Pregunta 6

November 20, 2019

1 Pregunta 6

(2 puntos) La imagen Ex3Preg6(a).tif muestra una imagen tomada con un microscopio de cultivo de bacterias identificadas por los círculos intensos:

a. (0.5 puntos) Usando una técnica de umbralización global, segmente la imagen y muestre el resultado de la segmetnación.

b. (0.5 puntos) A la imagenoriginal se le aplicó una umbralización con valores locales yal resultado se le realizó una apertura morbológica obteniendo la imagen Ex3Preg6(b).tif. Usando esta imagen,cuente y etiquete cuantos objetos de la segmentación pueden considerarse células independientes.

c. (1 punto) Continuando con la imagen anterior. Cuente y etiquete cuantos objetos de la segmentación pueden considerarse 2 células agrupadas, y cuantos y cuales más de 2 células.

[1]:
```
# Functional programing tools :
from functools import partial, reduce

# Visualisation :
import matplotlib.pyplot as plt
import matplotlib.image as pim
import matplotlib.patches as mpatches
import seaborn as sns

# Data tools :
import numpy as np
import pandas as pd

# Image processing :
import cv2 as cv
from skimage import data
from skimage.filters import threshold_otsu
from skimage.segmentation import clear_border
from skimage.measure import label, regionprops
from skimage.morphology import closing, square
from skimage.color import label2rgb

# Machine Learning :
```

```
from sklearn.cluster import KMeans

# Jupyter reimport utils :
import importlib
```

[2]:
```
# Custom :
import mfilt_funcs as mfs
importlib.reload(mfs)
import mfilt_funcs as mfs

import utils
importlib.reload(utils)
import utils
```

[3]:
```
#plt.style.available
```

[4]:
```
plt.style.use('seaborn-deep')
plt.rcParams['figure.figsize'] = (10, 5)
```

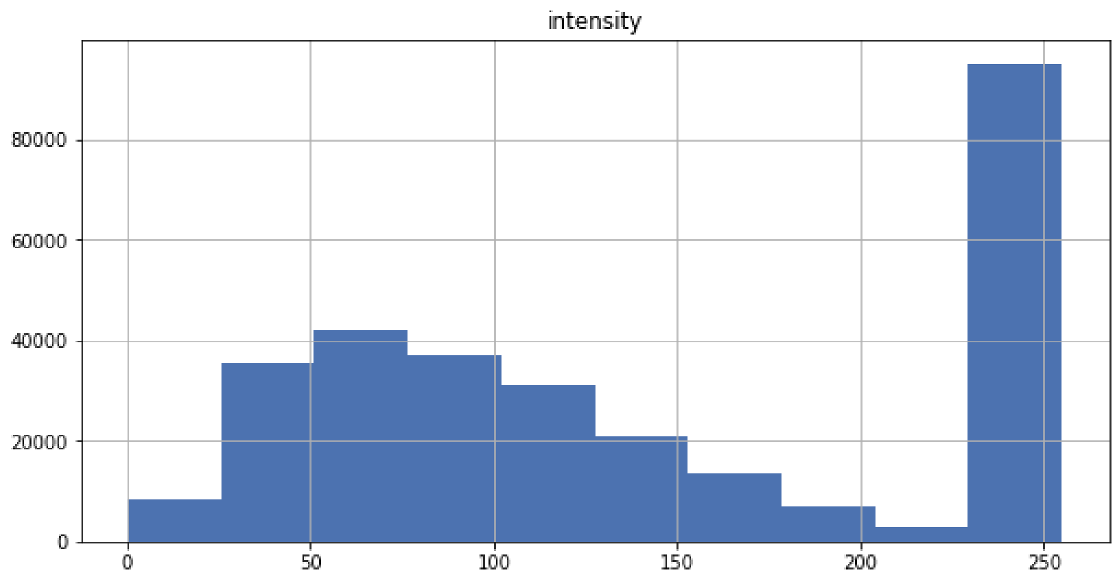
[6]:
```
img   = cv.imread('imagenes/Ex3Preg6(a).tif', cv.IMREAD_GRAYSCALE)
color = cv.cvtColor(img, cv.COLOR_GRAY2RGB) # Color copy, to draw colored circles
```

1.1 a. (0.5 puntos) Usando una técnica de umbralización global, segmente la imagen y muestre el resultado de la segmetnación.

[7]:
```
intensities = pd.core.frame.DataFrame(dict(intensity=img.flatten()))
```

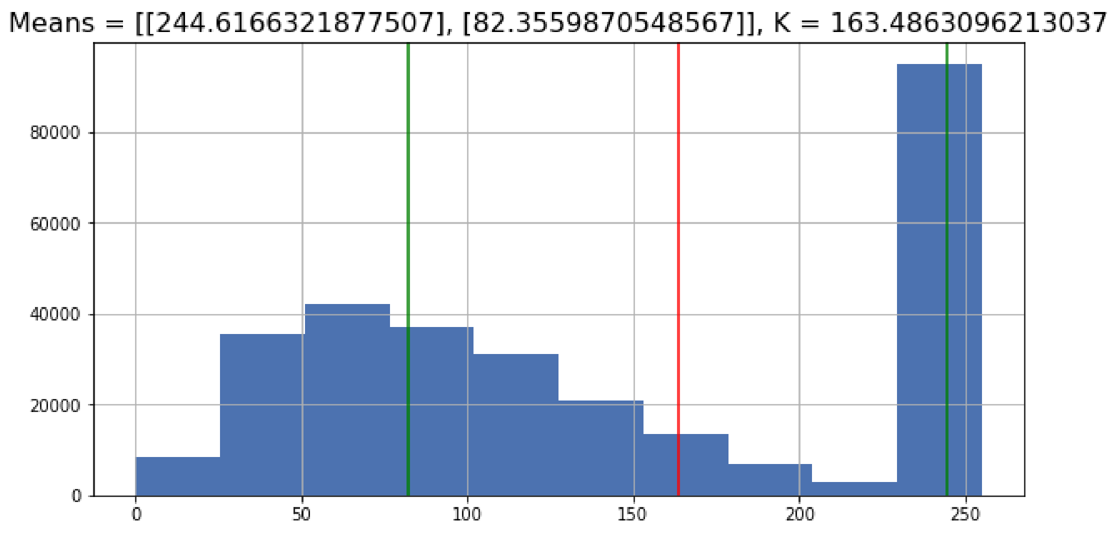
[8]:
```
intensities.hist()
```

[8]:
```
array([[<matplotlib.axes._subplots.AxesSubplot object at 0x1c252e0c90>]],
      dtype=object)
```



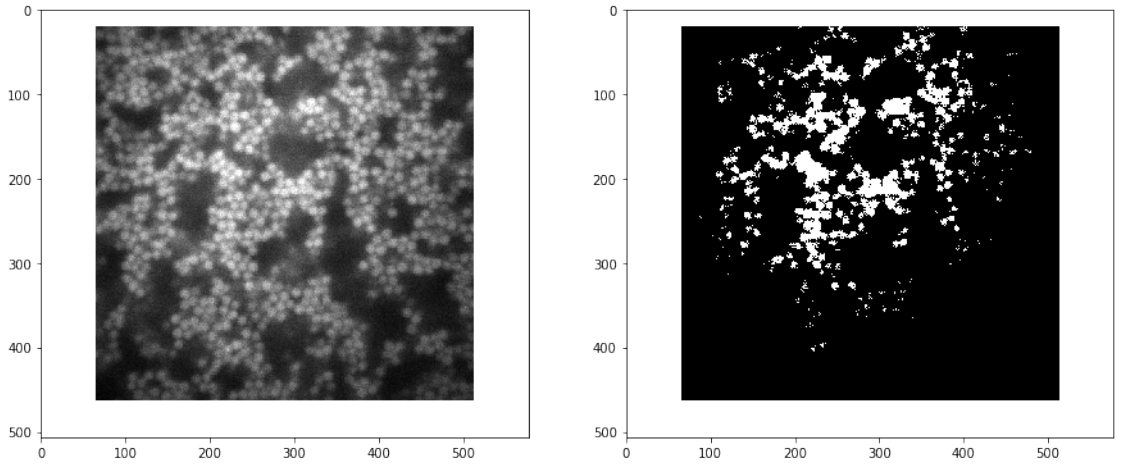
```
[9]: kmeans = KMeans(n_clusters=2, random_state=0, verbose=False).fit(intensities)
     K = kmeans.cluster_centers_.mean()
```

```
[10]: intensities.hist()
      plt.axvline(K, color='r')
      list(map(lambda x: plt.axvline(x, color='g'), kmeans.cluster_centers_))
      _ = plt.title(f"Means = {kmeans.cluster_centers_.tolist()}, K = {K}", size=16)
```



```
[11]: thresh1 = cv.threshold(img, K, 255, cv.THRESH_BINARY)[1]
```

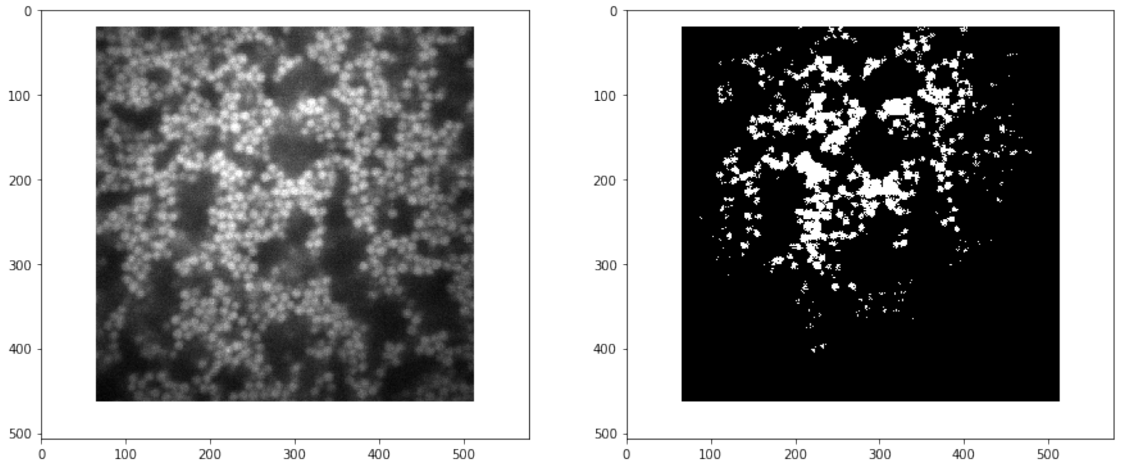
```
[12]: utils.side_by_side(img, thresh1)
```



Como podemos ver, una técinca de umbralización estándar como k-medias móviles, con dos medias, da resultados muy pobres.

```
[13]: otsu1 = cv.threshold(img,0,255,cv.THRESH_BINARY+cv.THRESH_OTSU)[1]
```

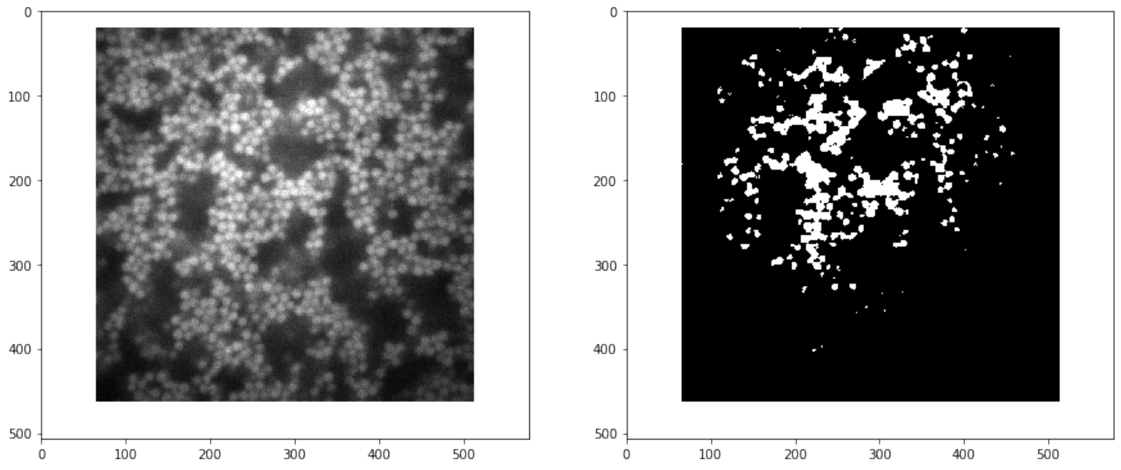
```
[14]: utils.side_by_side(img, otsu1)
```



El algoritmo de Otsu no logra mejorar mucho la segmentación (esto era de esperarse dado que el histograma original era claramente bimodal).

```
[15]: gblur = cv.GaussianBlur(img,(3,3),0)
      otsu2 = cv.threshold(gblur,0,255,cv.THRESH_BINARY+cv.THRESH_OTSU)[1]
```

```
[16]: utils.side_by_side(img, otsu2)
```



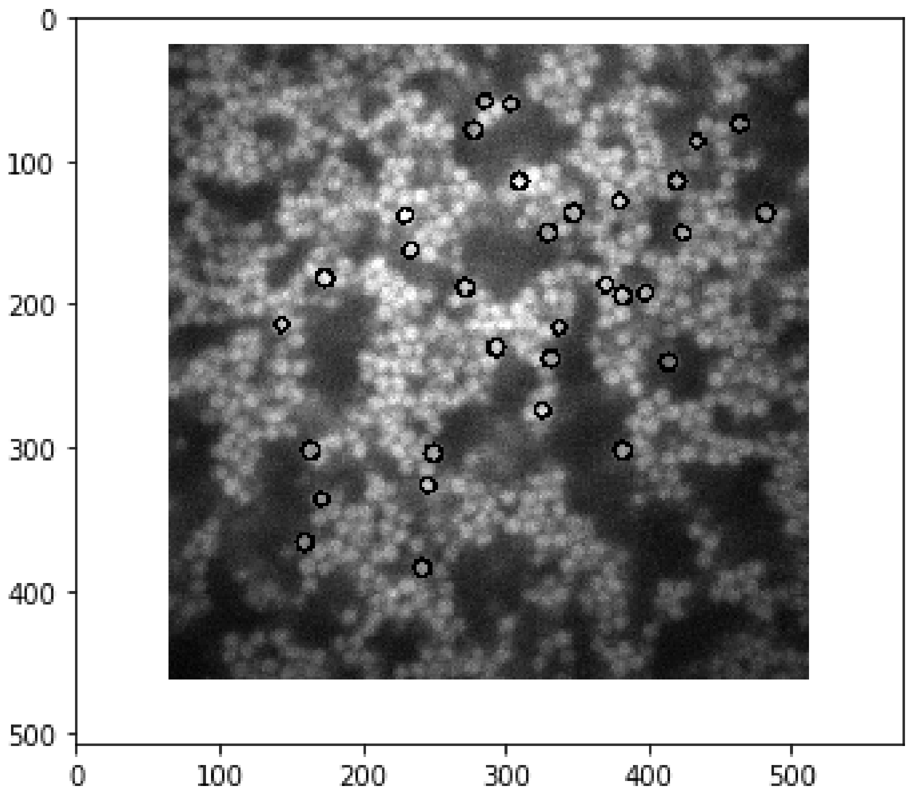
El algoritmo de Otsu no logra mejorar mucho la segmentación aún en combinación con un suavizado Gaussiano.

```
[17]: img_blur = cv.medianBlur(img, 5)
      circles = cv.HoughCircles(img_blur, cv.HOUGH_GRADIENT, 1, img.shape[0]/64,␣
       ↪param1=200, param2=10, minRadius=5, maxRadius=7)
```

```
[18]: if circles is not None:
          circles = np.uint16(np.around(circles))
          for i in circles[0, :]:
              cv.circle(img, (i[0], i[1]), i[2], (0, 0, 255), 2)
```

```
[19]: plt.imshow(img, cmap='gray')
```

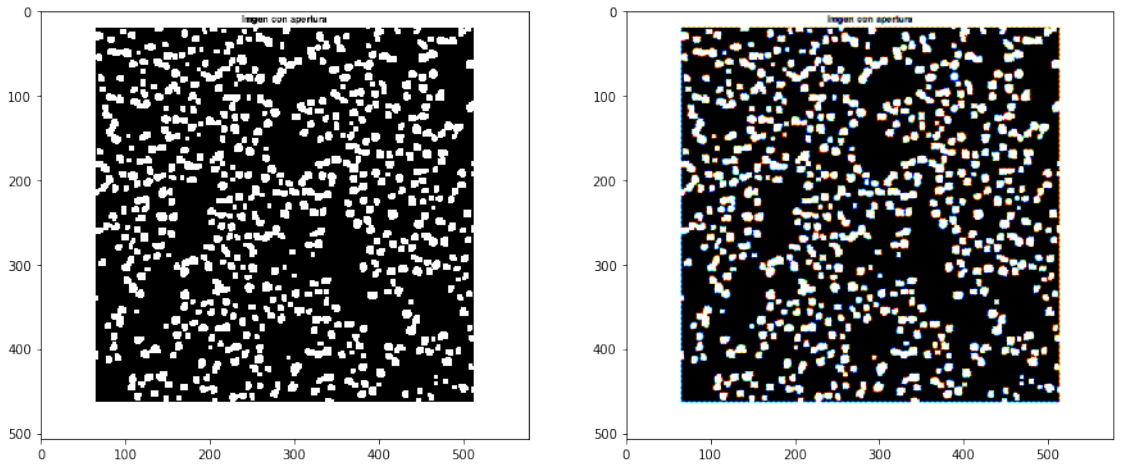
```
[19]: <matplotlib.image.AxesImage at 0x1c26f285d0>
```



Al ver que las células tenían una apariencia más o menos circular, parecía una buena idea usar una transformada de Hough para buscar los círculos de la imagen, pero esto entregó resultados muy pobres. Tal vez esto podría funcionar con la imagen binaria.

1.2 b. (0.5 puntos) A la imagenoriginal se le aplicó una umbralización con valores locales yal resultado se le realizó una apertura morbológica obteniendo la imagen Ex3Preg6(b).tif. Usando esta imagen,cuente y etiquete cuantos objetos de la segmentación pueden considerarse células independientes.

[20]:
```
imgb  = cv.imread('imagenes/Ex3Preg6(b).tif', cv.IMREAD_GRAYSCALE)
imgbc = cv.cvtColor(imgb, cv.COLOR_BAYER_GB2RGB)
utils.side_by_side(imgb, imgbc)
```



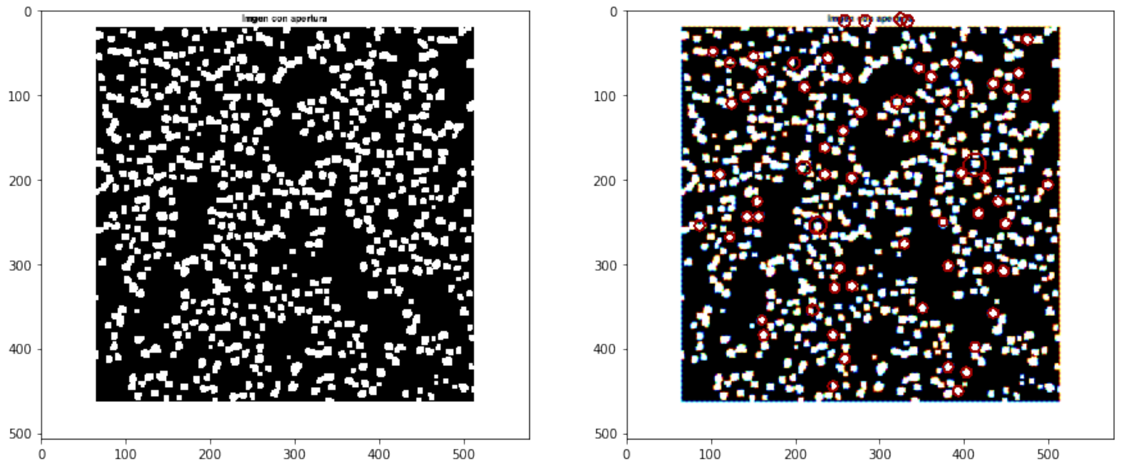
1.2.1 Primera aproximación :

El uso de la transformada de Hough para círculos, no para líneas. Al ver la imagen, uno podría pensar que un círculo es una buena aproximación de la forma de una célula, por lo tanto los cículos encontrados por una transformada de Hough serían las células que buscamos identificar, caracterizar y contabilizar

[21]:
```
circles = cv.HoughCircles(imgb, cv.HOUGH_GRADIENT, 1, img.shape[0]/64,␣
↪param1=200, param2=10, minRadius=5, maxRadius=15)
```

[22]:
```
if circles is not None:
    circles = np.uint16(np.around(circles))
    for i in circles[0, :]:
        cv.circle(imgbc, (i[0], i[1]), i[2], (155, 0, 0), 2)
```

[23]:
```
utils.side_by_side(imgb, imgbc)
```

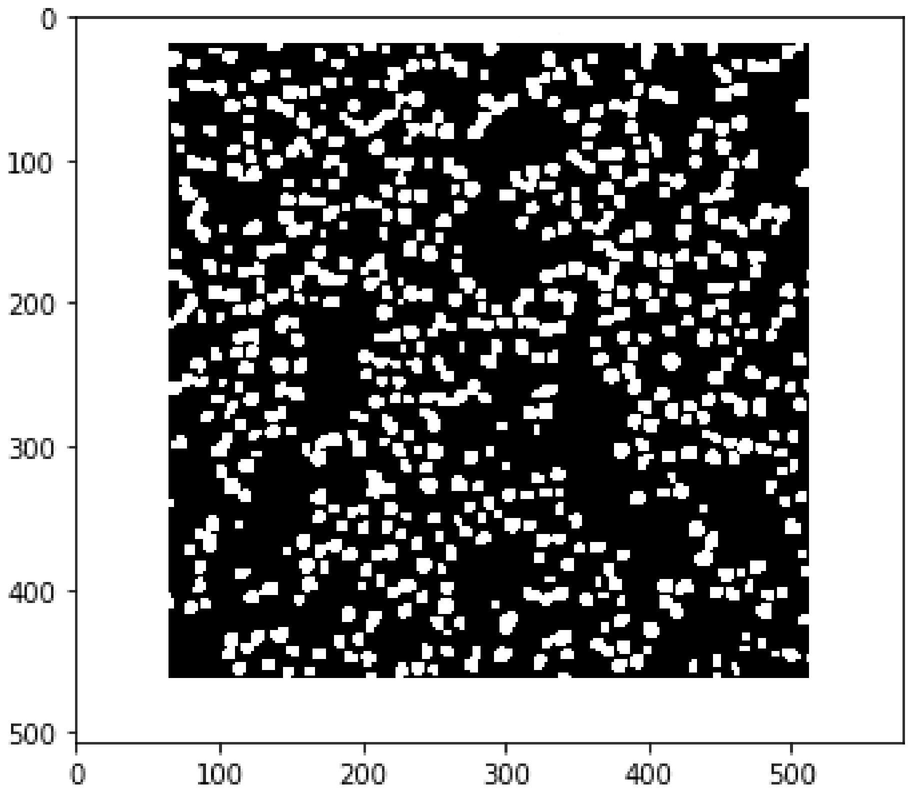


Aquí podemos ver que aunque la imagen principal carece de falsos positivos (es decir todos los círculos dibujados dentro de la región útil de la imagen contienen una célula) el **número de falsos negativos es altísimo** : sólo una pequeña parte de las células observadas fueron identificadas por `cv.HoughCircles()`.

Esto nos indica que tal vez las células no se asemejan tanto a un círculo. Por esta razón, no se explorará más a fondo esta vía de acción. Cabe mencionar que la transformada encuentra círculos en el texto de encabezado : **Imagen con apertura**. Por esta razón, en delante se trabajará con otra imagen recortada a mano para excluir este texto que podría causar problemas en la segmentación más adelante.

[28]:
```
imgb2  = cv.imread('imagenes/Ex3Preg6(b)3.tif', cv.IMREAD_GRAYSCALE)
plt.imshow(imgb2, cmap='gray')
```

[28]:
```
<matplotlib.image.AxesImage at 0x1c274d78d0>
```



[34]:
```
sns.distplot(imgb2.flatten())
```

[34]:
```
<matplotlib.axes._subplots.AxesSubplot at 0x1c28096bd0>
```

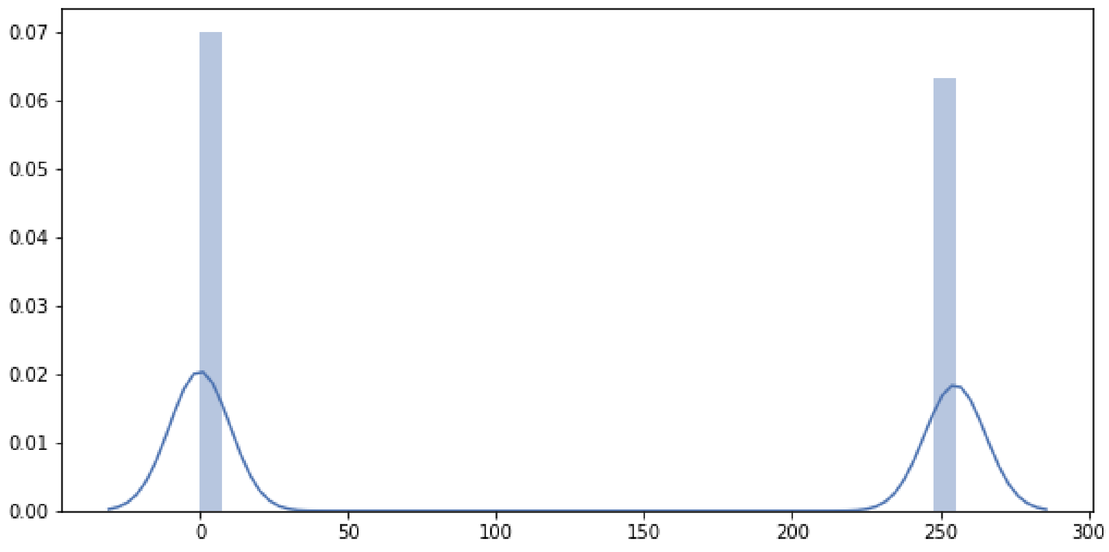
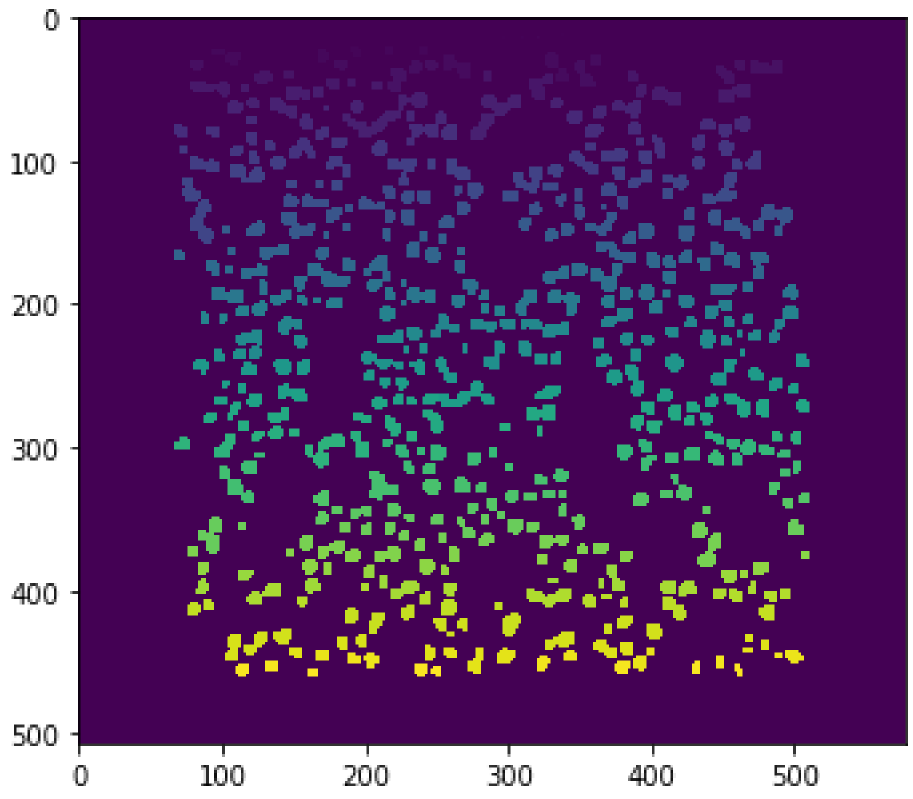


Imagen claramente binaria.

[29]:
```
label_image, n_objs = label(imgb2, return_num=True)
plt.imshow(label_image)
print(n_objs)
```

```
475
```

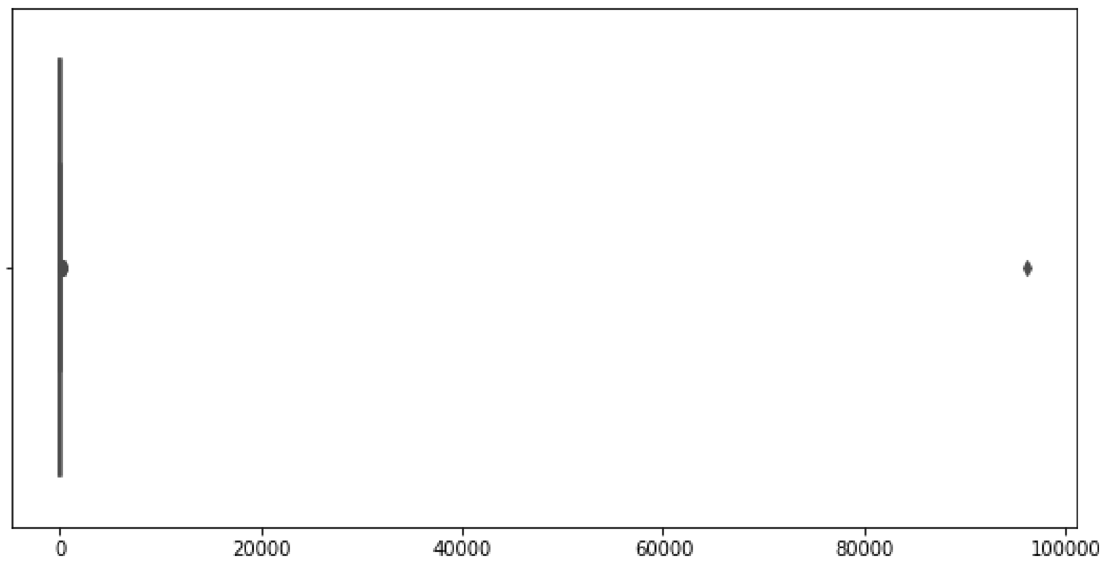


[74]:
```
objs = regionprops(label_image)
```

[75]:
```
areas = pd.core.frame.DataFrame({
    'area': map(lambda x: x.area, objs)
})
```

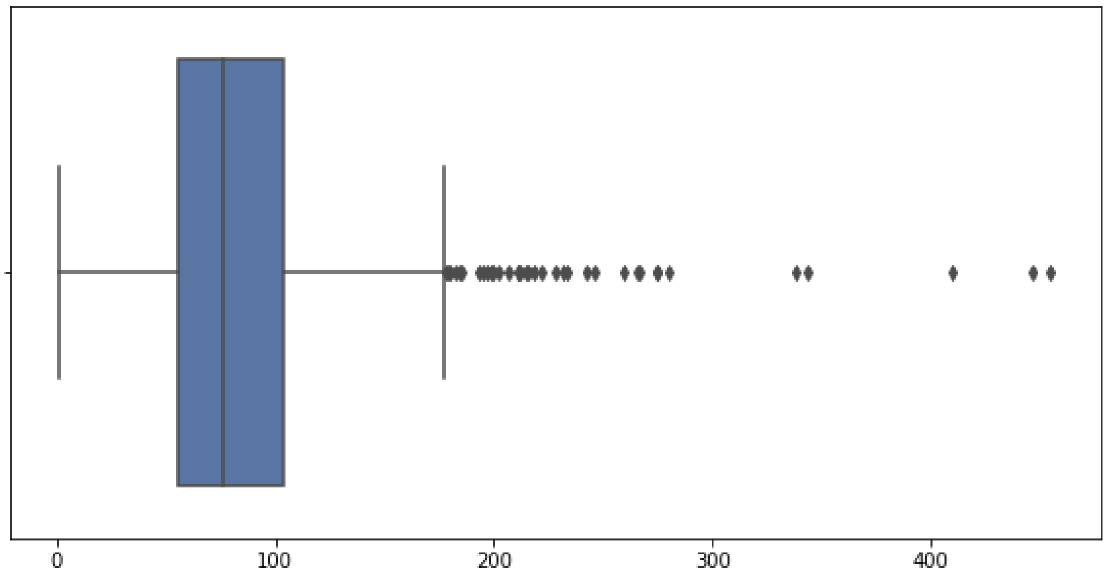
[76]:
```
areas.describe(), sns.boxplot(areas)
```

[76]:
```
(                area
 count    475.000000
 mean     292.715789
 std     4409.522293
 min        1.000000
 25%       55.500000
 50%       76.000000
 75%      104.000000
 max    96185.000000, <matplotlib.axes._subplots.AxesSubplot at 0x1c29e3b110>)
```



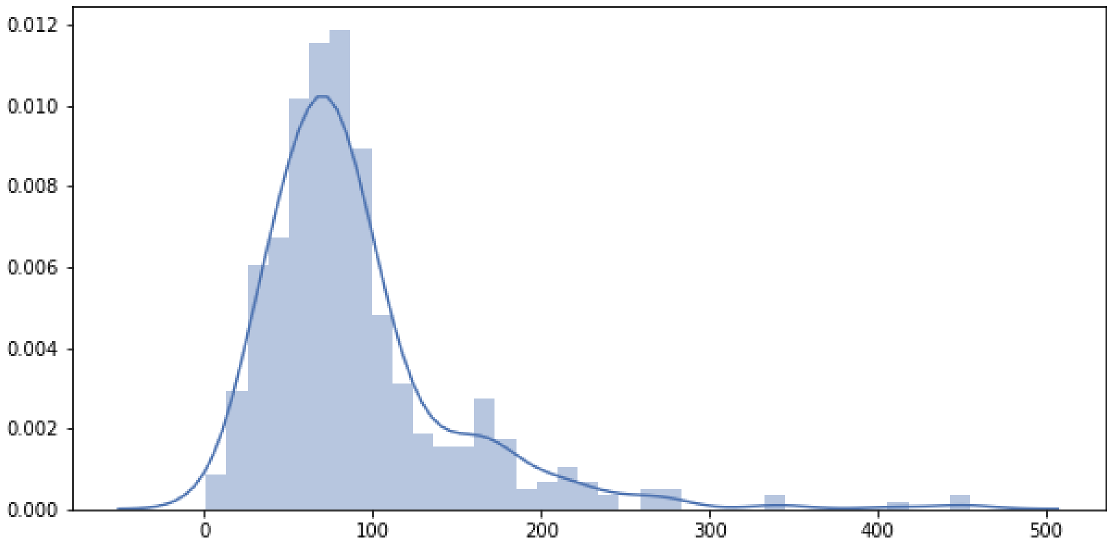
[77]:
```
areas2 = areas[ areas.area != areas.area.max() ]
areas2.describe(), sns.boxplot(areas2)
```

[77]:
```
(              area
 count  474.000000
 mean    90.411392
 std     59.468520
 min      1.000000
 25%     55.250000
 50%     76.000000
 75%    104.000000
 max    455.000000, <matplotlib.axes._subplots.AxesSubplot at 0x1c302ac450>)
```



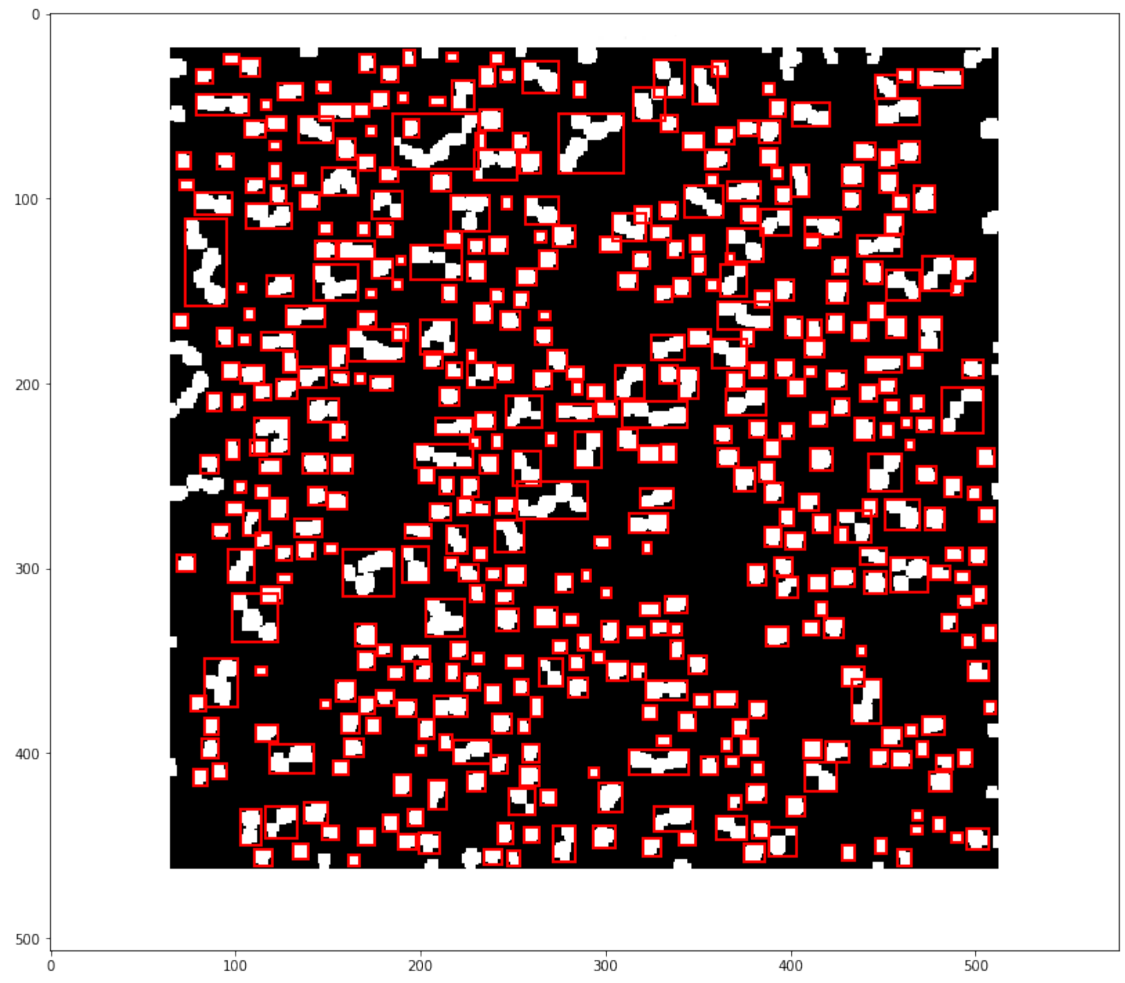
```
[78]: sns.distplot(areas2)
```

```
[78]: <matplotlib.axes._subplots.AxesSubplot at 0x1c30260490>
```



```
[93]: #image_label_overlay = label2rgb(label_image, image=imgb2)

fig, ax = plt.subplots(figsize=(15, 10))
ax.imshow(imgb2, cmap='gray')
```

```python
for region in objs:
    # take regions with large enough areas
    if region.area >= 10 and region.area < areas.area.max():
        # draw rectangle around segmented cells
        minr, minc, maxr, maxc = region.bbox
        rect = mpatches.Rectangle((minc, minr), maxc - minc, maxr - minr,
                                  fill=False, edgecolor='red', linewidth=2)
        ax.add_patch(rect)

#ax.set_axis_off()
plt.tight_layout()
plt.show()
```



[88]:

```
Help on method imshow in module matplotlib.axes._axes:
```

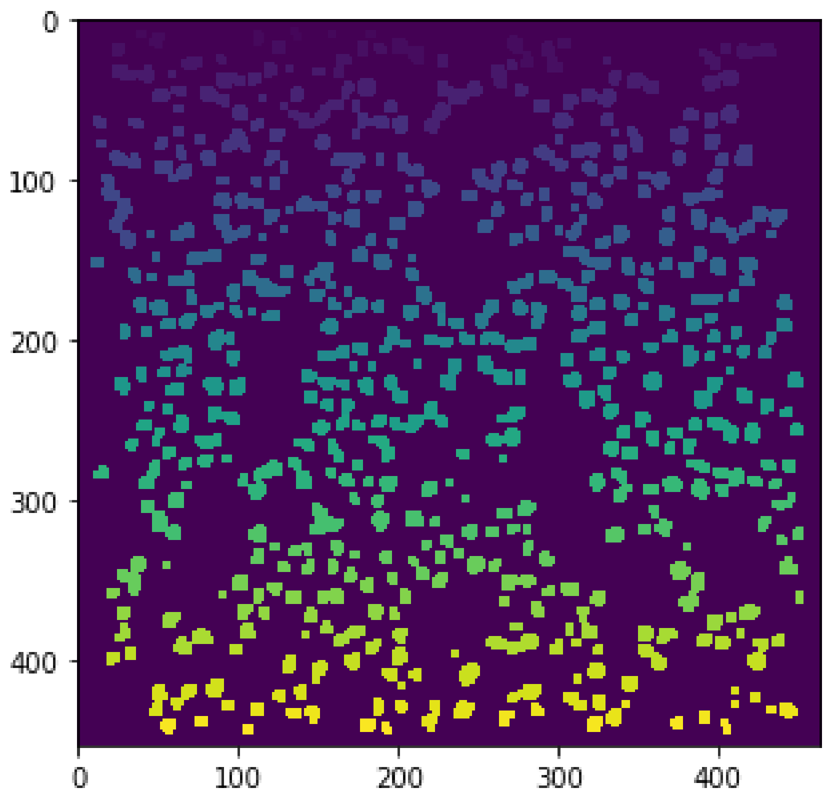
```
imshow(X, cmap=None, norm=None, aspect=None, interpolation=None, alpha=None,
vmin=None, vmax=None, origin=None, extent=None, shape=<deprecated parameter>,
filternorm=1, filterrad=4.0, imlim=<deprecated parameter>, resample=None,
url=None, *, data=None, **kwargs) method of
matplotlib.axes._subplots.AxesSubplot instance
    Display an image, i.e. data on a 2D regular raster.

    Parameters
    ----------
    X : array-like or PIL image
        The image data. Supported array shapes are:

        - (M, N): an image with scalar data. The data is visualized
          using a colormap.
        - (M, N, 3): an image with RGB values (0-1 float or 0-255 int).
        - (M, N, 4): an image with RGBA values (0-1 float or 0-255 int),
          i.e. including transparency.

        The first two dimensions (M, N) define the rows and columns of
        the image.

        Out-of-range RGB(A) values are clipped.

    cmap : str or `~matplotlib.colors.Colormap`, optional
        The Colormap instance or registered colormap name used to map
        scalar data to colors. This parameter is ignored for RGB(A) data.
        Defaults to :rc:`image.cmap`.

    norm : `~matplotlib.colors.Normalize`, optional
        The `Normalize` instance used to scale scalar data to the [0, 1]
        range before mapping to colors using *cmap*. By default, a linear
        scaling mapping the lowest value to 0 and the highest to 1 is used.
        This parameter is ignored for RGB(A) data.

    aspect : {'equal', 'auto'} or float, optional
        Controls the aspect ratio of the axes. The aspect is of particular
        relevance for images since it may distort the image, i.e. pixel
        will not be square.

        This parameter is a shortcut for explicitly calling
        `.Axes.set_aspect`. See there for further details.

        - 'equal': Ensures an aspect ratio of 1. Pixels will be square
          (unless pixel sizes are explicitly made non-square in data
          coordinates using *extent*).
        - 'auto': The axes is kept fixed and the aspect is adjusted so
          that the data fit in the axes. In general, this will result in
          non-square pixels.
        If not given, use :rc:`image.aspect` (default: 'equal').

    interpolation : str, optional
        The interpolation method used. If *None*
        :rc:`image.interpolation` is used, which defaults to 'nearest'.

        Supported values are 'none', 'nearest', 'bilinear', 'bicubic',
        'spline16', 'spline36', 'hanning', 'hamming', 'hermite', 'kaiser',
        'quadric', 'catrom', 'gaussian', 'bessel', 'mitchell', 'sinc',
        'lanczos'.

        If *interpolation* is 'none', then no interpolation is performed
        on the Agg, ps, pdf and svg backends. Other backends will fall back
        to 'nearest'. Note that most SVG renders perform interpolation at
        rendering and that the default interpolation method they implement
        may differ.

        See
        :doc:`/gallery/images_contours_and_fields/interpolation_methods`
        for an overview of the supported interpolation methods.

        Some interpolation methods require an additional radius parameter,
        which can be set by *filterrad*. Additionally, the antigrain image
        resize filter is controlled by the parameter *filternorm*.

    alpha : scalar, optional
        The alpha blending value, between 0 (transparent) and 1 (opaque).
        This parameter is ignored for RGBA input data.

    vmin, vmax : scalar, optional
        When using scalar data and no explicit *norm*, *vmin* and *vmax*
        define the data range that the colormap covers. By default,
        the colormap covers the complete value range of the supplied
        data. *vmin*, *vmax* are ignored if the *norm* parameter is used.

    origin : {'upper', 'lower'}, optional
        Place the [0,0] index of the array in the upper left or lower left
        corner of the axes. The convention 'upper' is typically used for
        matrices and images.
        If not given, :rc:`image.origin` is used, defaulting to 'upper'.

        Note that the vertical axes points upward for 'lower'
        but downward for 'upper'.

    extent : scalars (left, right, bottom, top), optional
        The bounding box in data coordinates that the image will fill.
        The image is stretched individually along x and y to fill the box.
        The default extent is determined by the following conditions.
        Pixels have unit size in data coordinates. Their centers are on
        integer coordinates, and their center coordinates range from 0 to
        columns-1 horizontally and from 0 to rows-1 vertically.

        Note that the direction of the vertical axis and thus the default
        values for top and bottom depend on *origin*:

        - For ``origin == 'upper'`` the default is
          ``(-0.5, numcols-0.5, numrows-0.5, -0.5)``.
        - For ``origin == 'lower'`` the default is
          ``(-0.5, numcols-0.5, -0.5, numrows-0.5)``.

        See the example :doc:`/tutorials/intermediate/imshow_extent` for a
        more detailed description.

    filternorm : bool, optional, default: True
        A parameter for the antigrain image resize filter (see the
        antigrain documentation).  If *filternorm* is set, the filter
        normalizes integer values and corrects the rounding errors. It
        doesn't do anything with the source floating point values, it
        corrects only integers according to the rule of 1.0 which means
        that any sum of pixel weights must be equal to 1.0.  So, the
        filter function must produce a graph of the proper shape.

    filterrad : float > 0, optional, default: 4.0
        The filter radius for filters that have a radius parameter, i.e.
        when interpolation is one of: 'sinc', 'lanczos' or 'blackman'.

    resample : bool, optional
        When *True*, use a full resampling method.  When *False*, only
        resample when the output image is larger than the input image.

    url : str, optional
        Set the url of the created `.AxesImage`. See `.Artist.set_url`.

    Returns
    -------
    image : `~matplotlib.image.AxesImage`

    Other Parameters
    ----------------
    **kwargs : `~matplotlib.artist.Artist` properties
        These parameters are passed on to the constructor of the
        `.AxesImage` artist.

    See also
```

```
    --------
    matshow : Plot a matrix or an array as an image.

    Notes
    -----
    Unless *extent* is used, pixel centers will be located at integer
    coordinates. In other words: the origin will coincide with the center
    of pixel (0, 0).

    There are two common representations for RGB images with an alpha
    channel:

    -   Straight (unassociated) alpha: R, G, and B channels represent the
        color of the pixel, disregarding its opacity.
    -   Premultiplied (associated) alpha: R, G, and B channels represent
        the color of the pixel, adjusted for its opacity by multiplication.

    `~matplotlib.pyplot.imshow` expects RGB images adopting the straight
    (unassociated) alpha representation.

    .. note::
        In addition to the above described arguments, this function can take a
        **data** keyword argument. If such a **data** argument is given, the
        following arguments are replaced by **data[<arg>]**:

        * All positional and all keyword arguments.

        Objects passed as **data** must support item access (``data[<arg>]``)
and
        membership test (``<arg> in data``).
```

2 Extra

[26]:
```python
label_image, n_objs = label(imgb2, return_num=True)
plt.imshow(label_image)
print(n_objs)
```

```
477
```

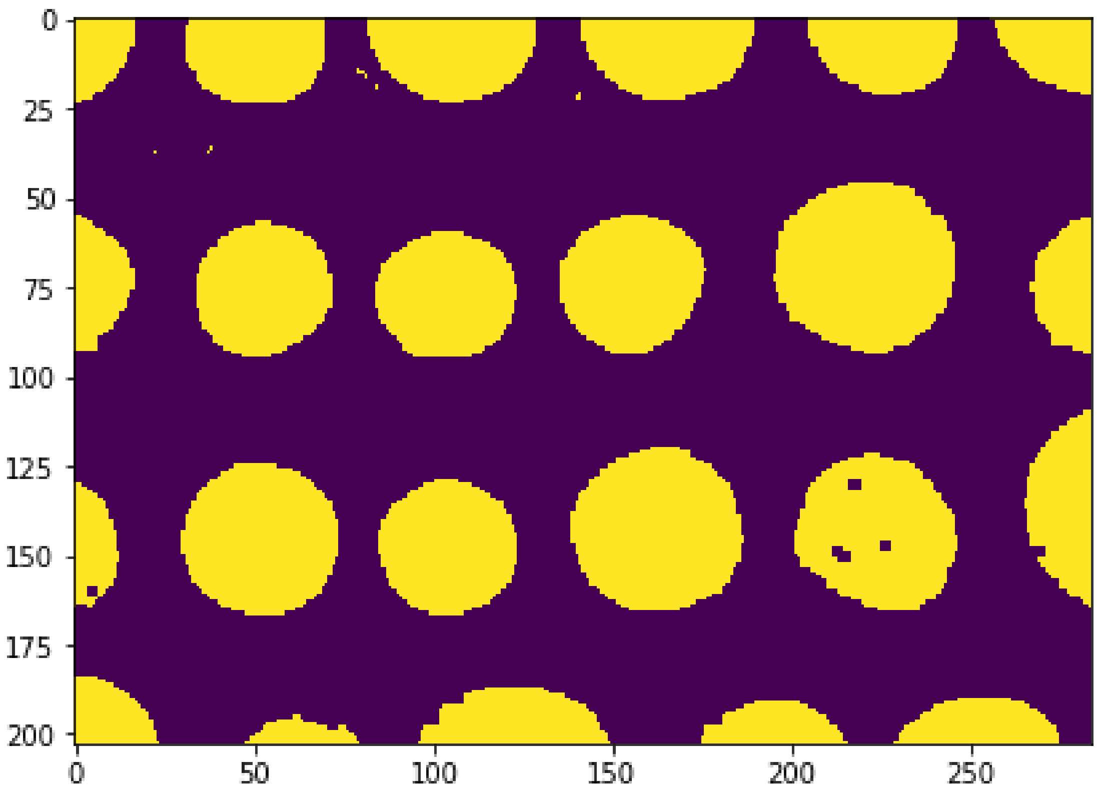


```
[ ]: cleared = clear_border()
     plt.imshow(cleared)
```

3 Scikit-Image example :

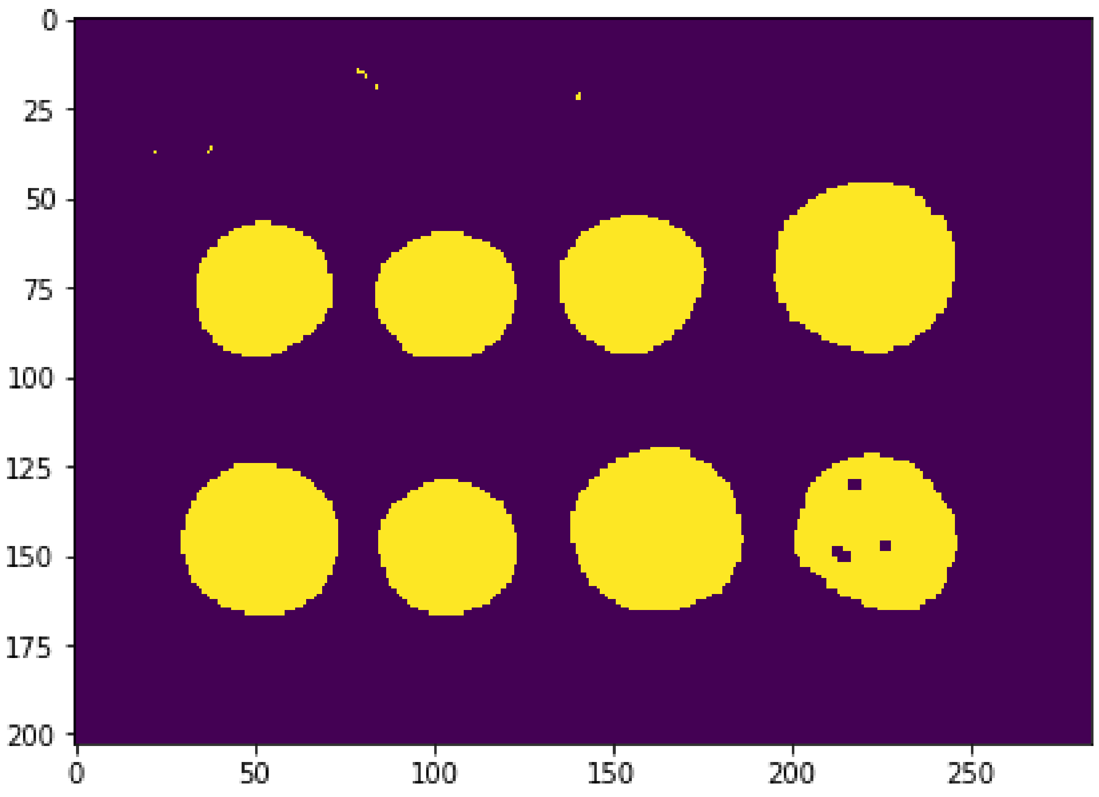
```
[151]: image = data.coins()[50:-50, 50:-50]

       # apply threshold
       thresh = threshold_otsu(image)
       bw = closing(image > thresh, square(3))
       plt.imshow(bw)
```

```
[151]: <matplotlib.image.AxesImage at 0x1c2ff27b90>
```



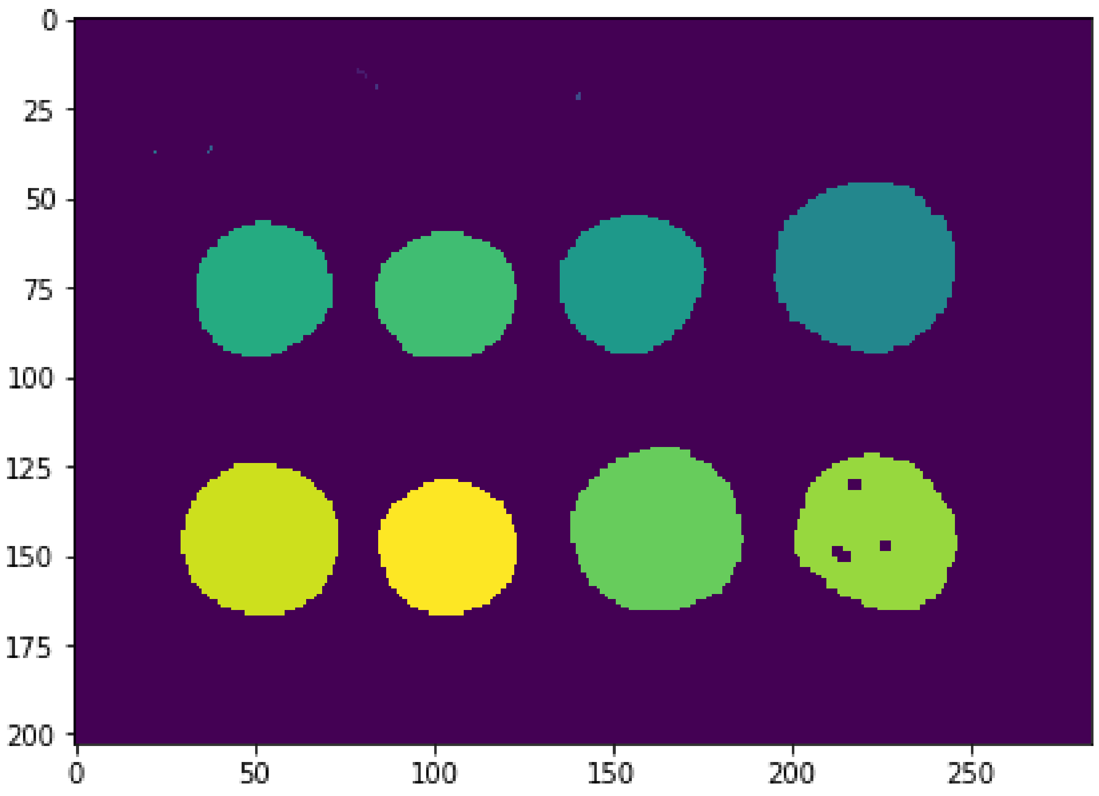
```
[152]: # remove artifacts connected to image border
       cleared = clear_border(bw)
       plt.imshow(cleared)
```

```
[152]: <matplotlib.image.AxesImage at 0x1c32f9a690>
```

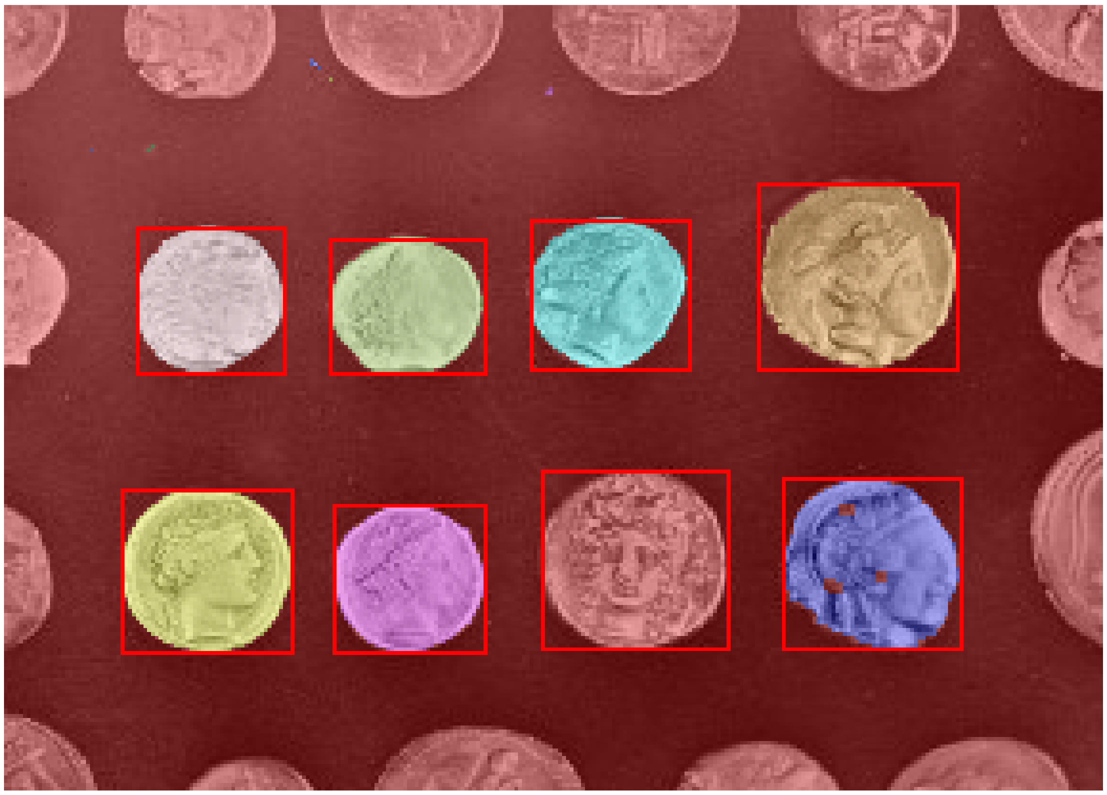


[154]:
```
# label image regions
label_image = label(cleared)
plt.imshow(label_image)
```

[154]:
```
<matplotlib.image.AxesImage at 0x1c31283190>
```



[155]:
```
image_label_overlay = label2rgb(label_image, image=image)

fig, ax = plt.subplots(figsize=(10, 6))
ax.imshow(image_label_overlay)

for region in regionprops(label_image):
    # take regions with large enough areas
    if region.area >= 100:
        # draw rectangle around segmented coins
        minr, minc, maxr, maxc = region.bbox
        rect = mpatches.Rectangle((minc, minr), maxc - minc, maxr - minr,
                                  fill=False, edgecolor='red', linewidth=2)
        ax.add_patch(rect)

ax.set_axis_off()
plt.tight_layout()
plt.show()
```



[147]:
```
#help(cv2.HoughCircles)
```

[148]:
```
#help(cv2.cvtColor)
```

[158]:
```
help(label)
```

```
Help on function label in module skimage.measure._label:

label(input, neighbors=None, background=None, return_num=False,
connectivity=None)
    Label connected regions of an integer array.

    Two pixels are connected when they are neighbors and have the same value.
    In 2D, they can be neighbors either in a 1- or 2-connected sense.
    The value refers to the maximum number of orthogonal hops to consider a
    pixel/voxel a neighbor::

      1-connectivity     2-connectivity     diagonal connection close-up

           [ ]           [ ]  [ ]  [ ]             [ ]
            |               \  |  /                 |  <- hop 2
      [ ]--[x]--[ ]      [ ]--[x]--[ ]        [x]--[ ]
```

```
        |              /  |  \            hop 1
       [ ]          [ ]  [ ]  [ ]

Parameters
----------
input : ndarray of dtype int
    Image to label.
neighbors : {4, 8}, int, optional
    Whether to use 4- or 8-"connectivity".
    In 3D, 4-"connectivity" means connected pixels have to share face,
    whereas with 8-"connectivity", they have to share only edge or vertex.
    **Deprecated, use** ``connectivity`` **instead.**
background : int, optional
    Consider all pixels with this value as background pixels, and label
    them as 0. By default, 0-valued pixels are considered as background
    pixels.
return_num : bool, optional
    Whether to return the number of assigned labels.
connectivity : int, optional
    Maximum number of orthogonal hops to consider a pixel/voxel
    as a neighbor.
    Accepted values are ranging from  1 to input.ndim. If ``None``, a full
    connectivity of ``input.ndim`` is used.

Returns
-------
labels : ndarray of dtype int
    Labeled array, where all connected regions are assigned the
    same integer value.
num : int, optional
    Number of labels, which equals the maximum label index and is only
    returned if return_num is `True`.

See Also
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regionprops

References
----------
.. [1] Christophe Fiorio and Jens Gustedt, "Two linear time Union-Find
       strategies for image processing", Theoretical Computer Science
       154 (1996), pp. 165-181.
.. [2] Kensheng Wu, Ekow Otoo and Arie Shoshani, "Optimizing connected
       component labeling algorithms", Paper LBNL-56864, 2005,
       Lawrence Berkeley National Laboratory (University of California),
       http://repositories.cdlib.org/lbnl/LBNL-56864

Examples
```

```
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>>> import numpy as np
>>> x = np.eye(3).astype(int)
>>> print(x)
[[1 0 0]
 [0 1 0]
 [0 0 1]]
>>> print(label(x, connectivity=1))
[[1 0 0]
 [0 2 0]
 [0 0 3]]
>>> print(label(x, connectivity=2))
[[1 0 0]
 [0 1 0]
 [0 0 1]]
>>> print(label(x, background=-1))
[[1 2 2]
 [2 1 2]
 [2 2 1]]
>>> x = np.array([[1, 0, 0],
…                 [1, 1, 5],
…                 [0, 0, 0]])
>>> print(label(x))
[[1 0 0]
 [1 1 2]
 [0 0 0]]
```

```
[ ]:
```