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# Improving Analysis of Gene Editing in Plants Through Open-Source Software Optimization

## Introduction

### Background

- Gene editing in cells helps increase the scientific understanding of the genome, improving crop health and even helping cure diseases.
- In this study, foreign DNA is introduced into plant tissue through particle bombardment, modifying the plant cell DNA. Cells with modified DNA glow with a green fluorescence.
- Hand-counting these cells is time consuming and leads to error.
- Using software to automate the cell-counting greatly improves efficiency and eliminates person-to-person variation in counts.
- CellProfiler is an open-source cell characterization software through which programs can be customized for the identification and analysis of cells in images.

### Objective

The goal of this project is to optimize the cell counting program by adjusting the different module settings such that the program accurately counts cells and outputs totals similar to our hand-counts.

## CellProfiler Pipeline

- Images
- Metadata
- NamesAndTypes
- Groups
- GaussianFilter
- EnhanceOrSuppressFeatures
- Crop
- Crop
- IdentifyPrimaryObjects
- IdentifyPrimaryObjects
- IdentifyPrimaryObjects
- MeasureObjectIntensity
- MeasureObjectIntensity
- MeasureImageIntensity
- RescaleIntensity
- OverlayObjects
- SaveImages
- SaveImages
- ExportToSpreadsheet

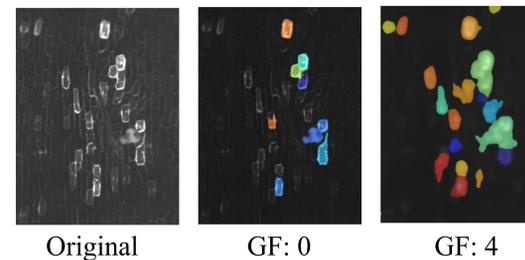
- CellProfiler program pipelines are made up of series of modules that process the images given as input.
- Settings within these modules can be adjusted to affect how the images are processed and how cells are identified and counted.
- The modules indicated by a blue arrow were the focus of testing.



## CellProfiler Modules

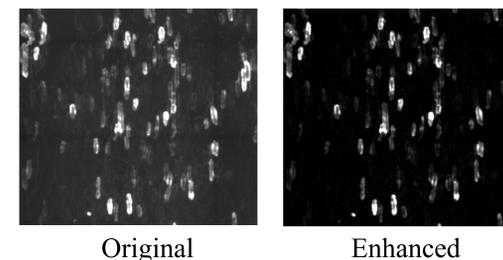
### Gaussian Filter

This applies an adjustable Gaussian blur to the image, smoothing out local brightness peaks and making dimmer cells more recognizable by the software.



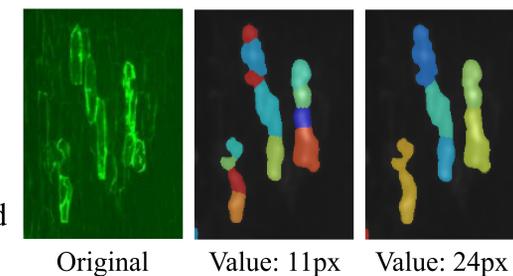
### Enhance Features Filter

The Enhance or suppress features filter brings forward prominent features such as bright objects while suppressing background noise.



### Declumping (IdentifyPrimaryObjects)

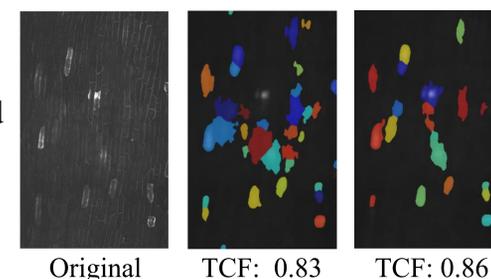
An adjustable declumping value suppresses local maxima closer than a given number of pixels. This governs how bright cells grouped together are split up by the algorithm. Lower values lead to higher counts and vice-versa.



### Thresholding (IdentifyPrimaryObjects)

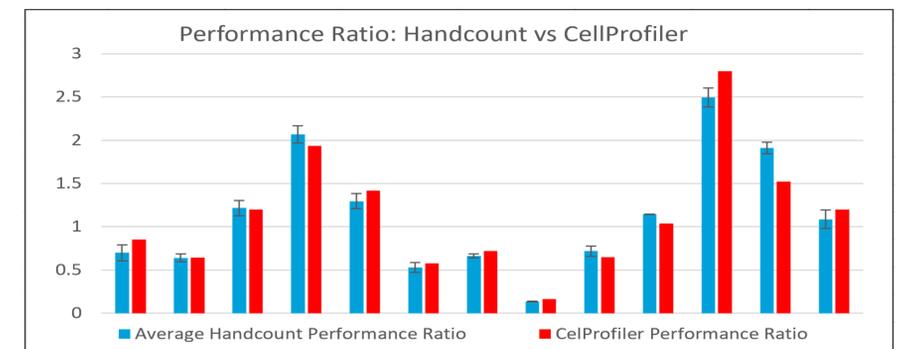
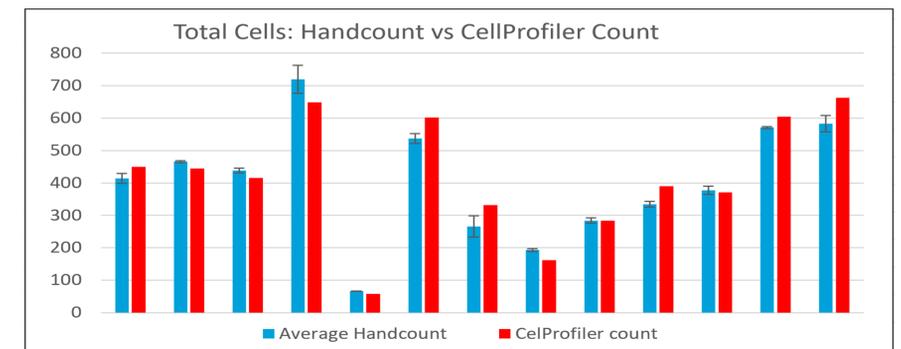
The program runs a thresholding algorithm on the image to determine the minimum brightness required for objects to be identified and counted.

An adjustable threshold correction factor can be applied to the algorithmically determined value. Changing this correction factor by even 0.01 can greatly affect how cells are identified.



## Results

The cell counts and performance ratios below were obtained and compared to hand-counts for 13 images using the following settings:  
Gaussian Filter: 4 | Enhance Filter | Object Size: 20-120 pixels  
Declumping Value: 30 pixels | Threshold Correction Factor: 0.86



## Conclusion

- The above settings gave comparable results to the hand-counts, generally to within 15%.
- Because all images are different, no single program will work consistently for every image
- Abnormalities in images such as folds or breakages in the tissue can cause the program to miscount by large amounts. These images are considered outliers and have to be hand-counted.
- This tool for assessing plant cell transfection can improve the accuracy and efficiency of data collection, ultimately increasing our understanding of gene editing in plants.