Towards Barcoding *C. elegans* Populations

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Problem Statement

C. elegans are broadly used in almost every field of biology, and large populations are processed as part of high throughput assays. Tracking individual or clusters of worms could assist in reducing noise in many standard protocols. Behavioral studies could benefit from mixing populations of worms with different genetic backgrounds. These challenges could be tackled if only there was a way to distinguish between individual worms or targeted subpopulations.

Methods

Our project focuses on identifying mechanisms to cluster subpopulations of *C. elegans*. To do this, we use internalized fluorescent nanoparticles to differentially label *C. elegans* worms. We hypothesize that by internalizing a unique combination of assorted colored nanoparticles, each worm or population cluster will have a "fluorescent barcode". To test our hypothesis, worms are fed with particles described below, mixed with bacterial food source, *E. coli* OP50.

Particle Name	Color, Size	Excitation Wavelength (nm)	Emission Wavelength (nm)
Sphero, by Spherotech	Red (Propidium Iodide) 0.04-0.09µm	590nm	620nm
Sphero, by Spherotech	Green (GFP) 0.04-0.09µm	440-470nm	490-510nm
Fluoresbrite, by Polysciences	Blue (CFP) 0.05µm	441nm (max)	485nm (max)

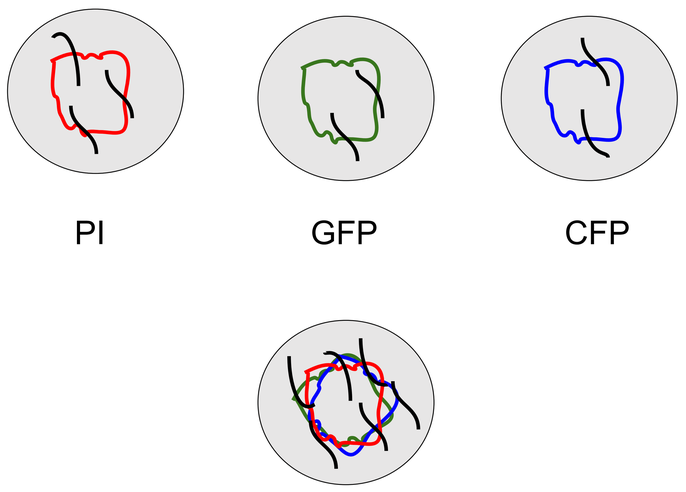
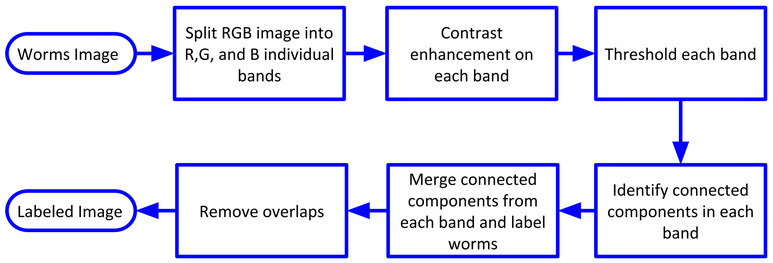
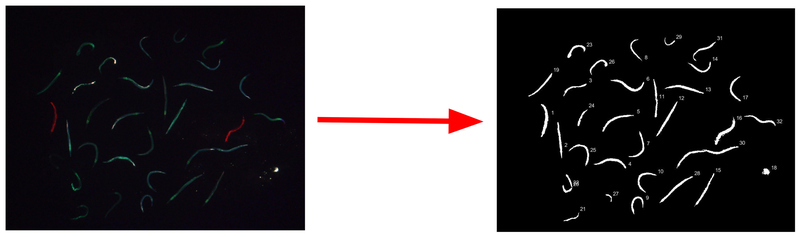


Image Segmentation

To cluster the worms into subpopulations, the first step is to separate the worm regions in the image from the background. The block diagram below shows the segmentation process:

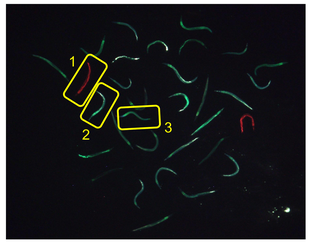




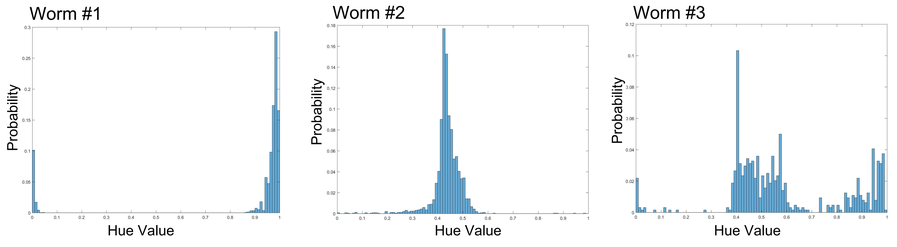
The block diagram above shows the process followed to convert the input RGB image on the left into the segmented binary image on the right. Each worm is randomly assigned a number label.

Histogram Analysis

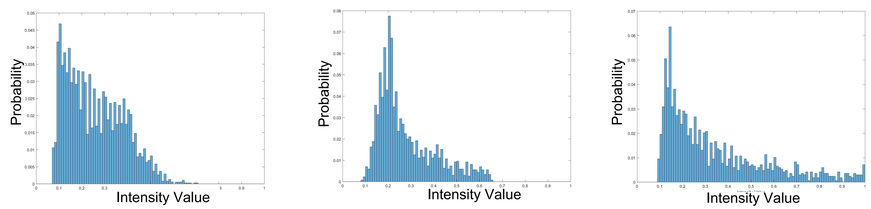
We then analyze the colors of each worm. To do this we convert the RGB image to an HSV (hue-saturation-value) image and then create a histogram of hue values for each worm. This distribution of hue values is used as an input to the k-means clustering algorithm to distinguish between worms.



Hue (Color):

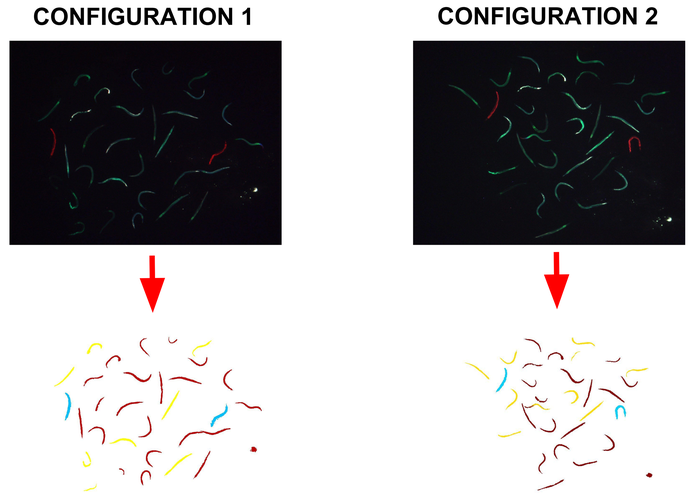


Value (Intensity):



K-Means Clustering

K-means is an iterative algorithm that partitions *n* observations into *k* clusters, each observation being placed in the cluster with the closest mean. Using the hue histogram data as features, we cluster the worms into the appropriate number of clusters. In the images below, we have worms with 3 different colors: red, blue, and blue-green. The images show the output from the clustering algorithm for the same worms in 2 different configurations.



Cluster #	# of Worms (Configuration 1)	# of Worms (Configuration 2)
1	2	2
2	19	17
3	8	10

Conclusions and Future Work

Our project is at a stage where we can segment and cluster worms that are fed with different combinations of the three fluorescent nanoparticles. Our next steps include:

- Developing image processing methods to separate overlapping worms
- Fine grain hue analysis of different regions of a worm to uniquely identify individual worms
- Use machine learning to train the algorithm
- Track and label freely moving worms in video recordings

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References

1. Kiyama, Y., Miyahara, K. & Ohshima, Y. Active uptake of artificial particles in the nematode *Caenorhabditis elegans*. *J Exp Biol* **215**, 1178-1183, doi:10.1242/jeb.067199 (2012)
2. *Image Processing Toolbox Documentation*, www.mathworks.com/help/images/