curiosity. In essence TLM allows the researcher to more thoroughly study the embryonic development in a more comprehensive manner.

The way that this research correlates with the research done by our group is that we plan to take the methods developed by Cicconet and create way for TLM to be used across various platforms, thus making this method of research available to more scientists and researchers. Currently the program which is currently used to process the data is written using Objective-C, a MAC OS program, thus making it incompatible with Linux, Windows, or other operating systems. The aims of this research team is to take the source code provided and code a program for our client in order that they can process data using the TLM data collected by Cicconet et al using which ever OS platform is preferred in their laboratory.

Another stated goal of this research team is to, if possible, improve upon the research methods provided by the Cicconet et al and Wong et al research teams. In terms of adaptation of

the source code to other platforms, an obvious improvement will be to translate the original source code and write a program that can then be used regardless of OS. In the process of doing so, there should be attempts to shave the embryo counting time from the reported 0.35 seconds to the lowest possible time [3]. Even though the paper states that there was no reported or observable lag between the images, removing time between frames and/or lowering the amount of processing type needed furthers the ability of the program that much more. Another improvement can be trying to make the 8 cell counter completely automatic, as is the 4 cell counter, rather than requiring regular human interaction. This frees the research team up to focus on other aspects of their project.

The topic of this paper is related to the use of morphokinetics as a predictor of embryo implantation and the techniques used in the implementation and application of related research. Morphokinetics helps researchers and doctors understand the success rate for embryo transfers with IVF. In this study TLM played a role in helping with the quantitative aspects to help narrow down the ability of a favorable outcome. The research basically increased the survival rate of embryos with or without implantation.

What are the exact applications of morphokinetics in the real world? One of the main applications, as previously mentioned, is the calculation of the survival success rate of an embryo being used to help an infertile couple conceive a baby [2]. The paper also raises the issue that many research teams investigating this particular topic have suggested additional evaluations in order to further determine the timing of cellular division contributes to embryonic viability.

As a result of these additional observations, the overwhelming consensus of the various research groups is that early cleavage embryos have a much higher rate of success than embryos that have a delayed division. However, the majority of these observations conducted were done so with more than one embryos and a mix of early and late embryos which may have contributed to the varying results achieved in the observations. To further determine this, a study conducted by Van Mootfoort et al. [14] investigated the success rates between early cleavage and late cleavage success rates; using both single and double embryo transfers. The results were overwhelming supportive of early cleavage embryos yielding a significantly higher pregnancy rate. The blastocyst formation rate for early cleaving embryos also increased, and the miscarriage rate decreased compared with the late cleaving group [14].

The results were inconclusive as to whether the higher success rate of pregnancy was due to the presence of multiple embryos or whether the early cleavage of the embryos was the sole causing factor. Another aim of this research was to determine if these factors worked in concert and were together responsible for delivering favorable results or whether this was the work of embryo morphology. Another result ascertained from the research is that higher cleavage embryos tend to have a significantly higher number of cells compared to their lower cleavage counterparts and this could possibly explain the discrepancy in the pregnancy success rates [14].

This study in particular looked to improve upon the results of the Van Mootfoort et al [14]. study along with others and create a method through which these various research results can be compiled into a program that can then be used to calculate

the ability of an embryo to survive the injection process and result in a successful pregnancy. It also resulted in the development of a classification system which can be used to determine the success rate based on status as an early or late cleavage embryo which doctors and/or researchers can then select based on that criteria for the insemination and injection process [11].

This is a synopsis of three articles regarding the analysis of embryonic viability, shape statistics for cellular division detection, and the superiority of the Hough transform method. The three articles being summarized are done so as follows: Shape Statistics For Cell Division Detection In Time-Lapse Videos Of Early Mouse Embryo, Application Of Computer Vision Methods In Automatic Analysis Of Embryo Development, and finally Complex-Valued Hough Transforms For Circles. The purpose is to provide a linear and streamlined summarization of the three articles for further reference in regards to current research development underway.

The topic of the first article is related to the usage of shape statistics and their usage in the detection of cellular division through usage of time lapse video recordings of embryos. The process used is based on a computation that calculates the likelihoods of certain radii ranges to be in each frame without counting each individual cell. The process for computing the likelihoods come from a process involving the usage of data from multiple points like the Randomized Hough Transform method [4].

This particular research team based their study on the observation of mouse embryos. Past research teams who have focused primarily on invitro fertilized human embryos different measurements have been used to measure the viability of embryos. In terms the observation of nonhuman embryos, such as those of mice, the statistics gleaned about the lifecycle of cells are important, especially for research into the initial stages of life [4].

The detection of divisions can be obtained via dynamic programming as it is the method most likely to find the best solution in terms of creating the model used to determine the likelihood of cellular division within mice embryos. One way this is done is through the observation of the radii range in certain pictures, the interval between the major and semi major axis of an ellipse, that can then assist the researcher in determining which embryos should be studied more closely [4].

The experiments conducted by the research team show that their dynamic programming method is successful in providing good statistical predictions of cellular division. This method has been proven to be more successful that it assists the researchers in understand the limits of the computation methods used and the performance in the various scenarios created to understand how cellular divisions function with the model subjects and in term how this may work with human embryos [4].

The topic of the second article is related to the topic of in vitro fertilization (IVF), infertility resulting from biological problems in both male and female reproductive systems, and the applied image processing methods used to detect and determine embryonic viability. The role of this method is to ensure that embryos resulting from the IVF process have the greatest potential to result in a successful pregnancy, through the understanding of how embryos develop in the early stages [8].

The relatively high infertility rate among human males and females, about 15% of the reproduction age with over 25% of couples, have necessitated the advancement of technology for achieving pregnancy through alternative methods. Because IVF has become a relied upon method for family planning and creation, further research and improvement of its success rates have become increasingly popular with research teams [8]. New techniques are constantly proposed on an annual basis with the common goal being successful transfer and increased rates of success. The current success rate hovers around 30% per single transfer although the present goal is to increase those odds.

The newest method being used to determine the viability of embryos is time lapse microscopy, which is the continues monitoring of embryonic development without disturbing the culture conditions. This allows the observer to see changes in the embryo in a more fluid manner than would be possible with time images taken at specific intervals. This is done through automatic image capturing which measures development through the positioning of the fetal positions of embryos through time-lapsed images to highlight critical stages that predict successful development of human embryos.

The conclusive theory generated from this study is that evaluation of embryonic viability based on image processing is a method that is showing to be a step in the direction of continued improvement on this front. The results have shown that high detection rates have been sufficiently determined using this method, although other similar methods and technologies exist [8].

The topic of the final article proposes the usage of complex variables to represent votes in the Hough transform for circle detection. Simply put this proposal recommends the replacing of positive numbers in favor of a more complex series of numbers to in turn allow cancellation effects when adding up votes within the aforementioned process. Such methodology has the potential to shape the likelihood of achieving more robust solutions than is achievable with the classic algorithms currently being used [5].

The usage of circles as a geometrical structure in computer vision applications is common place and the Hough transform is one of the main methods for locating these shapes within the context of experimental observations. The Hough transform is a method built around using image elements to vote for the parameters of a geometric object. This concept is based on the usage of complex numbers to replace the voting process through the implementation of wave functions which are modeled using said complex numbers to gain better and more reliable probability statistics; both to add and cancel out probabilities related to the observation [5].

As previously stated, the Hough transform is a voting scheme which “accumulates votes in the parameter space of the possible geometrical shapes, and the shapes with enough votes are recognized. It is known to be resistant to outliers” [5]. However, the research team notes that there are no cancellations within this system as all votes are added, thus replacing the system where cancellations were possible based on the voting system.

The conclusive results of this research demonstrate that the usage of complex numbers yield a much more reliable and favorable result than the continued usage of the classic system; real, constant numbers [5]. The research team also showed the results of the introduction of complex numbers and their

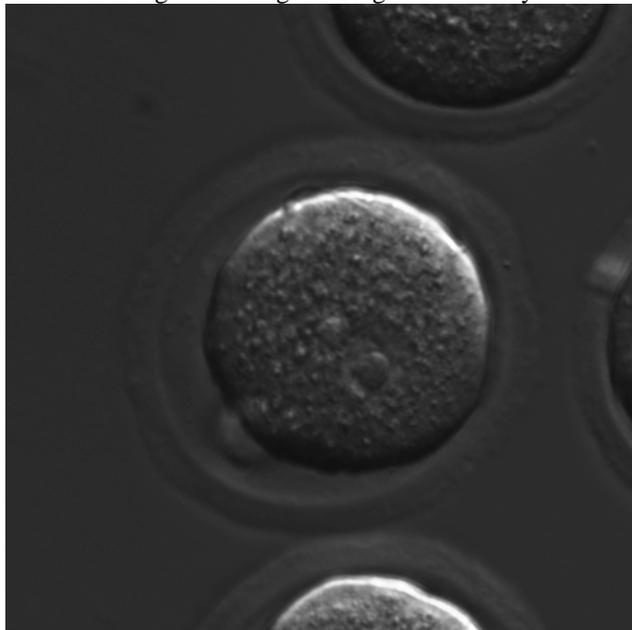
magnitude for voting in the Hough transform for circle detection when the radius was not known precisely and with the availability of tangent information. In conclusion, the Hough transform is touted as the superior and preferred system to use in future experiments [5].

III. METHODOLOGY

A. Database

For the purpose of reciprocating the results Cicconet achieved with his team and tracking application, the same dataset of images was used. Aside from Cicconet's data set, we will test our CellTracker application on Embryos were incubated using Embryoscope machines from Vitrolife. They work by inserting slides of 12 embryos each, which are then photographed every 15 minutes over 32.5 hours, resulting in 130 images of each embryo. We have a dataset consisting of 234 slides, that is 2808 embryos, or 365040 images. Figure 1 displays an example of an image containing a single cell embryo. Each image is also taken 3 times with different camera focus providing some depth. Including these we have over 1 million images, or about 25.8 Gb of data. Each image, see examples below, is 400x400 pixels and compressed with jpeg.

Figure 1. Image of Single Cell Embryo

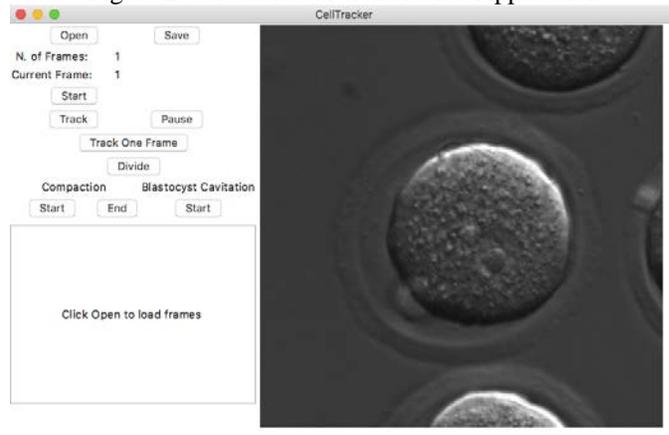


B. Graphical User Interface

To develop an application capable of running on any operating system and facilitate reproduction or advancement of this project, the graphical user interface (GUI) was built using Python's standard GUI package Tkinter. Tkinter allows developers to create platform independent GUIs. Since Celltracker was originally written in the Objective-C programming language using the Cocoa Framework, it was only executable on a Mac operating system. The GUI allows users to open a folder of images, click start to begin the detection process and select track to begin the automated

tracking process. To manually track cell division, instead of pressing the "Track" button, user must click the "Track One Frame" button. The "Divide" button allows a user to track the newly formed embryos. The text box will give instructions on how to proceed in each step. Once tracking is complete, the user can save a .txt file generated by the tracker detailing when certain cells split and from which parent cell did the children cell derive from.

Figure 2. Screenshot of CellTracker Application



C. Image Processing

Prior to tracking, the images are pre-processed in order to analyze them through segmentation. Segmentation will be helpful in detecting edges of the cells, finding the center of cells and detecting division among the cells. The OpenCV-python library, used for image processing in Python, was used to transform the images. First, textured images are created from the originals using equalize adaptive histogram function to apply adaptive histogram equalization to the image. Adaptive histogram equalization changes the contrast, rearranges the light parts of an image and augmenting the edges. After the texture image has been created, the image is convolved using the Gaussian filter. Applying the Gaussian filter allows for the blurring of an image which reduces the noise and Finally, the local maxima of the embryo in the image are found using the morphology local maxima function which indicates the center of the cell and a circle is drawn around the cell for tracking.

D. Tracking

Figure 3. Screenshot of Cell and Point class

```

class Point():
    def __init__(self):
        self.row = 0
        self.col = 0

class Cell():
    def __init__(self, theNLocations, theBandLength):
        self.theNLocations = theNLocations
        self.theBandLength = theBandLength
        self.active = False
        self.tracked = False
        self.generation = 0
        self.centerRow = 0
        self.centerCol = 0
        self.radius = 0
        self.boundary = Point()

```

In order to track cells as they divide, a Cell class was created. The Cell class contained nine attributes which describe whether or not the cell is active, where the boundary and center points are located and the radius measurement of the circle. A separate Point class was created to capture the row and column of pixels in the boundary of the cell.

When the track button is pressed, the tracking steps are set at either infinity for automatic tracking or one to track one frame at a time. Depending on the tracking steps variable, a while loop runs until keep tracking Boolean variable is false or steps variable is equal or greater than the tracking steps variable. During the while loop, the image in the current frame is processed using erosion, dilatation, and gradient functions. Afterwards, using the tracker object, add the point near the center of the cell, add the boundary, update tracked attribute of cell to true and display new image with its boundary drawn. The appropriate instance variables for the cell, such as radius, center row and center column, are set and button configurations are adjusted accordingly depending on whether or not there are more frames to view.

IV. CONCLUSION

Many parents are unable to experience difficulties in conceiving a baby due to infertility in both men and woman. For these parents, In Vitro fertilization is an option commonly used. In Vitro fertilization is the process of fertilization by manually combining an egg and sperm in a laboratory dish and then transferred to the uterus. As with any medical procedure, there are risks that are weighed against potential benefits. There is a risk of multiple births associated with IVF, related to transfer of multiple embryos. Multiple births greatly affect the health of a woman and her children. The risk can be mitigated by reducing the number of embryos transferred. The time lapsed recording of the embryos is very important for identifying the best quality embryos to transfer that ultimately improve IVF success rates [1]. The objective of this project was to port over the CellTracker application to a different programming language and graphical user interface in order to be able to run on any operating system. Python was the programming language of choice while Tkinter and OpenCV were the GUI and image processing libraries with Python. Please see user manual on how to run the application.

V. FUTURE WORK

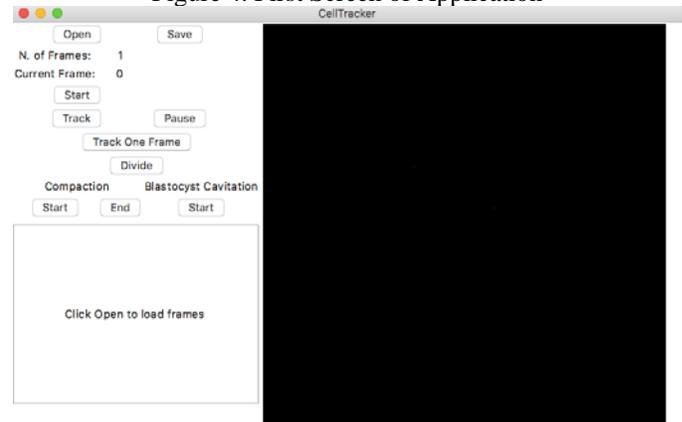
The time lapsed recordings of embryos have proved to be a great tool in evaluating, logging and conceptualizing embryos. While microscopes continue to improve the notion that this technology will somehow become obsolete isn't the case so the time lapse recording technique is the best and can be used in future even if there is some advancement with the technology.

Moreover, in time lapse microscopy; the scientist can gather a lot more information used in the valuation of the embryos can determine embryonic deficiencies and are able to make crucial decisions in regards to each embryos state. The value of time lapsed microscopy greatly increases the chances of birth rate successes. Identifying embryos with the highest implantation potential is no small feat, but with advance technology such as microscopy, the cost of IVF and subsequent relevancies continue to be low. In this way the time lapse microscopy can increase the success rate of IVF.

Currently, the cell tracker application that we have developed can track up to the 4-cell stage and then needs a manual intervention to track the remaining stages. However, this process is semi-automated so we can make the complete process automatic to track all the stages in future. There is a possibility of using a large set of embryo images and providing an appropriate statistical analysis of the image set.

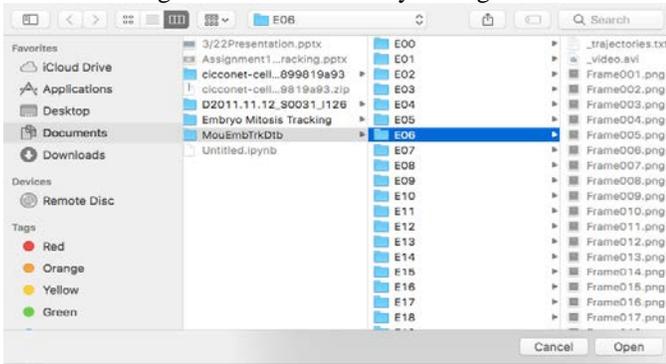
VI. USER MANUAL

Figure 4. First Screen of Application



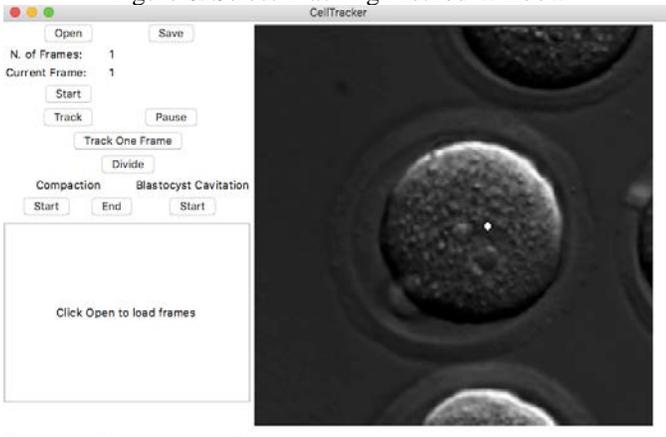
To run the CellTracker application, users must retrieve the program code from Github by either downloading the zip file or cloning the repository and using the "git pull" command in the terminal. Once the code base has been retrieve, user must change directories in their terminal until finding the program. Once you've located the program, run "python celltracker.py". Python 2.7 is required to run this application. Once the application runs, you'll see the window in Figure 3. Upon initially running the application, users see an image pane on the right side and buttons on the left side of the CellTracker window along with a text dialog box where instructions are presented as the application is being used.

Figure 5. Select Directory Dialog Box



To load images, click on the open button and the select a directory with images in .tiff or .png formats as seen in Figure 4. After selecting the folder of images and the first image appears in the viewing panel, press the start button to activate the tracking feature. After the start button has been clicked, you can either choose between pressing the track button for automatic tracking or the track one frame to manually track each frame as seen on Figure 6. As seen in the text box, users can use the command button along with the directional arrows to point the dot in the center of the cell if it is not there already as seen in Figure 6. The dot must be in the center in order for the track to draw the circle around the cell. Once the dot has been centered click the Track button for automatic tracking or Track One Frame for manual tracking. Once tracking begins, users can temporally stop by pressing the Pause button.

Figure 6. Select Tracking Method Window



For automatic tracking, press the track button and the tracker will go through all of the images in the selected directory. While the tracker goes through all of the images, it draws a circle on the boundary of the cell. If the tracker misses the division of a cell, the user can press the pause button to stop the track. Instructions on how to divide the cells manual can be found after Figure 7. For the semi-automated method for tracking, the user must press the track one frame button or press the right directional button in order to track one frame at a time. Once the the cells begin to multiply, it can become difficult for the automatic tracker to detect cell division and draw the circles around them and figure out which parent cell a child might have come from.

Figure 7. Single Cell Tracked

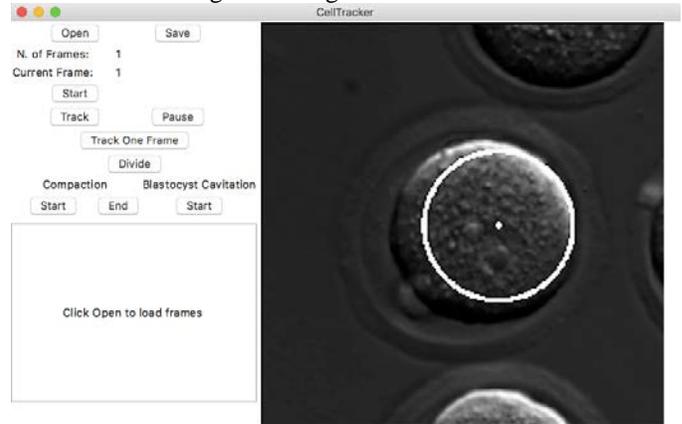
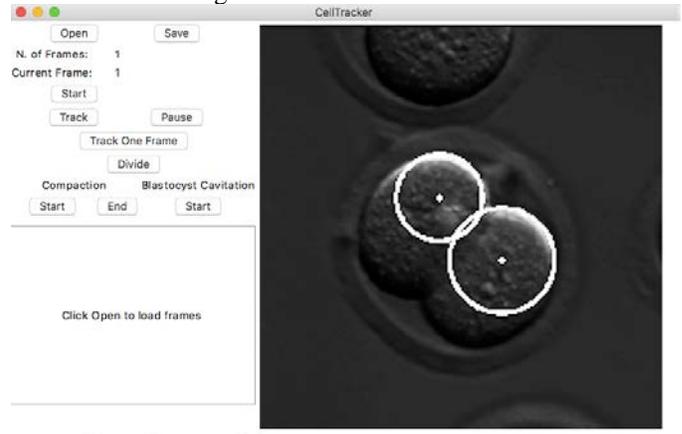


Figure 6 shows an image with its boundary drawn and tracking one frame at a time.

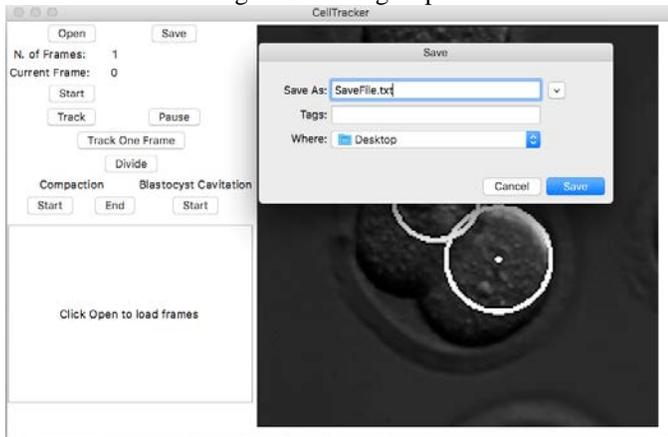
Whether the user is tracking automatically or semi-automatically, user intervention may be necessary when a cell has divided. To track a newly divided cell, press the space button to initiate the division of cell. Then, press the divide button to adjust the points of the centers of the cells. Using the directional arrows, move the two points, one on the old cell and the other on the new cell, to the cell and press enter to set. To continue tracking, click track one frame button to proceed. Figure 8 shows how the two cells are now being tracked and have their boundaries drawn after manually dividing.

Figure 8. Two Cells Tracked



Once the user has reached the last frame and is done tracking, they can save a report. When user presses the save button, a new dialog box displays. A report, which must be save at a .txt file, detailing the location of the point drawn on the center of the cell along with the length of the radius of the border drawn can be saved to the directory of the user's choice. Figure 9 shows the window displayed asking user to name the .txt file and select a destination to save the report. Once a report is saved, the application can be closed or a new directory of images can be chosen to start tracking a new directory of images.

Figure 9. Saving Report



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