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Embryonic Mitosis Detection Using Time-Lapsed Images

***Abstract*— This paper presents and discusses the application of 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, and extend the automated tracking to the 8-cell stage. Time-lapsed images along with Gaussian filters and edge detection 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 results of our project will be summarized here in the next draft of this paper along with a detailed description of how the CellTracker application runs and how to use it.**

**Keywords: Computer vision, embryo division detection, image analysis, in vitro fertilization, time-lapsed movie**

# 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 [5]. 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 [4]. 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 [9]. On the other hand, embryo scope is the world’s most used time lapse system for observation of embryo development [6].

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 [9]. 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 [6]. 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 [7].

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 [10]. 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, using a different set of embryo images and reporting the statistical results, and extending the automated tracking of cell up to the 8-cell stage. Currently, the Celltracker application can track up to the 4-cell stage automatically then needs manual intervention to track the later stages.

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 describes the results. Section 5 concludes the paper with a review of our results in comparison to previous works. Section 6 discusses the future work to be undertaken.

# 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 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 all, 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 [12]. 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 embryotic 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 all 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 all and Wong et all 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. [11] 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 [11].

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 [11].

This study in particular looked to improve upon the results of the Van Mootfoort et al [11]. study along with others and create a method through which these various research results can be compiled into a programed 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 [8].

# Methodology

## 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 500x500 pixels and compressed with jpeg.

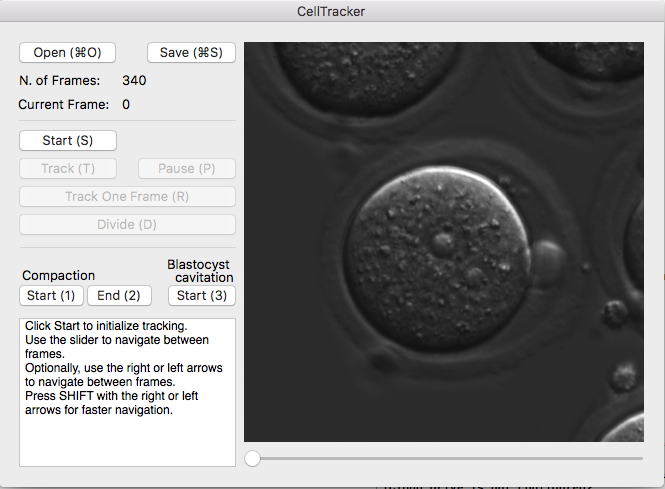
Figure 1. Image of Single Cell Embryo



## 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 in 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



## 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 scikit-image library, used for image processing in Python and it is part of the Scipy package, was used to transform the images. First, textured images are created from the originals using scikit-image’s equalize adaptive histogram function to apply adaptive histogram equalization to the image. Adaptive histogram equalization changes the contrast, rearranges the light parts of and image and augmenting the edges. After the texture image has been created, the image is convolved using the Gaussian filter using scikit-image’s Gaussian filter function. 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 scikit-image’s morphology local maxima function which indicates the center of the cell and a circle is drawn around the cell for tracking.

## Tracking

Coming soon.

## Statistics

Coming soon.

# Results

Coming soon.

# Conclusion

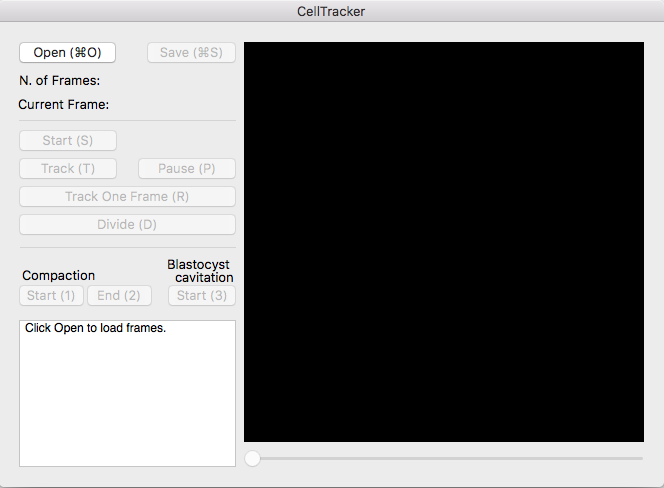
Coming soon.

# Future Work

Coming soon.

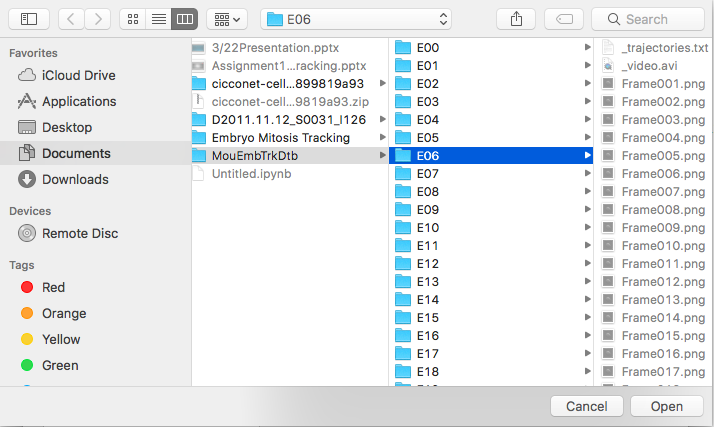
# User Manual

Figure 3. First Screen of Application



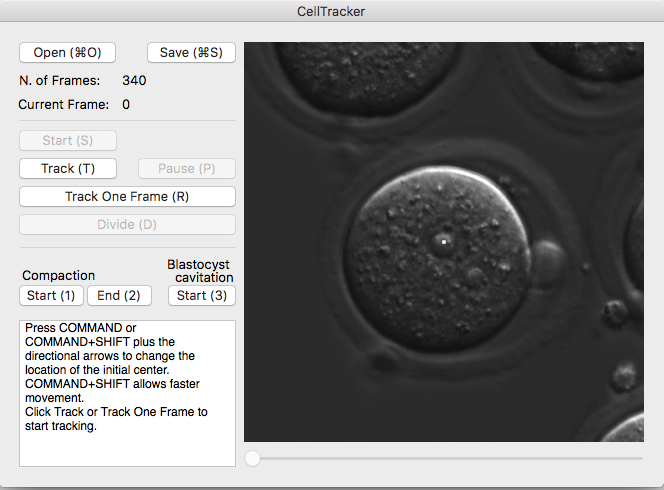
To run the CellTracker application, users must retrieve the program code from Github by either downloading the zip file or cloning the repository and using the “git pull” command in the terminal. Once the code base has been retrieve, user must change directories in their terminal until finding the program. Once you’ve located the program, run “python celltracker.py” if using Python 2 or “python3 celltracker.py” if using Python 3. 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 4. 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 5. As seen in the text box, users can use the command button along with the directoional arrows to point the dot in the center of the cell if it is not there already as seen in Figure 5. 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 5. Select Tracking Method Window



# References

[1] S. Armstrong, A. Vail, S. Mastenbroek, V. Jordan and C. Farquhar, "Reply: Time-lapse in the IVF lab: how should we assess potential benefit?", *Human Reproduction*, vol. 30, no. 5, pp. 1277-1277, 2015.

[2] Baczkowski T, Kurzawa R, Glabowski W. Methods of embryo scoring in in vitro fertilization. Reprod Biol 2004;4:5–22.

[3] Cicconet, Marcelo et al. "Label Free Cell-Tracking And Division Detection Based On 2D Time-Lapse Images For Lineage Analysis Of Early Embryo Development". Computers in Biology and Medicine 51 (2014): 24-34. Web.

[4] "FastStats", Cdc.gov, 2017. [Online]. Available: https://www.cdc.gov/nchs/fastats/infertility.htm. [Accessed: 08- Mar- 2017].

[5] "Infertility", HHS.gov, 2017. [Online]. Available: https://www.hhs.gov/opa/reproductive-health/infertility/index.html. [Accessed: 08- Mar- 2017].

[6] P. Kovacs, "Embryo selection: the role of time-lapse monitoring", *Reproductive Biology and Endocrinology*, vol. 12, no. 1, p. 124, 2014.

[7] M. Luengo-Oroz, M. Ledesma-Carbayo, N. Peyriéras and A. Santos, "Image analysis for understanding embryo development: a bridge from microscopy to biological insights", *Current Opinion in Genetics & Development*, vol. 21, no. 5, pp. 630-637, 2011.

[8] M. Meseguer, J. Herrero, A. Tejera, K. Hilligsoe, N. Ramsing and J. Remohi, "The use of morphokinetics as a predictor of embryo implantation", Human Reproduction, vol. 26, no. 10, pp. 2658-2671, 2011.

[9] R. Mikut, "Automated Processing of Zebrafish Imaging Data: A Survey", *Zebrafish*, vol. 10, no. 3, pp. 401-421, 2013.

[10] A. Rachmad Syulistyo, "Ellipse Detection On Embryo Imaging Using Random Sample Consensus (Ransac) Method Based On Arc Segment", *International Journal On Smart Sensing And Intelligent Systems*, vol. 9, no. 3, 2016.

[11] A. Van Montfoort, "Early cleavage is a valuable addition to existing embryo selection parameters: a study using single embryo transfers", Human Reproduction, vol. 19, no. 9, pp. 2103-2108, 2004.

[12] Wong, C. et al. "Time-Lapse Microscopy And Image Analysis In Basic And Clinical Embryo Development Research". Reproductive BioMedicine Online 26.2 (2013): 120-129. Web.