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Utilizing Computer Vision for Automated Cellular Microscopy

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Abstract. Post-acquisition data analysis of microscopy images is a vital yet time-consuming process for researchers. Quantitative fields such as biology and microbiology often require using images as primary data sources. Finding methods to automate this process would increase the throughput, quality, and reproducibility. This research aims to provide a novel end-to-end pipeline that reduces the workload on researchers in identifying cell cytoplasm and nuclei while creating a process that can scale to the researcher's needs. The proposed methodology utilizes various image-processing techniques to rapidly identify the boundaries of cells and nuclei, including filtering, thresholding, and deep learning. The results of this research indicate that the proposed methodology could be a valuable tool for microbiologists, saving time and effort for accurate data collection.

1 Introduction

Advanced light microscopy relies on collecting and analyzing images of cells. A vital part of this data collection procedure is post-acquisition analyses of microscopy images to understand cellular processes. The standard approach involves taking an image capture from its raw data format, applying a variety of image processing techniques, and extracting information on the organisms of interest. For many microbiologists, a crucial task is the identification, labeling and quantification of individual cells or subcellular structures. Large-scale image processing presents a significant challenge for researchers in that traditional microscopic image processing methods often involve many manual steps, are time-consuming, and may not always produce the most reliable results. Manual cellular image processing typically involves a researcher drawing boundaries around cells and counting upwards of 10,000 dots per image. Some of this work can be automated with varying degrees of success, depending on the experiment. Moreover, the heterogeneity of biological processes in individual cells requires single-cell information to be extracted from images

The target of this research involves working with single-molecule RNA fluorescence in situ hybridization (smRNA FISH), which is used by the research laboratory at The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology to observe viral infection's impact on human host cells. Messenger RNA (mRNA) provides a way for researchers to quantify gene expressions in the cell. In viral infections, this allows them to study the impact a virus infection has on a cell's gene expression. The impact can be seen through observations such as counts, saturation, or location to name a few. The benefits of this experiment are clear, but the data collection and analysis processes are time consuming and resource intensive.

A central issue is processing these images is tedious and time consuming. A researcher needs to manually load the images, change the contrast to make the cells visible, and attempt to use a variety of software tools to outline the cells and nuclei. If the software fails to perform this automatically, then additional manual work is required. Then, the mRNA molecules must be individually counted. The counting process has some options in various software, but reliable automation would be preferred to prevent unnecessary time sinks in the experimental pipeline.

Extensive research has previously been done for computer vision applications in biology. Nonetheless, there are several challenges affecting researchers in the field when applying advanced techniques or state-of-the-art artificial intelligence (AI) algorithms. Firstly, much of the existing research is difficult to integrate as a practical tool for common laboratory use. There are ongoing projects such as DeepImageJ (Gómez-de-Mariscal et al., 2021), which aims to bring deep learning algorithms to the widely used laboratory software, ImageJ. However, these tools are commonly developed as a "point-and-click" analysis tool and are not suitable for fully automated processing of images. Secondly, biologists are often not focused on the data science and computer science skills necessary to independently develop customized solutions using the latest algorithmic advances.

Widely known traditional image processing methods are difficult to automate, as they are often dependent on image-specific characteristics and manual input. Techniques such as thresholding typically require fine-tuning before they produce satisfactory results. There are more complex tools that perform better within the field of traditional computer vision, but learning how to use and tune these tools takes additional time on top of existing research workloads. Consequently, the cumbersome workflow to process and analyze experimental images delays research progress, as researchers and scientists must devote more time to data processing instead of analysis and further experiments.

The challenges arise from the complexity of the microscopy images rather than from tools or methods. Traditional computer vision has made significant advancements and achievements since its first development in the 1950's (Caspersson et al., 1962) and has provided a diverse range of algorithms for image processing tasks. However, the biology and microbiology fields produce highly variable images at various levels of detail and composition. For example, the content of the images

varies due to the vast diversity of cell morphology. Additionally, experimental conditions such as focus, zoom level, and lighting all contribute to the wide range of resulting images. The types of fluorescence and dyes used also vary between experiments. As a result, many algorithms struggle to provide consistent performance across all these differences.

The weakness of traditional computer vision algorithms is partially solved by recent advances in AI, they perform well in some cases but are not capable of generalizing their solutions as discussed earlier. However, well-trained computer vision AI models have shown promising improvements in this regard. The issue is either getting access to a model or training the model locally. Currently there are numerous public models that perform dependent on use case. If one's use case was not represented in the training set for the model, then it is not likely to produce desirable results. In this case a custom model would need to be made but might not be a viable solution. Model building is a far more complicated process that would require extensive time and effort. This is not a reasonable expectation for an individual lab for a specific use case.

Software exists that is tailored to the medical and biomedical fields, but these tools have limitations. The lack of an automated solution for processing cellular data in microbiology presents an opportunity for improvement using data science techniques. We present research that aims to address these issues by exploring methods to utilize computer vision techniques and deep learning algorithms to streamline the process of cellular image data processing.

The proposed strategy involves using both traditional and state-of-the-art AI computer vision approaches. The weakness of traditional computer vision, based in the highly varied reality of biological microscopy, can be remedied by AI. The result is a pipeline that takes smFISH data into a representation of the data that easily lends itself to traditional computer vision algorithms. Therefore, an array of traditional computer vision tools can be leveraged in addition to state-of-the-art AI algorithms.

2 Literature Review

There have been significant technological advances in computer vision techniques in recent years with the development of deep neural networks like convolutional neural networks (CNNs) and transformer networks. However, traditional image processing algorithms and machine learning approaches are still being used to provide efficient and accurate interpretation of cellular microscopy images. One of the primary efforts of this research involved assessing the range of available algorithms and customizing them to the selected use-case.

This literature review focuses on the techniques and methodologies extensively explored for automated cell detection, segmentation, and identification. The evolution

of microscopy hardware and imaging processing algorithms has contributed significant advances to understanding biological processes

2.1 Early Advances in Cellular Image Processing

The idea of improving biological research processes through automated image-processing has been a topic for researchers for decades. Early advances in automated microscopy data acquisition and interpretation became popular in the 1950's with improvements in hardware technology. This allowed for higher resolution of single-cell images and rapid developments in computer processing facilitated increasingly advanced algorithms. Caspersson et al. (1962) pioneered techniques for high resolution microscopy image scanning. This enabled researchers to begin shifting focus to developing and evaluating methods for automating the extraction of meaningful information from the images. Bartels et al. (1977) documented the ongoing development of supervised and unsupervised learning algorithms and their applications to biomedical image interpretation. However, there were still significant challenges for early data scientists with data storage and staging, image I/O, and the general availability of libraries and software packages (Bartels et al., 1977).

Prewitt et al. (1966) created a methodology for classifying single-cell images of stained white blood cells into one of four types. The process involved scanning microscopy images into a digital grayscale format and used optical density histograms to identify image regions. For example, the histograms showed three distinct peaks of varying optical density values, which corresponded to the image background, cytoplasm, and nucleus. To classify the specific type of white blood cell, thirty-five parameters were calculated from the optical density frequency distribution and clustered using a nearest-neighbors algorithm. Finally, a spatial differentiation method was developed to define the cell boundaries and separate the background.

A decade later, Borst et al. (1979) expanded on these ideas by creating an algorithm for automatic segmentation of single-cell images. In this method, a grayscale image is first median filtered using 29 neighboring pixels to reduce noise. The median filtered image is then filtered again using a smaller number of pixel neighbors to further distinguish the cell from the background. The neighboring pixel values are stored as three histograms, which are used for masking and thresholding. The thresholding process defines the cell boundary and separates the nucleus and cytoplasm from the background. Using this algorithm, post-processing steps, such as removal of small regions and merging of nearby regions, can be applied to the segmented cells to obtain the result. A similar cell segmentation methodology involving filters, masks, and thresholds was documented by Abmayr et al. (1979) for automated cancer cell detection. To determine if a cell was cancerous or not, the researchers included additional image processing steps for texture analysis, feature selection and classification.

An algorithm for multi-cell image segmentation was developed by Harms et al. (1986), with the idea of matching geometric features and colors in the image to the

organismal cell structure. The morphological methodology involved splitting the image into red, green, and blue color channels, and segmenting the nucleus and cytoplasm based on threshold probabilities from these channels. A novel method for splitting multiple overlapping cells was also introduced, which used isograms and geometric centers to create separation boundaries between cells.

In the 1990's, fluorescence in situ hybridization (FISH) signals became a popular method for detecting specific features in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Additionally, advances in imaging hardware with confocal laser scanning microscopes (CLSM) allowed for nondestructive three-dimensional imaging of biological organisms. Umesh et al. (1999) introduced an end-to-end pipeline for efficient cell segmentation of FISH signals. The algorithm was composed of traditional image processing techniques and a novel approach for segmentation of the 3D cell images. A graphical user interface (GUI) was also developed to make the research results available to a wider audience. The methods and algorithms described above were instrumental in laying the foundation for modern research and advancing biomedical cellular knowledge.

2.2 Artificial Intelligence in Image Processing

Previous advancements and applications in computer vision were undeniably impressive and valuable, but they were not without limitations. One significant drawback to these traditional methods is that they are not generalizable to the contents of the image, but rather the data present within the image. Fortunately, recent developments in AI introduced a novel solution to address these longstanding challenges through Convolution Neural Networks (CNNs). CNNs are a specialized type of neural network specifically designed for processing visual data such as images and videos. CNN architectures consist of convolutional layers, pooling layers, and fully connected layers. The convolutional layer is the main building block, creating trainable filters that detect certain properties in an image such as edges or shapes. Pooling layers are like convolutional layers in how they slide over the image matrix, but they are parameter-less down sampling calculations and thus are not trainable. One example of a pooling layer is max pooling, which simply takes the maximum value of the pixels in the window. Although the idea behind artificial neural networks such as CNNs has been around for decades, recent improvements in hardware and big data storage have created rapid advances in the field.

LeCun et al. (1990) developed one of first CNN architectures (LeNet) for image recognition to classify handwritten digits in the widely recognized MNIST dataset. CNNs gained popularity with the successful research conducted by Krizhevsky, A., Sutskever, I., and Hinton, G. E. in 2012. Their work was one of the first examples of using CNNs for widescale image classification, where they trained a deep CNN to classify over one million images into a thousand different classes. This experiment generated widespread interest in CNNs from data scientists and researchers across computer vision.

Image segmentation is a computational task that builds upon the fundamental ideas from CNNs. It involves partitioning an image into regions and separating each pixel within the image by class. Although it shares some fundamental concepts with CNNs, segmentation is regarded as one of the most challenging tasks in image processing and computer vision. The main distinction between the CNNs and segmentation is the output: CNNs classify an image into a multiclass result while segmentation typically outputs a masked image of the segments that is the same dimensions as the original image.

A naïve approach to segmentation is to convolve the image in place a few times and then have the final output be the final image. Unfortunately, this model fails to perform as it does not introduce a growing channel space which increases the expressiveness and generalizability of a model. Badrinarayanan, V., Badrinarayanan, V., & Cipolla, R. (2015) solved this problem by developing the encoder decoder network. They created a method that down samples just like classification CNNs and thus reaping the benefits of model expressiveness. The model is then sampled up to the same size as the original image. The result is a network that processes an image into high dimensional low-level space and then expands back to the original space and size. Even though the model worked, performance was far from optimal.

An example of the next iteration in model development was in the work of Ronneberger, O., Fischer, P., & Brox, T. (2015). They show a variation of the U-Net architecture which still used the overall encoder decoder methodology but added a novel component to the network. Instead of operating in a sequential matter the model stores the weights from the various down sample stages and reintegrates them into the network in the up sampled stage. The benefit of this approach is the model can maintain context and "remember" the previously created weights at each stage. These improvements helped the model's performance significantly such that the U-Net structure is still used to this day.

Recently a generalized approach to image segmentation called Segment Anything (SAM) has been released by Lei Ke, Mingqiao Ye, Martin Danelljan, Yifan Liu, Yu-Wing Tai, Chi-Keung Tang, & Fisher Yu. (2023). Their model resolves an issue that impacted every model that was previously discussed; models must be trained with data directly relevant to their problem. SAM does not have this issue and performs well at segmentation in general, but it can see performance gain through transfer learning on desired domain specific image segmentations. SAM offers two methods to work with the model. The first is the pure SAM approach where an image is fed into the model and the model outputs as many masks as possible. The second is the augmented SAM approach where the image is fed with additional data such as a point or a bounding box and SAM outputs the most confident mask given the context of the prompt.

3 Methods

The methods used in this analysis combine many traditional image processing techniques with SAM into an executable pipeline for semi-automated image processing. The cellular image processing pipeline was divided into steps shown in *Figure 1*. First, the image data is extracted from the proprietary file structure into a usable format. The image is then preprocessed to enhance the contrast and denoise the image. The first analysis phase starts with nuclei segmentation. Using the augmented SAM approach, the user interacts with the image within a GUI by drawing bounding boxes around nuclei of interest. The users bounding boxes are then processed and input into SAM with the image. Cytoplasm segmentation occurs in the same fashion as nuclei segmentation. After the nuclei and cytoplasm are segmented, a post processing step is performed to properly link the cytoplasm with their corresponding nucleus. Finally, the pipeline performs the mRNA “dot” counting step. The user specifies which z axis and staining channel combinations they would like to process and then the program automatically analyzes every combination. “Dot” counting is accomplished by utilizing pure SAM to produce segmentations on the isolated cell. Once complete the number found is simply recorded and returned to the user.

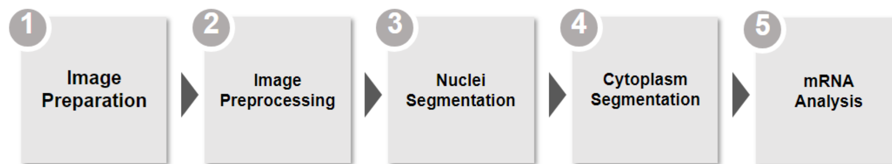

Figure 1. Overview of the image processing pipeline showing steps from image loading to postprocessing.

3.1 Data

The data used in this research was collected by Dr. James Burke’s team at the University of Florida’s Scripps Biomedical Research laboratory. The raw images are captured using a Nikon microscope and are available in proprietary Nikon ND2 format. The images were parsed using an ND2 processing Python library and converted into a NumPy array. The image file sizes range between 250 and 500 MB.

The result of loading the raw image file into NumPy is a 4-dimensional array. The 4 dimensions include the fluorescence channel, z-index of the 3-d z-stack, the x-axis, and the y-axis. Each experimental channel is used to highlight or enhance various aspects of the cell. For example, DAPI staining strongly binds to sections of DNA and thus acts as an indicator of where the nucleus is located. The z-stack contains levels of focus at different depths in the cell. Processing using this z-stack format is typically done by either manually selecting the z-stack of interest or combining them through a simple linear combination. The pixel values in the x-axis and y-axis of the image are light intensity values and can range from 0 to 64,000.

3.2 Image Preprocessing

Due to the high quality of the Nikon microscope camera, the resulting images can capture a wide range of intensity values which are stored in 16-bit format. Many algorithms in both traditional image processing and deep learning expect the image in an 8-bit format, so a preprocessing step must first be employed to scale down the image intensities. Initially, the image is prepared by clipping intensity values that are exceptionally higher or lower than the image mean value, effectively removing outliers. Following the clipping step, the image is rescaled from 16-bit to 8-bit for use in further analysis.

Background noise and excessive static can reduce the effectiveness of many segmentation algorithms. To enhance the image quality and remove background noise, a denoising algorithm such as Gaussian filter is applied to the image. Following this, the image contrast is enhanced through contrast limited adaptive histogram equalization (CLAHE). Contrast stretching or normalization is a technique that stretches the histogram of image intensity values to span a desired range with a linear scaling function. These methods both improve the image contrast, which results in significant improvements in boundary detection.

3.3 Cell Nuclei Segmentation

This research explored various methods for nuclei identification in microscopic images, ranging from traditional image filtering techniques to deep learning algorithms, with a particular focus on evaluating the effectiveness of architectures such as CNNs. Among the explored algorithms, SAM demonstrated exceptional performance, making it the core model for the specific use case.

To enhance SAM's capabilities, a human-assisted version was developed, which requires additional input in the form of bounding boxes. A Graphical User Interface (GUI) canvas was designed to allow users to draw bounding boxes within the image, providing additional information. These bounding boxes are then translated into the appropriate input format for SAM, and the resulting mask predictions are exported for the user's use. The human-assisted GUI offers researchers the flexibility and control to precisely define the regions of interest in the images, allowing them to incorporate their domain knowledge. This ensures that the program is accurately capturing the cell nuclei to the researcher’s satisfaction. This approach creates a balance between automation and human augmentation. This results in more reliable and interpretable results for nuclei segmentation.

3.4 Cell Cytoplasm Segmentation

The segmentation of the cell cytoplasm, or boundaries, presents a more significant challenge compared to nuclei segmentation. These challenges primarily arise from the lack of clearly defined cell boundaries and the irregular shapes and sizes of the cells in the study. The boundary identification process relies heavily on visual interpretation of multiple layers of data and can require further human input for

accurate segmentation. Despite these inherent challenges, SAM has shown promising results for this application. Therefore, the same human-assisted SAM processing procedure was applied to the cytoplasm segmentation as the nuclei segmentation.

3.5 Cell Mask Linkage

After completing both segmentation steps, the resulting output is two image masks for the nuclei and cytoplasm. However, these masks are not linked to each other. To establish this link, the cell's physical attributes are used to create rules. For example, a nucleus is always contained within the cytoplasm of the linked cell. Using these rules, linear transformations can be performed between the two segmentation matrices to produce a new matrix that contains the necessary linkage information. This linked-mask segmentation approach enables further analysis and interpretation of the cell during future processing steps.

3.6 Messenger RNA (mRNA) Analysis

In certain image channels, the mRNA molecules are visible as small areas of high intensity “dots” on an image and are far smaller than a cytoplasm or nucleus. Despite this size difference, SAM still demonstrates decent performance, but requires a new protocol for best results. In this adjusted procedure, the default SAM algorithm is utilized over the augmented approach with a process modification called SAM quilting. The original method involves inputting a single image into SAM, with SAM generating multiple segmentations for the image. However, in SAM quilting, the input image is broken up into a series of sub-images representing a grid-like structure. Each sub-image is then individually input into SAM, and the number of segmentations is counted for each. At the end of the process, the segmentation algorithm results are summed to yield the full count for the original image. The SAM quilting approach provides an effective way to improve the performance of the algorithm on small segmentations and analyze mRNA molecules.

4 Results

The full image processing pipeline produces multiple visualizations and CSV files, each relevant to specific tasks within the lab. A cell segmentation ID output image provides a visual reference linking other output data to the respective cell. The intensity CSV output yields pixel intensity sums and pixel areas for each cytoplasm and nucleus. This allows for calculations of mean intensity values for the overall segmented cell or its components. Lastly, the “dot” counting step outputs a CSV of mRNA counts within each nucleus and cytoplasm of the segmented cells. The mRNA counting step additionally outputs an image to display what the model counted to give insight into performance on the given cell. Performance will be demonstrated in a comparative fashion by displaying two cells that have a major difference in expression of mRNA counts in the mCherry channel along a singular z-axis. The difference between the two can also be clearly seen and represented in the analysis.

4.1 Cell ID Reference Image

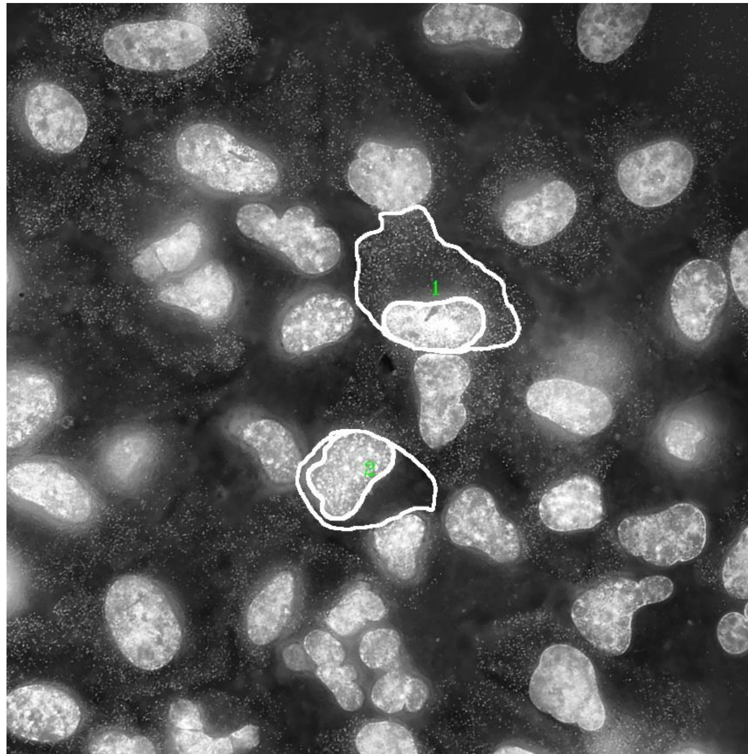

Figure 2. Cell ID reference image where two cells were selected for analysis. This image is a linear combination of the DAPI and mCherry channels at a singular z-index.

Figure 2 displays an example of the entire cell segmentation output. It provides a way for researchers to see the model's performance, while also providing a way to reference cells based on their IDs in future analysis steps. As shown in the image, Cell 1 displays significant mRNA presence while Cell 2 displays minimal mRNA presence. This indicates differences in the viral responses of the cells

4.2 Intensity CSV

Table 1. Raw Intensity Output

Cell ID	Cyto. Intensity	Cyto. Area	Nuc. Intensity	Nuc. Area	Z-Index	Channel
1	729,737,702	110,628	216,006,098	38,480	1	WF mCherry
2	211,956,984	48,931	183,428,574	43,834	1	WF mCherry

Table 1 presents the raw intensity output provided to the user. This output format offers the user the flexibility to decide the preferred type of mean intensity analysis for the study. The analysis options for the mean intensity values require minimal additional effort, and include formats for the entire cell, nucleus-only, and cytoplasm-only.

Table 2. Mean Intensity Output

Cell ID	Mean Cell Intensity	Mean Cyto. Intensity	Mean Nuc. Intensity	Z-Index	Channel
1	6,342	6,596	5,613	1	WF mCherry
2	4,262	4,332	4,185	1	WF mCherry

Table 2 displays the resulting mean intensity values. The cell mean intensity for Cell 1 is larger than Cell 2, which was expected based on visual analysis of the image.

4.3 mRNA Counts

The mRNA "dot" counting step generates two types of output. The first is a CSV file containing mRNA counts, similar in format to the intensity CSV output. The second output is a model performance visualization, presenting an image of the cell that highlights the segmented "dots" corresponding to the identified mRNA molecules.

4.3.1 mRNA Counts CSV

Table 3. mRNA Count Output

Cell ID	Cyto. Counts	Nucleus Counts	Z-Index	Channel
1	606	205	1	WF mCherry
2	85	117	1	WF mCherry

The output of the mRNA "dot" counting step in *Table 3* reveals that Cell 1 exhibits a significantly higher mRNA count compared to Cell 2, in both their nuclei and cytoplasm. These output formats allow researchers to aggregate these statistics from single cell analysis to images with dozens or even hundreds of cells. As such, the impact of cellular responses to viral infections can be monitored and researched on a large scale through mRNA analysis.

4.3.2 mRNA Counts Reference Image

To enable the user to quickly assess the pipeline performance, additional outputs of cellular image segmentations are provided on a per cell, per channel, and per z-axis

basis. *Figure 3A* and *Figure 3C* display Cell 1 and Cell 2 cytoplasm mRNA segmentation results, respectively. These visualizations offer valuable insights into the mRNA distribution within each cell, facilitating evaluation of cell responses and comparative analysis between cells.

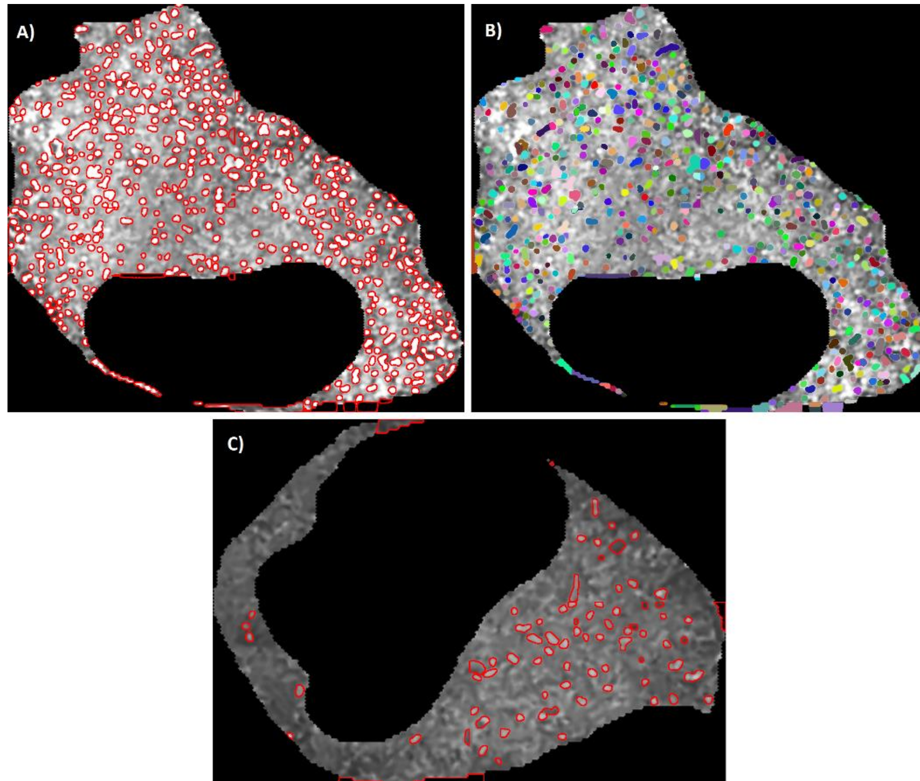

Figure 3. Cell "Dot" Segmentations and Segmentation Outlines. **(A)** Cell 1 Segmentation Outlines. (**B**) Cell 1 Segmentations. (**C**) Cell 2 Segmentation Outlines

Lastly, an additional segmentation image, *Figure 3B*, is provided for further examination of model performance. The outlines provide a quick view into the validity of the segmentations but the procedure to produce these outlines can group separate segmentations together under one entity. The segmentation image can help clarify this issue as every color is indicative of a unique segmentation.

5 Discussion

Through this research, implications, limitations, and ethical considerations were identified and are discussed below. Additionally, future research directions are outlined for further refinement and exploration.

5.1 Implications

The results shown above demonstrate the effectiveness of combining traditional image processing techniques with deep learning methods to streamline the segmentation and analysis of cellular images. The algorithms and methods used for segmentation are robust to the shape and boundary irregularities present in cell cultures. With the continued advances in deep learning methods and computational hardware, rapid adoption of these technologies within the biological field should be further explored.

The significance of these methodologies relates to the practical implications they have for laboratory researchers and scientists. For laboratory researchers, the proposed image processing pipeline translates into time savings in data preparation and results gathering, allowing for increased focus on the analysis of the experimental results. There are wider implications for the scientific community with the demonstrated success in terms of performance and robustness of deep learning algorithms for image processing.

A time efficiency metric is not provided as a single lab's workflow is not sufficient for a general claim. Still a rough estimate would be at least twice as fast as the normal workflow. Additional benefits are both consistency and automation. The model output will be consistent, reducing human error, which has a beneficial effect on scientific research. Additionally, there is the benefit of automation. Human researchers cannot process the data and work on other tasks, but human researchers could have the program process the data while they process other tasks.

Lastly, the success of Meta's Segment Anything Model over both traditional segmentation methods and U-Net neural networks that had been specifically trained for cell detection and segmentation was surprising.

5.2 Limitations

The current development of the image processing pipeline is configured to focus primarily on cell segmentation and mRNA detection and are aligned with the requirements of the research direction and objectives. However, techniques, methods, and algorithms used in this study could be generalized to a wide range of additional use cases in the field of microbiology, but time would need to be spent to develop additional process pipelines dedicated to these new objectives. Additionally, the current pipeline requires some manual adjustments and user input. Supplementary refinement of the image processing pipeline could be achieved to enhance efficiency, streamline workflows, and further reduce manual effort required from researchers.

Overall, the "dot" counting procedure is strong but not perfect. There are cases where "dots" are missed or objects that are not "dots" are segmented. For this reason, it is recommended to always inspect these images and adjust your output, as necessary. This hybrid approach is not perfect, but it saves more time than the manual approach.

5.3 Ethics

For continued development and expansion of this methodology to additional use-cases in the field of biology, ethical implications must be considered. The current data does not involve human subjects, but for additional applications this might not be the case. If the images contain information from human subjects in a clinical trial setting, researchers must ensure that informed consent is followed, and subjects understand the risks associated with the study and how the data will be used. Similarly, any image data containing personal or identifying information from human subjects requires processes in place to ensure data classification, confidentiality, and privacy.

5.4 Future Research

Future research directions primarily focus around investigating the potential benefits of using transfer learning to improve cytoplasm segmentation and further refine nuclei segmentation. In addition, improvements could be made by continuing to refine the mRNA analysis "dot counting" procedure through adjustments to the Segment Anything Model or by exploring other methods.

This research's objective was to provide a dedicated tool for cellular image processing tailored to microbiology research applications at the Scripps Biological Research Laboratory at the University of Florida. However, expanding the generalizability and accessibility of the proposed methods used could improve workflows of biological researchers working with similar use-cases. To achieve further accessibility and user-friendliness to the research community, continued efforts could be made to expand and improve the development of web applications and image processing methods. With the large file size of the images, the processing is computationally expensive. Further work could be done to improve computational efficiency or use a scaling method to reduce the size of the working images.

6 Conclusion

In conclusion, this research's objective was to create a novel pipeline for the identification, segmentation, and labeling of cellular microscopy image data for the application of mRNA analysis. The proposed pipeline and methods explored throughout this research demonstrated the effectiveness of combining traditional

image processing techniques with deep learning methodologies to create a streamlined workflow for laboratory researchers.

Future research directions involve improving the robustness of the implemented algorithms and making the pipeline more accessible to the broader scientific community.

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