Activity 4

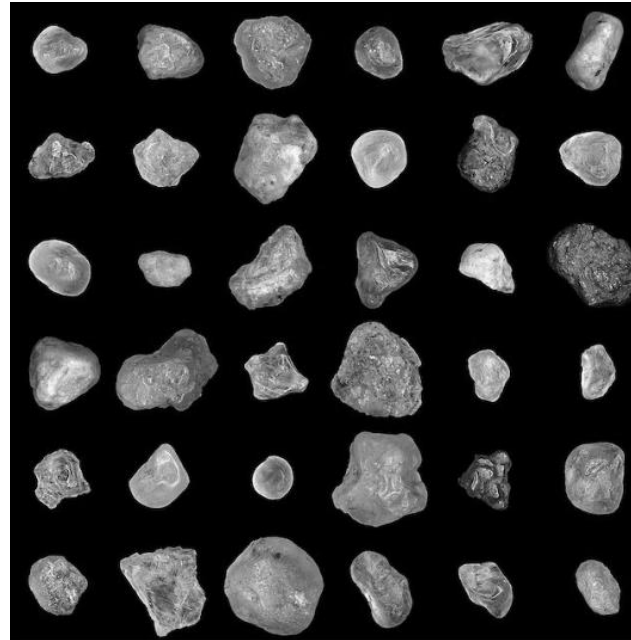# Feature Extraction using ImageJ

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Objectives

1. Perform image segmentation using ImageJ software

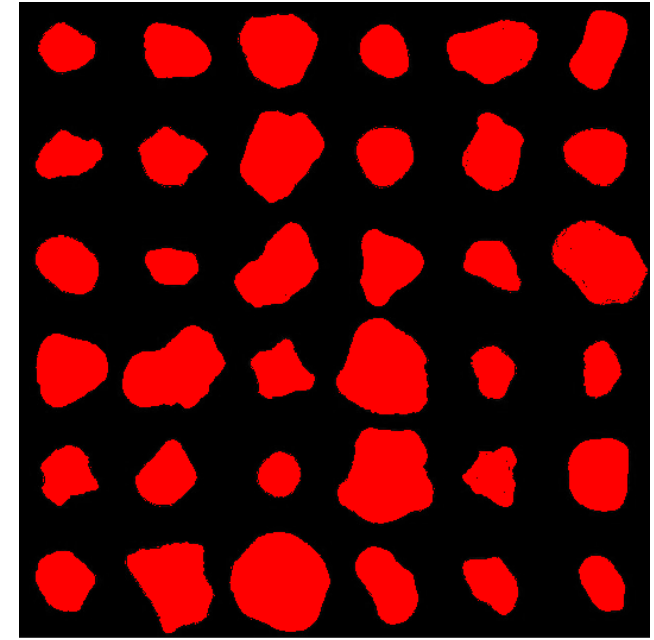2. Obtain values for the count, area, and perimeter of segmented images with an established scale.

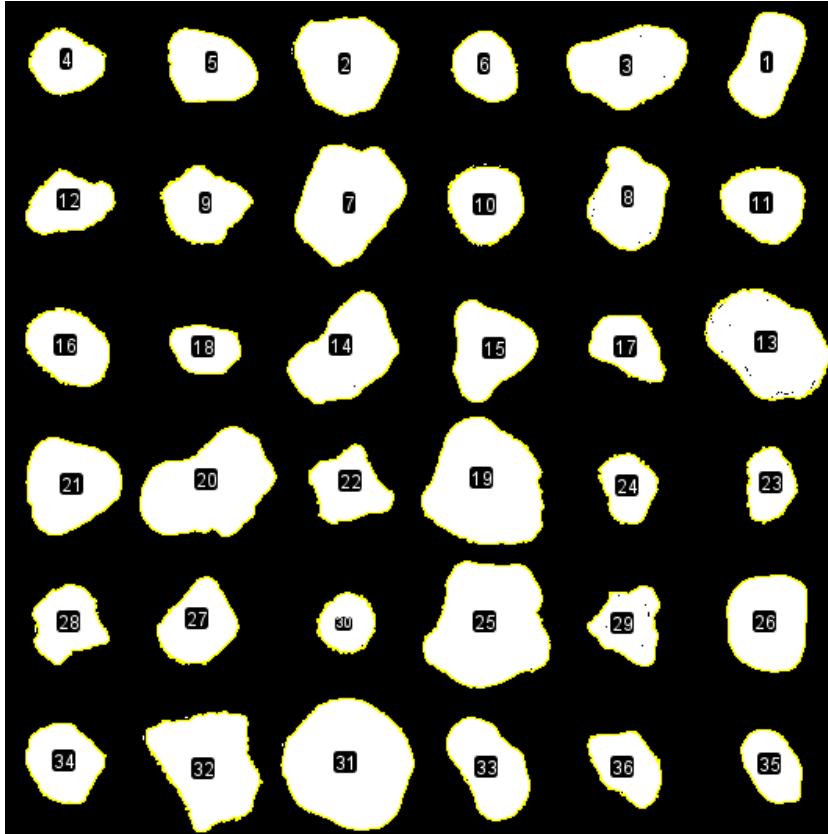# Image Segmentation Process

1. Original image

2. Conversion to grayscale

3. **Intensity threshold: 17 < Red < 255**

Image Segmentation Process

The image is then binarized, and ImageJ's "Analyze Particles" command is used.

Object count threshold: **>20 pixels^2** in size are counted. Alongside the threshold red values, this setting depends on the judgment of the user.

Note: The resulting binarized image may or may not be inverted, depending on whether ImageJ will throw an error when using the command.

Count	Total Area	Average Size	%Area	Mean	Perim.	Circ.	Solidity
36	84975	2360.417	29.968	254.825	195.475	0.739	0.935

Image Segmentation Process

Count	Total Area	Average Size	%Area	Mean	Perim.	Circ.	Solidity
36	84975	2360.417	29.968	254.825	195.475	0.739	0.935
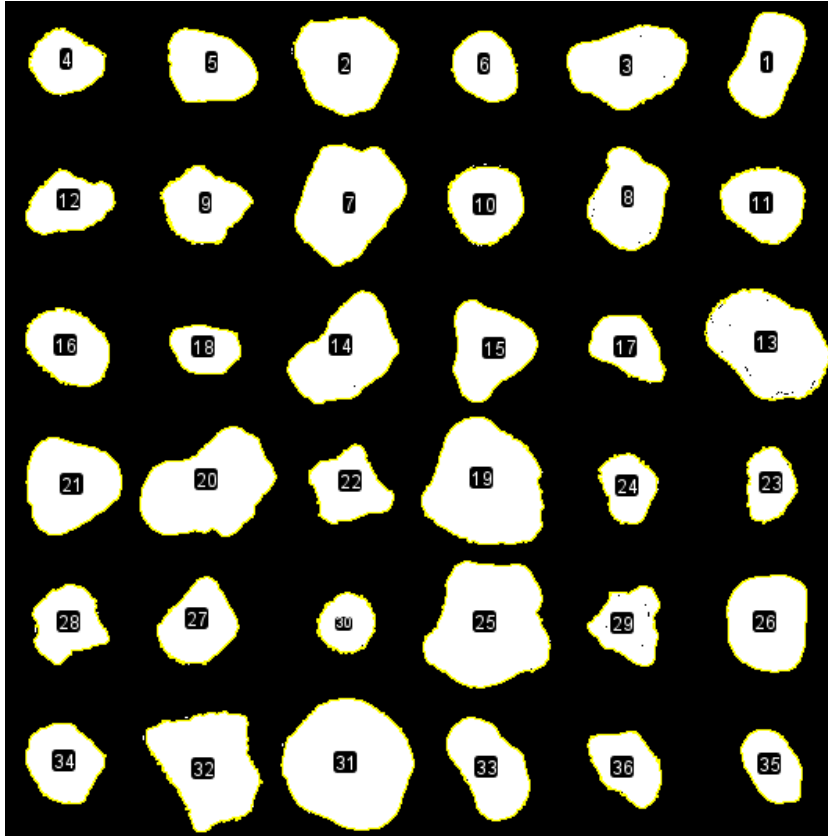
By manually adjusting the threshold, good segmentation can be obtained using ImageJ. Since no scale is given, the given area and sizes are in units of pixels.

It is important to judiciously select the correct threshold for the grayscale intensity and object size.

Intensity threshold: To properly separate the objects from the background. If the minimum threshold is too low, the objects may appear larger. If too high, some objects may be missed or may appear smaller.

When doing area or size calculations, the threshold must be just enough to not overestimate or underestimate the borders of the objects.

Image Segmentation Process

By manually adjusting the threshold, good segmentation can be obtained using ImageJ. Since no scale is given, the given area and sizes are in units of pixels.
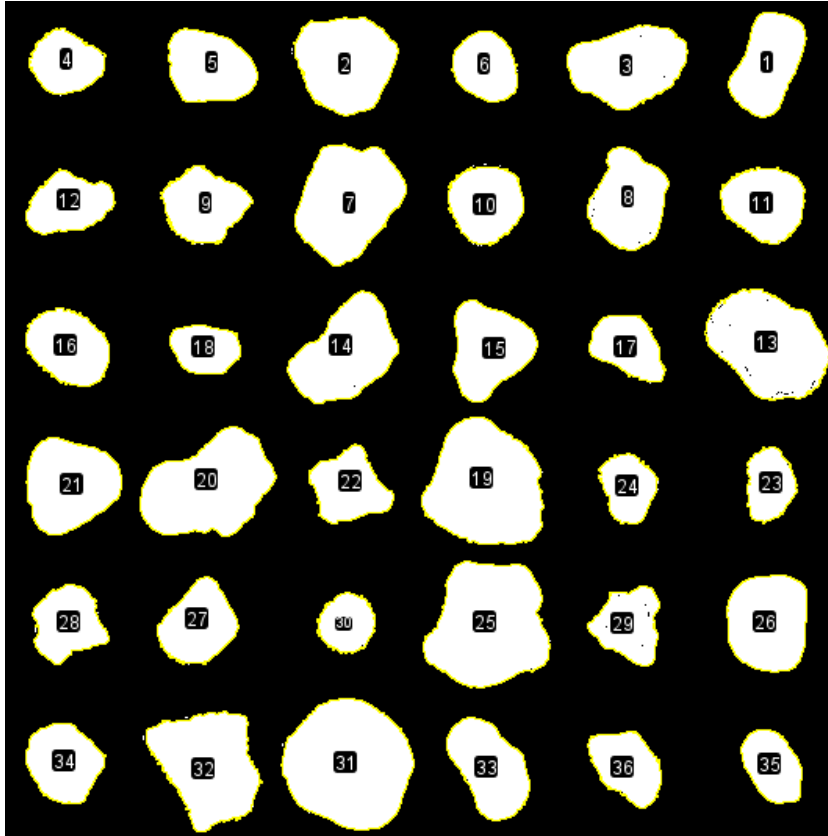
It is important to judiciously select the correct threshold for the grayscale intensity and object size.

Size threshold: To properly count the objects. Too small and small artifacts may be included in the count, too large and some objects may be missed.

Count	Total Area	Average Size	%Area	Mean	Perim.	Circ.	Solidity
36	84975	2360.417	29.968	254.825	195.475	0.739	0.935

Image Segmentation of Rice Grains

Intensity range: (118,255), size range: (20 pixels^2, infinity)

Good segmentation is observed when the objects in the image do not have contiguous pixels (i.e., do not touch each other).
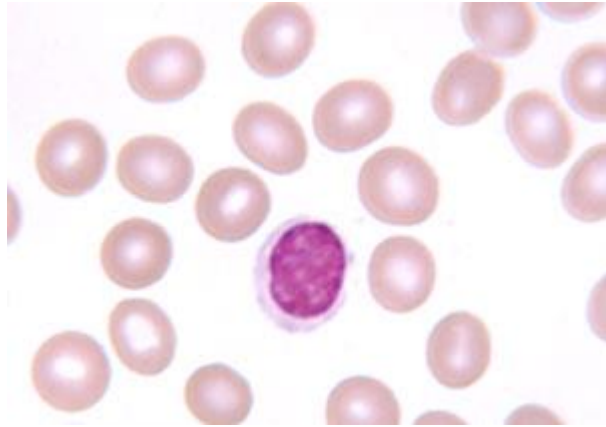
Limitation: Contiguous borders

Note that Object 38 (encircled) is identified by ImageJ as a single rice grain, which shouldn't be the case. This is because the pixels bordering the rice grains touch each other (contiguous).
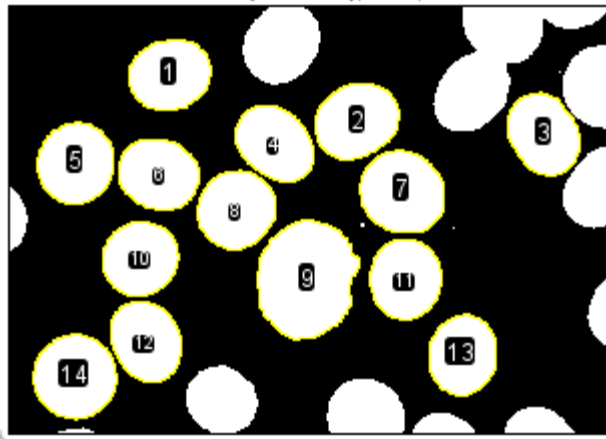
Count	Total Area	Average Size	%Area	Mean	Perim.	Circ.	Solidity
134	19596	146.239	13.093	255	61.144	0.494	0.863

Units: pixels

Application: Estimating the size of a white blood cell
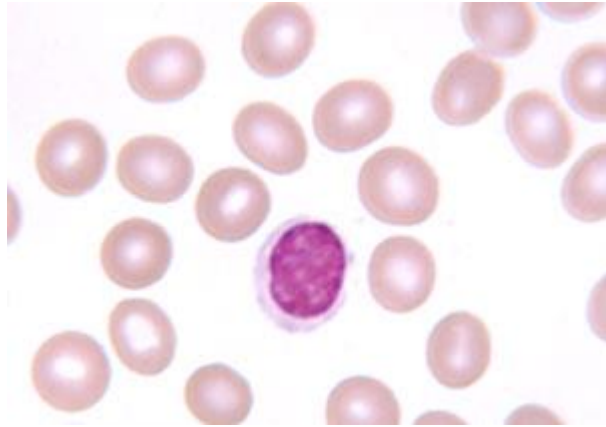

60.00x42.60 microns (300x213); 8-bit; 62K

Scale: 100 pixels/micron
Intensity: (247,255)
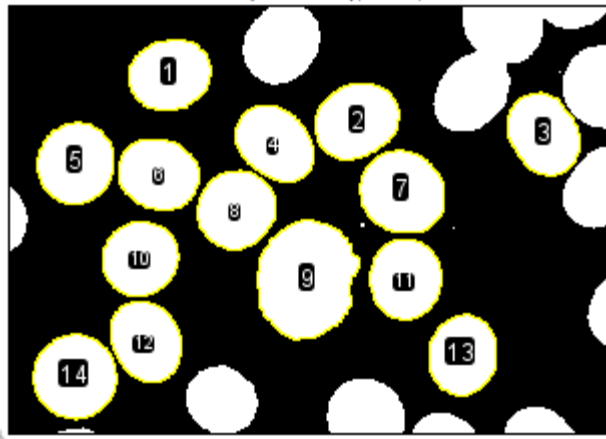Size: (0.20 microns^2, infinity)

	Label	Area	Mean	X	Y	Perim.	Circ.	AR	Round	Solidity
1	lymphocyte.jpg	45.519	255	15.872	6.698	25.011	0.914	1.199	0.834	0.961
2	lymphocyte.jpg	49.199	255	34.512	11.318	26.142	0.905	1.219	0.821	0.964
3	lymphocyte.jpg	44.880	255	53.190	12.643	25.225	0.886	1.293	0.773	0.957
4	lymphocyte.jpg	44.440	255	26.358	13.565	25.059	0.889	1.341	0.746	0.964
5	lymphocyte.jpg	48.679	255	6.473	15.550	25.859	0.915	1.079	0.927	0.962
6	lymphocyte.jpg	44.960	255	14.754	16.648	24.845	0.915	1.180	0.847	0.963
7	lymphocyte.jpg	54.599	255	39.080	18.446	27.225	0.926	1.096	0.912	0.968
8	lymphocyte.jpg	47.559	255	22.508	20.195	25.508	0.919	1.061	0.943	0.962
9	lymphocyte.jpg	91.519	255	29.508	27.136	36.482	0.864	1.219	0.820	0.952
10	lymphocyte.jpg	44.040	255	12.916	25.087	24.611	0.914	1.062	0.942	0.963
11	lymphocyte.jpg	45.319	255	39.400	27.129	24.893	0.919	1.125	0.889	0.963
12	lymphocyte.jpg	44.200	255	13.506	33.339	24.728	0.908	1.259	0.794	0.967
13	lymphocyte.jpg	42.720	255	45.117	34.732	24.328	0.907	1.198	0.835	0.964
14	lymphocyte.jpg	54.439	255	6.399	36.882	27.439	0.909	1.008	0.992	0.961

Using ImageJ, the lymphocyte in the background has an estimated area of 91.52 microns^2. Assuming that it is circular, the diameter is approximately 11 microns, which is within the typical size.

Application: Estimating the size of a white blood cell
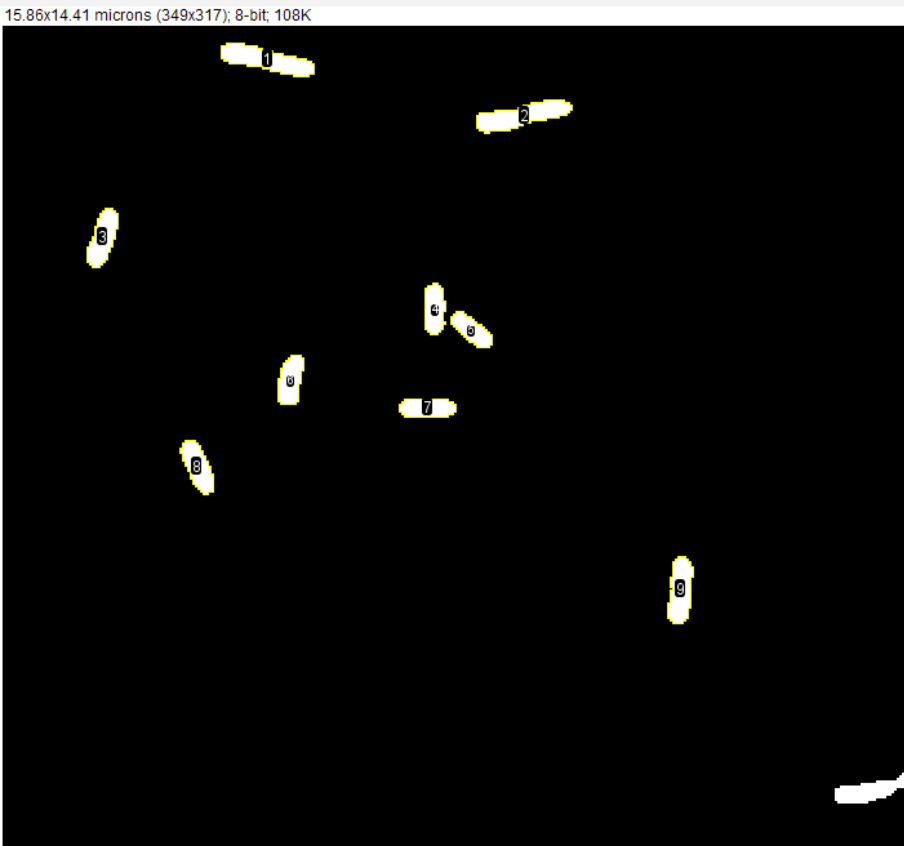

60.00x42.60 microns (300x213); 8-bit; 62K

Scale: 100 pixels/micron
Intensity: (247,255)
Size: (0.20 microns^2, infinity)

	Label	Area	Mean	X	Y	Perim.	Circ.	AR	Round	Solidity
1	lymphocyte.jpg	45.519	255	15.872	6.698	25.011	0.914	1.199	0.834	0.961
2	lymphocyte.jpg	49.199	255	34.512	11.318	26.142	0.905	1.219	0.821	0.964
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14	lymphocyte.jpg	54.439	255	6.399	36.882	27.439	0.909	1.008	0.992	0.961

Note: Regular thresholding would produce holes in the binarized image. ImageJ's "Fill Hole" command was used to fix this.

Application: Estimating the size of *E. coli*

15.86x14.41 microns (349x317); 8-bit; 108K

Limitations:
1. May not count objects at the edges.
2. Intensity thresholding isn't enough to resolve bacteria at the early or middle stages of binary fission (Objects 2 and 1). This is related to the contiguous border limitation I emphasized earlier.

Slice	Count	Total Area	Average Size	%Area	Mean	Perim.	Circ.	Solidity
e coli.png	9	3.244	0.360	1.420	255	2.722	0.634	0.899

Units: microns

Scale: 22 pixels/micron
Intensity: (64,255)
Size: (0.10 microns^2, infinity)

Image Segmentation of Chlorophyll a Molecules in Cyanobacteria PSI*
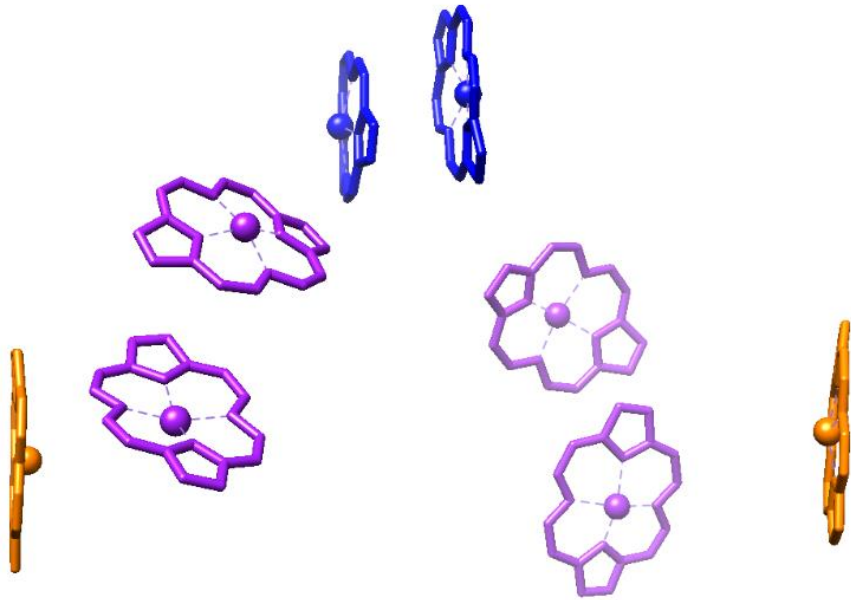
Figure generated using UCSF Chimera.

Segmented image.

PS: Currently my research topic (quantum dynamics of chlorophyll pigments in photosystems)
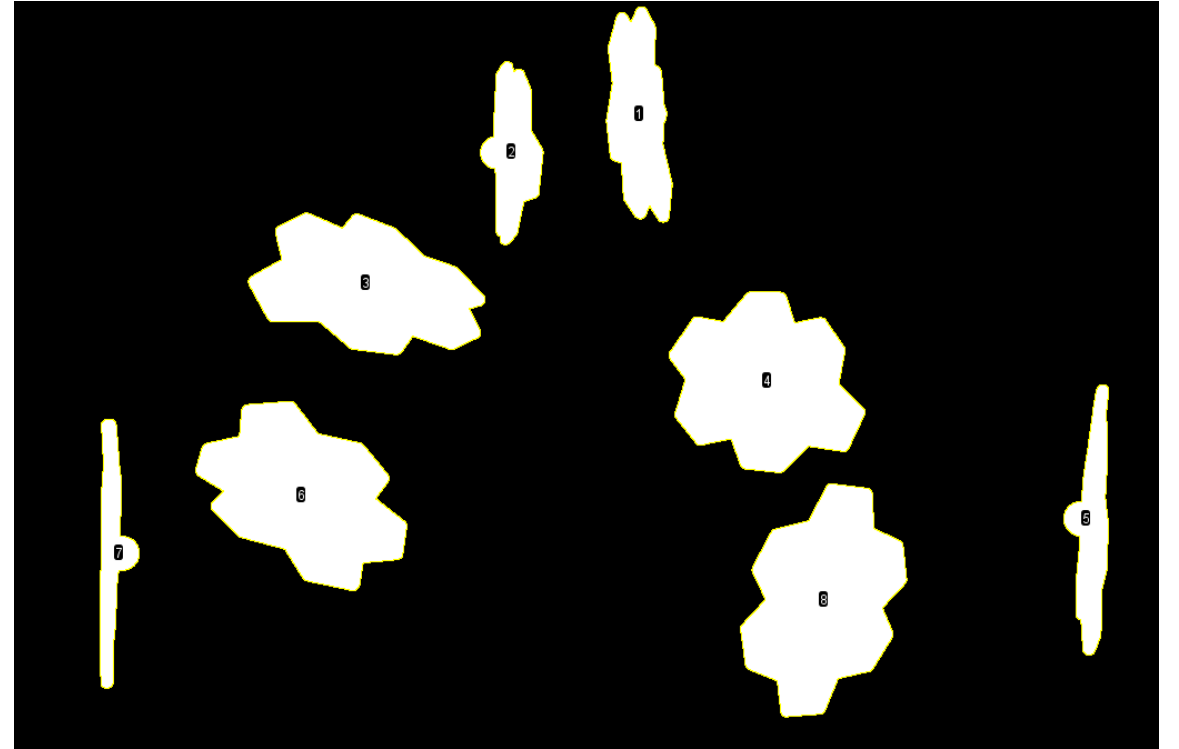
Image Segmentation of Chlorophyll a Molecules in Cyanobacteria PSI*
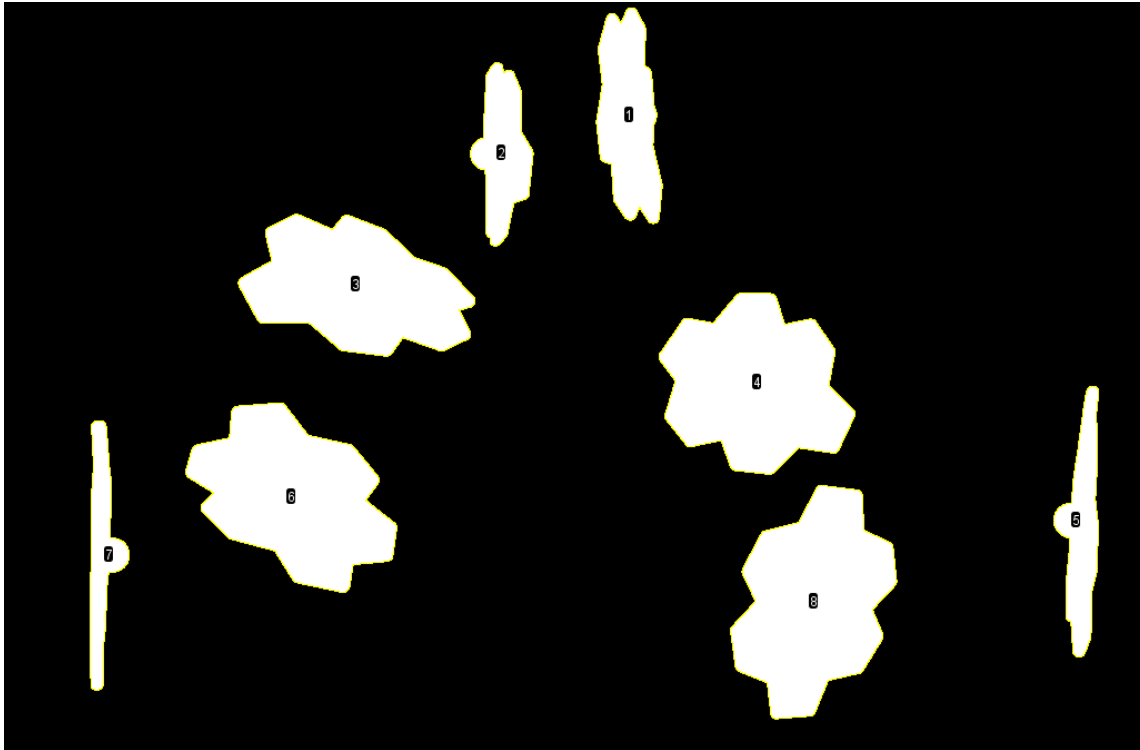
The chlorophyll molecules have the same sizes but they are arranged differently in 3D space, so it wouldn't make much sense to perform quantitative measurements.

The measurement of area, perimeter, and other features using image segmentation is most effective if a scale is established **and** the objects lie in the same plane. However, this isn't the case for the chlorophyll image.

PS: Currently my research topic (quantum dynamics of chlorophyll pigments in photosystems)

Self-Reflection

Score: 100/100

Reasons:

1. I think I sufficiently demonstrated real-life applications of image segmentation using ImageJ

2. Showed the threshold values to replicate the images.

3. Straightforward activity

References

Rice grains: Aulakh, J.S., & Banga, V.K. (2012). Grading Of Rice Grains By Image Processing. *International journal of engineering research and technology, 1.*

E. coli: Tavaddod, S., Naderi-Manesh, H. Evidence of Multi-Domain Morphological Structures in Living *Escherichia coli . Sci Rep* **7**, 5660 (2017). https://doi.org/10.1038/s41598-017-05897-7

Lymphocytes: Obtained from

https://www.histology.leeds.ac.uk/blood/blood_wbc.php