

Automated Extraction of Imaging Data for High-Content Screening

Abstract

Automated imaging has become the method of choice for extraction of data for drug discovery applications. Because this field has expanded so rapidly, the quantity of data collected has expanded from a few hundred images to tens of thousands.

The Digilab MIAS-2 is a fully automated, high-throughput microscope. This technology is ideal for collecting thousands of images without human intervention. It is efficient and very versatile because of its fully robotic controls. Given this equipment, there is a need to develop a protocol for the orderly extraction and analysis of the retrieved data.

We have used a program called *CellProfiler* that was designed to define pathways for data analysis. It was utilized because of its compatibility with automated microscopy and ability to analyze thousands of images.

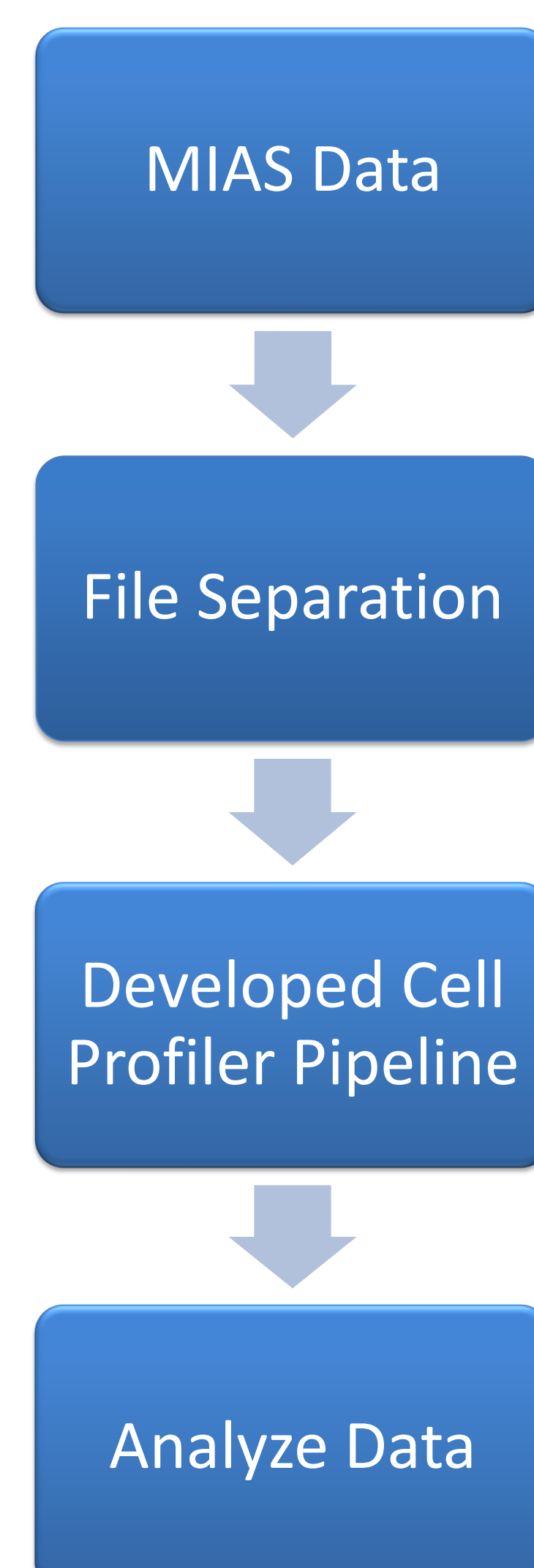
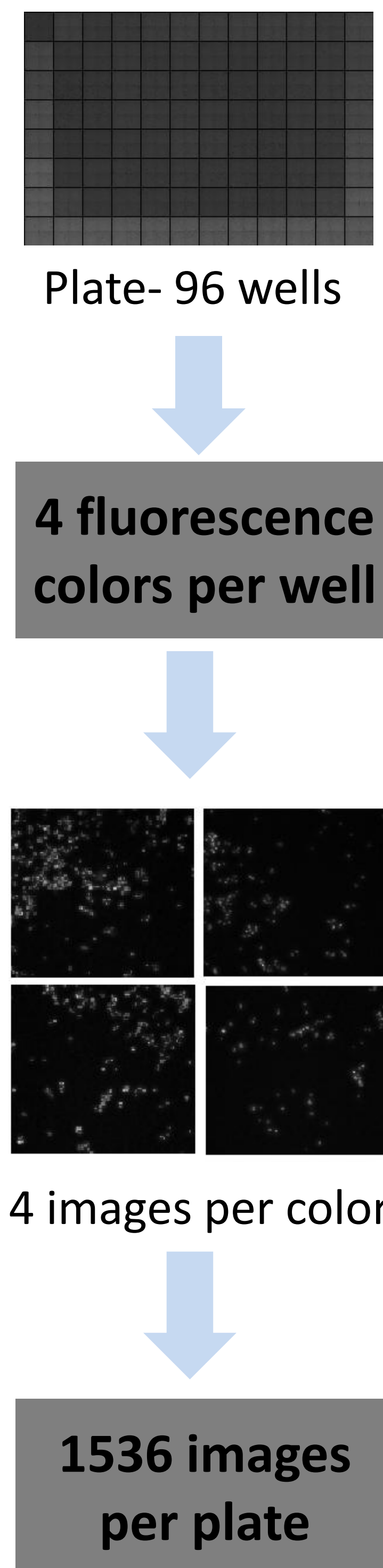
Introduction

To extract specific information from single cells, a series of analytical steps must be taken. When analyzing images, a specific protocol is most commonly developed for each new data set. The goal of this project was to create a general pipeline in *CellProfiler* that would analyze multiple sets of high-content images and deliver the information in a useful and recognizable format. *CellProfiler* requires file re-organization of the data, object definition within images, and a defined process in which the images should be analyzed and finally a declared methods for presentation of the results.

Data from the MIAS system was collected and organized to be compatible with *CellProfiler*. Multiple sets of data were run through a general pipeline that could be easily modified. Results were given in images and excel spreadsheets.

Methodology

- MIAS data was collected
- Unix program separated images from individual files and re-organized
- Pipeline:
 - Load images
 - Identify Primary Objects
 - Identify Secondary Objects
 - Measure Object Intensity
 - Measure Object Size Shape
 - Export to Spreadsheet
 - Save Images
- Final images and spreadsheets used for analysis



MIAS

- **Instrument:**
 - Zeiss fluorescence scope
 - High NA objectives
 - HG lamp
 - Auto focus
 - CO₂/heated stage
 - Robotic controlled
- **Data Collection:**
 - Plates (all types)
 - Well
 - Fluorescence (4 Colors)
 - Image



Cell Profiler

- Cell analysis software
- Developed pipeline for MIAS data

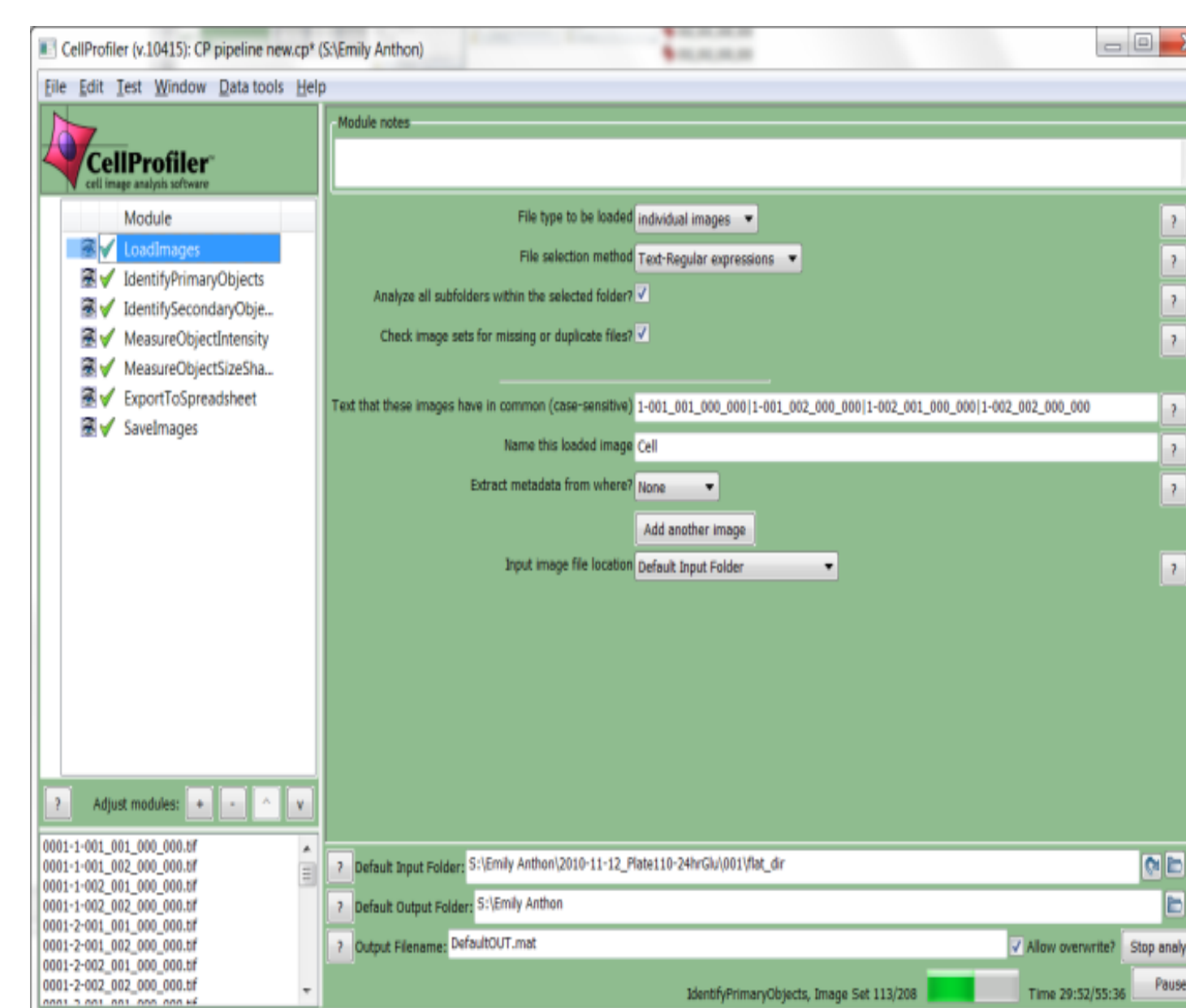


Image 1: CellProfiler Main

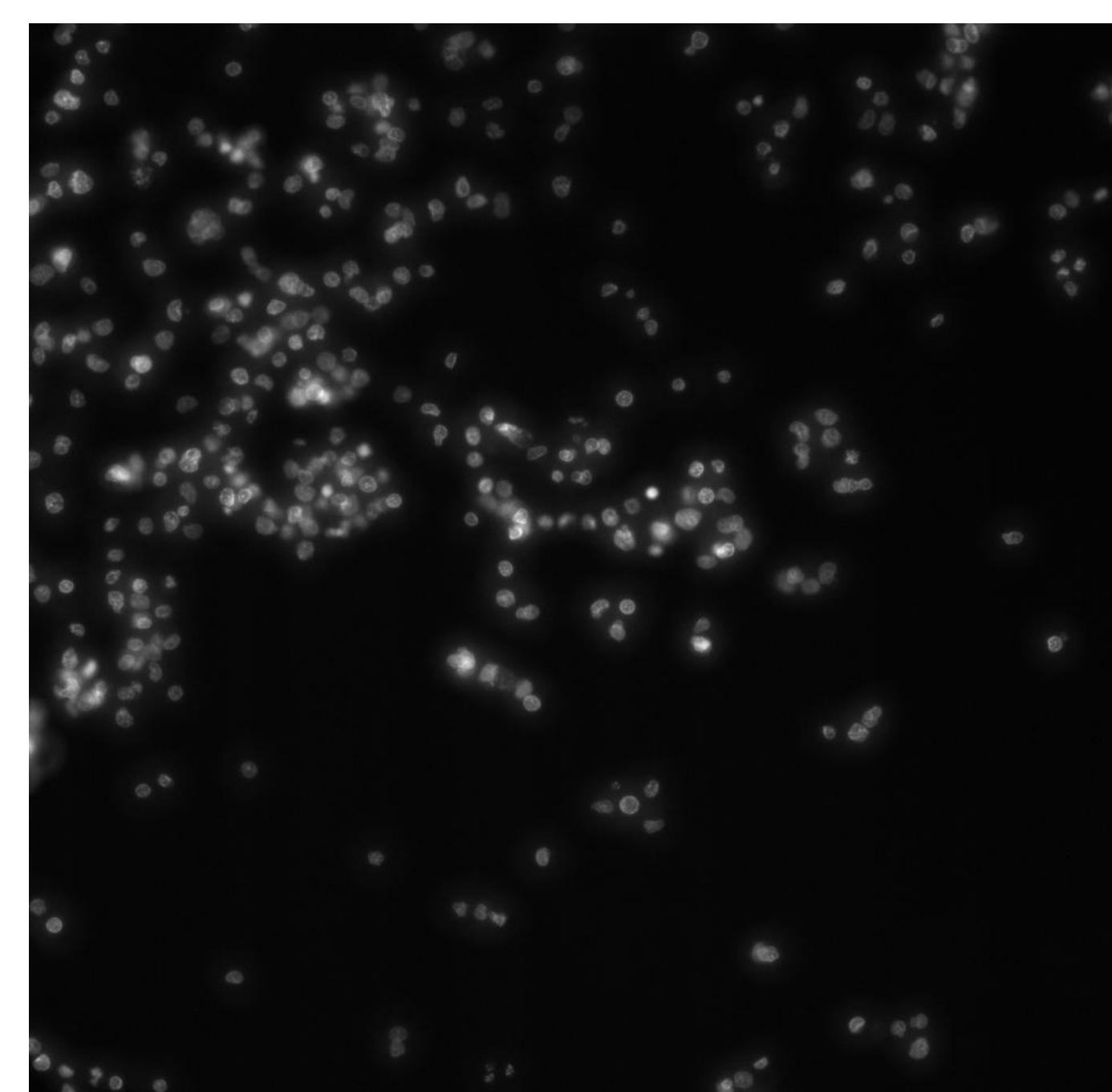


Image 2: Sample MIAS image analyzed

Results

- General data analysis protocol
- Usable images and spreadsheets

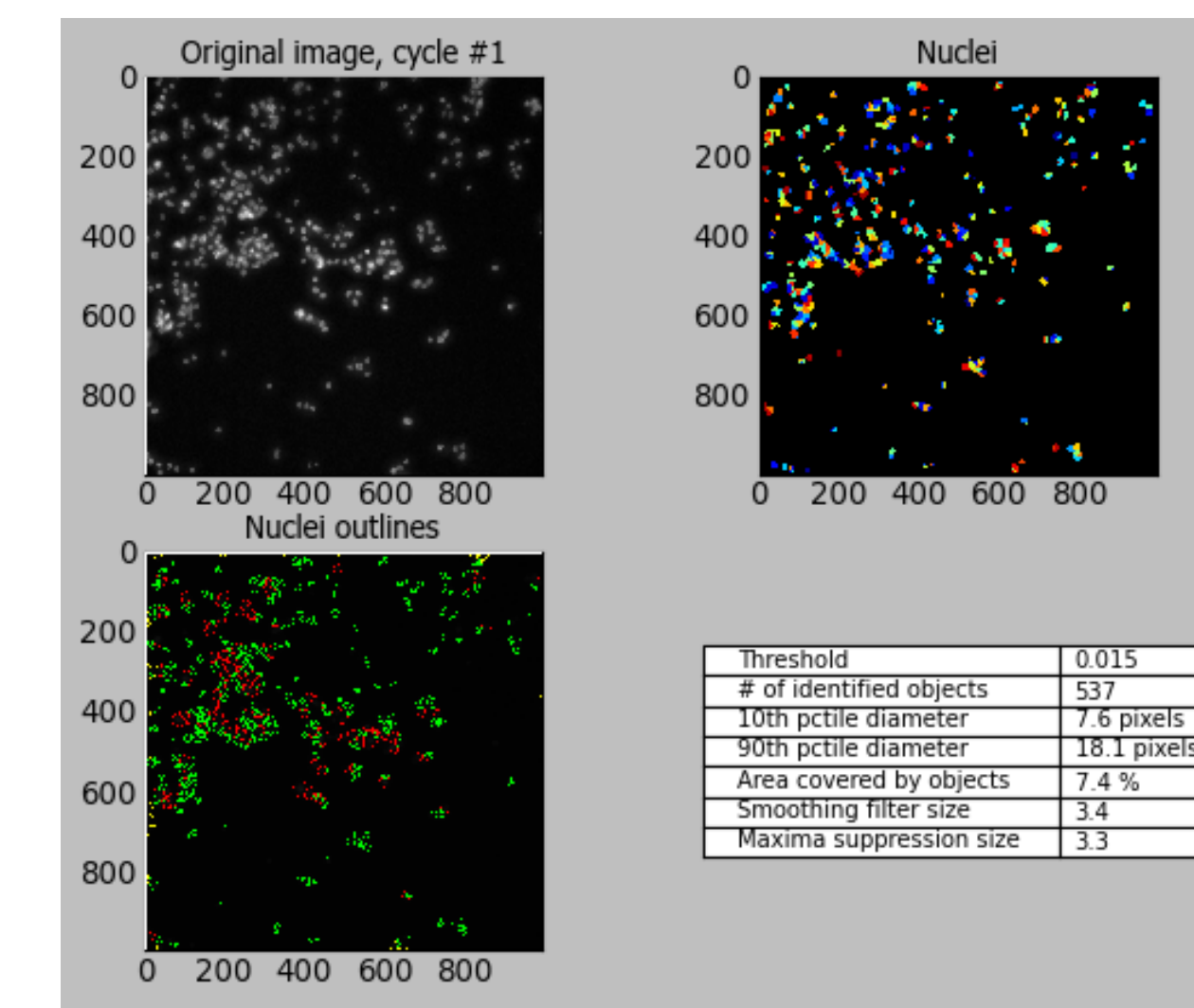


Image 3: Primary Objects

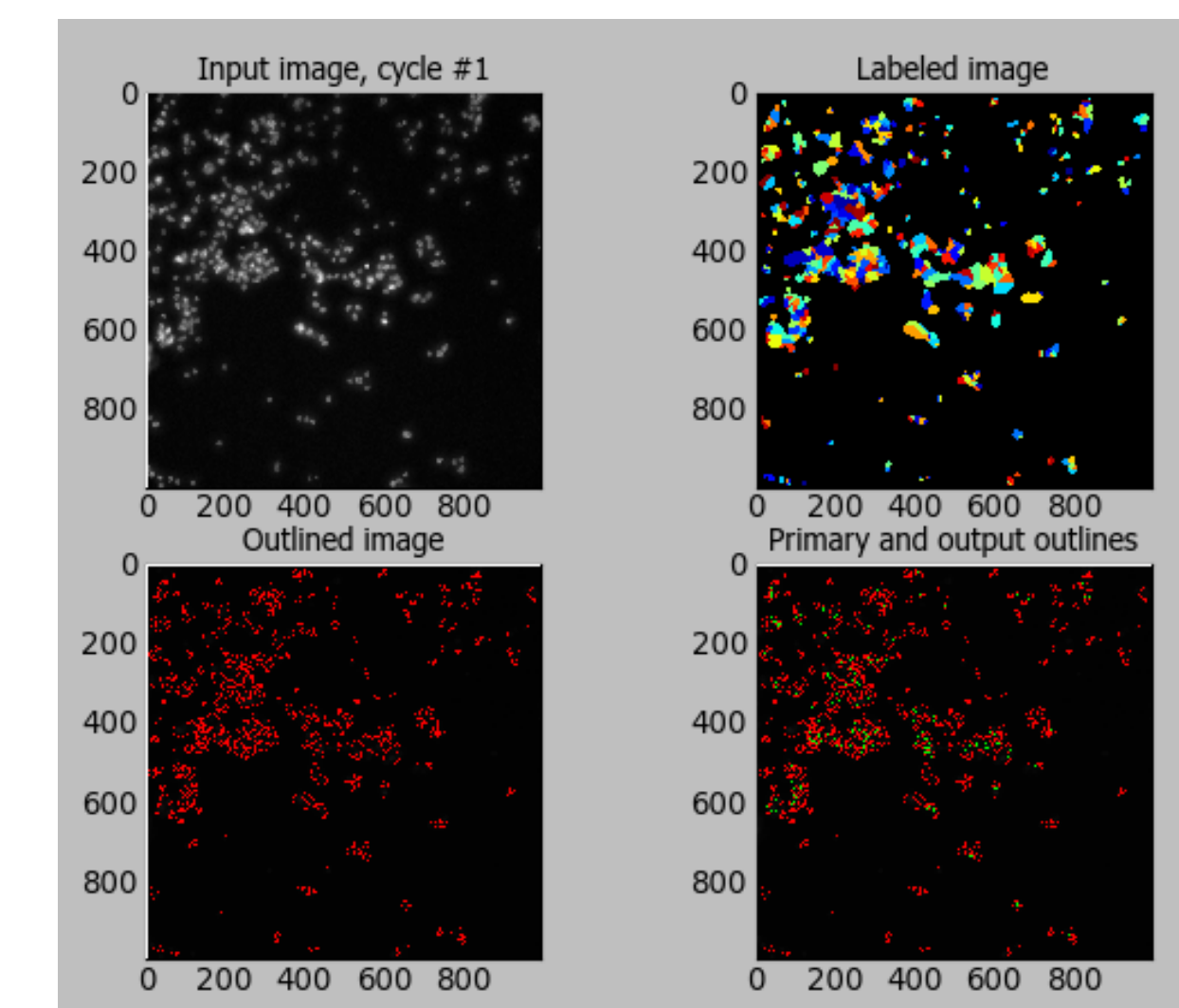


Image 4: Secondary Objects

Discussion

The thousands of raw images (image 2) were processed using the pipeline in *CellProfiler*. Image 3 shows primary objects (cells) and image 4 shows secondary objects (cytoplasm) identified. This allows separation of data from individual components within the cell. Other results produced with the pipeline are in a spreadsheet including the area, shape, and location information for all objects.

Conclusion

The pipeline created in this project allows us to quantitatively analyze images from the MIAS-2 microscope. The images that are most usable are those that include distinguishable nuclei. This protocol will aid in the analysis of thousands of images and further the movement towards complete automation, in turn improving efficiency and capabilities of drug discovery.

Applications

- Fully automated data collection and processing
- Run thousands of images per day
- Increases efficiency, quality control and reproducibility

References/Acknowledgements

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