Dual Channel Analytics and Tracking of Cells Experiencing the Dielectrophoretic Force

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Abstract

Quantifying the motion and morphology of cells through microchannels is a way to characterize and monitor cells and microdevice performance. This report presents cell tracking and analytics software for analyzing a video of cells flowing through a microchannel. This script calculates the average cell size, position, and circularity. It is able to segment a video into two different color channels allowing for analysis on multiple cell types. In addition, the channel is automatically detected and a cell tracking script with 93% accuracy was developed.

1. Introduction

It is critical to develop analysis methods of cells within devices in order to better characterize and monitor cell behavior and to develop better microdevices. Analyzing the motion of cells within microchannels is a way to characterize and monitor cell sorting and evaluate microdevice performance. Establishing a single cell evaluation method would allow for optimized device design and characterization of individual cells.

Cell sorting is one particular field of research that offers benefits both to the academic and clinical communities who have a need for isolating cell populations for further research and drug studies One such method for performing cell sorting, the one used to validate the developed script for this paper, is Dielectrophoresis (DEP). DEP is the motion of a dielectric, or polarized, particle in an electric field. It was first described in 1966 by Pohl and Hawk to sort live and dead cells [1]. Lately the technique has experienced a boom due to the development of inexpensive fabrication techniques. One such recent development has been contactless dielectrophoresis (cDEP) [2]. The electrodes are separated from the channel by a thin insulating membrane which acts to capacitatively couple the electric field with the channel. Removing contact between the sample and the electrodes prevents bubble formation due to hydrolysis and electrode delamination as well as the reduction of sample contamination.

The aim of this project was to develop cell tracking and analytics software in Matlab. This project extends the script created last semester (Fall ’13). Several elements remained the same, background subtraction and use of the Hough Transform for cell detection and tracking. In order to increase functionality and applicability the script was expanded to be able to measure cell circularity using Active Contours, automatically detect the channel, utilize a single step Kalman filter for tracking cells, and be able to perform dual analysis on two color channels (red and green). Red and green was chosen as those are the colors of the fluorescent dyes available for staining the cells.

There were many tools that were used in order to create this script.

Background subtraction is very important in order to isolate and detect moving objects, in our case biological cells flowing through a microchannel. Techniques can range from simplistic to very complex with varying computational requirements. A temporal median filter, where the median values across some number of frames is taken, has the advantage of running very quickly and provides an adequate background image as long as enough frames are used. As more frames are used the stability of the resultant background image increases. [3]

There are many different approaches for performing dynamic tracking. One of these is to use nearest neighbor, such as the one implemented in [4]. Another approach is to use a Kalman filter, where the motion of a particle is measured and used to predict future positions [5].

The Hough Transform is an algorithm patented by Paul Hough in 1962 [6]. It is a voting method for detecting specific shapes, most commonly lines or circles, where object candidates are counted and added to an accumulator space and the local maxima is used to find the bin with the most votes. The technique has also been expanded to detect arbitrary shapes [7].

Determining the circularity of an object can be very challenging. Active Contours is a powerful approach for finding object boundaries. It works by initializing a contour model near the desired object and then iteratively evolving the contour to exactly fit the object boundary. It does this by minimizing a defined energy function (Gradient Vector Flow is popular, where the field is computed as a spatial diffusion of the gradient of an edge map derived from the image). The internal energy encourages prior shape preference such as smoothness, elasticity, and a particular known shape, and the external energy encourages contour fit on areas with edges. The approach, in addition to being widely applied to computer

vision applications has been applied to tracking motile cells [8] and segmenting migrating cells for doing cell-based drug testing [9]. The most pertinent difference between this technique and the Hough Transform is that the Hough Transform in a single voting pass can detect multiple instances of an object whereas Active Contours in one optimization pass can fit a single contour and must be iteratively adjusted.

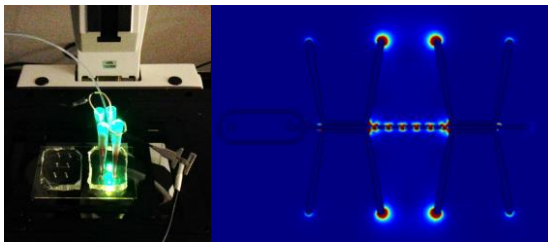

Figure 1: Shown is the microdevice used in this paper through which a cell sample was passed. Visual information is used to characterize the cells and determine efficacy of the devices. The actual microfluidic device used for experimentation on a microscope is shown on the left, and the model of the electric field is shown on the right.

2. Approach

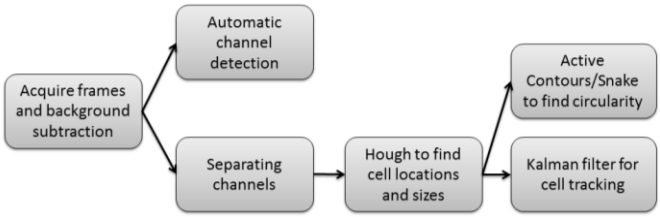

Figure 2: Schematic outline of the implemented code after a cell video has been loaded.

A script was written in MATLAB (Mathworks). MATLAB was chosen for its analytical power and computer vision support. The above algorithms were applied to build the script.

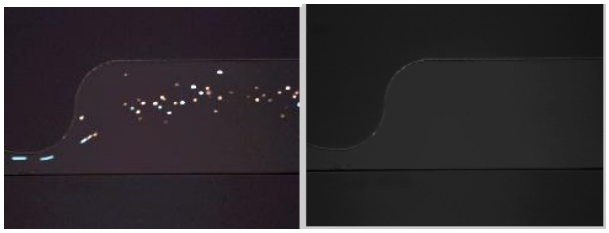

Figure 3: One, of 20 randomly selected, input frame used for calculating the background image using a median filter on left and resultant background image on right.

The script begins by reading in a video of cells taken at 10x magnification and performing background subtraction by applying a median filter across each RGB channel. This allows us to find the background image because the moving pixels, in this case cells, will be disregarded. The found background image is then subtracted from every frame to isolate the moving cells.

The channel is automatically detected by performing the Hough transform to find all lines in an image after canny edge detection has been performed. In particular it finds the top 20 lines that are detected and finds those lines that are parallel and a certain distance apart, set using the known width of the microchannel.

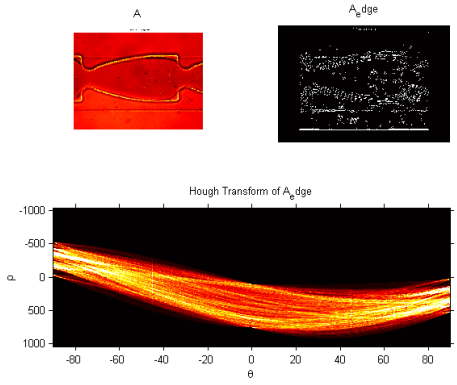

Figure 4: Input image top left, image after canny edge detection top right, and Hough transform of all lines in the image on the bottom.

The video is then separated into its red and green channels. Because the cells are fairly similar in color, meaning that the cells that have been dyed red do show up on the green RGB channel, simply separating the RGB channels isn't sophisticated enough to separate the cells. Instead the cell colors are separated by comparing pixel values. Those pixel values where the red RGB channel has a higher value than the green RGB channel are set to the red channel image, and vice versa.

Once the red channel images and the green channel images were determined they were converted to a binary image to threshold the image. This also had the advantage of removing from analysis cells/features which had a low intensity, which indicates non-viable cells.

Those individual channels are run through the Hough Transform to detect circles to find the cell locations and sizes. Once the centers and radius information have been obtained the average cell size, radius, and standard of deviation for both channels are calculated and the number of cells that went into making those calculations are recorded. Because the videos are taken using a fluorescent microscope with a fixed lens at 10X magnification it is trivial to convert from pixels to microns.

The positions of all cell centers that pass through evenly spaced windows in the image were recorded and used to calculate the average cell position. This data allows us to determine the amount of DEP force the cells experienced and gives us critical characterization information. This was plotted over the mean image across all frames.

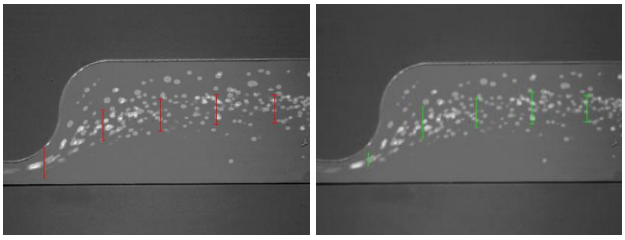

Figure 5: Calculated mean cell trajectory with evenly spaced windows and the standard of deviation. Red channel, left, and the green channel on right.

Cell circularity was calculated by using the cell locations found by the Hough Transform to initialize the Active Contours script. 50 iterations per cell location were used to minimize the energy function.

Last semester a cell tracking script was developed that relied on finding the nearest neighbor cell locations. It ran into problems with high cell densities and fast fluid flow rates. Dynamics were incorporated into tracking in order to improve tracking accuracy. Those locations found using Hough were used to developed a single step Kalman filter for tracking cells moving through the channel. To visualize the results the frames were translated into the RGB color space where the previous frame was the red channel, and the next frame was the blue channel. The connections between frames were plotted as blue lines.

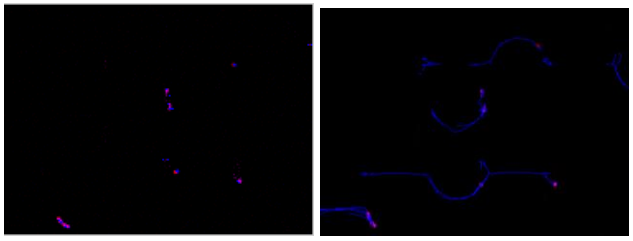

Figure 6: The Kalman filter worked on more complicated device geometries where the trajectory of the cells through the channel was less uniform. Left shows the match between two frames, right shows the resultant trajectory image.

3. Experiments and Results

For the following figures several testing videos with different geometries were used in order to illustrate the robustness of the newly developed script.

The background subtraction script using 20 randomly chosen frames performed well at removing all moving pixels. This result can be used to show how randomness helps the median filter because random frames have a low degree of correlation and ultimately result in better moving pixel elimination than using consecutive frames which have a higher degree of correlation.

The automatic channel detection script did a good job finding the main cell channel which always has a characteristic shape of two parallel lines that are a known distance apart. Performing Canny edge detection prior to the extraction of Hough lines was found to improve performance.

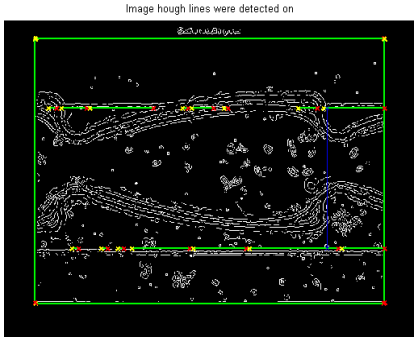

Figure 7: Example showing good performance of the automatic channel detection script. Green lines show the top 20 detected lines using Hough. Blue shows the ultimate detection of the channel.

The automatic channel detection script is sensitive to its presets and to debris in the channel. Therefore it is necessary to input the correct channel width requirements and for the background image to be sufficiently clean.

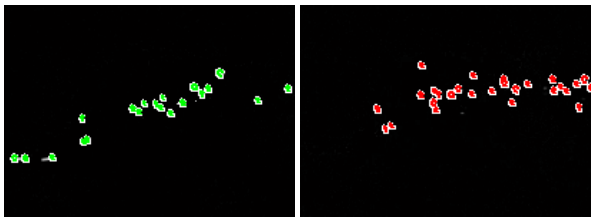

Figure 8: Images showing successful channel separation between the red and green channels which are two different cell lines dyed with red and green flourescent dyes. The overlaid circles are the detected cells using the Hough Transform in Matlab.

A Vicell machine (*Beckman Coulter*) was used to perform a size and circularity measurement baseline on the cell lines used. The true cell radius for the red channel was found to be 9.235 microns and 8.51 microns for the green channel. This yields an analysis error of 10.9% and 20.0%, which is reasonable for biological data.

Table 1: Cell measurements taken using the developed script and a commonly used biology technique.

Cell type	Measured radius size (um)	Actual radius size (um)
MOSE-FFL (red)	10.2484 +- 1.4325 (298 cells)	9.235 (10.9% error)
MOSE-FFL chemo res (green)	10.2159 +- 1.4398 (252 cells)	8.51 (20.0% error)

The true circularity measurements for both channels was found to be 88%. The measured circularity, using Active Contours was 99% for the red channel and 95% for the green channel. This error may be because the chosen script that was implemented was very sensitive to initialization

and the contour had a tendency to "walk away" from the boundary despite parameter optimization.

Figure 9: Results from implementation of active contours script on a frame with cells. The green lines show the initialized contour and the red line show the final contour.

Table 2: Results showing the circularity as measured by the developed script and a popular biology technique.

	MOSE-FFL (red)	MOSE-FFL chemo res (green)	**Actual (ViCell)**
Circularity	.9976	.9571	.88
Stdev	0.0021	0.0441	N/A
Number of cells	353	495	N/A

For 5 frames for each channel the correct pair matches and incorrect pair matches were recorded. Last semester, using the nearest neighbor approach, the cell tracking script had an accuracy that varied between 68.1% to 90.1% with an average of 80.6%. The Kalman filter improved the accuracy to an average of 93%, a 13% improvement from last semester.

Table 3: Cell tracking results using the Kalman filter

Red channel			**Green channel**		
Pairs in frame	**Pairs missed**	**Accuracy**	**Pairs in frame**	**Pairs missed**	**Accuracy**
14	1	92.8%	40	1	97.5%
14	0	100%	42	2	95.2%
13	1	92.3%	41	3	92.7%
15	0	100%	40	3	92.5%
15	2	86.7%	42	6	85.7%

4. Conclusion and Future Work

This report presents software for channel detection, channel segmentation, and cell tracking in addition to measurements of cell size, circularity, and average position. This script will prove a valuable tool for future quantification of cell movement and morphology within a microchannel and has already been used in the BMES lab.

There are two main areas for further extension to this work.

The first is to continue developing the Kalman filter. A more extensive algorithm that takes into account all prior measurement movements, more than just the most recent movement, could further increase the tracking accuracy.

The second largest extension would be to completely replace the Hough Transform with Active Contours. In order to do this a more rigorous algorithm would need to be developed. An algorithm such as one by Chan-Vese [10] where initialization is not required could be used as a basis for implementation.

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