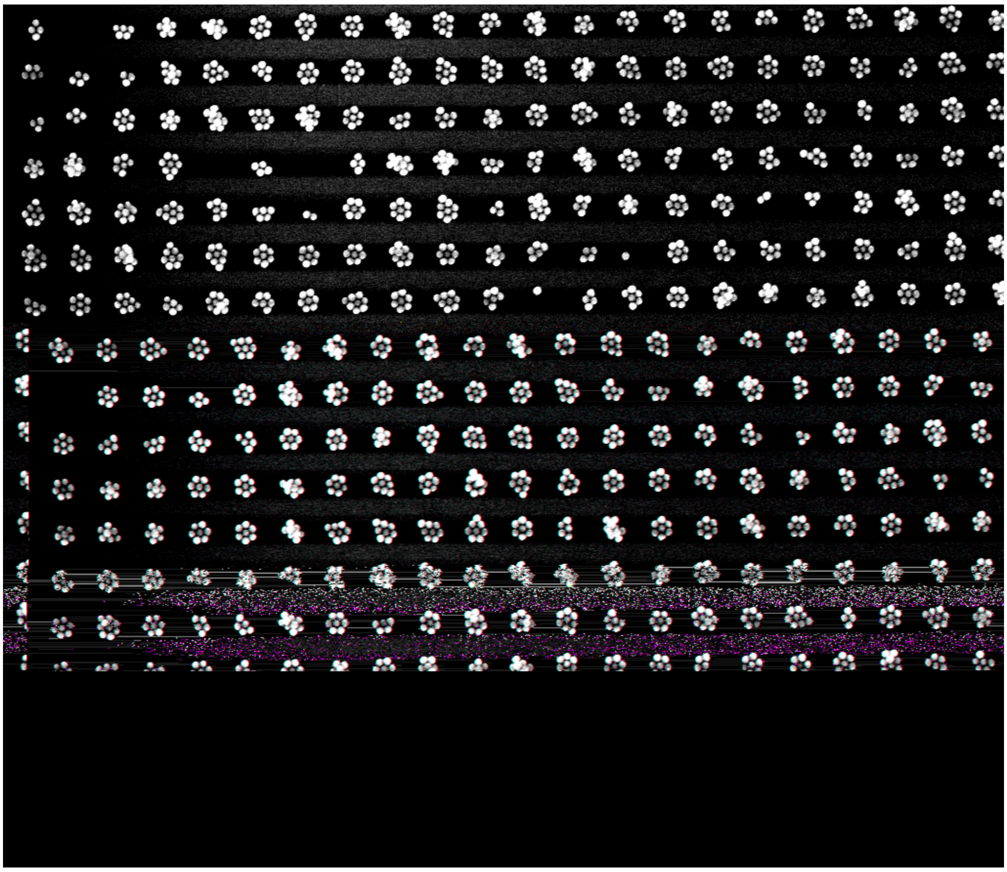
Optimization and Image Processing Project 1

Project Description

In this project you are going to derive an image processing approach to statistical analysis on microscopy images showing so-called (regular) heptamer arrangements ("clusters") of gold(Au) nano-particles on a substrate.

The project goal is to answer relevant statistical morphology questions automatically. All questions below should be answered in for of a histogram, displaying the quantity on question over the (reasonably binned) number of particles/clusters fulfilling this quantity.

Reference image



Tasks

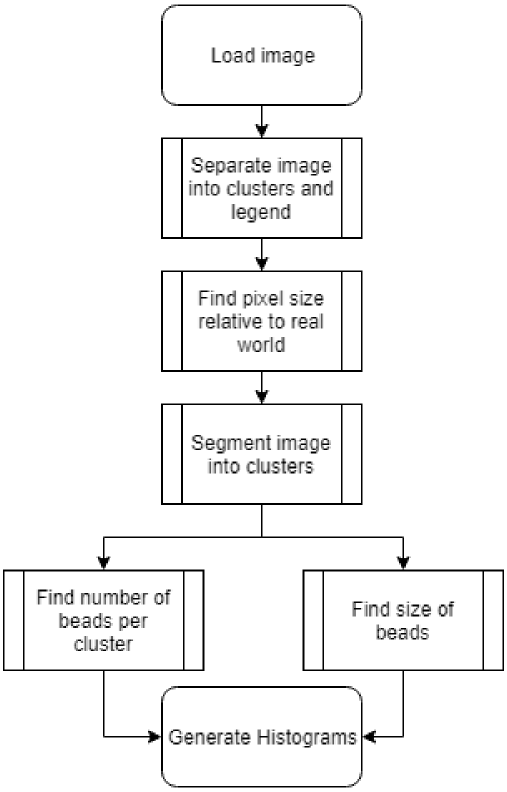
The target tasks to be performed are: Use (and extend) you image processing library to automatically measure:

1. The diameter of all single particles shown on the provided microscopy images.
2. The number of particles per heptamer cluster (ideally 7).
3. (VOLUNTARY): for the regular clusters (7 particles): their orientation, i.e., the angle about which they are turned relative to one reference orientation.

Overview of Process

Flowchart of Process

Process Flowchart

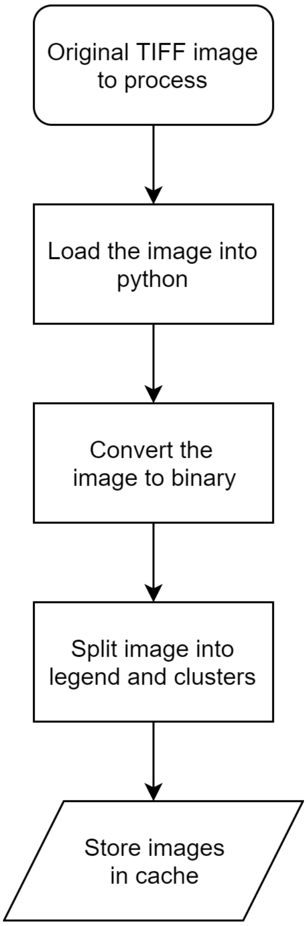

Load image
Separate image into clusters and legend
Find pixel size relative to real world
Segment image into clusters
Find number of beads per cluster
Find size of beads
Generate Histograms

The steps taken to complete the tasks are as follows:

1. Load and crop image

```
1. Load the image into Python
2. Split the image into the legend and the clusters
3. Save images to disk/cache
```

Load and Crop Image

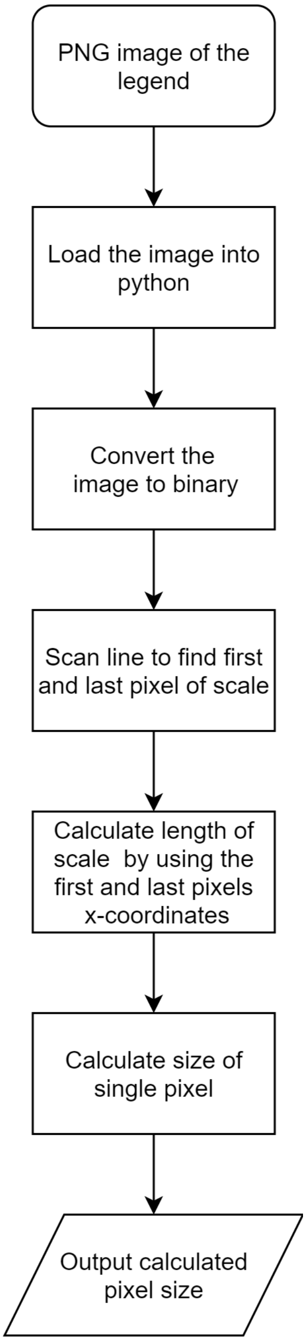

Original TIFF image to process
Load the image into python
Convert the image to binary
Split image into legend and clusters
Store images in cache

2. Find the pixel size relative to the real world

For this step, we use the legend image we created in step 1.

```
1. Use a line scan to get the first and last pixel of the scale
2. Find the length using the first and last pixel
3. Given that we know the size of the scale (4 micrometers), we calculate the si
ze of a single pixel
```

Finding the Pixel Size Relative to Real World

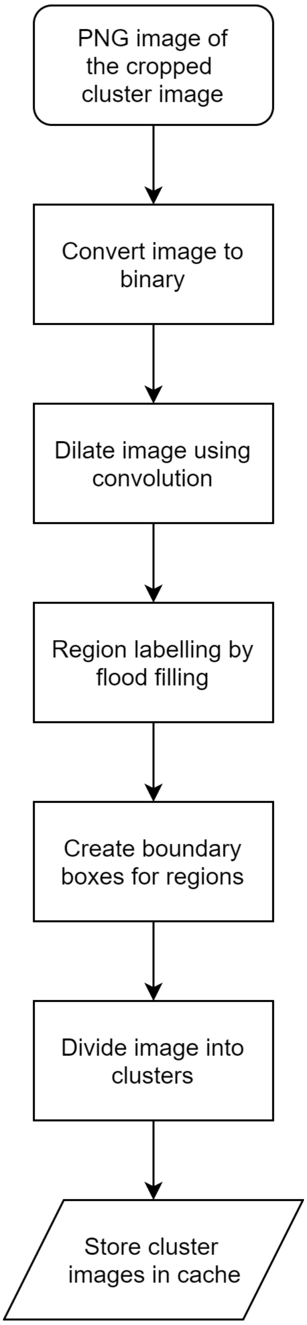

PNG image of the legend
Load the image into python
Convert the image to binary
Scan line to find first and last pixel of scale
Calculate length of scale by using the first and last pixels x-coordinates
Calculate size of single pixel
Output calculated pixel size

3. Segment the image into clusters

For this step, we use the cluster image we created in step 1.

```
1. Convert the image to binary (using thresholding)
2. Dilate the image using convolution
3. Label regions within the image using flood fill
4. Get the boundary boxes for region
5. Using the boundary boxes, divide the image into clusters
```

Segment Image Into Clusters


PNG image of the cropped cluster image
Convert image to binary
Dilate image using convolution
Region labelling by flood filling
Create boundary boxes for regions
Divide image into clusters
Store cluster images in cache

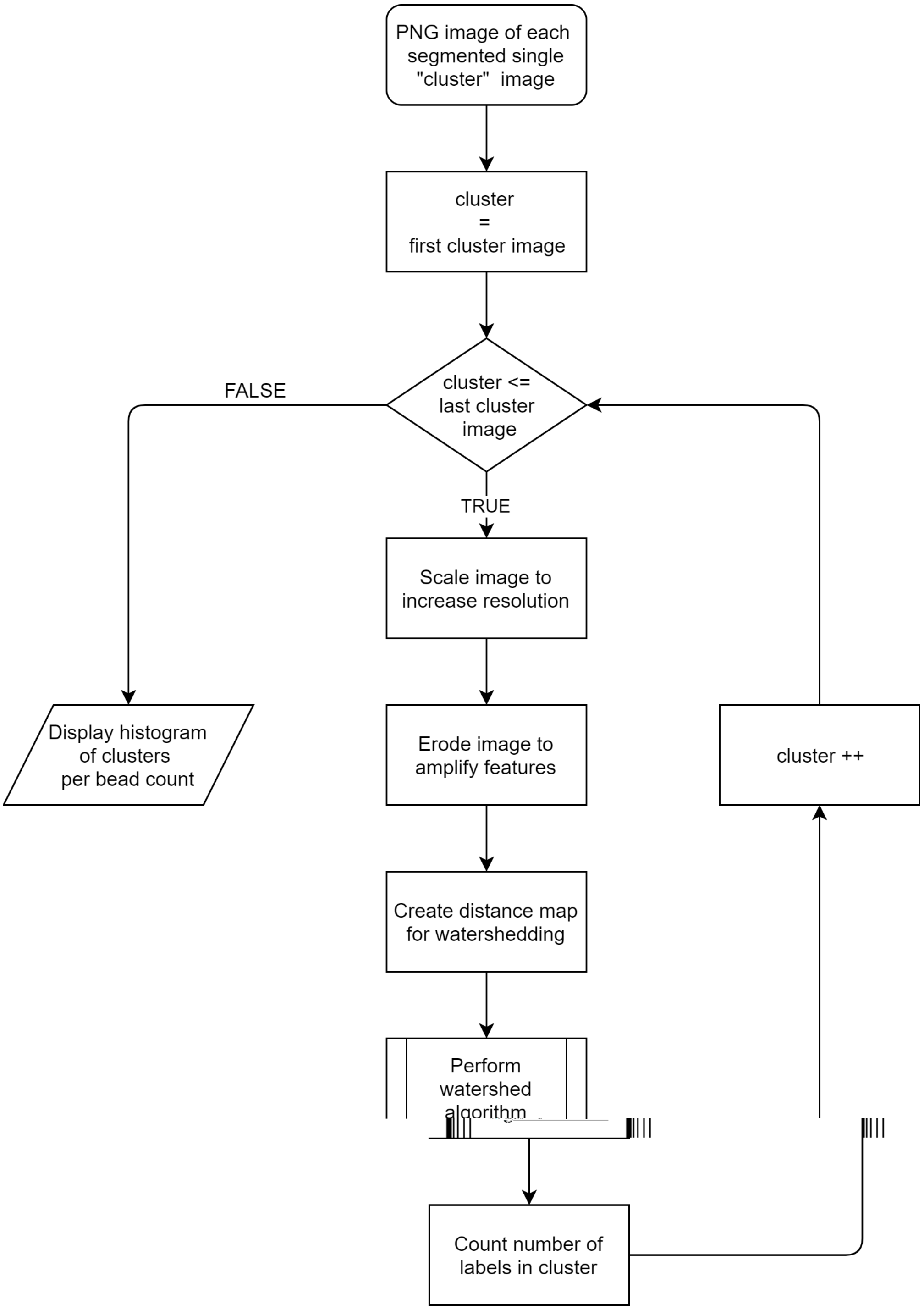
4. Find the number of beads in each cluster

For this step, we use the cluster images from step 3. The steps are performed for each cluster.

```
1. Scale image to increase resolution
2. Erode image to amplify features
3. Create distance map for watershedding
4. Perform the watershed algorithm
5. Count the number of labels in cluster
```

After these steps are performed for every cluster, the values are used to create a histogram of number of beads per cluster.

Find the Number of Beads In Each Cluster


PNG image of each segmented single "cluster" image
cluster = first cluster image
cluster <= last cluster image
FALSE
TRUE
Scale image to increase resolution
Erode image to amplify features
Create distance map for watershedding
Perform watershed algorithm
Count number of labels in cluster
cluster ++
Display histogram of clusters per bead count

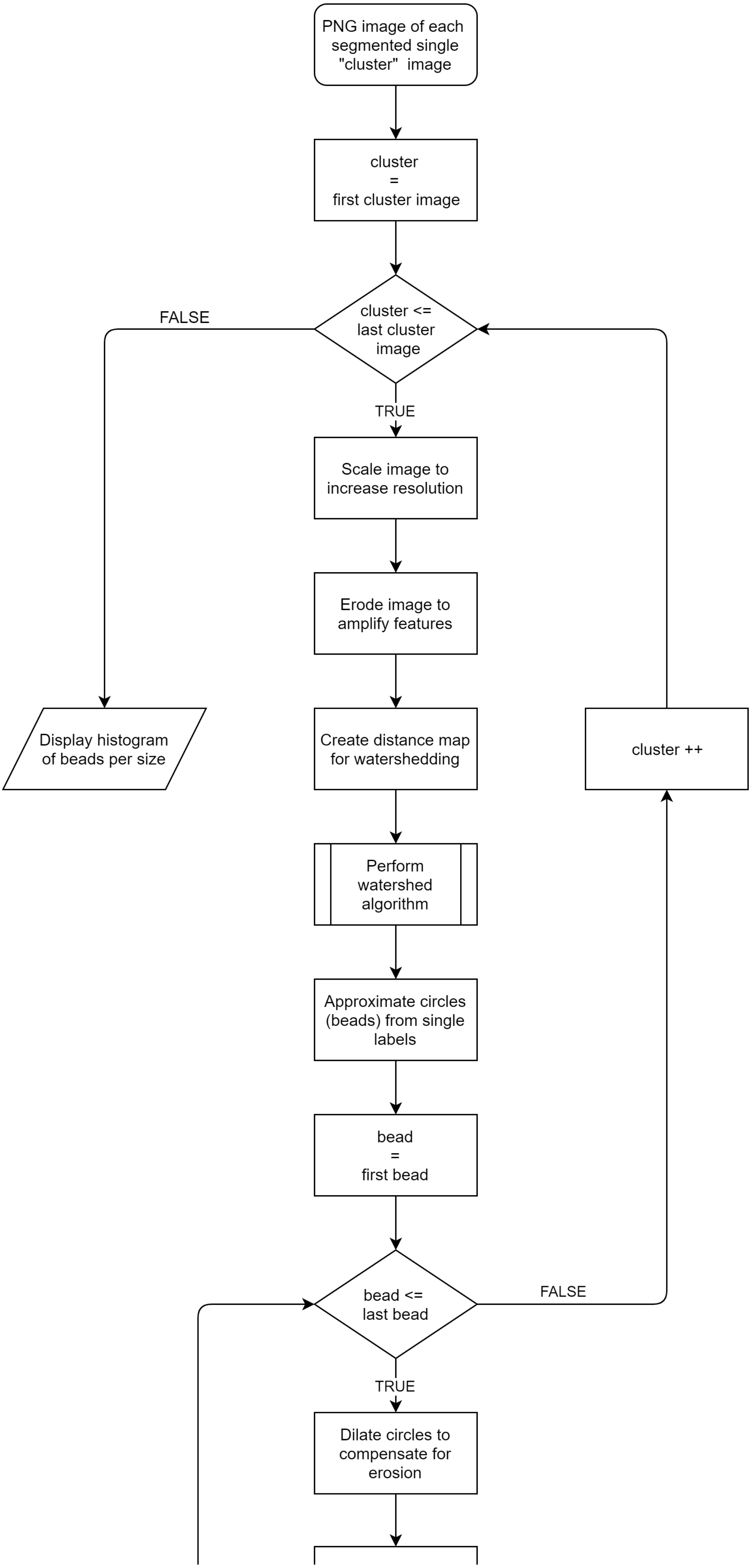
5. Find the size of each bead in each cluster

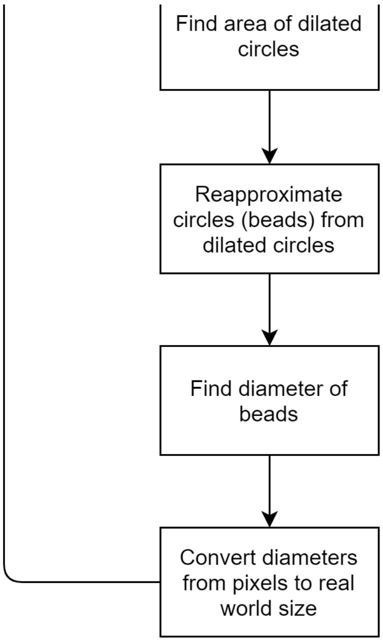
For this step, we use the cluster images from step 3. The steps are performed for each cluster.

```
1. Scale image to increase resolution
2. Erode image to amplify features
3. Create distance map for watershedding
4. Perform the watershed algorithm
5. Using the area of each label, approximate a circle with the same area.
6. Dilate circles to compensate for the erosion in step 5.2.
7. Find area of dilated circles
8. Use dilated areas to approximate new circles
9. Find the diameters of these circles
10. Convert the diameters from pixels to real world size using the value found i
n step 2.
```

After these steps are performed, the values are used to create a histogram of number of beads per cluster.

Find the Size of Each Bead In Each Cluster


PNG image of each segmented single "cluster" image
cluster = first cluster image
cluster <= last cluster image
FALSE
TRUE
Scale image to increase resolution
Erode image to amplify features
Create distance map for watershedding
Perform watershed algorithm
Approximate circles (beads) from single labels
bead = first bead
bead <= last bead
FALSE
TRUE
Dilate circles to compensate for erosion
Display histogram of beads per size
cluster ++


Find area of dilated circles
Reapproximate circles (beads) from dilated circles
Find diameter of beads
Convert diameters from pixels to real world size

Demonstration

1. Load and crop image

```
1. Load the image into Python
```

In [1]:

```
import modules.oiplib as oiplib

originalImage = oiplib.loadImage("../images/heptamers.tiff")
oiplib.showImage(originalImage, figsize=(10,10), title="Original Image")
```

Out[1]:

```
(<Figure size 1000x1000 with 1 Axes>,
 <matplotlib.axes._subplots.AxesSubplot at 0x1d328850>)
```

1. Split the image into the legend and the clusters

In [2]:

```
cropped, legend = oiplib.separateLegend(originalImage)
```

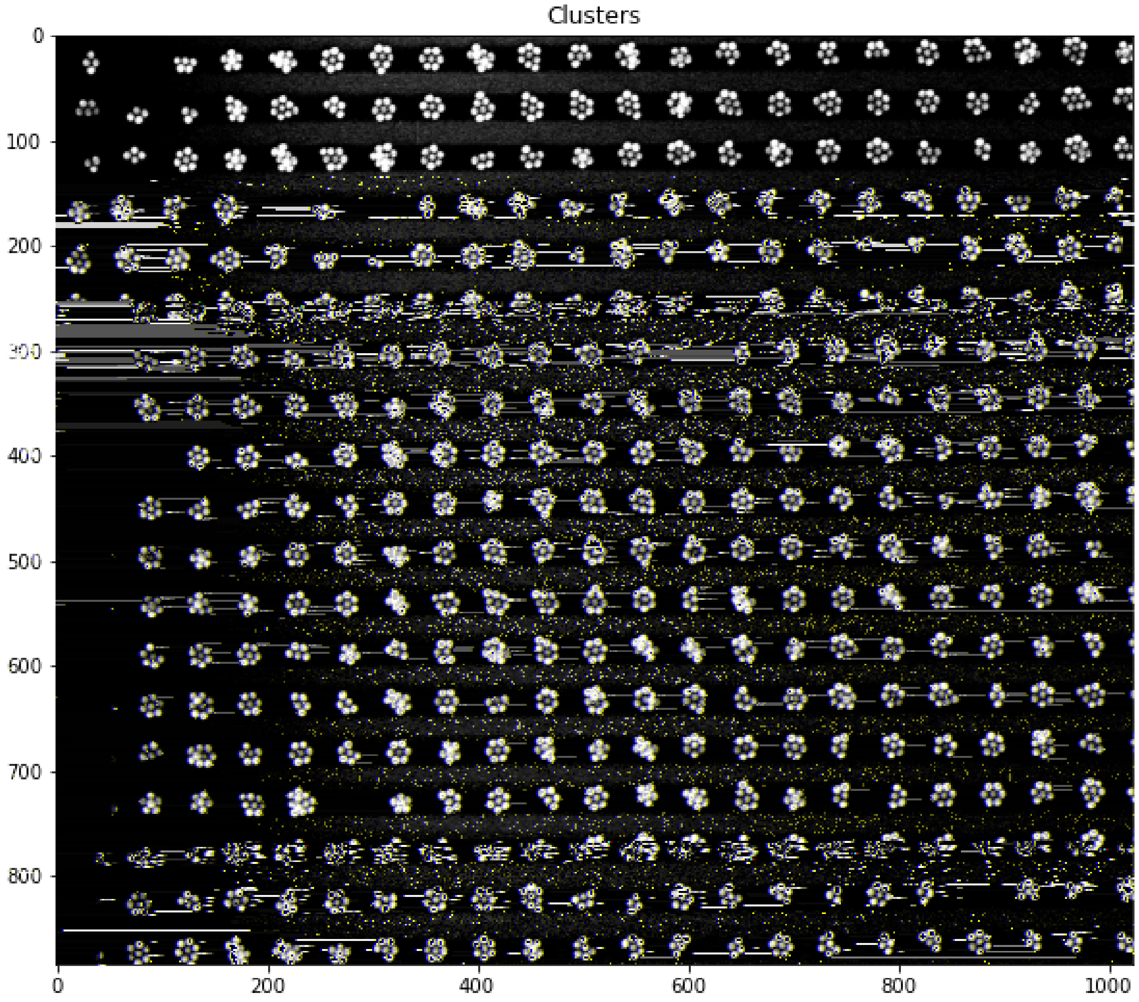
Now we have two images, one of the clusters alone and one of the legend. We save these images for later use.

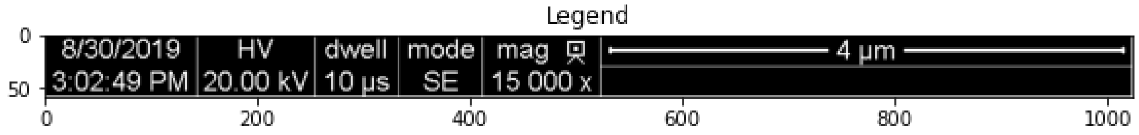
In [3]:

```
oiplib.showImage(cropped, figsize=(10,10), title="Clusters")
oiplib.showImage(legend, figsize=(10,10), title="Legend")
```

Out[3]:

```
(<Figure size 720x720 with 1 Axes>,
 <matplotlib.axes._subplots.AxesSubplot at 0x1d9d7d50>)
```




Legend
0
50
8/30/2019
3:02:49 PM
HV
20.00 kV
dwell
10 µs
mode
SE
mag
15 000 x
4 µm
0
200
400
600
800
1000

2. Find the pixel size relative to the real world

For this step, we use the legend image we created in step 1. Since the legend is generated by the microscope, we know the anchorpoint and value of the scale as these are constant. They are coordinates (514, 15) and 4 micrometers.

```
1. Use a line scan to get the first and last pixel of the scale
2. Find the length using the first and last pixel
3. Given that we know the size of the scale (4 micrometers), we calculate the si
ze of a single pixel
```

In [4]:

```
pixelSize = oiplib.measurePixelSize(legend)
print(pixelSize, "meters")
```

```
8.247422680412371e-09 meters
```

We find that the size of a pixel is approximately 8.25 nanometers in real life.

3. Segment the image into clusters

For this step, we use the cluster image we created in step 1.

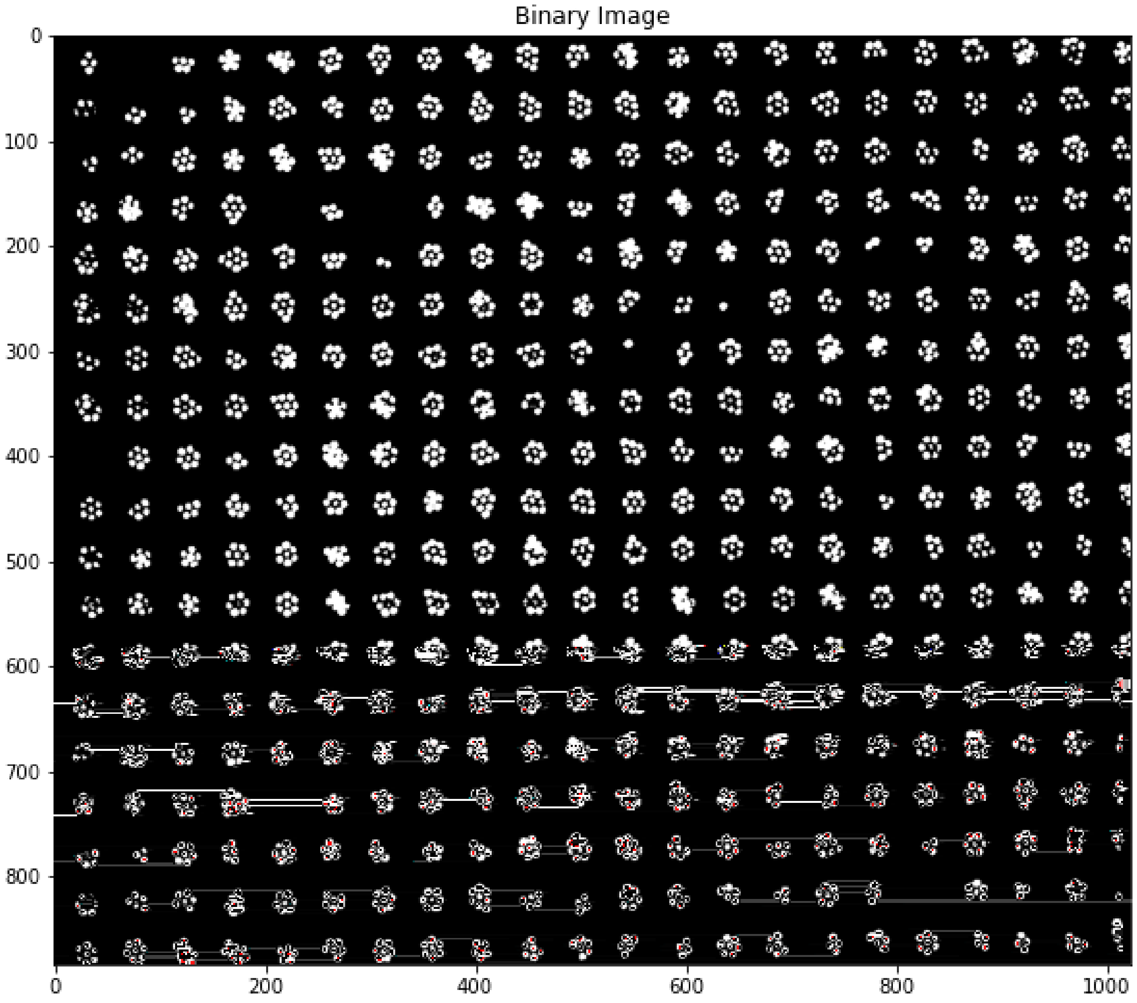
```
1. Convert the image to binary (using thresholding)
```

In [5]:

```
croppedBin = oiplib.gray2Binary(cropped)
oiplib.showImage(croppedBin, vmax=1, figsize=(10,10), title="Binary Image")
```

Out[5]:

```
(<Figure size 720x720 with 1 Axes>,
 <matplotlib.axes._subplots.AxesSubplot at 0x1dd39290>)
```



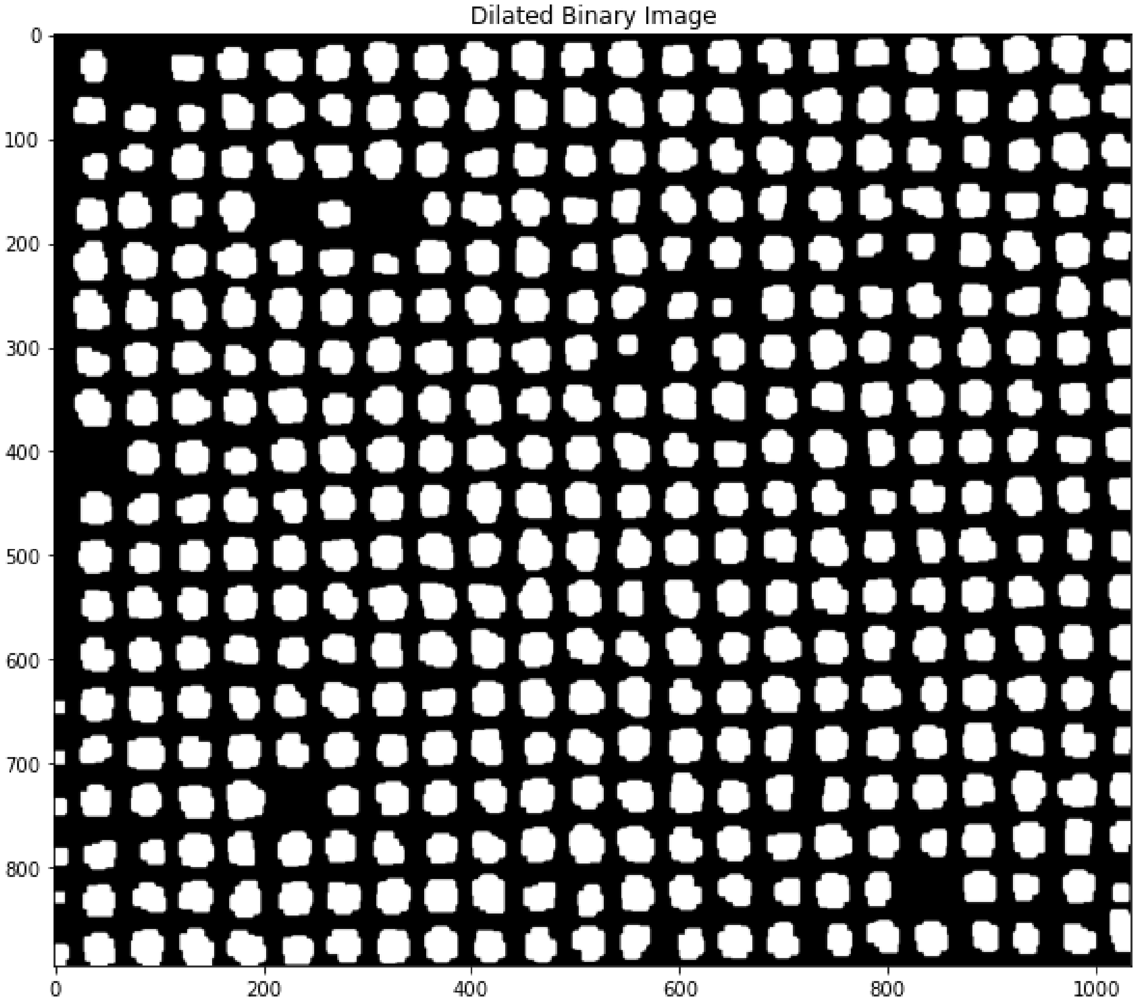
```
2. Dilate the image using convolution
```

In [6]:

```
croppedBin = oiplib.growBinary(croppedBin, 11)
oiplib.showImage(croppedBin, vmax=1, figsize=(10,10), title="Dilated Binary Image")
```

Out[6]:

```
(<Figure size 720x720 with 1 Axes>,
 <matplotlib.axes._subplots.AxesSubplot at 0x6013970>)
```



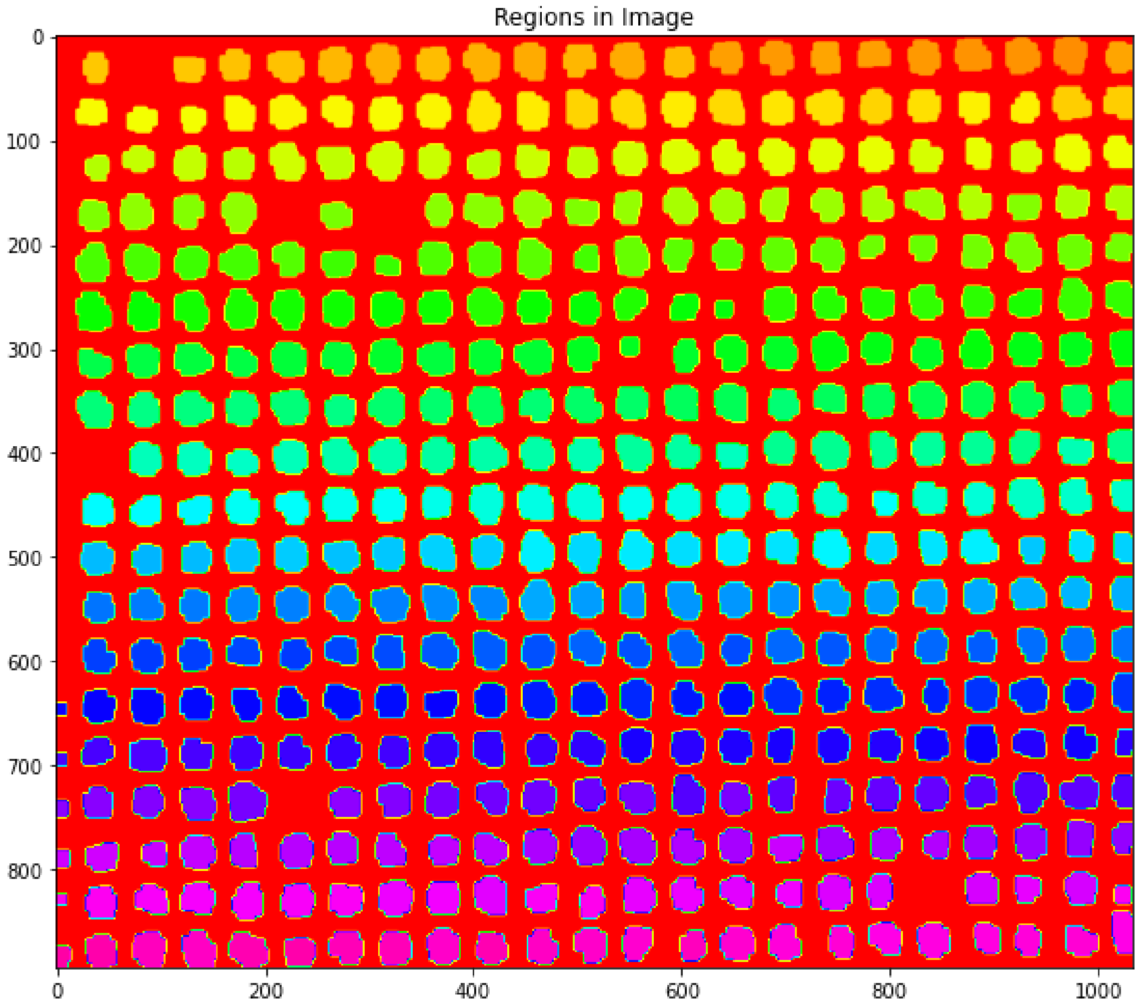
```
3. Label regions within the image using flood fill
```

In [7]:

```
regionImg = oiplib.labelRegions(croppedBin, lmin=50)
oiplib.showImage(regionImg, figsize=(10,10), cmap="hsv", title="Regions in Image", vmax
=oiplib.arrayMax(regionImg)+50)
```

Out[7]:

```
(<Figure size 720x720 with 1 Axes>,
 <matplotlib.axes._subplots.AxesSubplot at 0x1d3310b0>)
```



4. Get the boundary boxes for region

In [8]:

```
anchors, boxSize = oiplib.getBoundaryBoxes(regionImg)
```

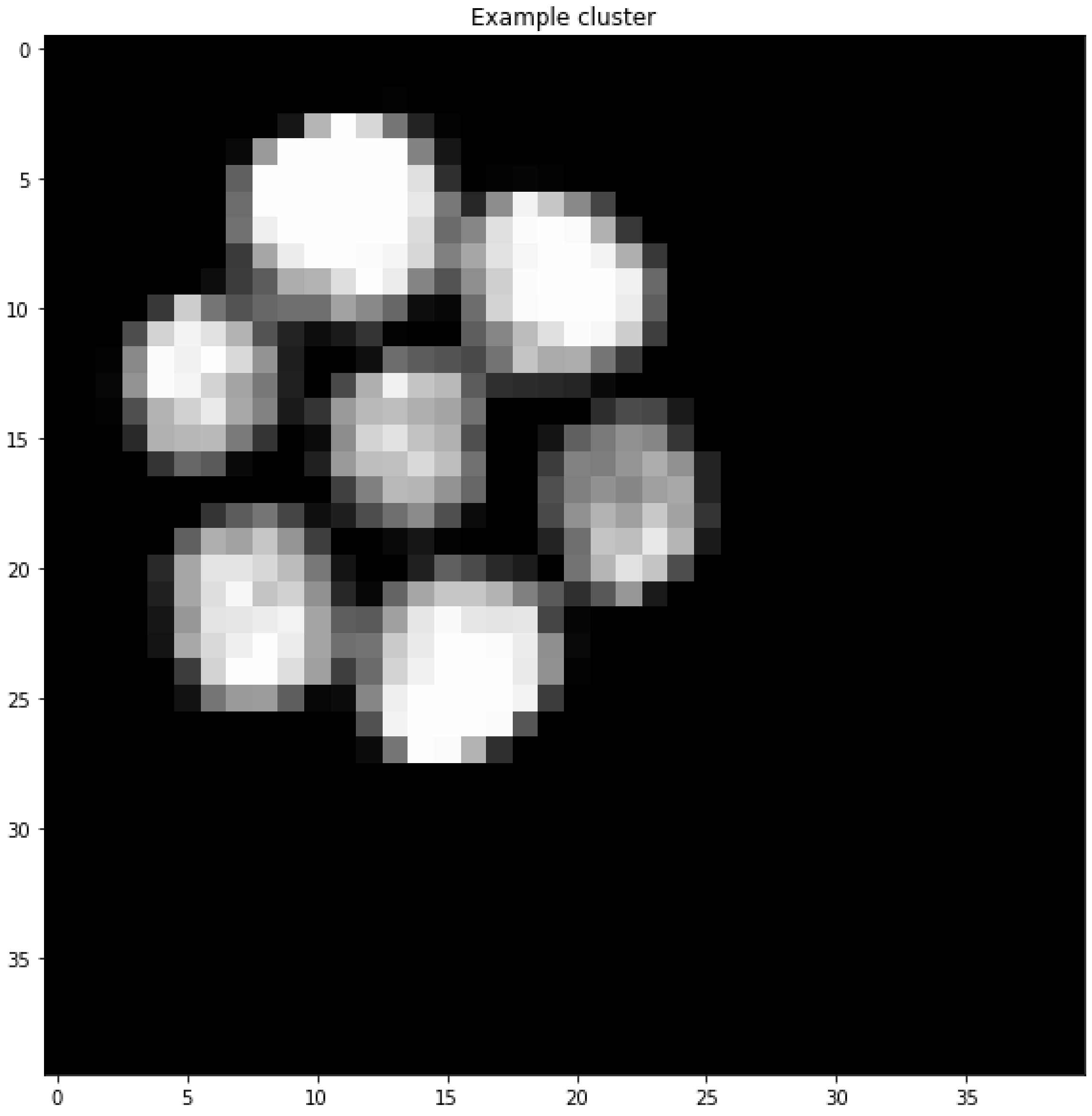
5. Using the boundary boxes, divide the image into clusters

In [9]:

```
clusters = []

for anchor in anchors:
    x, y = anchor

    # Crop image and remove background noise
    clusters.append(oiplib.removeBackgroundNoise(cropped[x:x + boxSize, y:y + boxSize
]))

oiplib.showImage(clusters[0], figsize=(10,10), title="Example cluster")
```

Out[9]:

```
(<Figure size 720x720 with 1 Axes>,
 <matplotlib.axes._subplots.AxesSubplot at 0x1de50ef0>)
```



4. Find the number of beads in each cluster

For this step, we use the cluster images from step 3. The steps are performed for each cluster.

```
1. Scale image to increase resolution
2. Erode image to amplify features
3. Create distance map for watershedding
4. Perform the watershed algorithm
5. Count the number of labels in cluster
```

In [10]:

```python
beadCounts = {}

for cluster in clusters:
    labelImg = oiplib.labelRegionWatershed(cluster)
    labels = oiplib.unique(labelImg)

    beadCount = len(labels) - 1

    if beadCounts.get(beadCount) is None:
        beadCounts[beadCount] = 1
    else:
        beadCounts[beadCount] += 1
```

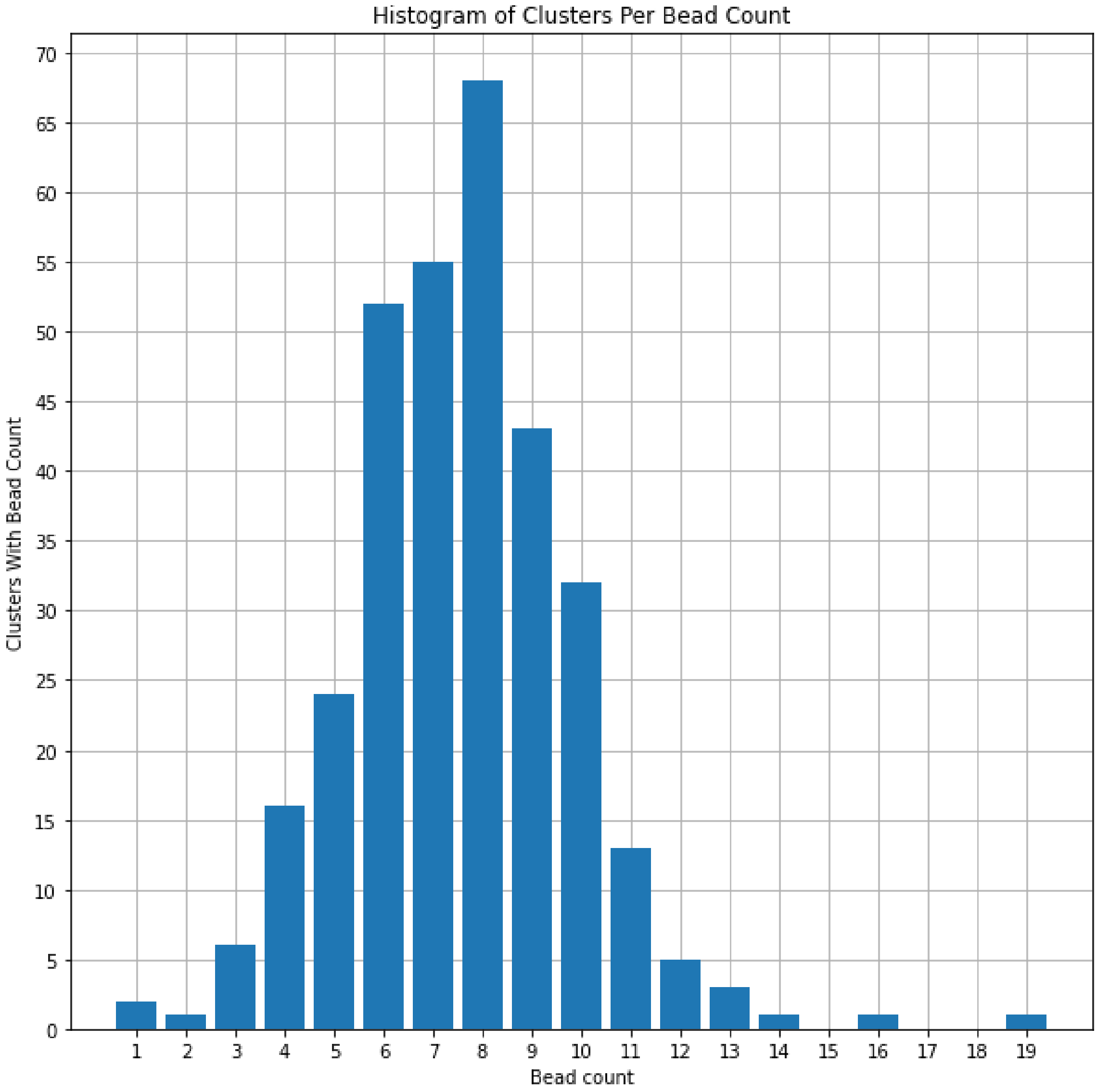
Create histogram of bead counts

In [11]:

```
oiplib.beadCountHist(beadCounts)
```

```
Out[11]:
```

```
<BarContainer object of 19 artists>
```


Histogram of Clusters Per Bead Count
Clusters With Bead Count
Bead count
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

We see that the bead count is centered around 7-8, which is to be expected.

5. Find the size of each bead in each cluster

For this step, we use the cluster images from step 3. The steps are performed for each cluster.

```
1. Watershed the cluster using the same process as step 4.
5. Using the area of each label, approximate a circle with the same area.
6. Dilate circles to compensate for the erosion in step 5.2.
7. Find area of dilated circles
8. Use dilated areas to approximate new circles
9. Find the diameters of these circles
10. Convert the diameters from pixels to real world size using the value found i
n step 2.
```

In [12]:

```
sizes = []

for cluster in clusters:
    # Find beads

    labelImg = oiplib.labelRegionWatershed(cluster)
    beads, areas = oiplib.unique(labelImg, return_counts=True)

    # Approximate the diameter of each bead

    diameters = []

    for area in areas[1:]: # We skip the first element as it is the background
        diameters.append(oiplib.areaToDiameter(area))

    # Find the area of each bead, compensating for erosion

    compAreas = []

    for diameter in diameters:
        compAreas.append(oiplib.dilateBead(diameter, labelImg.shape))

    # We approximate the diameters using the compensated areas

    diameters = []

    for area in compAreas:
        diameters.append(oiplib.areaToDiameter(area)/10) # We divide by 10, as we scale
d the image up 10

    # Write the diameters to the global array, converting it to real world size in the
 process.
    #We floor the value so the data is arranged in bins with integer intervals

    for diameter in diameters:
        sizes.append(oiplib.pixelToLength(diameter, pixelSize * pow(10, 9)))
```

We create a dictionary to better organize the data.

In [13]:

```
sizes = oiplib.unique(oiplib.floor(sizes), return_counts=True)

sizesDict = dict()

for i in range(int(oiplib.arrayMax(sizes[1]))):
    sizesDict[i] = 0

for i, size in enumerate(sizes[0]):
    sizesDict[size] = sizes[1][i]
```

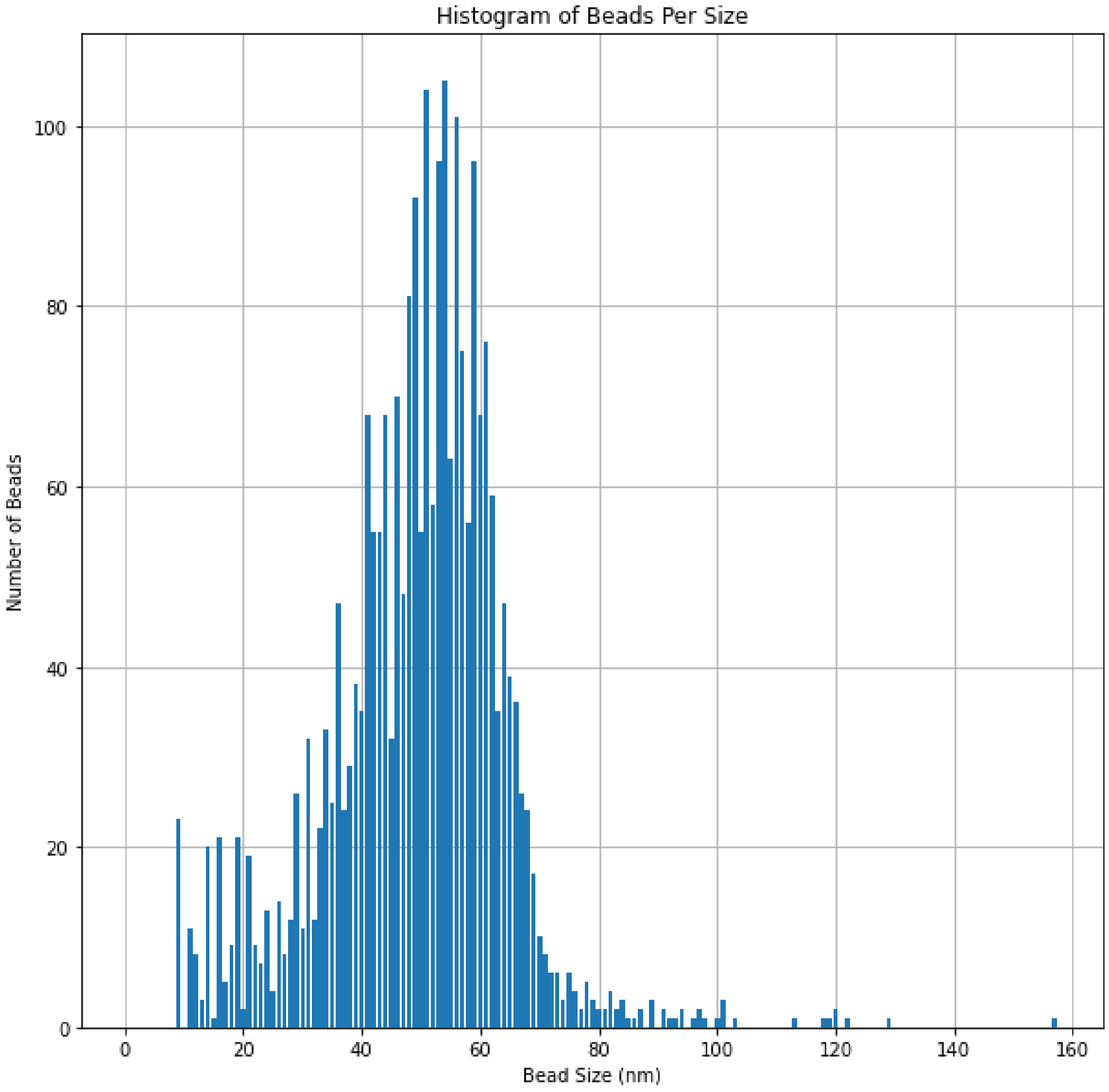
Create histogram of bead sizes

In [14]:

```
oiplib.beadSizeHist(sizesDict)
```

Out[14]:

```
<BarContainer object of 157 artists>
```



The bead sizes are collected mainly around the 50-60 nm area. This is a bit lower than the expected 60-70 range. This is likely due to the dilation and erosion steps performed when seperating the beads within the clusters. The outliers are from clusters that were not separated correctly.