

Image segmentation of microglia cells in zebrafish

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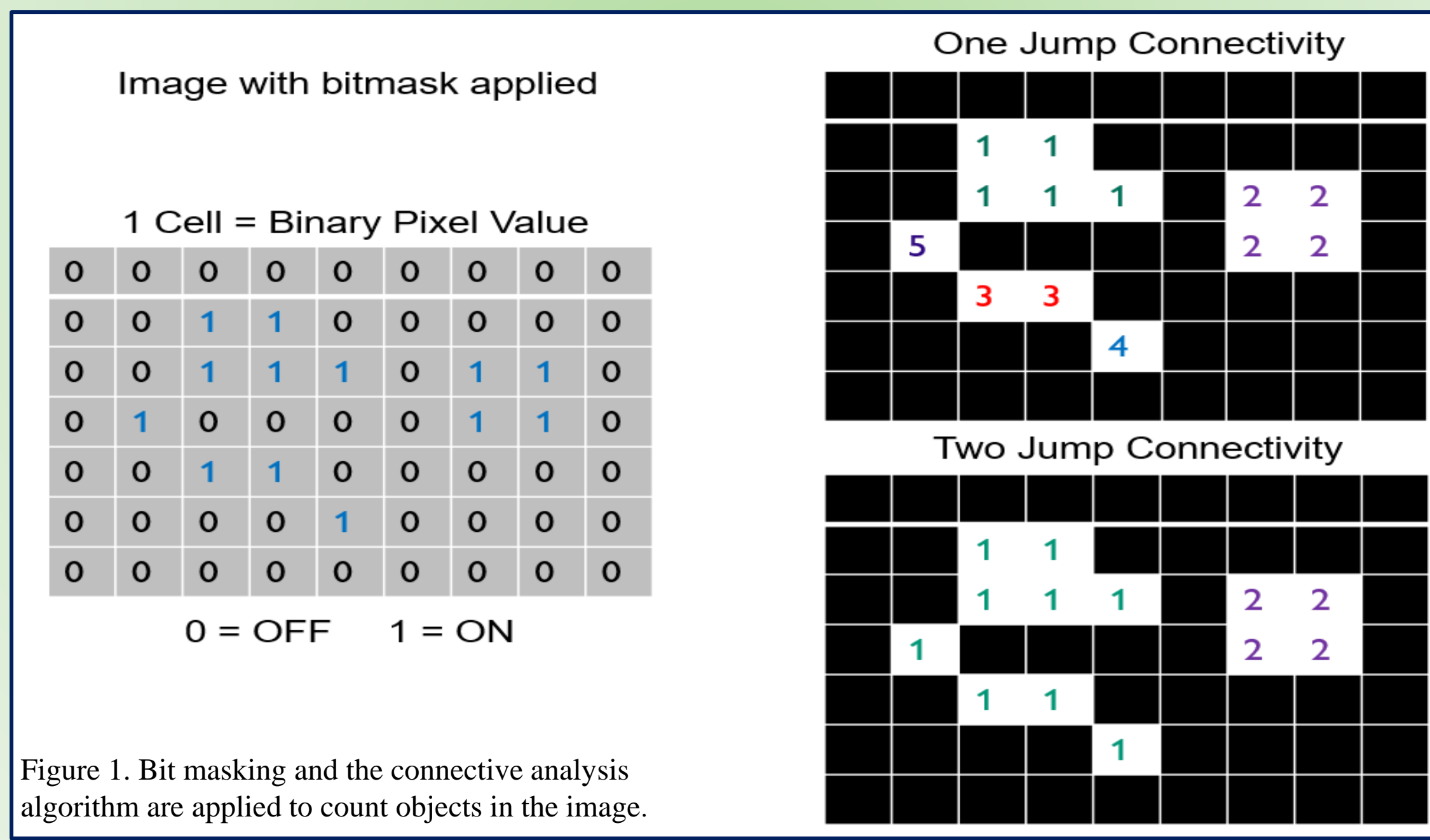
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ABSTRACT

Microglia research of the central nervous system (CNS) is a rapidly growing field in biology. Dr. Diana Mitchell's² research investigates wild-type retinal microglia in zebrafish and a mutation that triggers the regeneration of retinal neurons. Further research on this mutation may lead to medical treatments that repair the damaged retinas of patients. Cell data is recorded as a digital video file at 20x magnification using a spinning disk confocal microscope. A 488nm laser excites green fluorescent proteins (GFPs) within the microglia while an optimized filter set detects GFP emissions. This type of imaging allows researchers to observe cell behavior. Using Python, the Long³ computer science lab aids researchers by automating image analysis. The software stores the data as image files by extracting half-second frames from the zebrafish video file while reporting the frames-per-second (FPS). Through user input, a second routine identifies the microglia cell's red, green, and blue (RGB) pixel values and then isolates them. After its runtime, the software records cell count and applies a color fill filter to each cell. This expedites the research process and provides reliable data. Future features can include computational metrics such as microglia cell tracking, cell speed, and total distance traveled.

BACKGROUND

- In an image, pixels are comprised of red, green, and blue channels. Each channel has an assigned numeric range between 0 and 255 bits (0 = black, 255 = brightest pigment for the color channel).
- Color compositions are represented in a computer programming as a tuple in the following notation: (R, G, B).
- Bit Masking is the method of isolating specific color value combinations (objects of interest) from the rest of a digital image.
- The connective analysis algorithm allows the software to separate identified objects or combine them based on adjacency or diagonality. This is commonly referred to as one jump and two jump connectivity.



MATERIALS

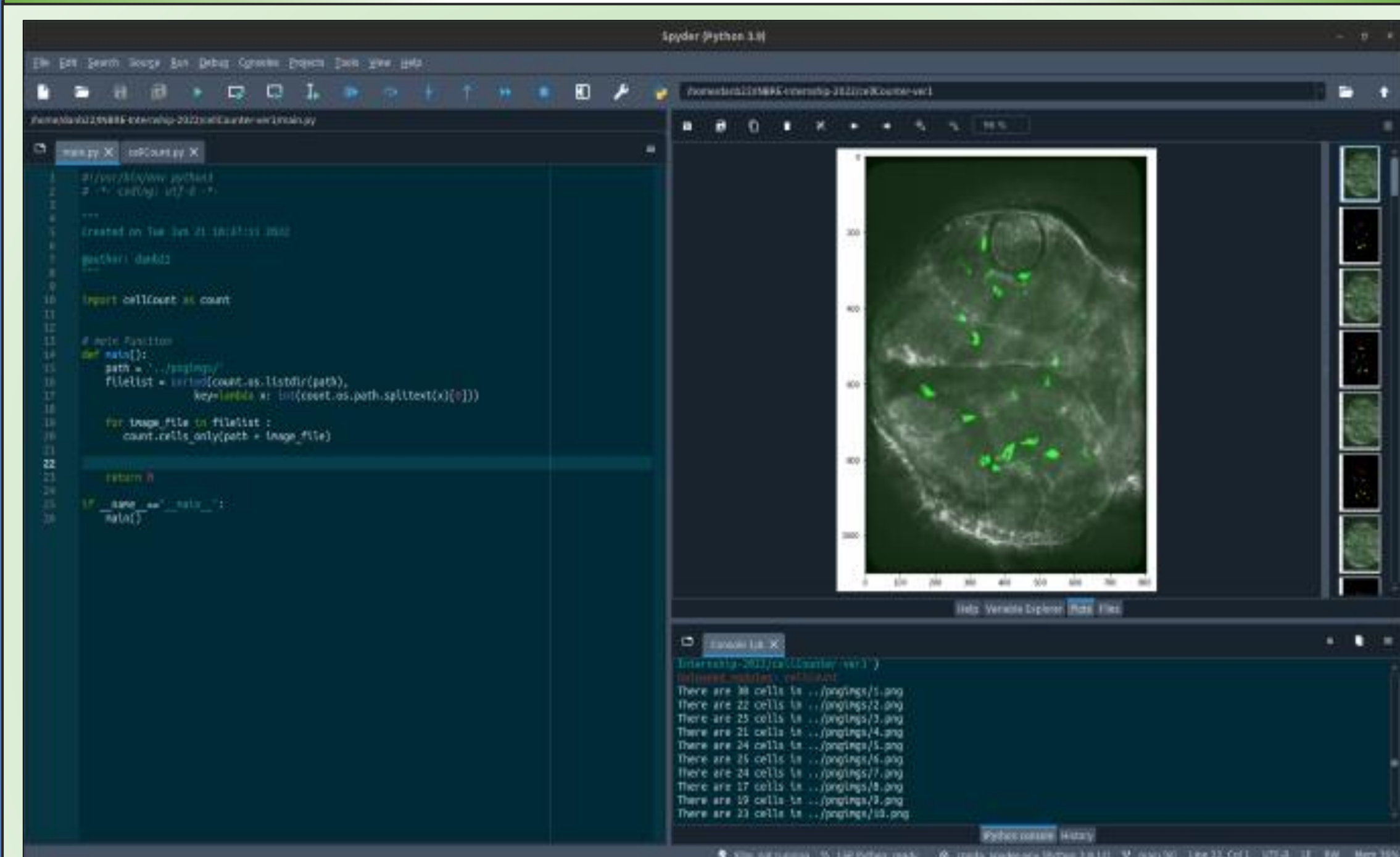


Figure 2. Spyder integrated development environment for image analysis software testing and data visualization. <https://www.spyder-ide.org/>

Cell counter program technical specifications:
Hard disk space (programs and Spyder IDE): 659.1 MB Operating System: Linux/Ubuntu 22.04
Memory: 8GB, DDR4 SDRAM (recommended) Compatible GPU: NVIDIA GeForce RTX 2060 6GB RAM



METHODS

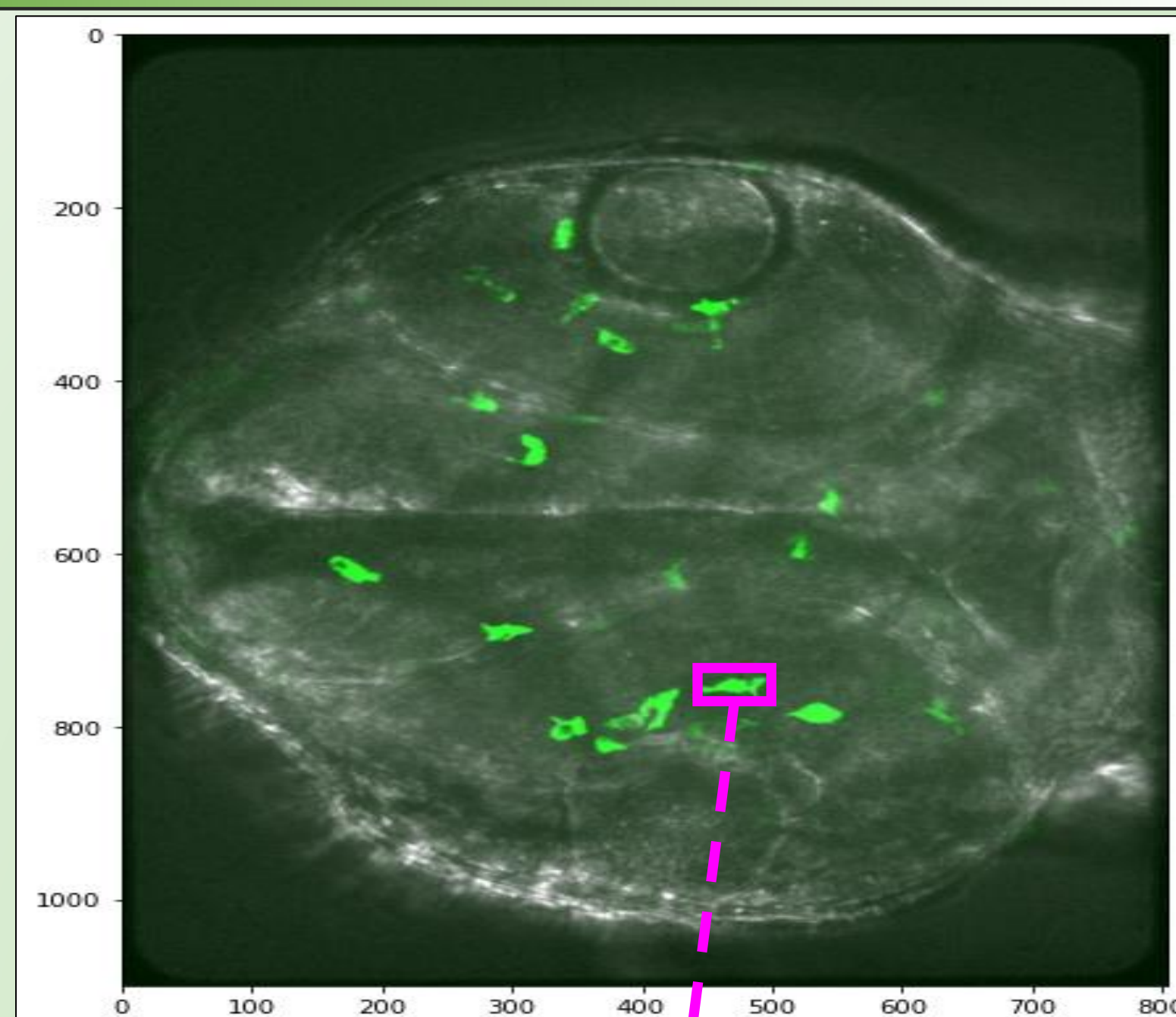


Figure 3. Zebrafish image (1.png) with pixel dimensions (ry and cx axis).

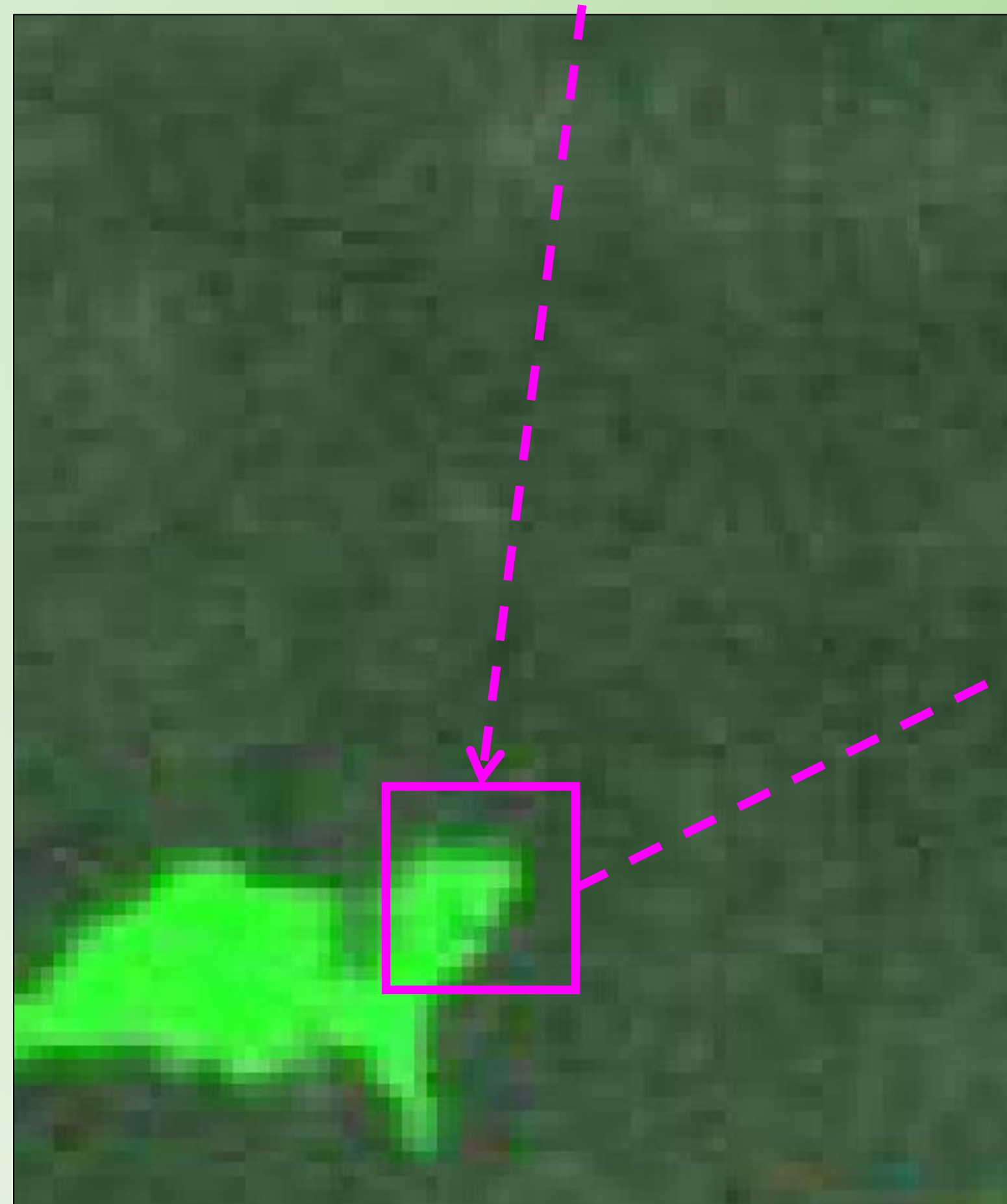


Figure 5. Sampling R, G, and B pixel values for the threshold algorithm to isolate.

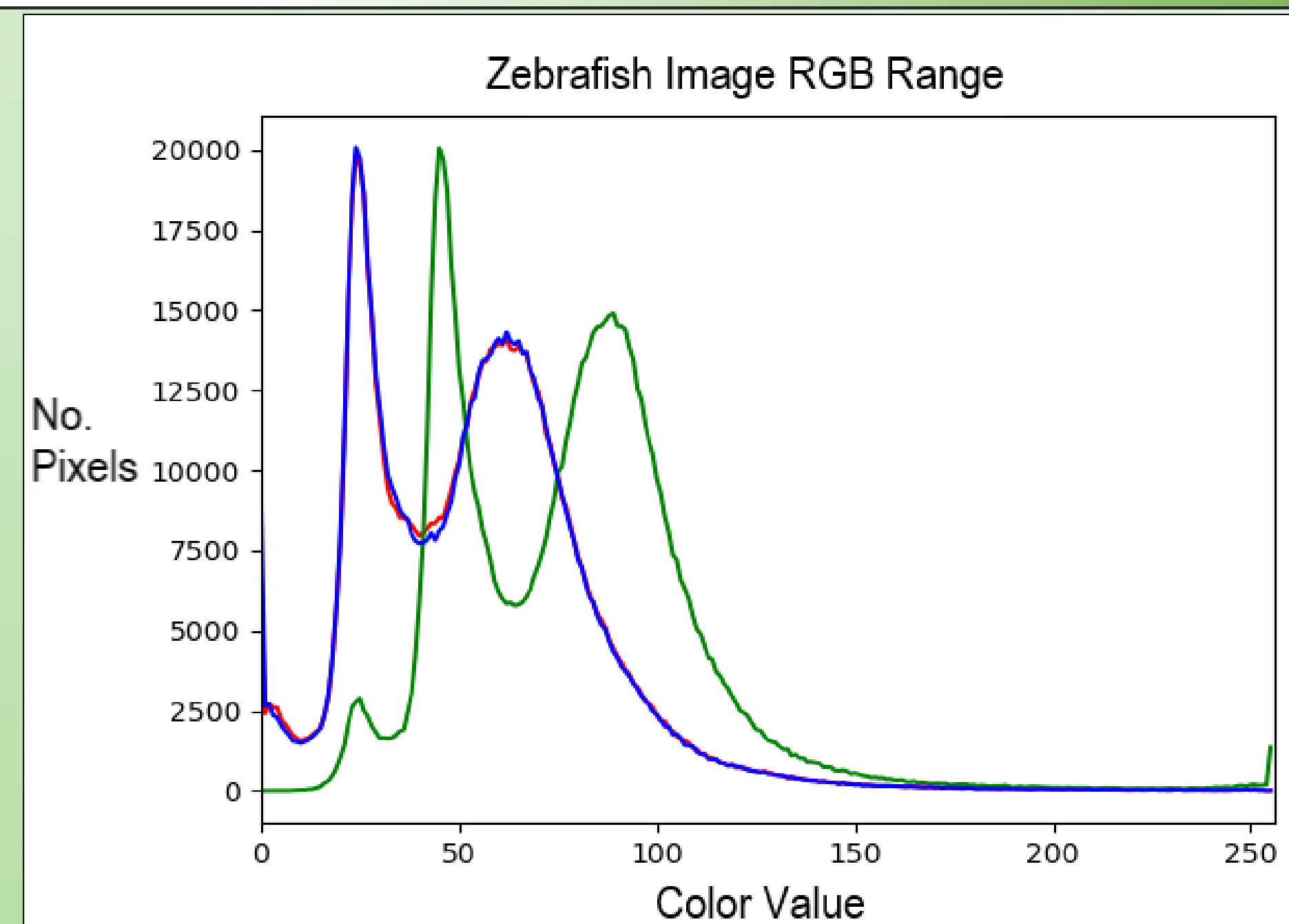


Figure 4. Histogram analysis of the RGB channels used to determine a bit mask threshold.

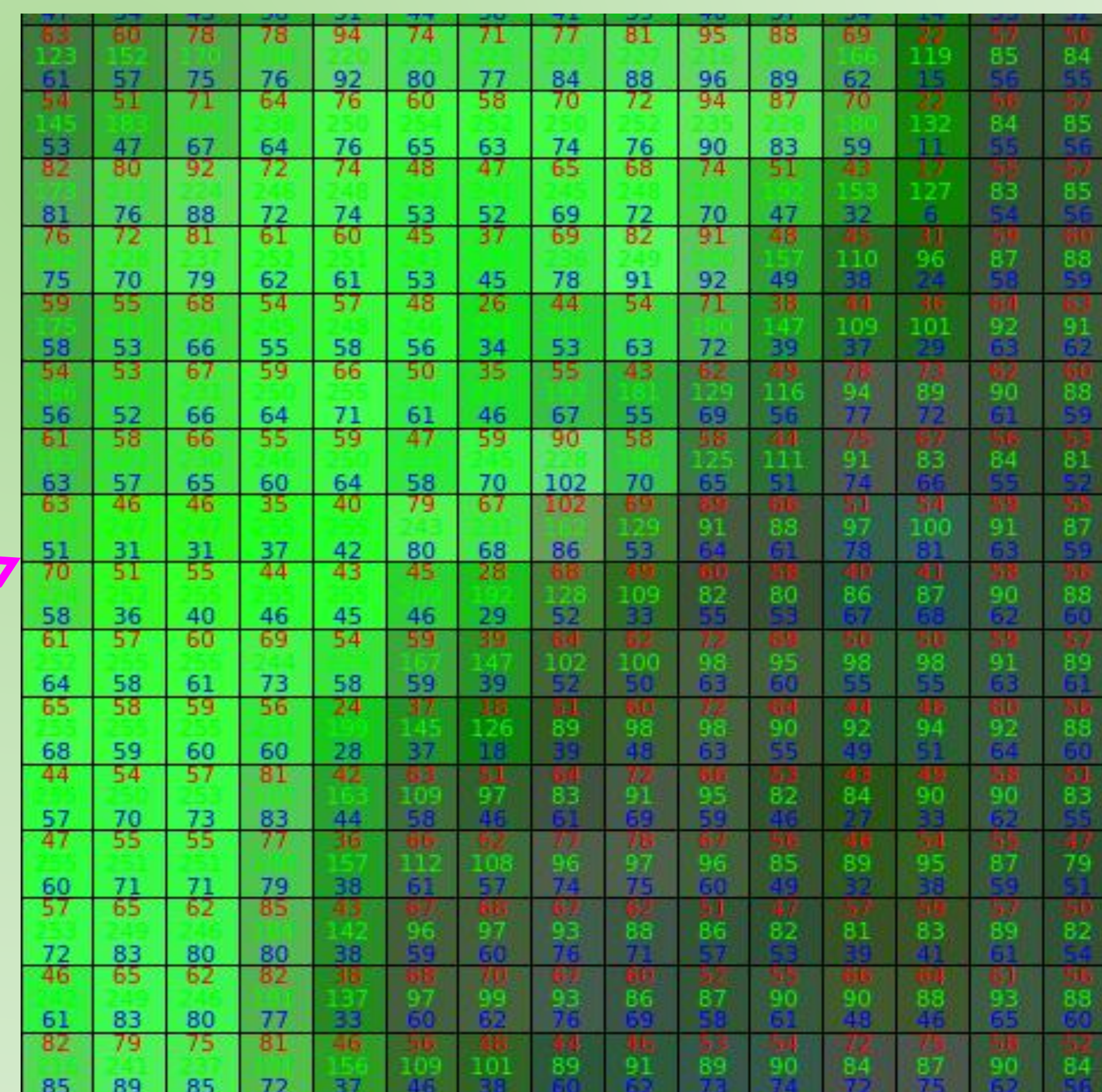


Figure 6. Enhanced view of the pixel ranges from the chosen cell to sample.

RESULTS

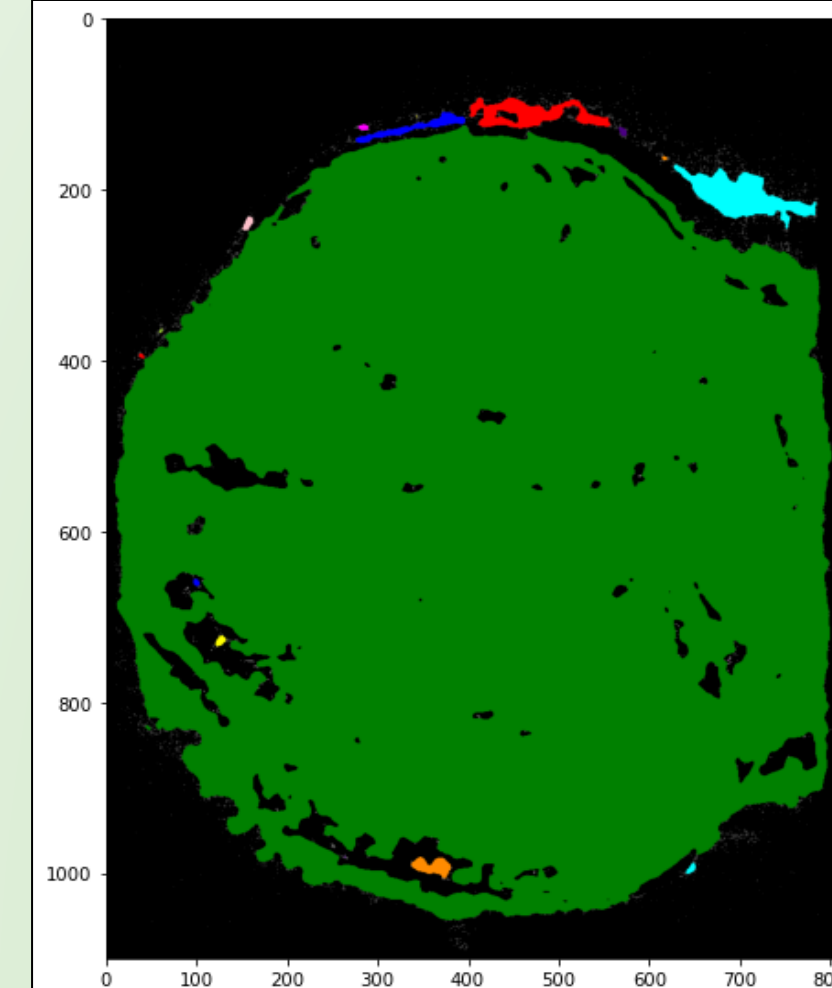


Figure 7. Low green range threshold
Threshold value = (97, 60, 97)

There are 18 cells in ../pngimgs/1.png
There are 10 cells in ../pngimgs/2.png
There are 12 cells in ../pngimgs/3.png
There are 14 cells in ../pngimgs/4.png
There are 11 cells in ../pngimgs/5.png
There are 9 cells in ../pngimgs/6.png
There are 15 cells in ../pngimgs/7.png
There are 11 cells in ../pngimgs/8.png
There are 14 cells in ../pngimgs/9.png
There are 13 cells in ../pngimgs/10.png
There are 8 cells in ../pngimgs/11.png
There are 2 cells in ../pngimgs/12.png
There are 5 cells in ../pngimgs/13.png
There are 10 cells in ../pngimgs/14.png

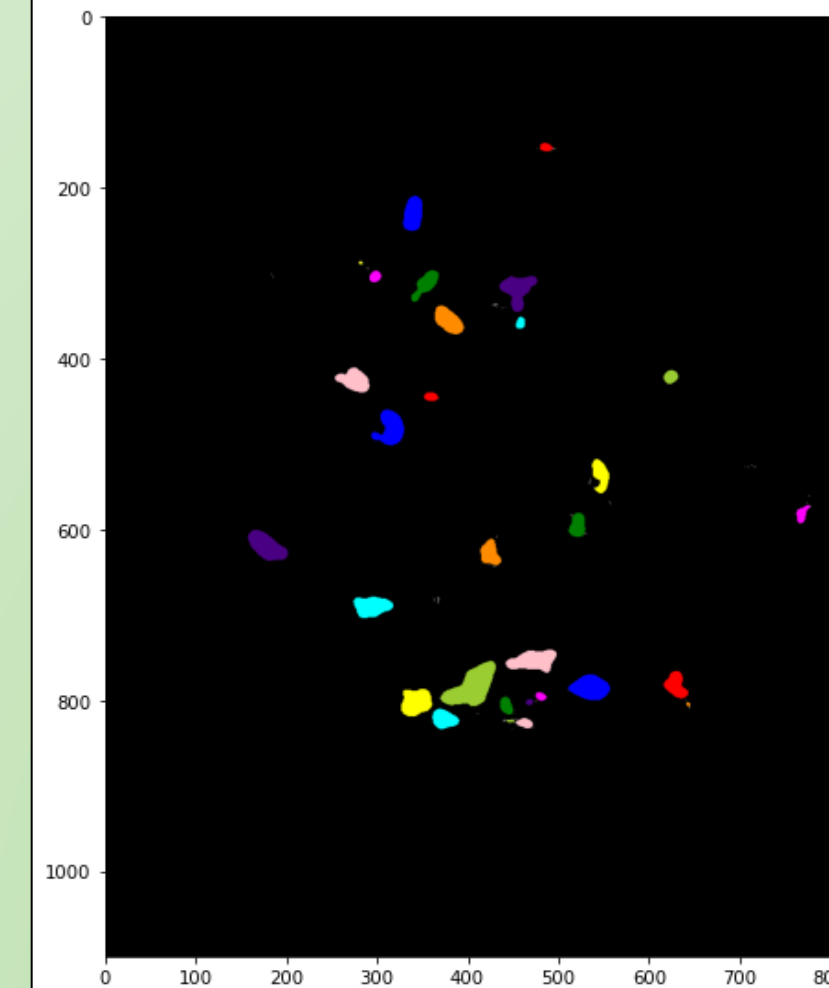


Figure 8. Calibrated green range threshold
Threshold value = (97, 132, 97)

There are 29 cells in ../pngimgs/1.png
There are 24 cells in ../pngimgs/2.png
There are 29 cells in ../pngimgs/3.png
There are 24 cells in ../pngimgs/4.png
There are 27 cells in ../pngimgs/5.png
There are 27 cells in ../pngimgs/6.png
There are 23 cells in ../pngimgs/7.png
There are 19 cells in ../pngimgs/8.png
There are 20 cells in ../pngimgs/9.png
There are 25 cells in ../pngimgs/10.png
There are 22 cells in ../pngimgs/11.png
There are 21 cells in ../pngimgs/12.png
There are 18 cells in ../pngimgs/13.png
There are 23 cells in ../pngimgs/14.png

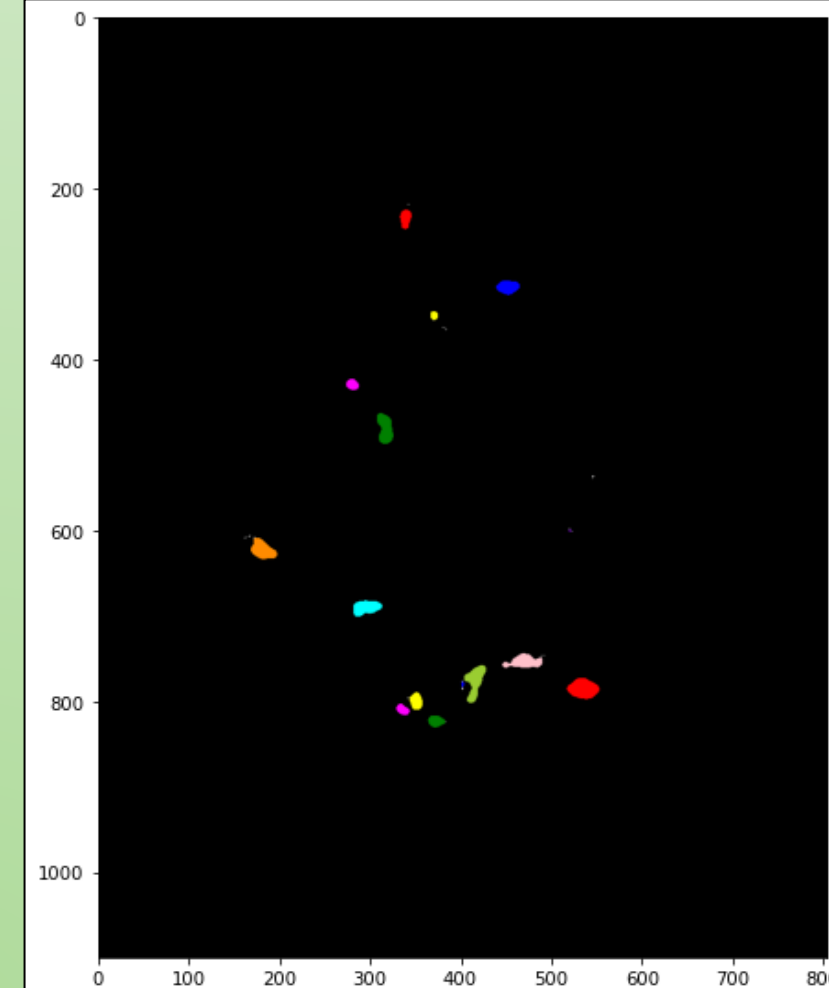


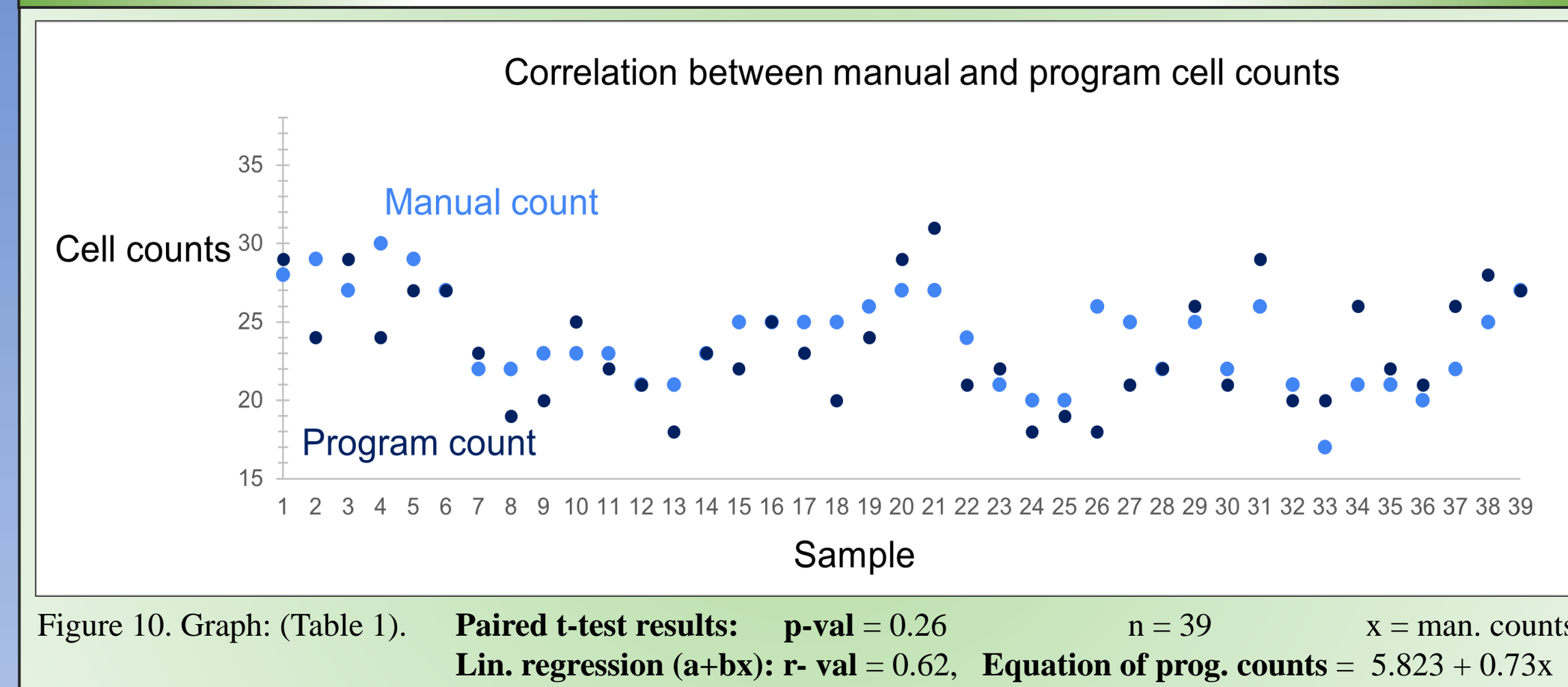
Figure 9. High green range threshold
Threshold value = (97, 250, 97)

There are 15 cells in ../pngimgs/1.png
There are 22 cells in ../pngimgs/2.png
There are 18 cells in ../pngimgs/3.png
There are 19 cells in ../pngimgs/4.png
There are 17 cells in ../pngimgs/5.png
There are 15 cells in ../pngimgs/6.png
There are 18 cells in ../pngimgs/7.png
There are 11 cells in ../pngimgs/8.png
There are 12 cells in ../pngimgs/9.png
There are 9 cells in ../pngimgs/10.png
There are 14 cells in ../pngimgs/11.png
There are 13 cells in ../pngimgs/12.png
There are 12 cells in ../pngimgs/13.png
There are 10 cells in ../pngimgs/14.png

Microglia Cell Count			
Image name	Man. Count	Prog. Count	Difference
1.png	28	29	-1
2.png	29	24	5
3.png	27	29	-2
4.png	30	24	6
5.png	29	27	2
6.png	27	27	0
7.png	22	23	-1
8.png	22	19	3
9.png	23	20	3
10.png	23	25	-2
11.png	23	22	1
12.png	21	21	0
13.png	21	18	3
14.png	23	23	0
15.png	25	22	3
16.png	25	25	0
17.png	25	23	2
18.png	25	20	5
19.png	26	24	2
20.png	27	29	-2
21.png	27	31	-4
22.png	24	21	3
23.png	21	22	-1
24.png	20	18	2
25.png	20	19	1
26.png	26	18	8
27.png	25	21	4
28.png	22	22	0
29.png	25	26	-1
30.png	22	21	1
31.png	26	29	-3
32.png	21	20	1
33.png	17	20	-3
34.png	21	26	-5
35.png	21	22	-1
36.png	20	21	-1
37.png	22	26	-4
38.png	25	28	-3
39.png	27	27	0

Table 1: cell count comparison

RESULTS (CONT.)



DISCUSSION AND CONCLUSIONS

- Thresholding is a valuable technique that influences the accuracy of the bit mask routine to isolate objects of interest from the rest of the image.
- RGB threshold values should not be set too low or too high to ensure the best results.
- Bit mask results are not limited to the segmentation (counting) of microglia cells, it is also used to calculate pixels concentration per cell. Cell surface area can be estimated as a result.
- For images that are less complex, grayscale thresholding is used to quickly isolate objects with a bit mask.
- A linear regression test and paired t-test confirm that the manual cell counts and the program cell counts are not significantly different from one another. This analysis shows promise for the program to be accepted as manual cell counting alternative.

FUTURE WORK

- Finishing the manual calibration routine for the program which will identify objects based on the dominant pixel channel's average value.
- Reporting cell pixel counts to estimate cell surface area.
- Tracing the microglia cell's path of travel across multiple images.
- Calculating each identified microglia cell's average velocity.
- Calculating each identified microglia cell's total distance travelled.
- Creating an open-source distribution of the software with documentation.

For a video demonstration (via YouTube) of the software, feel free to scan the provided QR code. See the video comments section for the Github repo link to the program's source code.



ACKNOWLEDGEMENTS

Special Thanks to:
Professor Rhena Cooper, for coordinating us with our fellowship/industry mentors and providing support both in professionalism and scientific decorum over the summer.
Professor Kirsten Blanchette, for facilitating our professional development meetings each Friday and her critiques for this project.
Professor Brandi Fries, for taking the time to provide the professional development seminar on interview etiquette and preparation.
Professors David Foster and Susanne Bromley, for their taking the time to bestow their wisdom on scientific etiquette and professional data representation for our posters.
Jerome Pollos, for his time to photograph each fellow and intern to help chronicle our summer experiences.
Mellisa Clemons, for her insight on information concerning microglia for my abstract. Also, ensuring I kept the question of "why is this project important to others?" at the forefront my mind.
My Family, for supporting me and listening to all my programmer jargon over the summer. Also, for teaching me that failure is never the end of something, but the beginning of something else.
And the rest of the NIC INBRE cohort for their feed back and peer reviews of this project.

This project was made possible by the INBRE Program, NIH Grant No. P20GM103408 (National Institute of General Medical Sciences).

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