Identifying Accurate and Efficient Data Analysis Tools for Wound Healing Assays

Andres Quintero, Karli Wittenberg, Dr. Jessica K. Bell
Chemistry & Biochemistry Department
University of San Diego

Abstract

Cell migration assays are an important technique in analyzing how altering different variables can affect cell motility. In the laboratory, cells are grown in a confluent layer on a 35mm dish then, a “wound” or “scratch” is introduced across this layer of cells by drawing a pipet across the field of cells to create an open space. The movement of cells into this open area is monitored for ~24 h by microscopy, most often brightfield images taken every 2-10 minutes. To quantify the movement of cells as a function of time and the overall closure of the open area, the researcher must define the open area on each image. This constitutes a major difficulty with these assays as several software programs are available for this analysis, but their reliability and efficiency vary. In this study, three different software packages were used to assess cell migration assays: MiToBo (an ImageJ plugin), SketchAndCalc (an iPad app), and ibidi’s Automated Cellular Analysis System (ACAS). Migration assays for Hap1 cells, WT or having a single protein knockout, were completed in triplicate. Data, images taken every 10 min for a 24 h time period (149 images) were analyzed by each of the three programs. MiToBo was a very fast image analysis program but was not able to consistently distinguish between the cells and the open area. This led to over- or under-estimation of the open area. The SketchAndCalc approach was an extremely accurate image analysis program but required the user to trace the edges of the open area on each image, requiring several hours to completely analyze each data set. The ACAS was also a very fast automated program like MiToBo but was much more consistent in distinguishing the cells from the open area. In directly comparing the SketchAndCalc with the ACAS analysis, the WT cells had a 25% closure for the ACAS and a 26% closure for the SketchAndCalc and for the knocked-out cell line it was reported to have a 15% closure by the ACAS system and 13% by the SketchAndCalc program. These results support that the efficient analysis by the ACAS software provides reliable analysis of cell migration assay data.

Goal

Our goal is to identify the ***most accurate*** and ***time efficient*** program for analysis of would healing data.

Introduction

Wound healing assays are a unique way to study the effects of any cellular change on the overall movement of the cell compared to its basal movement. Wound healing assays are a fundamental technique in measuring change in cellular movement. Wound healing experimental design is well characterized and after practice can be performed quickly and efficiently, but the difficulty is what comes after, data analysis. Previously, the images from wound healing assays taken over a 24 hour period would have been printed, the wound area excised by cutting, and area calculated by weight of cut out compared to standardized weight of defined area. With the advances in technology and image recognition software, the images from wound healing assays can now be uploaded into a program and analyzed as fast as a few minutes to a few hours. Much faster than the days it would take to sift through and manually determine the area of cell coverage. With new image processing software being released that makes data analyses much quicker the question arises , are all approaches equivalent? Are the new advances in technology accurate as well as efficient in analyzing wound healing assay data? We set up a series of experiments to try to determine if different image analysis programs are able to accurately determine the change in cell movement over a 24 hour period. The three programs tested were MiToBo (an ImageJ plugin), Automated Cellular Analysis System (ACAS) through ibidi, and SketchAndCalc (an app that can be downloaded on any touch screen device). Each program comes with its advantages and disadvantages. Sketch and Calc is highly accurate but requires the user to manually outline the background area in each image to determine the overall change in 24 hours. Thus it is time intensive for the user. ACAS is extremely fast, approximately 2-5 minutes per data set, in terms of analyses, but the accuracy of the program is unknown. Similarly, MiToBo is also an extremely fast program taking about an hour to analyze a full data set, but its accuracy is also unknown. In this study, we compared the accuracy of the SketchAndCalc approach analyses to ACAS and MiToBo to determine if accuracy of analysis was maintained while improving time efficiency.

Results

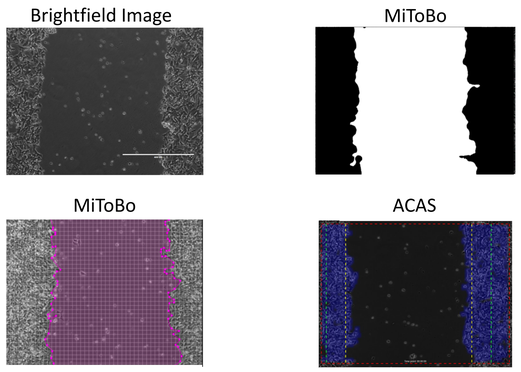
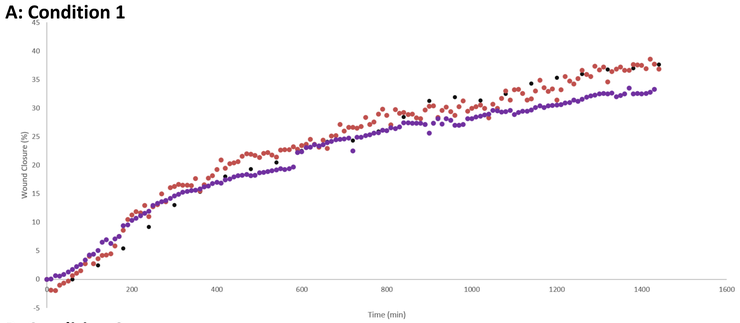
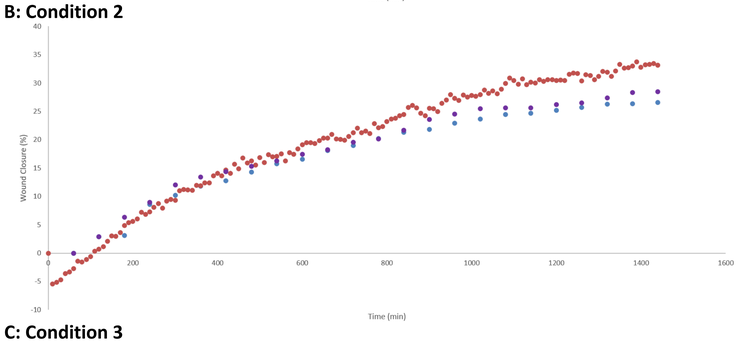


Figure 1: Wound healing assay and analyses. Brightfield image shows wound at start of 24 h assay. MiToBo (ImageJ plugin) masks black to indicate cell coverage while white represents the background or open wound area. SketchAndCalc of wounded area bounded to calculate area of wound. A second box would be draw over entire area for total area. Cell coverage would be the difference between total and wound areas. ACAS analysis uses red dashed lines to show the full area that the program is considering when analyzing each image. The yellow line is an estimation as to where the leading edge is. Between the two green dashed lines is where the program determines coverage.

A: Condition 1



B: Condition 2



C: Condition 3

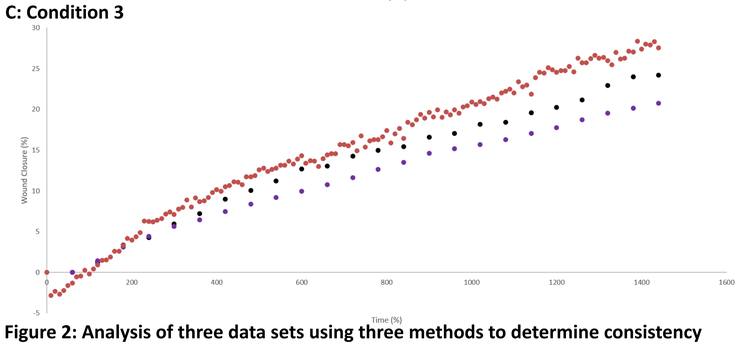


Figure 2: Analysis of three data sets using three methods to determine consistency between approaches. Wound healing assays from three conditions were assessed. In each graph, analysis from each method is shown as follows: SketchAndCal, Black; ACAS, orange; MiToBo, purple. For clarity, a single representative data set of three is shown for each condition.

Methods

ACAS was used to track movement of cells across a wound in a 35mm plate. The program uses the cell migration images taken over a 24 hour period of time using a microscope to determine the rate and total movement of cells across the wound. To do this, it requires that the resolution of pixel/µm be input so the program can determine cell size, adjust leading edge range, and overall picture size.

ImageJ plugin MiToBo functions similar to ACAS, but inputting the resolution of the image is optional. The wound healing assay image was run as is, without changing any of the resolution parameters within the program. Only required the images to be uploaded into ImageJ in a file format that it was able to understand (tif).

SketchAndCalc does not have any parameters that need to be set in order for the program to run properly. Requires that the image is in a format that an iPad or handheld device understands so that the image from the microscope can be downloaded onto the device and uploaded into the program. SketchAndCalc also requires a touch screen in order to outline the cell leading edge by hand.

Discussion

Each program was used to analyze three different data sets to determine differences between the programs. This approach examined how the programs biased the data or if they generally agreed with one another. Overall the data shows that each program is within 5% difference between one another. This originally seems like a large difference but when working with actual data to generate a triplicate set, the standard error ranges between 5%-10%. The difference between programs falls within the error that is seen between trials of the same conditions. Accuracy of each program doesn’t seem to be an issue unless the error is smaller between trials. With similar accuracy, the next parameter of interest was speed of analyses. SketchAndCalc as mentioned previously is an app that runs on a handheld device that requires the user to draw between the leading edges and enclose the open area. It seemed previously like the most accurate choice, but as shown in figure 2 that isn’t necessarily the case. SketchAndCalc usually takes anywhere between 4-6hours to complete 24 images. MiToBo takes roughly an hour to an hour and a half to complete 145 images and ACAS take 10 min to complete 145 images. With all other things being equal, ACAS is the fastest wound assay analysis software and would be the best option for an accurate and efficient method.

Conclusion

In conclusion, it would appear that ACAS would be the most time efficient and sufficiently accurate program to analyze wound healing assays over a 24 hour period. SketchAndCalc was accurate, but would take 4-6 hours to analyze only a fraction of the data completed by ACAS in the same time period. MiToBo was also relatively quick only taking about an hour to run a full data set with no notable change in accuracy.

Future Directions

The future directions of this project are to move forward with using the most accurate software for image analysis and use it to further our studies in wound healing assays in a more time efficient and robust manner. It would seem that ACAS would be the ideal choice for speed and efficiency, but, depending on the error between trials, a new more precise program may have to be used to distinguish between more subtle changes in cell movement.