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CSC 664/864
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FINAL PROJECT REPORT

INTRODUCTION

For our assignment we chose Project-5 "Image Analysis for Biometric Feature Identification", which focuses on feature identification, specifically targeting zebrafish larvae. The goal of the project is to develop a solution that can automatically identify the center point of a fish's head in still frames of the larvae, utilizing intensity-based analysis and considering the geometry of the larvae.

The drug discovery process is a long testing process that drugs go through before they are available to the market. Potential drugs are tested on animals first before conducting them in humans. Zebrafish are one of such experimental subjects. They have close properties to mammals, grow relatively fast, and cheap which makes them important subjects (Nikam VS). Heart, liver, brain, pancreas, and kidneys are targeted to experiment to find for safety and efficacy of drugs. As for our project, finding the head of the zebrafish can be useful for future studies. Once we have the location of the fish's head, in future study it can be used to study on how drugs affect the fish's activity brain by looking at the area of the head. This is possible because the zebrafish's body are translucent in the early infancy which allows us to see the organs.

STRUCTURE OF THE REPORT

Our original plan was to work together by first working on two separate algorithms to tackle the problem. Where David's primary focus was to come up with a good contour algorithm to find the body of the fish. Then, David would also study on the intensity values of the fish and try to separate the fish's organs and eyes. Jessy would incorporate blob analysis to find the head of the fish. Then, we would combine our findings to come up with one algorithm to see how well both algorithms would perform.

However, we had conflict in schedule which led us to work on the project separately. This meant that we didn't combine our algorithms.

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Project Goals

- Understanding image analysis techniques for biometric identification.
- Exploring intensity-based analysis methods for identifying key points in images.
- Incorporating geometric information to improve the accuracy of feature identification.
- Developing an automated solution that reduces dependence on manual threshold values.
- Evaluating the accuracy of the solution by comparing results with manually defined centers.

Proposed problem formulation

The objective of the code I will use is to automatically identify the center point of a zebrafish larva's head in a video. The problem consists of several components: frame cropping to focus on the larva's head region, intensity-based thresholding in the HSV color space, morphological opening to refine the head region, blob analysis to identify connected regions and centroids, centroid calculation, and visualization of the results using markers. My formulation addresses the general problem of automatic head center identification without manual thresholding. The code's effectiveness can be evaluated by comparing the determined head positions with manually defined centers. It is important to examine the implementation details, such as cropping, thresholding, morphological operations, blob analysis settings, and centroid calculation, while also considering alternative methods and optimizing for larger datasets.

Jessy's High-Level Description

To conduct our research, we utilized several key resources, including the lectures provided in class, MATLAB as our primary image processing tool, and relevant papers sourced from online. Two notable papers that informed our work were "Detection of Blob Objects in Microscopic Zebrafish Images based on Gradient Vector Diffusion" and "Computational Techniques in Zebrafish Image Processing and Analysis". Drawing from these resources, we followed a series of steps to arrive at our findings.

We are enthusiastic about undertaking on this project because neither of us have much experience in image analysis and it'll be an opportunity get more familiar with it. For Jessy this course was an introduction. Only having a few weeks to finish this project, we are eager to learn as much as possible and challenge our creativity. Our approach make sense because incorporating intensity-based analysis methods enables us to identify key points accurately, while incorporating geometric information improves the accuracy of feature identification. The novelty of our approach lies in combining these techniques and conducting a rigorous evaluation by comparing our results with manually defined centers. We are excited about the knowledge we will gain in the process.

Jessy's Prior Work

As aforementioned, Jessy had limited knowledge about the content the course would entail but was not discouraged. Fortunately, we were given plenty of material to guide us throughout the course. From the class slides, we acquired an understanding of thresholding and pixel neighborhoods, including how thresholding allows for separation of objects of interest and pixel neighborhoods provide contextual information about the surrounding pixels which enable analysis.

In the textbook "Fundamentals of Multimedia," the distinction between lossy and lossless compression techniques were explained. Lossy compression methods sacrifice some image details to achieve higher compression ratios, while lossless compression methods retain all the original image information (Li et al., 2016).

In addition, we referred to several articles to give us direction in our assignment. First, in “Computational techniques in zebrafish image processing and analysis” the authors highlighted ROI’s methods, including image alignment and quantification, to help them detect zebrafish embryos (Xia et al., 2013). In another, methods were used on diffused gradient vector field and elastic deformable models as the researchers sought to accurately detect and locate blob objects in the zebrafish images (Li et al., 2007).

Jessy’s Method

I took an approach of utilizing the concepts of thresholding, morphological operations, and blob analysis. In processing the zebrafish video, each frame of the video is read and cropped, and then converted to the HSV color space. Thresholds are defined for each channel of the HSV image, and a binary mask is created based on these thresholds. Morphological opening is applied to remove noise or any small regions. Blob analysis is performed on the opened binary image, extracting blob areas and centroid coordinates. Markers are inserted at the centroid positions, and the centroid coordinates are calculated. Finally, the processed frames with inserted markers are displayed. This analysis helps identify and track the centroids of zebrafish in the video.

I encountered challenges in developing an algorithm to detect the center of the zebrafish’s head without relying solely on MATLAB libraries. My research involved exploring different approaches to determine the optimal method for this task. Additionally, I needed to devise a means of evaluating the accuracy of my automated results in comparison to manually defined centers. Overcoming these challenges required a combination of problem-solving, experimentation, and careful analysis.

FIGURE 1: CROPED IMAGED OF ZEBRAFISH

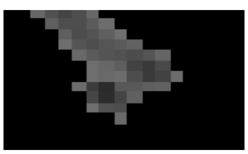


FIGURE 2: STRUCTURAL ELEMENT SHAPED

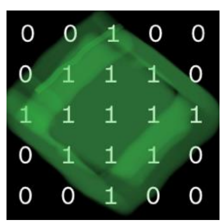
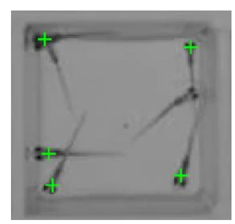


FIGURE 3: FRAME OF RESULTS



Jessy’s Experimental Evaluations

Through experimental and learning, I developed a custom structure element in the shape of a diamond (fig.2) specifically tailored to the diamond-like appearance of the zebrafish’s head when isolated from the surrounding image This approach yielded more accurate results compared to my previous use of a disk-shaped element (fig.3). I was able to output a centroid location, however I couldn’t quantify the accuracy of my detection in numerical terms at this stage. I have relied on a visual inspection of the processed images indicates that the detected centroids align closely with the actual positions. The markers overlaid on the detected objects exhibit a strong correspondence with the ground truth centroids of the fish’s head.

Jessy’s Conclusion

Exploring the detection of zebrafish head centroids proved to be a valuable learning journey, providing us with valuable insights and knowledge. Throughout the project, we gained proficiency in various image processing techniques tailored specifically to zebrafish analysis. This included manipulating color spaces, setting threshold values, and creating mask to isolate regions of interest. Moreover, we delved into the realm of object detection and tracking, employed blob analysis techniques to identify and locate zebrafish centroids within video frames. Having met most of the goals and running out of time, I am content with submitted the project as it is. The skills and understanding acquired through this process have equipped us with the ability to apply similar techniques to detect and track objects in diverse applications in the future.

I have gained so much knowledge in image processing, even in machine learning, that I aim to continue to use these new skills in future endeavors as well as contributing to its advancement.

David’s Proposed Problem Formulation

FIGURE 4: GRAYSCALED IMAGE

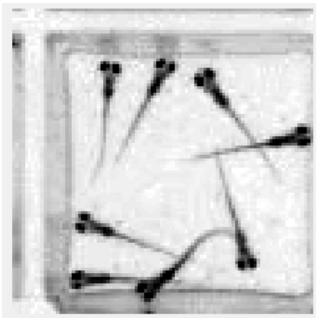
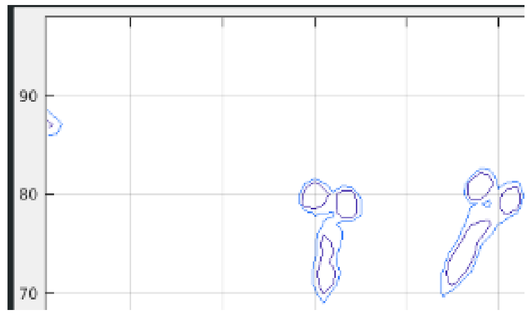


FIGURE 5: MATLAB 3D CONTOUR



My goal for this project was to find an algorithm that would find the head the fish by finding the center where the eyes are. We’re working with grayscaled images so the eyes stands out because they have are very dark. However, the zebrafish’s stomach is also very dark. So differentiating eyes from stomach is one challenge we face.

The second challenge is to figure out which eyes and stomach belong to which zebrafish. The fish have a translucent body and have a slightly higher intensity compared to the eyes and stomach.

David’s Prior Work

Prior work that I’ve tried was finding the body of the fish using edge detection algorithms. The first edge detection algorithm that I applied was the Sobel Operator. When trying to implement the Sobel operator, I’m taking the derivative along the x direction and then the y direction. Then I would calculate the magnitude in figure 6. However, this gave us a wide range of values that went over 500. So, I normalized the magnitude as shown in figure 7 and noticed looked at the histogram of that there was about two humps. So I applied the Otsu’s algorithm on the normalized Sobel Operator to give a clear edge of the fish in figure 8.

FIGURE 6: MAGNITUDE

$$M = \sqrt{s_x^2 + s_y^2}$$

FIGURE 7:(CUSTOM) NORMALIZED SOBEL OPERATOR TO 8-BIT

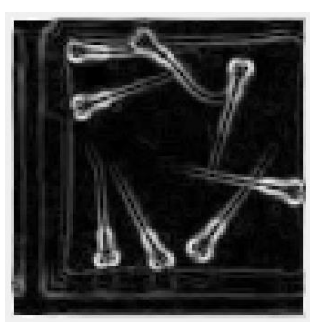


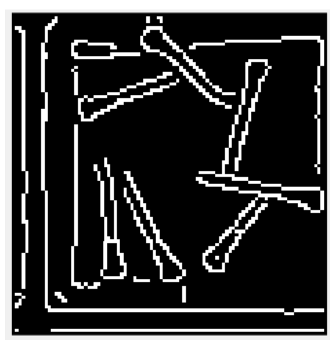
FIGURE 8: APPLYING OTSU’S ALGORITHM ON THE SOBEL OPERATOR

I’ve also tried the Laplacian of Gaussian algorithm (figure 9). The algorithm looked very good when performing it on grain of rice where there were clear separation of background and foreground. However, when trying it on zebrafish, the edges were segmented. Because of that our image look segmented and needed edge linking to be able to make use of it.

FIGURE 9: CUSTOM LAPLACIAN OF THE GAUSSIAN EDGE DETECTION

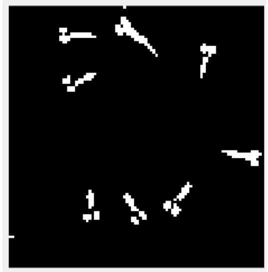
The canny edge detection have provide great edge but unlike LoG, it is sensitive to background edges as well.

FIGURE 10: MATLAB'S CANNY EDGE DETECTION



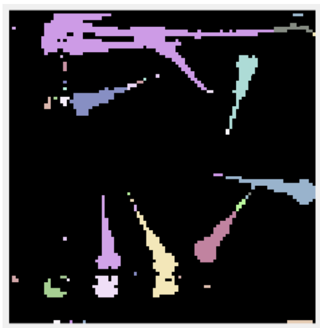
Adaptive threshold is overall the more useful thresholding algorithm. It was originally thought to be used for segmenting the eyes and stomach. However, the algorithm wasn't separating all the eyes and stomach. This meant that

FIGURE 11: MATLAB'S ADAPTIVE THRESHOLDING



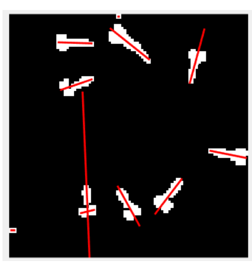
One of the difficulties with not being able to completely separate the eyes from the stomach, I looked at analyzing the fish's body by normalizing its position. The idea is to transform the region of the zebrafish to lie on the x-axis. If done correctly, we can process the cross section while analyzing the intensity along the y-axis and find the global maximum which indicates the center of the head.

FIGURE 12: (BUG) CUSTOM REGION SEGMENTATION USING 4-CONNECTIVITY



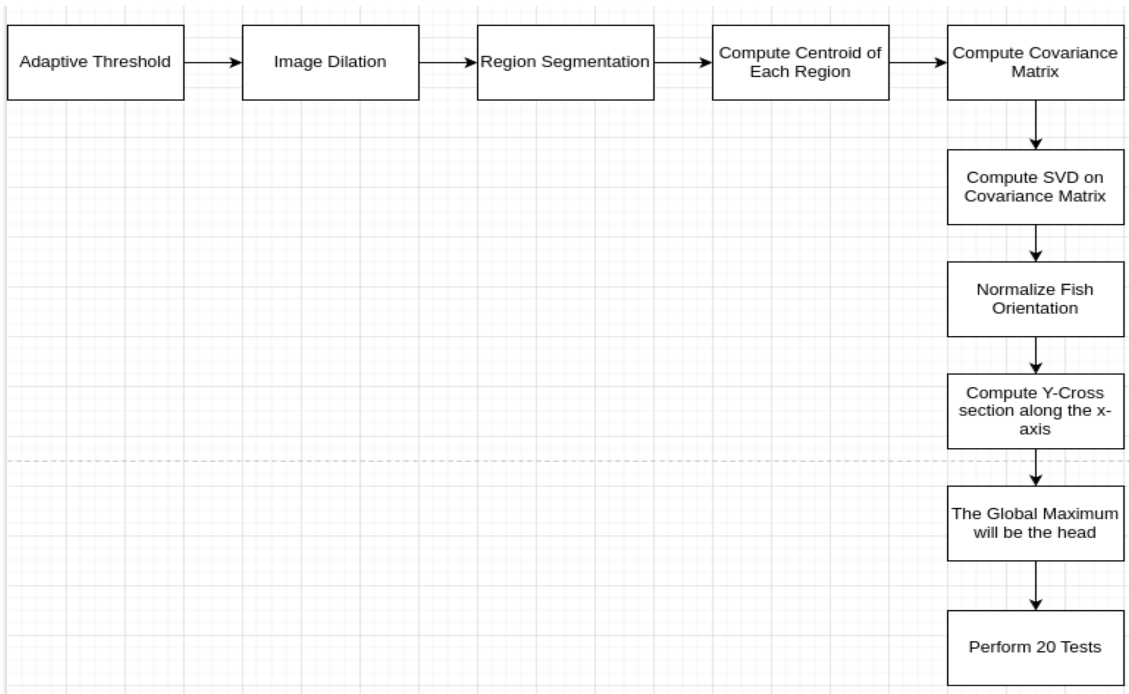
For us to tell body parts apart, we want to know which region they belong to. My implementation didn't fully produce the result that I wanted but it was very close (Figure 13). So I've been using Matlab's region segmentation for the meantime.

FIGURE 13: CUSTOM ORIENTATION VECTOR (DISPLAY)



To come up with such custom orientation was a challenge because using SVD on an image wasn't the right choice. I had to do several preprocessing before applying SVD. Basically, we had to apply a region segmentation and each region will store the coordinate of the fish's pixels (x,y). Then we would take those coordinate and compute the covariance matrix which describes the spread of the region. Then we apply the SVD on the covariance matrix to decompose into three matrices. The one that we're interested is the left singular matrix since they contain the vector of least spread. The first vector represents the orientation of the region and it is shown in figure 12.

David's Description of the Work



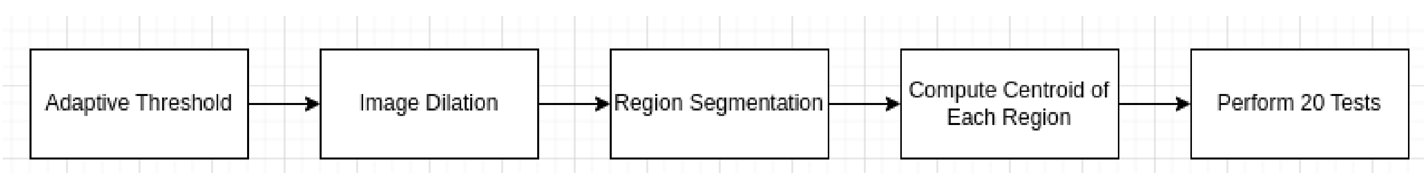
The figure above was the main algorithm that I wanted to compute. The idea is to separate the eyes from the stomach through the trick of normalizing the orientation along the x-axis.

Because I wasn't able to complete the main algorithm involving with normalizing the orientation, I've decided to use what I have.

I came up with 3 alternative algorithms that were meant to test against the main algorithm:

1. Finding the head using the centroid of the zebrafish's body

FIGURE 14: CENTROID OF THE BODY ALGORITHM PIPELINE

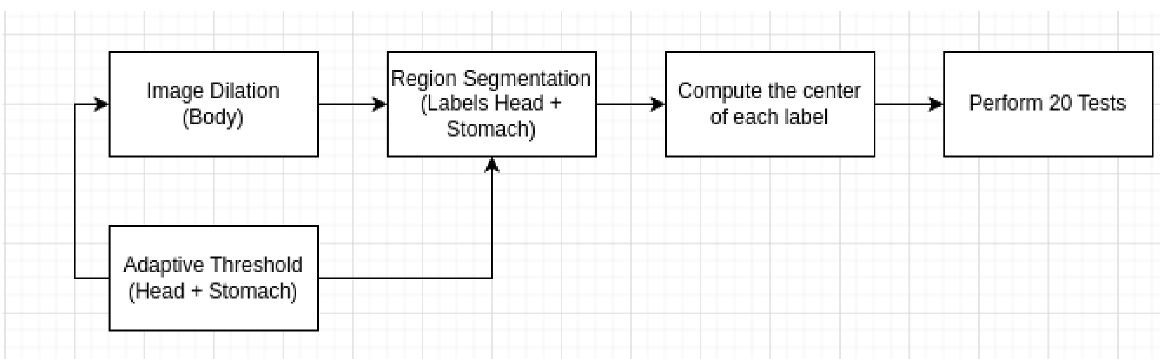


The idea is to compute the body of the fish and compute the center of that region. Performing the adaptive threshold will give us the eyes and stomach and then applying the image dilation will often give us the body of the fish. In the figure 15, it is applying image dilation to expand the the body and stomach which will often connect them to represent the body of the fish. Then we label each region to later perform the centroid of the fish. Then lastly, perform 20 tests to get the average precision and recall.

FIGURE 15: APPLYING DILATION USING 2X2 (ALL 1'S) STRUCTURED ELEMENT ON MATLAB'S ADAPTIVE THRESHOLD

Figure 15 Applying dilation using 2x2 (all 1s) structured element on Matlab's adaptive threshold

FIGURE 16: CENTROID OF THE (EYE + STOMACH) ALGORITHM PIPELINE

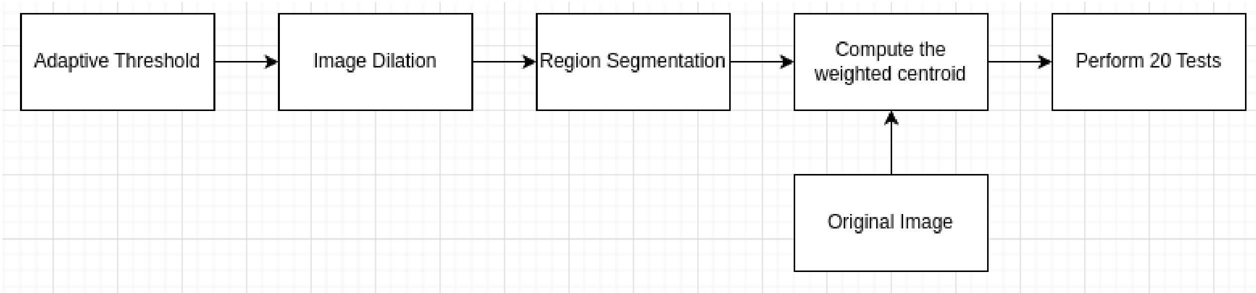


2. Finding the head using the centroid of the fish's eye and stomach

The second algorithm isn't too different from the first. Instead of computing the center of the body's region. This algorithm computes the center of the eye and stomach of the fish. It does by first labeling the body of each fish and then each eye and stomach get assigned to the label of the body. Lastly, it will compute the center of the eye and stomach. lastly, perform 20 tests to get the average precision and recall.

3. Finding the head using the body's weighted (intensity) centroid

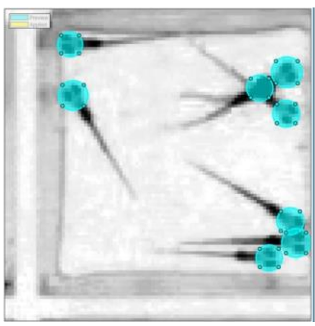
FIGURE 17: WEIGHTED CENTROID OF THE FISH'S BODY ALGORITHM PIPELINE



This is similar to the first algorithm where we're processing the body of the fish. The difference is that we're taking into account of the intensity of the fish rather than relying solely on the shape of the region.

David's Experimental Evaluation

FIGURE 18: MANUAL



For my test, I've manually drawn filled circles that represent the relevant data. All of our algorithm results a binary image where 1 is the head represents where our algorithm thinks the head is. It is designed such that each circle can only be counted once which will later come with challenges.

FIGURE 19: TEST 3 RESULTS ON 20 SAMPLES

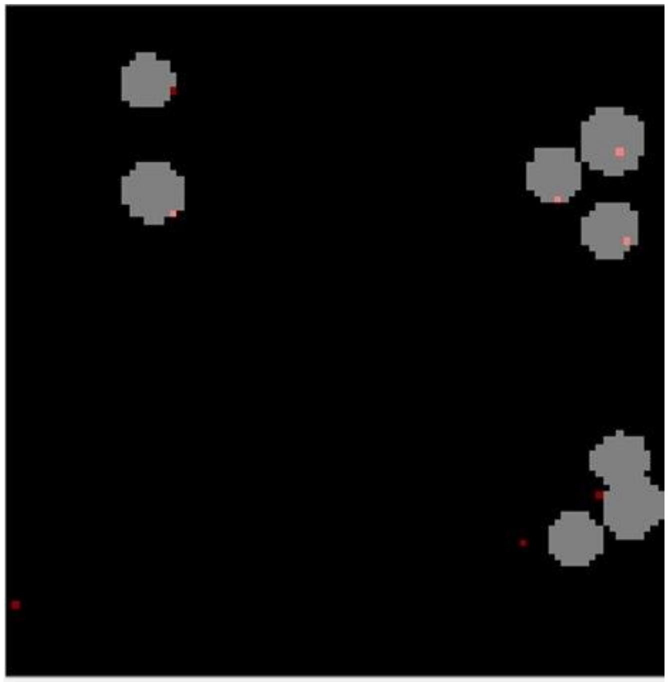
```
>> main
----- Weighted Centroid -----
Size: 20
---- Sample Summary ----
Sample size = 20
(Mean) Precision: 42.23%
(Standard Deviation) Precision: 15.85%
(Mean) Recall: 55.71%
(Standard Deviation) Recall: 23.51%
----- Centroid -----
Size: 20
---- Sample Summary ----
Sample size = 20
(Mean) Precision: 53.37%
(Standard Deviation) Precision: 11.27%
(Mean) Recall: 69.82%
(Standard Deviation) Recall: 18.96%
----- Centroid of the Body Parts -----
Size: 20
---- Sample Summary ----
Sample size = 20
(Mean) Precision: 50.71%
(Standard Deviation) Precision: 13.27%
(Mean) Recall: 67.95%
(Standard Deviation) Recall: 21.03%
>>
```

Considering that the three algorithms were meant to be compared with the main algorithm, they did pretty good. One of the reasons that the algorithm have low precision is because there are false positive results due to dark shadows. On figure 20, the bottom left dot was a dark shadow coming from another cell so that is a false positive result. Another problem is that all of the algorithm includes the stomach of the fish, it shifts the centroid away from the head. Another problem that I saw was that some of the fish’s eyes and stomach didn’t merge which our algorithm treat them as two separate entities.

One of the reasons that the numbers are so high could be that I drew the circles too big for the fish. As we can see in figure 20, some red pixels are right at the border of the head. If I drew each circles smaller, our numbers would’ve been much lower.

Based on the numbers, finding the centroid of the fish is a decent algorithm to test.

FIGURE 20: SINGLE TEST WEIGHTED CENTROID



David's Conclusion

In this project, I've learned several algorithms and also helped me apply algorithms that we learn in class. Even though I couldn't finish the main algorithm, I've learned valuable lesson about being creative on coming up with an approach and keep on trying.

I've come to learn that exploring and failing is part of the learning process. I was often discouraged when I get stuck. However, discussing about it with professor Singh and my peers helped with me keep my motivation up. I've come to realize that there are many ways to achieve similar results to these problems.

Works Cited

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