

Stem Cell Project Final Report

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1. The chem/bio problem

Stem cells have the capacity to both self-renew and to differentiate into the various cell types that comprise a complete organism. Much research has been conducted to understand the process of differentiation. A better understanding of this process will allow development of new therapies for tissue repair and replacement, which could be applied to a wide range of medical problems, from wound healing to full organ replacements.

Traditionally, biochemical signaling molecules have been thought to transmit information that is necessary for cell fate decisions. Another signaling mode, biophysical signaling, has only recently been given more attention in the context of stem cell development. Biophysical signaling has been previously studied in detail in developing and regenerating amphibian limbs. It was found that developing and regenerating amphibian limbs establish a voltage gradient between the limb tissue and the surrounding environment, and disruption of the gradient results in abnormal development. In particular, for regenerating limbs, the amphibian establishes an "injury current," an electric current flowing out of the limb stump, which is necessary for regeneration to occur. If the current is reversed or eliminated, a new limb does not develop. If a current is artificially imposed on a limb stump of a normally non-regenerative organism, a small degree of regeneration is induced.

Both development and regeneration events involve differentiation of stem-like cells which are not yet terminally fated to one specific cell type. Thus, it appears that biophysical signaling plays an important role in differentiation. The goal of the current study in the Kaplan lab is to determine whether electrophysiology is also tightly regulated during differentiation of human bone marrow-derived stem cells (BMSC) *in vitro*. BMSCs were cultured and differentiated toward bone and adipose lineages, and their membrane voltages were measured by staining with a voltage-sensitive fluorescent dye. The cells were then imaged using a confocal microscope, and the fluorescence intensity was measured using computer software.

Measured fluorescence intensities appeared to vary depending on user selection of cell boundaries. The goal of the collaboration was to develop a program that could automate the process of detecting boundaries around cells of interest and calculating pixel intensities within those boundaries. Pixel intensities can then be used to calculate membrane voltages using calibration data. Data can be collected over time to obtain a profile of membrane voltage during the process of differentiation.

2. The computer science problem

To complete the project we needed an algorithm that identified the pixels in the images that belonged to the eukaryote cell's cytoplasm. Pixels from the background or nuclei must be not be included. This is not trivial because:

- Cells come in a variety of sizes and each has a unique shape

- Cells undergoing mitosis have two nuclei.

- In the cytoplasm there is a significant variability of intensity.

- As the cells grow and divide, they increase in density and even overlap.

- When cells undergo lysis, the brightness of their cytoplasm doesn't not immediately change.

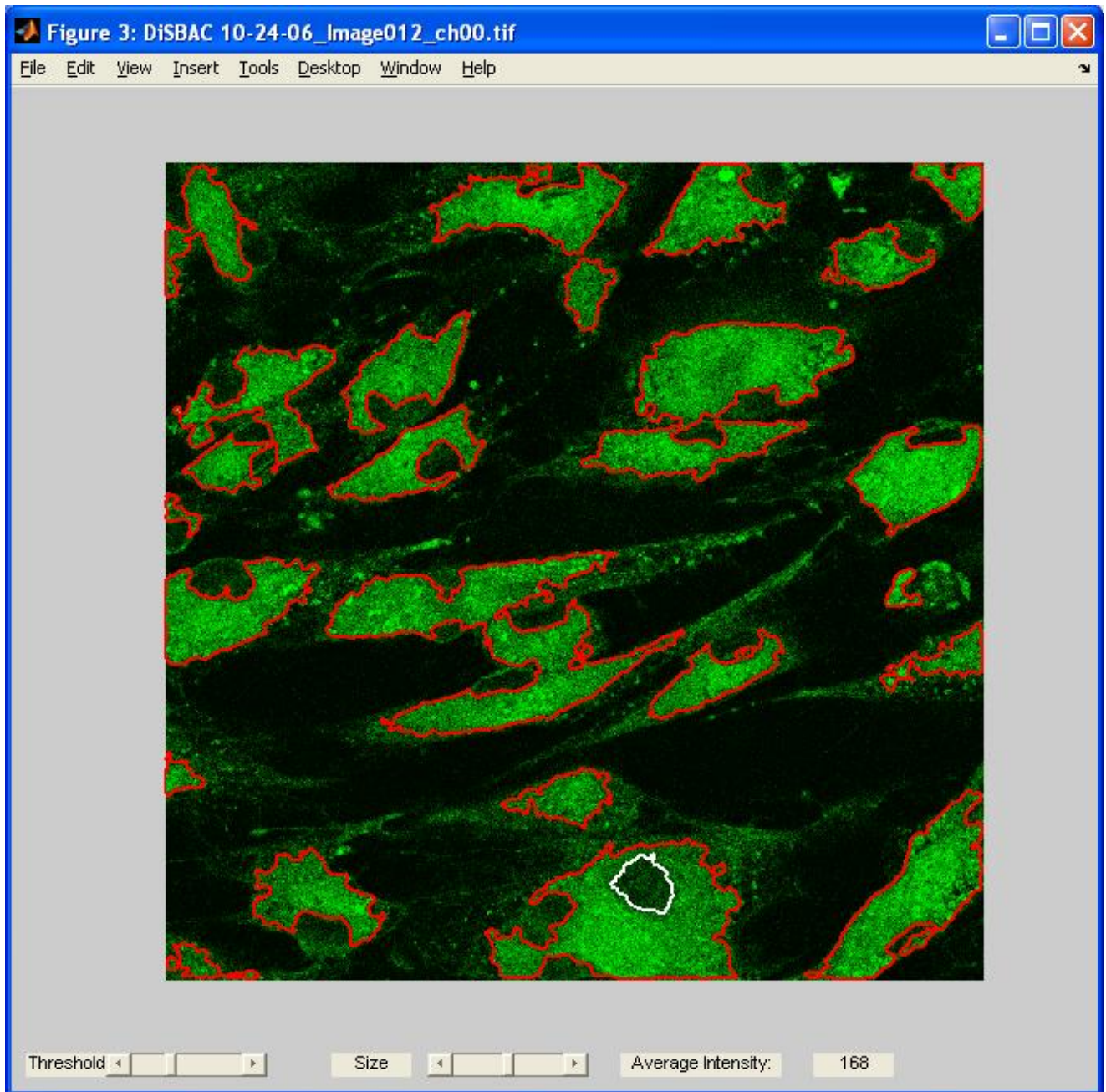
3. The computer science solution (method, experimental results, etc)

Following Professor Brodley's suggestion we applied a threshold to the image, dividing the cytoplasm from both the nuclei and background material. This technique proved very effective and was straight forward to implement in MATLAB. Lysed cells resulted in small fragments of bright cytoplasm. Since they are smaller than whole cells they can be excluded based on their size.

In an effort to make the software more easily used, a GUI was created. It includes a short help page and the ability to load either individual files or all images in a directory. There was some concern with the algorithm's sensitivity to small changes in the value of the threshold or the minimum size of a cell. To support investigation of the issue, adjustment sliders for these two values were added to the interface.

To investigate another alternative, a Sobel edge detection was algorithm was used identify cell boundaries. Due to the high frequency noise in the cytoplasm it was not very effective.

For a more detailed description of the development effort or to obtain source code, visit the project's technical Wiki at <https://wikis.uit.tufts.edu/confluence/display/CS150Steve/Projects>.



4. What this means for the chem/bio solution

Using the above MATLAB program, cell boundaries can be outlined in a consistent, reproducible way, which is important for reducing user-to-user variability when performing data analysis. Since the program allows the user to set the threshold for boundary detection, the program can be adjusted to be more or less selective when drawing boundaries. This is important for these differentiation experiments, since different differentiation treatments can cause the membrane voltage (and thus pixel intensity) to change significantly. In addition, the user can choose a cutoff size for the selected boundaries, which is necessary for eliminating unwanted selections of cell debris from the

set of data. Thus, the program effectively automates the process of cell selection for further analysis, while allowing some user control. When the program is adjusted to calculate correct pixel intensities within these selected regions, we will be able to obtain a profile of the membrane voltage of these stem cells over time as they differentiate.

5. Next steps in either chem/bio or computer science research

Once we construct a membrane voltage profile for these stem cells, it would be necessary to verify our results by repeating these differentiation experiments and using the MATLAB program to analyze the new images. Also, it may be of interest to determine whether user selection of threshold and cutoff size significantly affects the final membrane voltage calculated for each image and to determine how much variation can be tolerated when attempting to compare results from multiple experiments. Perhaps there may be a way to have the program itself determine the best values for these parameters based on some initial user input.

This program can also be used for a similar study of intracellular pH. In this study, the cells are also dyed with a fluorescent dye, and the resulting intensity is an indication of the pH. Two images of the same cell field must be collected at two different emission wavelengths, and the intracellular pH is calculated by taking ratios of the fluorescence intensities from the two images. In order to have accurate ratios, the cell selections must be the same for both images. This MATLAB program can be used to consistently apply cell boundary selections to both sets of images.

The next release of the MATLAB program is planned for mid-January. It will use a larger testing data set and incorporate suggestions generated by the current release. Also planned is the ability to process a collection of image files and create an compatible Excel file containing the image file name, computed intensity and the equivalent cell voltage or pH.