Image segmentation for proliferating retinal cells in zebrafish

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Abstract

Zebrafish have the remarkable ability to regenerate their retina after injury. The Mitchell lab is currently investigating how an inflammatory signaling cascade in microglia (mediated by a protein called MYD88) affects the Muller glial regenerative response in zebrafish retinas. The lab uses confocal microscopy to capture images of regenerating zebrafish retinas stained for markers of proliferation (PCNA), Muller glia (MG), microglia, and cell nuclei (DAPI). The resulting images show densely packed proliferating MG-derived progenitor cells that are challenging to quantify. Manual counts of the cells are tedious, time consuming, and subject to bias, so a semi-automated process using ImageJ's Fiji software had been applied in an attempt to count the proliferating cells but was not able to sufficiently segment clusters of cells. Therefore, an image segmentation algorithm was developed by the Long computer science lab to better distinguish individual proliferating cells by using computer vision techniques and overlaying the DAPI stain with he PCNA stain. The computer vision techniques used were: unsharp masking, Canny edge detection, Otsu and multi-Otsu thresholding, local thresholding, and a morphological opening operation. The algorithm can segment cells in an image within 6-12 seconds using common PC hardware and can identify more cells than the current Fiji implementation. The algorithm provides a viable alternative to manual counting that is fast and efficient. Further work is recommended on manual counts, to accurately identify the program accuracy, and potentially developing segmentation algorithms for the DAPI and PCNA stains separately.

Background

- The Mitchell lab at the University of Idaho captures images that can contain hundreds of proliferating Muller glia derived progenitor cells.
- Quantifying the proliferating cells is an important step in understanding how microglia signals affect the Muller glia regenerative response.
- The lab currently uses a semi-automated Python script with ImageJ's Fiji software to segment and count cells, which utilizes contrast enhancement and thresholding techniques.
- The Fiji process could identify where cells were located in the images, but could not correctly separate cells that were in large clumps, leading to incorrect counts.

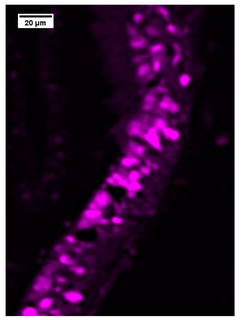

Figure 1: Original image of PCNA marker

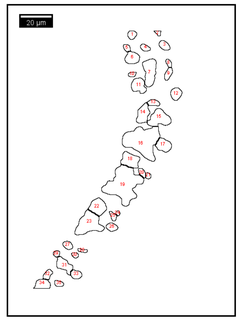

Figure 2: Fiji segmentation of PCNA marker

- Thresholding is an image manipulation technique that separates out the low intensity pixels from the high intensity pixels of an image based on a threshold

Materials

Computer Hardware

Processor: Intel(R) Core(TM) i3-8145U CPU @ 2.10GHz

RAM: 16 GB

Operating System: Windows (Linux also tested)

Python 3.8.10 Libraries used

Nd2reader, Numpy, Matplotlib, matplotlib_scalebar, OpenCV, Yaml, Scikit-image, OS

Methods

Each stain (DAPI and PCNA) went through the same image segmentation process. The final PCNA and final DAPI images were overlapped to see the corresponding regions, then counted to receive the final result.

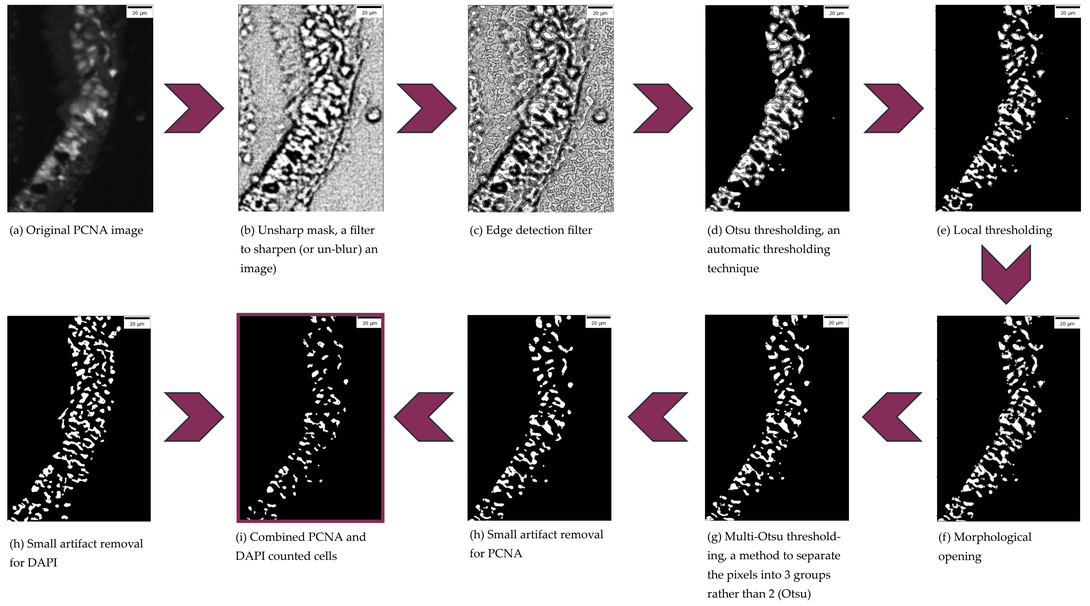

Figure 3: Example of the numerous image manipulation techniques, using the PCNA stain, that were applied to images of nuclei (DAPI stain) and of proliferating cells (PCNA stain) to identify cells

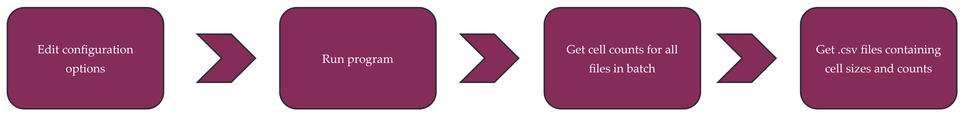

Figure 4: The steps to run the program developed and the outputs received from the program.

Results

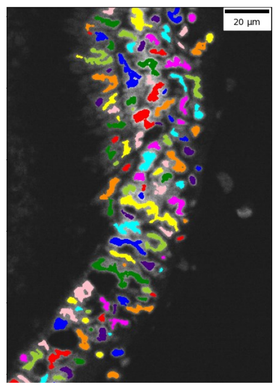

Figure 5: Final result of the DAPI cell counting process

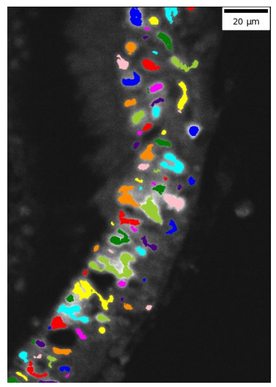

Figure 6: Final result of the PCNA cell counting process

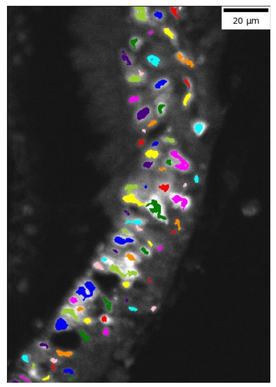

Figure 7: Final result of the program that overlaps the PCNA stain with the DAPI stain

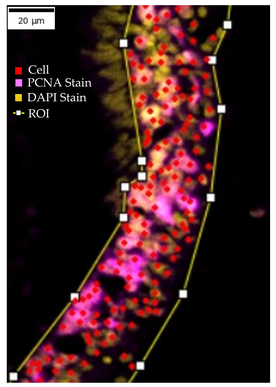

Figure 8: Manual counts performed by Mitchell lab. The region of interest is outlined in yellow.

Discussions and Conclusion

- This algorithm segments cells much better than the Fiji implementation.
- Images are able to be segmented within 6-12 seconds on common PC hardware.
- While the program can identify areas where cells are located, it is still struggles to segment large clusters of densely packed cells.
- The final program can be run on Windows and Linux.

Future Research

- More comparison is needed with manual counts to provide a better estimate of accuracy.
- Different segmentation algorithms should be further developed for each stain to better handle segmentation of densely packed cells.
- A machine learning model could be implemented to potentially identify cells better while being developed for a more generalized use.
- A graphical user interface can be implemented when program is in final state to aid technicians in quickly and efficiently running the program.

Acknowledgements

- Rhena Cooper for connecting the interns and fellows with an appropriate mentor and providing support for scientific and professional decorum.
- Kirsten Blanchette for leading the Friday meetings and making sure we are prepared for each step of the INBRE summer experience.
- NIC faculty for the guest presentations on statistics, graphs, interviews, etc.
- Jerome Pollos for his time spent photographing interns and fellows.
- Dan Blanchette for introducing me to INBRE and being a sounding board for algorithm ideas.
- Jordan Rumford for explaining the biological aspects of the project and testing program iterations.

Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408

References

IdahoINBRE IDeA Network of Biomedical Research Excellence

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