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## Detection and Tracking of T Cells in Time-lapse Imaging

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### Comments

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# Detection and Tracking of T Cells in Time-lapse Imaging

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## ABSTRACT

The effective classification and tracking of cells obtained from modern staining techniques has significant limitations due to the necessity of having to train and utilize a human expert in the field who must manually identify each cell in each slide. Often times these slides are filled with noise cells that are not of particular interest to the researcher. The use of computational methods has the ability to effectively and efficiently enhance image quality, as well as identify and track target cell types over large data sets. Here we present a computational approach to the in vitro tracking of T cells in time-lapse imagery capable of scaling to hundreds of cells and applicable to multiple staining techniques.

## Categories and Subject Descriptors

J.3 [Life and Medical Science]: Biology and Genetics

## General Terms

Algorithms

## Keywords

imaging, automated cell tracking

## 1. INTRODUCTION

In order to successfully automate cell tracking and identification there are significant hurdles that must be overcome. These issues include but are not limited to: varying cell population density due to cells entering/leaving field of view, low signal to noise ratio of various microscopy techniques, complex cellular topologies (cell shape change, cell to cell contact, partial overlap) and the overall large volume of data. There are several methods that are currently available for use in automated cell tracking, both open source and private, however many of these solutions have been created with a specific experiment or cell type in mind. We propose a novel approach that greatly simplifies the cell tracking and

identification process in order to allow for greater generalization of cell tracking algorithms and use across many types of high-throughput experiments.

The system that we propose has several advantages over current methods. It has a built-in image enhancement algorithm, which allows for the direct utilization of images obtained through phase-contrast microscopy or other methods, without the need for manual enhancement or the use of separate image enhancement programs. Additionally it utilizes an image segmentation function that prevents large cell clusters from being identified as one large cell, a common issue in cell tracking algorithms; this function has a default size but can be customized to separate the image into sub-images, which are the size of a single cell, in order to enhance accuracy. The use of the "tracking by detection" [2] [3] [4] technique employed by this algorithm also provides the advantage of being able to cope with inconsistent and highly variable cell movement. A unique function of this algorithm is the ability to distinguish amongst cell types, based on their overall shape and size.

Our study focuses on images containing leukocytes, which crawl faster than any other cell in the body. This migration is essential for immune function. Leukocyte migration was first described over 30 years ago, however the underlying molecular mechanisms that facilitate this process are incompletely characterized. T cell migration within the lymph nodes is required for efficient recognition of cognate antigen and inflammatory cellular activation [5] and therapeutics that inhibit T cell migration to sites of inflammatory pathology have proved effective in human autoimmune disease [1].

## 2. DATA

Human peripheral blood lymphocytes (PBLs) were isolated from blood of voluntary healthy donors by Ficcoll-Hypaque density gradient centrifugation. Human T cells were then transfected by nucleofection using the high-viability protocol. To visualize human T cell migration in vitro, we utilized an established video microscopy protocol [6]. For in vitro T cell migration video microscopy, 35-mm glass bottom microwell dishes were coated overnight with 3 ug/ml intercellular adhesion molecule-1 (ICAM-1)-Fc, and then blocked with 2% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Transfected T cells were added and allowed to settle for 30 min at 37 °C, and nonattached cells were removed by gentle washing. Images were taken at 37 °C using an Olympus FluoView FV10i microscope.

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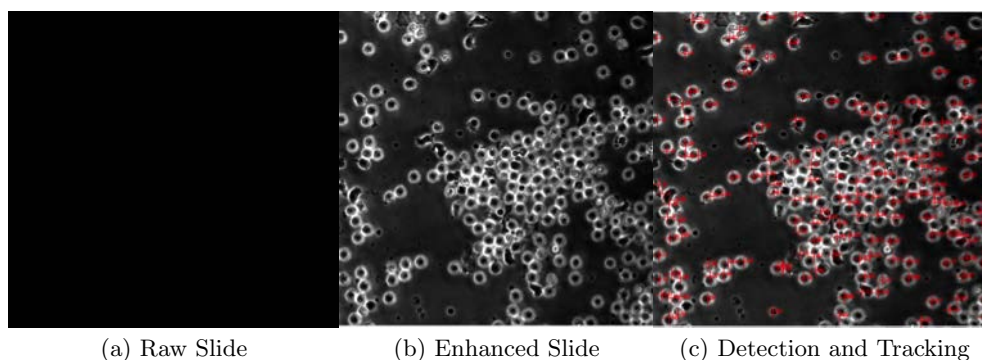


Figure 1: Image Processing Steps

### 3. METHODS AND RESULTS

Following collection of the live-cell microscopy data, each time series of images was analyzed individually. Each image was first enhanced with image saturation and unsharp masking. Following the initial treatment of the image, each individual image was segmented into sub-arrays to allow further enhancement without contributing significant amounts of noise to the overall data. Upon completion of image enhancement, each sub-array of the image was analyzed for the presence of cells using edge detection and blob detection algorithms. In order to ensure that the creation of sub-arrays did not alter the results, the frame was shifted and those cells near the border of the sub-array were recounted. Once each cell has been captured, parameters such as location, area, eccentricity, major/minor axis length and perimeter length are stored in a master array. Upon successful identification of every cell in every image of a particular time series, the information stored in the master array is used to determine the movement of the cell throughout its time in frame. Each cell at each time point is treated independently, cells within a given area in previous and subsequent time points are analyzed, the cells that are most similar to the target cell in these other time points are associated creating an accurate cell tracking algorithm. This method allows for cells to enter and leave the frame of the image at any time point in the experiment and be successfully tracked for their time in frame. Figure 1 depicts an example frame at each point in the software pipeline, starting with raw data in which nothing is visible to the human eye, then with cells visible after enhancement, and culminating with cells of interest being identified and tracked.

The algorithm was employed over two different time series, one with 242 consecutive time-lapse imaging planes and the other with 372 planes. Manual identification and tracking of all cells through all slides was impractical, so the accuracy of the algorithm was tested using small 3 to 5 slide increments, in the same manner that the algorithm was initially developed. Using these spot checks it was determined that the large-scale implementation of the algorithm did not hinder its accuracy or ability to deal with changing contrast and density throughout the entirety of a time series. Spot checks of the cell recognition portion of the algorithm confirmed that the accuracy of the algorithm exceeded 90%, and this portion of the algorithm was able to successfully identify over 20,000 cells throughout the length of each time series.

Additionally, the program was able to associate these individual captures to the tracks of various cells throughout the time-series, resulting in several thousand cell tracks. Cells that lasted over 100 time points were manually verified and it was determined that algorithm had an accuracy of greater than 95% in tracking these long-moving cells. Additional manual validation is necessary to further verify the accuracy of the algorithm, however the combination of spot-checks, the success of the preliminary analysis of the algorithm, and the confirmed accuracy of the long-term tracks demonstrates the potential of our software pipeline as a general purpose solution for automated cell detection and tracking.

### 4. CONCLUSION

The overall success of the algorithm supports further research into the area of creating a highly scale-able and generalizable cell tracking program. Going forward efforts to modify the algorithm to allow it to be run in parallel, allowing for even greater scalability, as well as testing with other cell types and the creation of a web portal to allow for usage by the general public will be undertaken.

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