

Evaluating and Optimizing Machine Learning Techniques for Automatic Nuclei Detection

1. Motivation

Many people die from cancer every year. This is terribly sad. :(We would like to prevent this. One option is to identify the cancer early; treatment of nascent cancers tends to be more successful. Often, cells suffering from cancer display different biomarkers in their nuclei. These biomarkers can be seen in immunohistochemical (IHC) imaging. If there were a rapid and accurate way to screen images for cancerous cells, many cancer prognoses could be improved. We aim to develop an automated cell nuclei detection technique.

2. Challenges

Imaging conditions can vary tremendously - illumination, contrast, fluorescence and staining will all affect the appearance of the cell. We would also like our technique to be generalizable to many cell types because cancer is insidious and affects many cell types. Depending on the biological sample that was imaged, cells may also be aggregated; where a trained eye could distinguish individual nuclei, an algorithm might falter.

3. Pre-treatment and Initial Segmentation (“Detection”)

Colour is usually either normalized or thresholded to remove noise and background [1]. A variety of pre-processing techniques can be used to find objects, from conventional blob detection, to morphological and/or contour resolution, to watershed segmentation [2-4]. We will use sensible discretion to pick a pre-treatment technique.

4. Segmentation and Identification

There are two primary approaches to cell segmentation:

Traditional methods segment nuclei from single or overlapping cells

Several traditional methods, often involving a-priori knowledge of cell shape and size [5]. First, cell clusters are segmented from the background by concavity [6-7]. Next, cell clusters can be separated into individual cells based on the concavity of the intensity distribution [7]. After individual cells have been identified, cell boundaries are often approximated using elliptical curve-fitting techniques [5,7]. Further segmentation can be applied to separate the cell nucleus from the cytoplasm. A gradient vector flow active contour model (GVF-ACM) has been shown to find boundaries between the nucleus and cytoplasm [8].

In this work, we can begin performing segmentation using the scikit-image package for python. This package includes methods for ellipse and boundary fitting, as well as edge detection and active contour modeling.

Machine learning methods identify nuclei via classification algorithms

Machine learning and pattern recognition have been successfully used to identify and segment cells in IHC images [9]. Whether using techniques such as cluster analysis [10], random forests [9], or deep neural networks [11-13], the workflow is similar. First, initial segmentation is performed (often in the pre-treatment step) to find cells and agglomerates. Then, training data is fed through a classifier to extract the most important features. Repeating this process while keeping only the most important feature vectors establishes a model, which is finally used to classify new test data.

We will start with cluster analysis and random forest classifiers (from the scikit-learn package), but likely will also employ traditional GVF or ellipse-fitting algorithms (which we will implement in python) to refine initial segmentation. If necessary, we also intend to look into convolutional neural networks implemented with the TensorFlow package.

References

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- [13] N. Kumar, R. Verma, S. Sharma, S. Bhargava, A. Vahadane, and A. Sethi. **(2017)** A Dataset and a

```
In [6]: ## change the following to where you have stored and/or extracted the files
path = './project/'
# path = '/Users/arrakis/Dropbox/Tool - Classes/ChBE 8803/Project'

n_samples = 3 #/665 total datasets in train1

#####
```

UPDATED 3/28 ideas for future work

Updates (3/29 4 am)

- KMEANS clustering to separate into different cell/conditions type - will fix tomorrow (tired)
- Watershed performance metrics calculated (comparable to the random forest now)
- SVM classifier for detection (slow to train) - took 30 min and wasn't finished so I removed it(same Xtrain, Ytrain as random forest)
- Random forest classifier using HSV instead of RGB values
- Example of an active contour model, but so far it seems too slow to be practical
- Plan to add chan-veese level set segmentation

Detection

- try splitting three channels as HSV instead of RGB
- do dimensional reduction / PCA on all training set images to parse into different cell/conditions type, then threshold each one with best method for group

Segmentation

Package Requirements

- numpy version 1.13.3
- pandas version 0.20.3
- matplotlib version
- sklearn version 0.19.1
- skimage version 0.13.0
- cv2 (used for image processing)
 - See lines 1-3 in the next block for installation on mac (version 3.2.0.6)
 - For PC, installation worked with pip install opencv-python from the anaconda prompt (version 3.4.0)

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In [49]: #import sys
        #!{sys.executable} -m pip install opencv-python==3.2.0.6 # for mac
        # >> https://stackoverflow.com/questions/47963386/image-not-found-error-after-installing-opencv-python-wheel-on-mac

        ## Load all packages used below
        from skimage.color import rgb2gray
        from skimage.filters import threshold_otsu
        import zipfile, io
        import numpy as np
        import pandas as pd
        import pylab as plt
        import sklearn, cv2
        import matplotlib.image as mpimg
        from scipy import ndimage
        from scipy.ndimage import label
        from skimage import feature
        from skimage.filters import sobel, laplace
        from skimage.morphology import watershed
        from sklearn import linear_model
        from sklearn import svm
        from sklearn.metrics import confusion_matrix
        from sklearn.model_selection import train_test_split
        from sklearn.ensemble import RandomForestRegressor
```

Loading Images and Masks from Training Set

- load_zipped_img takes an image and set of masks from the .zip
- A working example of extracting an individual image and associated masks is shown below

```

In [8]: ## STEP 1: Load an image (by index) and corresponding masks from ZIPPED stage1_train as np array

def load_zipped_img(path, img_index): # Load an image and all its masks
    z = zipfile.ZipFile(path, 'r') # access zip folder
    zlist = z.namelist() # list of files in zip folder

    img_name = zlist[img_index] # get selected image
    img_name = img_name[0:-1] # eliminate "/"

    # get image and return as np array
    img_raw = z.read('{}images/{}.png'.format(img_name, img_name)) # get raw image
    img = io.BytesIO(img_raw) # convert image
    img = mpimg.imread(img) # numpy array
    img = np.flip(img, 0) # flip image

    # get all masks and return as np array
    mask_list = []
    for string in zlist:
        if string.startswith(img_name + '/mask'):
            mask_list.append(string)
    mask_list = mask_list[1:-1] # list of masks

    masks = []
    for m in mask_list:
        mask_raw = z.read(m) # get raw mask
        mask = io.BytesIO(mask_raw) # convert mask
        mask = mpimg.imread(mask) # numpy array
        mask = np.flip(mask, 0) # flip mask
        masks.append(mask)

    return img, masks

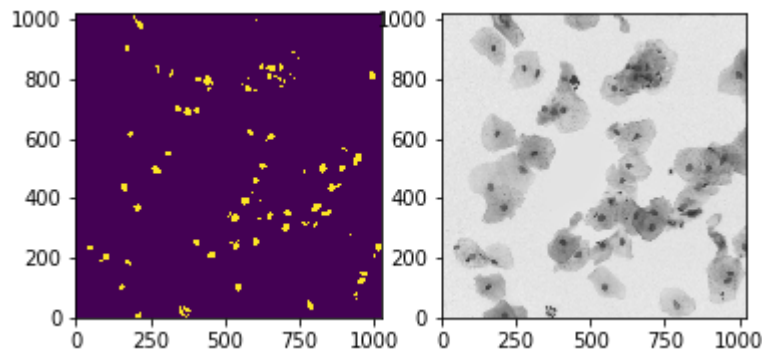
# WORKING EXAMPLE OF load_zipped_img

(img, masks) = load_zipped_img(path + '/stage1_train.zip', 177) # The one example that the model performs poorly for

fig, ax = plt.subplots(1, 2)

ax[0].imshow(sum(masks), origin='lower')
ax[1].imshow(img, origin='lower')
plt.show()

```



Detection: Separating Object from Background

Before determining the positions of nuclei in an image, it is key to separate the image from the background using a process called image segmentation. Image segmentation is typically performed on grayscale images.

- The `grayscale` function converts images from `rgb` to `grayscale`
- The `otsu` function selects an optimal threshold for equal inter-/intra-class variance
- The function `float2int8` converts the data type to `int8`, which is required for the `cv2` package
- The function `watershed` applies watershed segmentation to a given image to determine objects and background with the following outputs
 - `img_guess`: masked objects identified by watershed segmentation
 - `sure_bg`: background pixels identified with high confidence
 - `sure_fg`: object pixels identified with high confidence
 - `uncertain`: the region between `sure_bg` and `sure_fg` where pixels are identified with low confidence

```
In [9]: # convert to grayscale
def grayscale(im):
    return rgb2gray(im)

# Otsu's Method, calculates optimal threshold for equal inter-/intra-class variance
def otsu(image_gray):
    threshold_val = threshold_otsu(image_gray) #Select threshold from Otsu's method
    img_masked = np.where(image_gray > threshold_val, 1, 0)

    if np.sum(img_masked==0) < np.sum(img_masked==1):
        img_masked = np.where(img_masked, 0, 1)
    return img_masked

# Function to convert float32 raw images to int8 single channel
def float2int8(img_float):
    img_int8 = (img_float * 255).round().astype(np.uint8)
    return img_int8
```

```

In [51]: # Function to watershed segment images
def watershed(img_float32):
    img = img_float32[:, :, :3]

    # convert input image (float32) to 3-channel int8
    ch1 = float2int8(otsu(img[:, :, 0:1][:, :, 0]))
    ch2 = float2int8(otsu(img[:, :, 1:2][:, :, 0]))
    ch3 = float2int8(otsu(img[:, :, 2:3][:, :, 0]))
    img_guess = cv2.merge([ch1, ch2, ch3])

    # greyscale and otsu threshold original image
    img_grey = grayscale(img)
    int8_thresh = float2int8(otsu(img_grey))

    # noise removal
    kernel = np.ones((3,3), np.uint8)
    opening = cv2.morphologyEx(int8_thresh, cv2.MORPH_OPEN, kernel, iterations=2)

    # find sure background area
    sure_bg = cv2.dilate(opening, kernel, iterations=3)

    # find sure foreground area
    dist_transform = cv2.distanceTransform(opening, cv2.DIST_L2, 5)
    ret, sure_fg = cv2.threshold(dist_transform, 0.2*dist_transform.max(), 255, 0
)
    sure_fg = np.uint8(sure_fg)

    # finding uncertain region
    uncertain = cv2.subtract(sure_bg, sure_fg)

    # marker labelling
    ret, markers = cv2.connectedComponents(sure_fg)
    markers = markers+1 # add one to all labels so sure background is 1 (not
0)
    markers[uncertain==255] = 0 # mark unknown region as 0

    # apply watershed and mark boundary as -1
    markers = cv2.watershed(img_guess, markers)
    img_guess[markers == -1] = [255, 0, 0]

    return img_guess, markers, sure_bg, sure_fg, uncertain

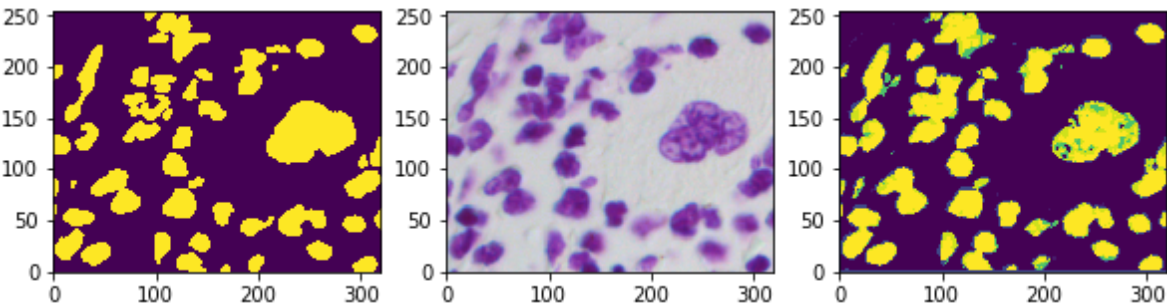
#### TESTING
(img, mask) = load_zipped_img(path+'/stage1_train.zip', 510)

img_guess, markers, sure_bg, sure_fg, uncertain = watershed(img)

fig, ax = plt.subplots(1,3, figsize = (10,10))

ax[0].imshow(sum(mask), origin='lower')
ax[1].imshow(img, origin='lower')
ax[2].imshow(grayscale(img_guess), origin='lower')
plt.show()

```

In [43]: *# Watershed Testing*

```
for i in range(0,560):
    (img, masks) = load_zipped_img(path+'/stage1_train.zip', i)

    (img_guess, markers, sure_bg, sure_fg, unknown) = watershed(img)
    yp = np.round(grayScale(img_guess)).reshape(-1,1)
    y_real = sum(masks).reshape(-1,1)
    accuracy = sklearn.metrics.accuracy_score(y_real, yp)
    precision = sklearn.metrics.precision_score(y_real, yp)
    f1 = sklearn.metrics.f1_score(y_real, yp)
    print(i, accuracy, precision, f1)
    acc.append([accuracy, precision, f1])

    #output = yp.reshape(shap[0], shap[1])

precision = np.mean(np.array(acc)[: ,1])
accuracy = np.mean(np.array(acc)[: ,0])
f1 = np.mean(np.array(acc)[: ,2])
print('The average precision of the model is {}'.format(precision*100))
print('The average accuracy of the model is {}'.format(accuracy*100))
print('The average f1-score of the model is {}'.format(f1*100))
```

0 0.97004699707 0.941236068896 0.791059073976
1 0.99040222168 0.960157516794 0.929476398699
2 0.907202148438 0.934694875871 0.857800224467
3 0.917321777344 0.693524020546 0.802634262902
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552 0.994232177734 0.771599657827 0.826764436297
553 0.996139526367 0.902649006623 0.91507217187
554 0.957484567901 0.950760230354 0.91887036928
555 0.978868258179 0.977553531229 0.951294690051
556 0.917556762695 0.988331495774 0.799271835643
557 0.95861882716 0.916947636414 0.908585746672
558 0.956080246914 0.934598378777 0.899117365567
559 0.942908950617 0.947619047619 0.892285743402
The average precision of the model is 78.3639440129741%
The average accuracy of the model is 95.20080933923725%
The average f1-score of the model is 78.23251191371496%

Classify Images for Future Segmentation

- Imaging conditions can vary from image to image. It can be difficult to determine a general method for nucleus segmentation under the variety of conditions. This section provides a method for separating images by type using a KMeans clustering algorithm.
- The function `img_info` determines a

```
In [35]: # one-indexes a 2d array into 1d, top down then left right, output is np 1d array
def one_index(arr2d):
    h, w = arr2d.shape[0:2]

    arr1d = []
    for col in range(0, w):
        for row in range(0, h):
            arr1d.append(arr2d[row][col])
    return np.array(arr1d)

# pads all vectors in array to have max_len, returns np array
def pad_normalize(array, max_len):
    for i in range(0, len(array)):
        vec = array[i]
        print(len(vec))
        if len(vec) < max_len:
            array[i] = np.concatenate(( np.array(vec).reshape(1,-1), np.zeros
            ((1, (max_len-len(vec)))) ), axis=1)
        else:
            array[i] = np.array(vec).reshape(1,-1)
    return np.array(array)
```

```
In [71]: # Get image information, apply clustering
def get_image_info(samples):
    # complete img ,rgb mode
    full_img_list = []
    # just grey mode
    img_list = []

    # average value of gray pixels per image
    average_list = []

    # max Contour area value per image
    max_cnt_area = []

    # mean Contour area value per image
    average_cnt_area = []

    # how many Contour areas per image
    num_cnt = []

    # width per image
```

```

wid_list = []

# length per image
len_list = []

# red per image
r=[]

# green
g=[]

# blue
b=[]

for i in samples:
    (img, masks) = load_zipped_img(path+'/stage1_train.zip', i)
    full_img_list.append(i)
    r.append(np.average(img[:, :, 0]))
    g.append(np.average(img[:, :, 1]))
    b.append(np.average(img[:, :, 2]))

    img = grayscale(img)
    img = float2int8(img)
    #img = cv2.imread(img, cv2.IMREAD_GRAYSCALE)
    print(i)
    # in some cases, image background is bright and cell darker, there need
    ds a inverse of pixel value
    if np.average(img) > 125:
        img = 255 - img
    img_list.append(img)

    lenth = img.shape[0]
    len_list.append(lenth)
    width = img.shape[1]
    wid_list.append(width)
    average_list.append(np.average(img))

    # use opencv to find contour and get some stactistic data
    img = cv2.GaussianBlur(img, (3, 3), 1)
    ret, thresh = cv2.threshold(img, 0, 255, cv2.THRESH_OTSU)

    _, cnts, _ = cv2.findContours(thresh, cv2.RETR_TREE, cv2.CHAIN_APPROX_
SIMPLE)
    cnts = sorted(cnts, key=cv2.contourArea, reverse=True)
    max_cnt_area.append(cv2.contourArea(cnts[0])/lenth/width)

    av = 0
    for i in cnts:
        av = av + cv2.contourArea(i)
    av = av/len(cnts)

    # since different pic has different size, we'd better normalise it
    average_cnt_area.append(av/lenth/width)
    num_cnt.append(len(cnts))

df = pd.DataFrame({'img':full_img_list,'max_area':max_cnt_area,'average_ar
ea':average_cnt_area,
                   'num_cnt':num_cnt,'average':average_list,'wid':wid_list

```

```
, 'len': len_list,
    'r': r, 'g': g, 'b': b
})

return df

df = get_image_info(range(0, 560))
```


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```
In [87]: df.to_csv('KMEANS_summary.csv')  
df
```

Out[87]:

	average	average_area	b	g	img	len	max_area	num_cnt	
0	6.142227	0.002843	0.024087	0.024087	0	256	0.008263	20	0.024
1	9.018600	0.001648	0.035367	0.035367	1	256	0.004219	36	0.035
2	93.074377	0.006824	0.756961	0.604796	2	256	0.031854	45	0.695
3	81.215198	0.004652	0.802636	0.662910	3	256	0.033124	53	0.702
4	92.987366	0.008269	0.786623	0.604949	4	256	0.021552	8	0.686
5	15.242493	0.002391	0.059774	0.059774	5	256	0.006172	17	0.059
6	16.667801	0.004530	0.065364	0.065364	6	256	0.022232	39	0.065
7	10.765440	0.011759	0.042217	0.042217	7	360	0.026779	18	0.042
8	16.871002	0.001431	0.066161	0.066161	8	256	0.003487	11	0.066
9	11.848765	0.013118	0.046466	0.046466	9	360	0.030363	18	0.046
10	10.967122	0.011311	0.043008	0.043008	10	360	0.028152	22	0.043
11	41.003713	0.001948	0.160799	0.160799	11	260	0.014038	78	0.160
12	17.424622	0.002013	0.068332	0.068332	12	256	0.006447	69	0.068
13	17.616776	0.002971	0.069085	0.069085	13	256	0.006477	24	0.069
14	8.932731	0.011968	0.035030	0.035030	14	360	0.036238	19	0.035
15	19.130478	0.001559	0.075021	0.075021	15	256	0.006889	20	0.075
16	6.385361	0.003363	0.025041	0.025041	16	256	0.008949	15	0.025
17	13.160849	0.009568	0.051611	0.051611	17	360	0.034024	26	0.051
18	4.869278	0.001752	0.019095	0.019095	18	256	0.004295	9	0.019
19	13.656174	0.001205	0.053554	0.053554	19	256	0.001526	5	0.053
20	4.103210	0.001934	0.016091	0.016091	20	256	0.002579	7	0.016
21	44.637525	0.001336	0.175049	0.175049	21	520	0.017916	158	0.175
22	11.913171	0.007606	0.046718	0.046718	22	360	0.023819	27	0.046
23	49.972590	0.000765	0.804029	0.804029	23	1024	0.042792	285	0.804
24	13.539978	0.002114	0.053098	0.053098	24	256	0.003540	7	0.053
25	39.509685	0.000499	0.845060	0.845060	25	1024	0.031569	313	0.845
26	19.444366	0.000888	0.076252	0.076252	26	256	0.003250	48	0.076
27	8.581192	0.003105	0.033652	0.033652	27	256	0.011017	29	0.033
28	15.459991	0.002969	0.060627	0.060627	28	256	0.005096	9	0.060
29	10.707253	0.000910	0.041989	0.041989	29	520	0.001405	7	0.041
...
530	14.649954	0.013698	0.057451	0.057451	530	360	0.051968	19	0.057

	average	average_area	b	g	img	len	max_area	num_cnt	
531	11.667593	0.011300	0.045755	0.045755	531	360	0.042519	21	0.045
532	5.318527	0.001477	0.020857	0.020857	532	256	0.003426	14	0.020
533	4.768578	0.000778	0.018700	0.018700	533	603	0.141384	198	0.018
534	109.992847	0.001993	0.804628	0.525771	534	256	0.052612	111	0.633
535	105.444226	0.002133	0.802869	0.547219	535	256	0.015259	73	0.645
536	10.283626	0.001660	0.040328	0.040328	536	520	0.005555	134	0.040
537	12.737670	0.012654	0.049952	0.049952	537	360	0.024618	20	0.049
538	3.725769	0.002308	0.014611	0.014611	538	256	0.004410	8	0.014
539	18.685562	0.001206	0.073277	0.073277	539	256	0.003036	50	0.073
540	16.692749	0.002115	0.065462	0.065462	540	256	0.007034	24	0.065
541	9.459320	0.001871	0.037095	0.037095	541	256	0.008713	35	0.037
542	11.870787	0.012394	0.046552	0.046552	542	360	0.045467	22	0.046
543	12.611875	0.011758	0.049458	0.049458	543	360	0.030096	24	0.049
544	16.025085	0.002214	0.062843	0.062843	544	256	0.003166	8	0.062
545	18.036499	0.001205	0.070731	0.070731	545	256	0.003441	16	0.070
546	13.798157	0.001833	0.054110	0.054110	546	256	0.005379	14	0.054
547	20.384412	0.006652	0.079939	0.079939	547	256	0.113544	24	0.079
548	18.372604	0.002701	0.072049	0.072049	548	256	0.004974	16	0.072
549	5.101028	0.001887	0.020004	0.020004	549	256	0.003365	11	0.020
550	5.940491	0.002115	0.023296	0.023296	550	256	0.004662	10	0.023
551	10.402206	0.001809	0.040793	0.040793	551	256	0.005920	31	0.040
552	4.996063	0.002335	0.019592	0.019592	552	256	0.003830	7	0.019
553	4.743835	0.002055	0.018603	0.018603	553	256	0.003914	10	0.018
554	10.327932	0.013414	0.040502	0.040502	554	360	0.048264	19	0.040
555	8.276042	0.001775	0.032455	0.032455	555	520	0.004642	113	0.032
556	13.301880	0.002986	0.052164	0.052164	556	256	0.008171	52	0.052
557	11.551404	0.011880	0.045300	0.045300	557	360	0.025058	19	0.045
558	8.408935	0.011111	0.032976	0.032976	558	360	0.023954	19	0.032
559	9.683364	0.011822	0.037974	0.037974	559	360	0.042052	22	0.037

560 rows × 13 columns



```
In [83]: from sklearn.cluster import KMeans

# train seperately
input_x = np.array(df[['max_area', 'average_area', 'num_cnt', 'average', 'wid',
'len']])

fkmeans = KMeans(n_clusters = 3).fit(input_x)

df['flabel'] = fkmeans.labels_

input_c = np.array(df[['r', 'g', 'b']])

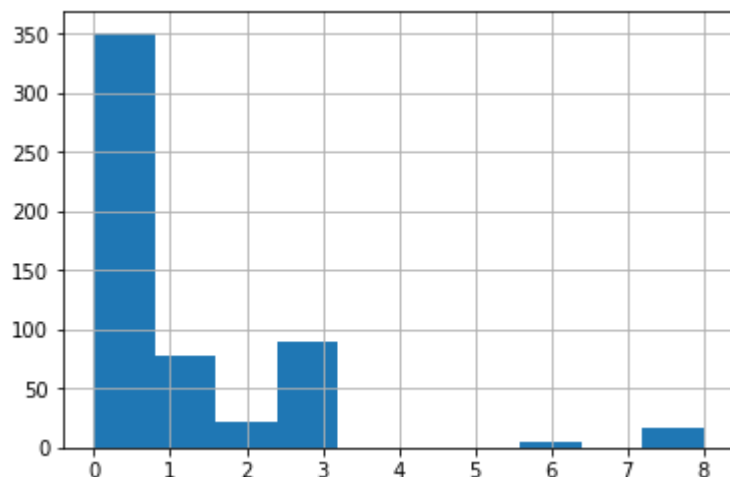
ckmeans = KMeans(n_clusters = 3).fit(input_c)

df['clabel'] = ckmeans.labels_

# and then make an combination
df['cflabel'] = 3 *df['flabel']

df['cflabel'] = df['cflabel'] + df['clabel']

df['cflabel'].hist()
plt.show()
df['cflabel'].values
```



```
Out[83]: array([0, 0, 1, 2, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 3, 0,
      8, 0, 8, 0, 0, 0, 3, 0, 0, 1, 0, 2, 0, 0, 1, 0, 1, 1, 0, 3, 0, 0, 3,
      0, 3, 0, 1, 0, 0, 1, 3, 0, 2, 3, 0, 2, 0, 0, 3, 0, 3, 0, 8, 0, 0, 0,
      8, 3, 0, 0, 0, 0, 3, 2, 0, 0, 0, 1, 0, 0, 2, 0, 0, 3, 0, 0, 6, 3, 3,
      3, 0, 0, 1, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 3, 8, 0, 0, 3,
      0, 0, 0, 2, 3, 0, 3, 0, 3, 1, 0, 3, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0,
      2, 1, 0, 0, 3, 0, 3, 2, 0, 0, 0, 8, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 1,
      0, 0, 0, 0, 1, 0, 0, 0, 3, 3, 1, 3, 0, 0, 1, 2, 8, 0, 0, 3, 1, 3, 3,
      0, 0, 1, 3, 3, 0, 1, 0, 0, 0, 3, 0, 1, 0, 0, 0, 0, 1, 1, 3, 1, 3, 0,
      0, 1, 3, 8, 3, 1, 2, 3, 0, 0, 0, 0, 0, 1, 6, 3, 0, 8, 3, 0, 0, 0, 2,
      0, 0, 0, 0, 0, 0, 1, 0, 0, 1, 0, 0, 0, 0, 3, 0, 0, 0, 0, 0, 3, 0, 0,
      1, 0, 3, 0, 8, 1, 0, 8, 1, 3, 3, 0, 0, 0, 1, 0, 0, 0, 3, 3, 0, 0, 0,
      0, 0, 1, 0, 0, 0, 1, 0, 0, 0, 1, 0, 1, 3, 3, 3, 0, 0, 0, 1, 0, 1, 0,
      3, 0, 0, 3, 3, 0, 1, 1, 2, 3, 0, 0, 1, 0, 0, 8, 0, 0, 3, 2, 0, 1, 0,
      0, 0, 0, 3, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 3, 2, 8, 0, 1, 3, 3, 0, 1,
      0, 0, 0, 0, 1, 3, 0, 3, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 3, 3, 8, 3, 0,
      0, 1, 0, 0, 3, 1, 0, 0, 8, 0, 0, 0, 2, 0, 0, 1, 2, 0, 3, 1, 3, 0, 3,
      0, 0, 0, 3, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 3, 2, 1, 0,
      0, 0, 6, 0, 0, 0, 0, 1, 0, 0, 3, 2, 0, 3, 1, 0, 0, 0, 3, 0, 3, 1, 1,
      0, 3, 1, 3, 0, 0, 0, 1, 0, 3, 0, 0, 1, 0, 0, 1, 3, 0, 1, 0, 1, 2, 0,
      3, 0, 0, 1, 0, 0, 0, 3, 1, 3, 0, 0, 0, 0, 0, 0, 0, 0, 3, 0, 0, 0, 0,
      3, 0, 0, 0, 1, 0, 0, 0, 2, 3, 0, 3, 0, 3, 0, 0, 1, 1, 0, 0, 0, 0, 0,
      1, 0, 0, 0, 1, 3, 0, 1, 0, 3, 0, 0, 8, 0, 3, 0, 0, 0, 0, 0, 0, 0, 0,
      6, 0, 0, 0, 6, 1, 1, 3, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
      0, 0, 0, 3, 0, 0, 0, 0])
```

Segmentation: Separating Individual Objects

- The function `separate_obj` separates the objects in an image after a thresholding method has been applied
- The function `convert2runlength` finds the objects in an image (1 corresponds to object, 0 to background) and finds runs of continuous object pixels
- The function `rle` generates a dataframe of images in run-length format. This is the output format required by the Kaggle competition

```

In [38]: ## STEP 3: Separate individual objects and encode in run-length format

# separate objects in image into individual masks
def separate_obj(img_masked):
    labels, nlabels = ndimage.label(img_masked)

    label_arrays = []
    for label_num in range(1, nlabels+1):
        label_mask = np.where(labels == label_num, 1, 0)
        label_arrays.append(label_mask)
    return labels, nlabels, label_mask

# convert path to run-length encoding (RLE) output format
def convert2runlength(x):
    obj = np.where(x.T.flatten()==1)[0] #1 corresponds to object, 0 to background
    run_lengths = []
    prev = -2
    for b in obj: # find continuous set of object pixels
        if (b>prev+1): run_lengths.extend((b+1, 0))
        run_lengths[-1] += 1
        prev = b
    return " ".join([str(i) for i in run_lengths])

def rle(img_masked, im_id):
    (labels, nlabels, label_mask) = separate_obj(img_masked)
    im_df = pd.DataFrame()
    for label_num in range(1, nlabels+1):
        label_mask = np.where(labels == label_num, 1, 0)
        if label_mask.flatten().sum() > 10:
            rle = convert2runlength(label_mask)
            s = pd.Series({'ImageId': im_id, 'EncodedPixels': rle})
            im_df = im_df.append(s, ignore_index=True)
    return im_df

```



```
In [39]: # Function to contour segment images
def contouring(img_float32, borderSize, gap, draw='off'):
    img = float2int8(img_float32[:, :, :3])

    # perform a BGR->HSV conversion and use the V channel for processing
    hsv = cv2.cvtColor(img, cv2.COLOR_BGR2HSV)

    # threshold, apply morphological closing, then take the distance transform (dist)
    th, bw = cv2.threshold(float2int8( hsv[:, :, 2] ), 0, 255, cv2.THRESH_BINARY | cv2.THRESH_OTSU)
    kernel = cv2.getStructuringElement(cv2.MORPH_ELLIPSE, (3, 3))
    morph = cv2.morphologyEx(bw, cv2.MORPH_CLOSE, kernel)
    dist = cv2.distanceTransform(morph, cv2.DIST_L2, cv2.DIST_MASK_PRECISE)
    distborder = cv2.copyMakeBorder(dist, borderSize, borderSize, borderSize, borderSize, cv2.BORDER_CONSTANT | cv2.BORDER_ISOLATED, 0)

    # create a template, take its distance transform and use it as the template (temp)
    kernel2 = cv2.getStructuringElement(cv2.MORPH_ELLIPSE, (2*(borderSize-gap
```

```

p)+1, 2*(borderSize-gap)+1))
    kernel2 = cv2.copyMakeBorder(kernel2, gap, gap, gap, gap, cv2.BORDER_CONSTANT | cv2.BORDER_ISOLATED, 0)
    distTemp1 = cv2.distanceTransform(kernel2, cv2.DIST_L2, cv2.DIST_MASK_PRECISE)

    # template matching (dist*temp)
    nxcor = cv2.matchTemplate(distborder, distTemp1, cv2.TM_CCOEFF_NORMED)

    # find local maxima of the resulting image, positions correspond to circle centers and values correspond to radii
    mn, mx, _, _ = cv2.minMaxLoc(nxcor)

    # thresholding template matched image
    th, peaks = cv2.threshold(nxcor, mx*0.5, 255, cv2.THRESH_BINARY)
    peaks8u = cv2.convertScaleAbs(peaks)
    _, contours, _ = cv2.findContours(peaks8u, cv2.RETR_CCOMP, cv2.CHAIN_APPROX_SIMPLE)
    # peaks8u = cv2.convertScaleAbs(peaks) # to use as mask

    # detecting circles as local maxima
    # if draw == 'on':
    for i in range(len(contours)):
        x, y, w, h = cv2.boundingRect(contours[i])
        _, mx, _, mxloc = cv2.minMaxLoc(dist[y:y+h, x:x+w], peaks8u[y:y+h, x:x+w])
        cv2.circle(img, (int(mxloc[0]+x), int(mxloc[1]+y)), int(mx), (255, 0, 0), 2)
        cv2.rectangle(img, (x, y), (x+w, y+h), (0, 255, 255), 2)
        cv2.drawContours(img, contours, i, (0, 0, 255), 2)

    img_contoured = img

    return img_contoured

#### TESTING
(img, mask) = load_zipped_img(path+'/stage1_train.zip', 177)
#177
img_guess, markers, sure_bg, sure_fg, uncertain = watershed(img)

# one way of determining borderSize and gap
num_markers = np.max(markers)
obj_size = []
for i in range(1, num_markers):
    obj_size.append(len(markers[markers==i]))

min_size = min(a for a in obj_size)
max_size = max(a for a in obj_size)
avg_size = np.sum(obj_size)/num_markers

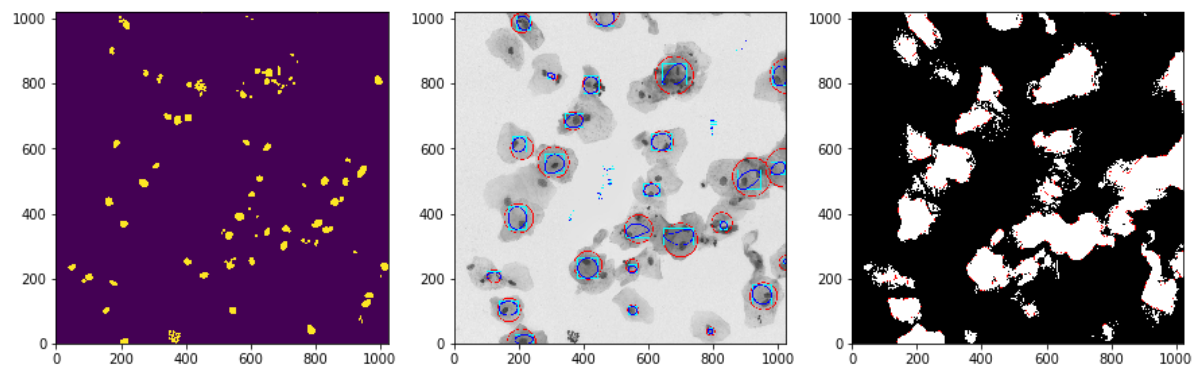
borderSize = int(np.sqrt(5000))
gap = int(borderSize/10)

img_contoured = contouring(img, borderSize, gap, draw='off')

fig, ax = plt.subplots(1,3, figsize = (15,15))

```

```
ax[0].imshow(sum(mask), origin='lower')  
ax[1].imshow(img_contoured, origin='lower')  
ax[2].imshow(img_guess, origin='lower')  
plt.show()
```



Active Contour Modeling

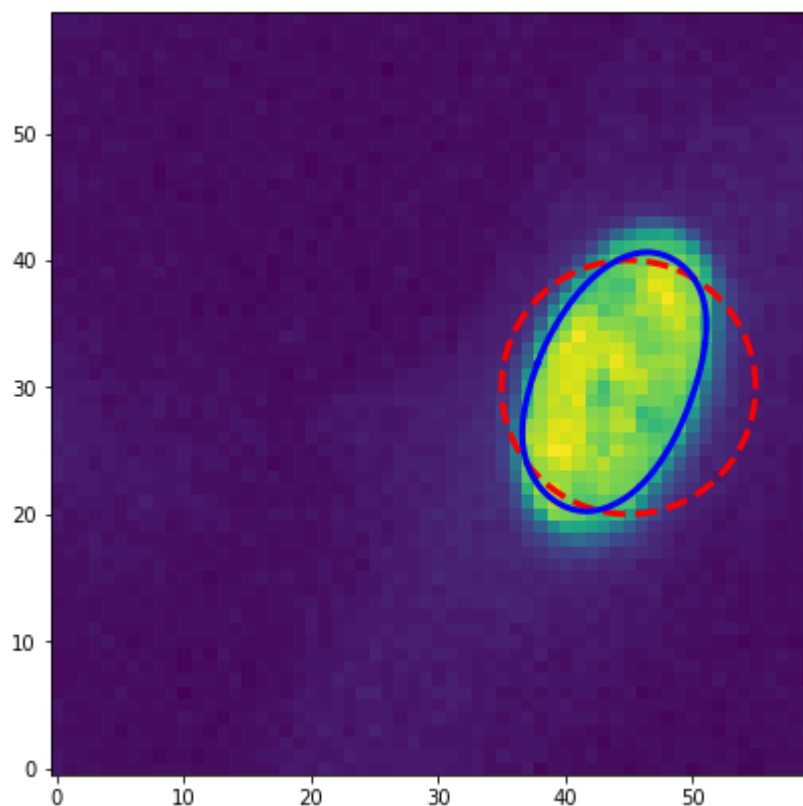
- This method applies level set methods for nucleus segmentation

```
In [24]: from skimage.segmentation import active_contour
from skimage.filters import gaussian

(img, mask) = load_zipped_img(path+'/stage1_train.zip', 1)
img = grayscale(img)[100:160,100:160]

s = np.linspace(0, 2*np.pi, 400)
x = 45 + 10*np.cos(s)
y = 30 + 10*np.sin(s)
init = np.array([x, y]).T

snake = active_contour(gaussian(img, 3), init, alpha=0.015, beta=10, gamma=
0.001)
fig, ax = plt.subplots(figsize=(7, 7))
ax.imshow(img, origin = 'lower')
ax.plot(init[:, 0], init[:, 1], '--r', lw=3)
ax.plot(snake[:, 0], snake[:, 1], '-b', lw=3)
#ax.set_xticks([]), ax.set_yticks([])
#ax.axis([0, img.shape[1], img.shape[0], 0])
plt.show()
```



Data Shape Manipulation

- The function `one_index` takes an image (2d array) and converts it to a 1d array. They are indexed from top to bottom then left to right
- The function `pad_normalize` helps account for variation in image sizes. It determines the maximum length in a set of one-indexed images and "pads" all other one-indexed images with zeros so that all images have the same length.

```
In [40]: # one-indexes a 2d array into 1d, top down then left right, output is np 1d array
def one_index(arr2d):
    h, w = arr2d.shape[0:2]

    arr1d = []
    for col in range(0, w):
        for row in range(0, h):
            arr1d.append(arr2d[row][col])
    return np.array(arr1d)

# pads all vectors in array to have max_len, returns np array
def pad_normalize(array, max_len):
    for i in range(0, len(array)):
        vec = array[i]
        if len(vec) < max_len:
            array[i] = np.concatenate(( np.array(vec).reshape(1,-1), np.zeros
            ((1, (max_len-len(vec)))) ), axis=1)
        else:
            array[i] = np.array(vec).reshape(1,-1)
    return np.array(array)
```

```
In [35]: #Separate the cells
```

Training a Model

- X = a vector of the one-indexed images in the training set
- Y = a vector containing the sum of the one-indexed masks for each image (The correct nuclei)
- Currently fits a Random Forest Classifier with a maximum depth of 4. The feature vector contains the following information:
 1. The grayscale pixel intensity (continuous)
 2. The watershed prediction for a pixel (discrete)
 3. The magnitude of the gradient of pixel intensity (calculated using the Sobel operator) 4-6. The intensity for the rgb color channels respectively (continuous)

Model Validation

- Tests the performance of the model
- Uses cross-validation for tuning of hyperparameters?
- Uses confusion matrix to quantify types of errors (fp, fn, tp, tn)

```

In [53]: from sklearn.ensemble import RandomForestRegressor
         from sklearn.ensemble import RandomForestClassifier

         # Not rigorous but I picked a few images to train on - it went pretty well

         n_samples = [0, 19, 50, 100, 149, 150, 175, 177, 200, 250, 300, 350, 400, 510, 560, 650]
         #n_samples = range(0,560)
         x_train = np.zeros(7).reshape(1,7) # predicted segmentation using Otsu's thresholding

         y_train = np.zeros(1) # "correct" segmentation from sum of masks

         max_len = 0
         conm = []
         acc = []
         for i in n_samples:
             (img, masks) = load_zipped_img(path+'/stage1_train.zip', i) # Loads image and associated masks

             print(i)
             (img_guess, markers, sure_bg, sure_fg, uncertain) = watershed(img)

             intensity=grayscale(img)
             #img_guess = otsu(intensity)
             img_guess=grayscale(img_guess)
             #img_guess = np.dot(0,intensity)
             laplac = laplace(grayscale(img))[uncertain==255].reshape(-1,1)
             fil = sobel(grayscale(img))[uncertain==255].reshape(-1,1)
             #fil = sobel(grayscale(img)).reshape(-1,1)
             intensity_raw = intensity[uncertain==255].reshape(-1,1)
             #intensity_raw = intensity.reshape(-1,1)
             watershed_raw = img_guess[uncertain==255].reshape(-1,1)
             #watershed_raw = img_guess.reshape(-1,1)

             img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
             ch1,ch2,ch3 = cv2.split(img3)
             #ch1 = ch1.reshape(-1,1)
             #ch2 = ch2.reshape(-1,1)
             #ch3 = ch3.reshape(-1,1)

             ch1 = ch1[uncertain==255].reshape(-1,1)
             ch2 = ch2[uncertain==255].reshape(-1,1)
             ch3 = ch3[uncertain==255].reshape(-1,1)

             feature = np.concatenate((intensity_raw,watershed_raw, fil, laplac, ch1, ch2, ch3), axis = 1) # Features include grayscale intensity, watershed, sob

```

el, rgb channels

```
y_raw = sum(masks)
y_raw = y_raw[uncertain==255].reshape(-1,1)

x_train = np.append(x_train,feature).reshape(-1,7)
y_train = np.append(y_train,y_raw).reshape(-1,1)

Xtrain, Xtest, ytrain, ytest = train_test_split(x_train,y_train)
# Kept a split training set around but not needed

#clf = svm.SVC(C = 1.0)
clf = RandomForestClassifier(max_depth=5, random_state=0)
%time clf.fit(Xtrain, ytrain[:,0]) #599 ms for a training set of this size!

#y_pred = clf.predict(Xtest)
#cm = confusion_matrix(ytest, y_pred)
#print(cm)
#conm.append(cm)
#accuracy = sklearn.metrics.accuracy_score(ytest, y_pred)
#print(accuracy)
#acc.append(accuracy)

#(img_guess, markers, sure_bg, sure_fg, unknown, reduced_area) = watershed(img)

#fig, ax = plt.subplots(1,4, figsize = (10,10))

#ax[0].imshow(sum(masks), origin='lower')
#ax[1].imshow( grayscale(img), origin='lower')
#ax[2].imshow(output, origin='lower')
#ax[3].imshow(img_guess, origin='lower')
#plt.show()

#A = np.array(acc)
#Avg_acc = np.mean(A)
#print(Avg_acc)
```


0
19
50
100
149
150
175
177
200
250
300
350
400
510
560
650

Wall time: 4.87 s

```
Out[53]: RandomForestClassifier(bootstrap=True, class_weight=None, criterion='gini',  
                                max_depth=5, max_features='auto', max_leaf_nodes=None,  
                                min_impurity_decrease=0.0, min_impurity_split=None,  
                                min_samples_leaf=1, min_samples_split=2,  
                                min_weight_fraction_leaf=0.0, n_estimators=10, n_jobs=1,  
                                oob_score=False, random_state=0, verbose=0, warm_start=False)
```

```
In [28]: # Random Forest Except using HSV instead of RGB

# Not rigorous but I picked a few images to train on - it went pretty well
from sklearn.ensemble import RandomForestClassifier
n_samples = [0, 19, 50, 100, 149, 150, 175, 177, 200, 250, 300, 350, 400, 510, 560, 650]
#n_samples = range(0,560)
x_train = np.zeros(6).reshape(1,6)

y_train = np.zeros(1) # "correct" segmentation from sum of masks

max_len = 0
conm = []
acc = []
for i in n_samples:
    (img, masks) = load_zipped_img(path+'/stage1_train.zip', i) # Loads image and associated masks

    print(i)
    (img_guess, markers, sure_bg, sure_fg, uncertain) = watershed(img)

    intensity=grayscale(img)
    #img_guess = otsu(intensity)
    img_guess=grayscale(img_guess)
    #img_guess = np.dot(0,intensity)
    fil = sobel(grayscale(img))[uncertain==255].reshape(-1,1)
    #fil = sobel(grayscale(img)).reshape(-1,1)
    intensity_raw = intensity[uncertain==255].reshape(-1,1)
    #intensity_raw = intensity.reshape(-1,1)
    watershed_raw = img_guess[uncertain==255].reshape(-1,1)
    #watershed_raw = img_guess.reshape(-1,1)
```

```

img_cv = float2int8(img[:, :, :3])

# perform a BGR->HSV conversion and use the V channel for processing
hsv = cv2.cvtColor(img_cv, cv2.COLOR_BGR2HSV)

#img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
ch1,ch2,ch3 = cv2.split(hsv)
#ch1 = ch1.reshape(-1,1)
#ch2 = ch2.reshape(-1,1)
#ch3 = ch3.reshape(-1,1)

print(ch1.shape)

ch1 = ch1[uncertain==255].reshape(-1,1)
ch2 = ch2[uncertain==255].reshape(-1,1)
ch3 = ch3[uncertain==255].reshape(-1,1)

feature = np.concatenate((intensity_raw,watershed_raw, fil, ch1, ch2, ch
3), axis = 1) # Features include grayscale intensity, watershed, sobel, rgb
channels

y_raw = sum(masks)
y_raw = y_raw[uncertain==255].reshape(-1,1)

x_train = np.append(x_train,feature).reshape(-1,6)
y_train = np.append(y_train,y_raw).reshape(-1,1)

Xtrain, Xtest, ytrain, ytest = train_test_split(x_train,y_train)
# Kept a split training set around but not needed

#s_v = svm.SVC(C = 1.0)
clf = RandomForestClassifier(max_depth=5, random_state=0)
%time clf.fit(Xtrain, ytrain[:,0]) #599 ms for a training set of this size!

#y_pred = clf.predict(Xtest)
#cm = confusion_matrix(ytest, y_pred)
#print(cm)
#conm.append(cm)
#accuracy = sklearn.metrics.accuracy_score(ytest, y_pred)
#print(accuracy)
#acc.append(accuracy)

#(img_guess, markers, sure_bg, sure_fg, unknown, reduced_area) = watersh
ed(img)

#fig, ax = plt.subplots(1,4, figsize = (10,10))

#ax[0].imshow(sum(masks), origin='lower')
#ax[1].imshow(grayscale(img), origin='lower')
#ax[2].imshow(output, origin='lower')
#ax[3].imshow(img_guess, origin='lower')
#plt.show()

```

```
#A = np.array(acc)
#Avg_acc = np.mean(A)
#print(Avg_acc)
```

```
0
(256, 256)
19
(256, 256)
50
(256, 256)
100
(256, 256)
149
(1024, 1024)
150
(256, 256)
175
(256, 320)
177
(1024, 1024)
200
(256, 256)
250
(520, 696)
300
(256, 256)
350
(520, 696)
400
(256, 256)
510
(256, 320)
560
(256, 256)
650
(256, 256)
Wall time: 8.93 s
```

```
Out[28]: RandomForestClassifier(bootstrap=True, class_weight=None, criterion='gini',
                                max_depth=5, max_features='auto', max_leaf_nodes=None,
                                min_impurity_decrease=0.0, min_impurity_split=None,
                                min_samples_leaf=1, min_samples_split=2,
                                min_weight_fraction_leaf=0.0, n_estimators=10, n_jobs=1,
                                oob_score=False, random_state=0, verbose=0, warm_start=False)
```

```
In [55]: # Testing the classifier on the remaining images
acc = []
for i in range(0,560):
    (img, masks) = load_zipped_img(path+'/stage1_train.zip', i)

    (img_guess, markers, sure_bg, sure_fg, unknown) = watershed(img)
    intensity = grayscale(img)
    shap = intensity.shape
    intensity_raw = intensity.reshape(-1,1)
    #watershed_raw = img_guess[unknown==255].reshape(-1,1)
    watershed_raw = grayscale(img_guess).reshape(-1,1)
    fil = sobel(grayscale(img)).reshape(-1,1)
    laplac = laplace(grayscale(img)).reshape(-1,1)
    #img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
    hsv = cv2.cvtColor(img, cv2.COLOR_BGR2HSV)

    #img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
    ch1,ch2,ch3 = cv2.split(hsv)

    #ch1,ch2,ch3 = cv2.split(img3)
    ch1 = ch1.reshape(-1,1)
    ch2 = ch2.reshape(-1,1)
    ch3 = ch3.reshape(-1,1)
    #print(ch1.shape)
    #print(intensity_raw.shape)
    feature = np.concatenate((intensity_raw,watershed_raw, fil, laplac, ch1,
ch2, ch3), axis = 1)

    yp = clf.predict(feature)
    y_real = sum(masks).reshape(-1,1)
    accuracy = sklearn.metrics.accuracy_score(y_real, yp)
    precision = sklearn.metrics.precision_score(y_real, yp)
    f1 = sklearn.metrics.f1_score(y_real, yp)
    print(i, accuracy, precision, f1)
    acc.append([accuracy, precision, f1])

    #output = yp.reshape(shap[0], shap[1])

precision = np.mean(np.array(acc)[: ,1])
accuracy = np.mean(np.array(acc)[: ,0])
f1 = np.mean(np.array(acc)[: ,2])
print('The average precision of the model is {}'.format(precision*100))
print('The average accuracy of the model is {}'.format(accuracy*100))
print('The average f1-score of the model is {}'.format(f1*100))
```

0 0.975708007813 0.894091187896 0.846005029986
1 0.988327026367 0.898442367601 0.91876393756
2 0.714721679688 0.89941083489 0.348807400802
3 0.835083007813 0.676328502415 0.212244897959
4 0.934167480469 0.728658536585 0.0814171350707
5 0.979019165039 0.692124105012 0.808362369338
6 0.93537902832 0.905896242795 0.860337037892
7 0.97013117284 0.979405737705 0.936774193548
8 0.97004699707 0.229555236729 0.245870149827
9 0.97112654321 0.971032041729 0.943552766548
10 0.952330246914 0.891814006544 0.905817427892
11 0.911483041454 0.823177758571 0.741084165478
12 0.961273193359 0.831437032419 0.893700787402
13 0.974761962891 0.786783854167 0.853912736266
14 0.954444444444 0.947524069267 0.907134767837
15 0.959701538086 0.432483474976 0.409568522245
16 0.985092163086 0.8735395189 0.886434964547
17 0.961404320988 0.989761290135 0.93171331058
18 0.991958618164 0.655839668279 0.782680412371
19 0.980895996094 0.153968253968 0.236585365854
20 0.994262695313 0.702702702703 0.824626865672
21 0.844473916888 0.826363929306 0.703184982071
22 0.963163580247 0.928815142046 0.915498442368
23 0.945902824402 0.297616653258 0.454970838898
24 0.980590820313 0.406043437205 0.57486631016
25 0.976623535156 0.328990820129 0.469781527147
26 0.9609375 0.685367702805 0.679358717435
27 0.953964233398 0.875250166778 0.813038359051
28 0.980194091797 0.5842994369 0.731040198923
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359 0.979187011719 0.476001586672 0.637619553666
360 0.995742797852 0.811178247734 0.885043263288
361 0.975112915039 0.442361111111 0.609715242881
362 0.987930297852 0.742529676627 0.821000226296
363 0.951848474801 0.899850226465 0.851255964015
364 0.957125884173 0.876488796868 0.846410436607
365 0.984228134155 0.618700552726 0.73414994856
366 0.961803713528 0.915171006674 0.88449197861
367 0.990203857422 0.898479427549 0.926019820235
368 0.980438232422 0.64020006252 0.761621420602
369 0.990051269531 0.758620689655 0.0974529346622
370 0.984466552734 0.688530104272 0.800860719875
371 0.980575561523 0.376051459673 0.544217687075
372 0.904014699381 0.898391382166 0.82308965447
373 0.855151367187 0.681637519873 0.224241631799
374 0.975021362305 0.104852686308 0.128791910591
375 0.962890625 0.790017567428 0.862769439115
376 0.994248390198 0.80897171958 0.831982170219
377 0.961196899414 0.857840590253 0.866530205217
378 0.965118408203 0.952353616533 0.85306594678
379 0.975814819336 0.556490384615 0.700321421819
380 0.976049804688 0.724293785311 0.395191122072
381 0.980178833008 0.178217821782 0.279534109817
382 0.951461791992 0.802157140608 0.864977291056
383 0.781164550781 0.822158749248 0.3789364282
384 0.862854003906 0.754491017964 0.100840336134
385 0.993850708008 0.700696055684 0.818058690745
386 0.936645507813 0.884174880238 0.755735968938
387 0.845642089844 0.620448179272 0.0654792698248
388 0.972853116711 0.937186040363 0.945408477938
389 0.958404541016 0.828771849126 0.840956826138
390 0.942600574713 0.808936467303 0.870011388239
391 0.955455246914 0.914855875831 0.914680105819
392 0.975662231445 0.466822429907 0.556081269134
393 0.97883605957 0.319396051103 0.442299959791
394 0.976856763926 0.947921371016 0.950111974079
395 0.920257568359 0.907395974812 0.737703272435
396 0.992202758789 0.777709736681 0.832513929859
397 0.989517211914 0.708352561144 0.817141336172
398 0.878297495012 0.830493069757 0.666362807657

399 0.95002746582 0.615858843537 0.638879700077
400 0.974578857422 0.37156448203 0.457682291667
401 0.980270385742 0.130153597413 0.199380804954
402 0.955795288086 0.785211267606 0.850662405279
403 0.993621826172 0.772861356932 0.862409479921
404 0.965637207031 0.643843336151 0.669891527411
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406 0.979385375977 0.756209400076 0.854213877199
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409 0.98469543457 0.897172236504 0.900466408653
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416 0.757319273653 0.162502702898 0.279326018445
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421 0.563427734375 0.660377358491 0.0019534520288
422 0.988372802734 0.775072224515 0.831341301461
423 0.975402832031 0.3870789619 0.465162574652
424 0.977641467728 0.926112288136 0.912062595088
425 0.87060546875 0.860032804811 0.372484016102
426 0.992431640625 0.697836706211 0.801282051282
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430 0.963146972656 0.666077217801 0.599549011805
431 0.981536865234 0.55335661622 0.702117183653
432 0.971562776304 0.940893852111 0.939352512051
433 0.960779320988 0.930239601561 0.915801156223
434 0.974300950486 0.955430821991 0.938786255372
435 0.589453125 0.82 0.0024322240019
436 0.700109863281 0.846153846154 0.0044575920898
437 0.974319458008 0.522310756972 0.609059233449
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441 0.945205688477 0.859563846558 0.879346840036
442 0.976501464844 0.942809083263 0.879215686275
443 0.9931640625 0.715249662618 0.82554517134
444 0.831225585937 0.680888114933 0.404308487721
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446 0.963168656057 0.899867294004 0.924870933562
447 0.970260620117 0.886743371686 0.900940279543
448 0.996185302734 0.911869587366 0.934725848564
449 0.880554199219 0.739583333333 0.0282053828583
450 0.970038580247 0.971872227152 0.944210571687
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487 0.900646972656 0.341772151899 0.00659099231051
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490 0.96369934082 0.730390951561 0.833181403829
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501 0.939666748047 0.570523507836 0.463937093275
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504 0.969039916992 0.302684563758 0.307744796998
505 0.984237670898 0.862114248194 0.910461991852
506 0.76748046875 0.561403508772 0.0165220983065
507 0.992721557617 0.84632034632 0.867683772538
508 0.955612182617 0.809523809524 0.795587098588
509 0.970840454102 0.4186866461 0.498556809236
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524 0.996994018555 0.833898305085 0.882247459653
525 0.939590454102 0.714589371981 0.651343020696
526 0.918731689453 0.948353164339 0.805079783341
527 0.971572875977 0.648418156809 0.716740155086
528 0.97388117284 0.96717967736 0.948694241933
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531 0.961041666667 0.912897770945 0.921639532538
532 0.996810913086 0.909254807692 0.935394126739
533 0.903479197305 0.283200946589 0.440977701932
534 0.689599609375 0.877218934911 0.0445630119486
535 0.807629394531 0.845010615711 0.0480821504077
536 0.974021883289 0.929026774331 0.948980367046
537 0.978279320988 0.970341035357 0.960313544149
538 0.993621826172 0.79854368932 0.862950819672
539 0.9716796875 0.736435501258 0.815396856972
540 0.974990844727 0.740240863787 0.813091572585
541 0.981842041016 0.841610500177 0.888576779026
542 0.965601851852 0.937087633805 0.938861155302
543 0.964359567901 0.986428909579 0.941994951715
544 0.980529785156 0.493899683687 0.631426920855
545 0.973007202148 0.423702149974 0.477400295421
546 0.976608276367 0.533198517021 0.673621460507
547 0.936413574219 0.878502577897 0.818647077255
548 0.970611572266 0.555474095797 0.702410383189
549 0.994216918945 0.828220858896 0.886831890116
550 0.992645263672 0.742441209406 0.846202935546
551 0.990676879883 0.931308093403 0.930780559647
552 0.991882324219 0.661301140174 0.787539936102
553 0.993133544922 0.774959525094 0.864539434076
554 0.96475308642 0.937917778352 0.934699945679
555 0.9752127542 0.914383205136 0.94630619415
556 0.937835693359 0.941780568895 0.862215909091
557 0.965470679012 0.902324985589 0.926430696894
558 0.963194444444 0.923262881613 0.918094714791
559 0.953302469136 0.941174757564 0.914386759089
The average precision of the model is 76.34429243351293%
The average accuracy of the model is 93.71913791606703%
The average f1-score of the model is 67.14543699222496%

Working Example of the Code

- Demonstrates the methods involved using a smaller set of 30 training images
- Evaluates performance based on a confusion matrix and accuracy (compared to the true masks)

```
In [53]: precision = np.mean(np.array(acc)[: ,1])
accuracy = np.mean(np.array(acc)[: ,0])
f1 = np.mean(np.array(acc)[: ,2])
print('The average precision of the model is {}'.format(precision*100))
print('The average accuracy of the model is {}'.format(accuracy*100))
print('The average f1-score of the model is {}'.format(f1*100))
```

```
The average precision of the model is 81.38706474131074%
The average accuracy of the model is 94.68844817046038%
The average f1-score of the model is 74.83144803204694%
```



```

In [42]: for i in range(173,174):
          (img, masks) = load_zipped_img(path+'/stage1_train.zip', i)

          (img_guess, markers, sure_bg, sure_fg, uncertain) = watershed(img)

          intensity = grayscale(img)
          shap = intensity.shape
          intensity_raw = intensity.reshape(-1,1)
          #watershed_raw = img_guess[unknown==255].reshape(-1,1)
          watershed_raw = grayscale(img_guess).reshape(-1,1)
          fil = sobel(grayscale(img)).reshape(-1,1)

          img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
          ch1,ch2,ch3 = cv2.split(img3)
          ch1 = ch1.reshape(-1,1)
          ch2 = ch2.reshape(-1,1)
          ch3 = ch3.reshape(-1,1)
          feature = np.concatenate((intensity_raw,watershed_raw, fil, ch1, ch2, ch3
          ), axis = 1)

          yp = clf.predict(feature)
          y_real = sum(masks).reshape(-1,1)

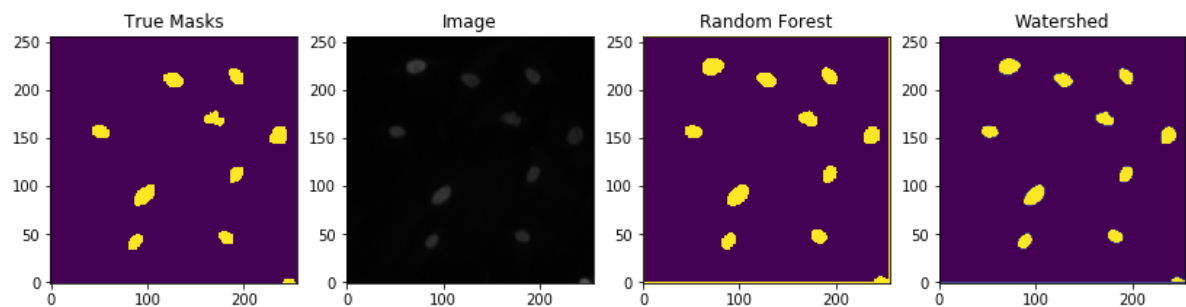
          labels, nlabels, label_mask = separate_obj(grayscale(yp.reshape(shap[0], s
          hap[1])))

          #print(labels, nlabels, label_mask)
          #print(len(masks))

fig, ax = plt.subplots(1,4, figsize = (14,14))

ax[0].imshow(sum(masks), origin='lower')
ax[0].set_title('True Masks')
ax[1].imshow(img, origin='lower')
ax[1].set_title('Image')
ax[2].imshow(grayscale(yp.reshape(shap[0], shap[1])), origin='lower')
ax[2].set_title('Random Forest')
ax[3].imshow(grayscale(img_guess), origin='lower')
ax[3].set_title('Watershed')
plt.show()

```



In [46]: *# Compare mask by mask*

```
def mask_by_mask_score(img, masks):  
    mask_scores = []  
    mask_num = len(masks)  
    for i in range(0,mask_num):  
        individual_mask = img[masks[i] == 1]  
        score = np.sum(individual_mask)/individual_mask.size  
        mask_scores.append(score)  
  
    return mask_scores  
  
print(mask_by_mask_score(yscale(yp.reshape(shap[0], shap[1])),masks))
```

```
[0.89323843416370108, 0.97409326424870468, 0.9543568464730291, 0.919540229885  
05746, 1.0, 0.98469387755102045, 0.96385542168674698, 0.95580110497237569, 0.  
99521531100478466, 0.9285714285714286, 0.94545454545454544]
```

```

In [40]: import numpy as np
from scipy.ndimage.morphology import distance_transform_edt as dtx
import scipy.ndimage.filters as filters

def ConvertMask(Mask):
    # convert binary mask to signed distance function
    Phi0 = dtx(1-Mask) - dtx(Mask) + Mask - 1/2
    return Phi0

def Kappa(Phi):
    dPhi = np.gradient(Phi) # calculate gradient of level set image
    xdPhi = np.gradient(dPhi[1])
    ydPhi = np.gradient(dPhi[0])
    K = (xdPhi[1]*(dPhi[0]**2) - 2*xdPhi[0]*dPhi[0]*dPhi[1] +
        ydPhi[0]*(dPhi[1]**2)) / ((dPhi[0]**2 + dPhi[1]**2 + 1e-10)**(3/2))
    K *= (xdPhi[1]**2 + ydPhi[0]**2)**(1/2)
    return K

def Impulse(X, Epsilon):
    # Smooth dirac delta function.

    # calculate smoothed impulse everywhere
    Xout = (1 + np.cos(np.pi * X / Epsilon)) / (2 * Epsilon)

    # zero out values |x| > Epsilon
    Xout[np.absolute(X) > Epsilon] = 0

    return Xout

def ChanVese(I, Mask, Sigma, dt=1.0, Mu=0.2, Lambda1=1, Lambda2=1, It=100):

    # smoothed gradient of input image
    I = filters.gaussian_filter(I, Sigma, mode='constant', cval=0)
    # dsI = np.gradient(sI)
    # I = 1/(1 + dsI[0]**2 + dsI[1]**2)

```

```
# generate signed distance map
Phi = ConvertMask(Mask)

# evolve level set function
for i in range(0, It):

    # calculate interior and exterior averages
    C1 = np.sum(I[Phi > 0]) / (np.sum(Phi > 0) + 1e-10)
    C2 = np.sum(I[Phi <= 0]) / (np.sum(Phi <= 0) + 1e-10)
    Force = Lambda2 * (I - C2)**2 - Lambda1 * (I - C1)**2

    # curvature of image
    Curvature = Kappa(Phi)

    # evolve
    Phi += dt * Force / np.max(np.abs(Force)) + Mu*Curvature

return Phi

print(ChanVese( grayscale(img), sum(masks), 3))
```

```
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:14: RuntimeWarning: overflow encountered in square

C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:15: RuntimeWarning: overflow encountered in square
  from ipykernel import kernelapp as app
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:15: RuntimeWarning: overflow encountered in power
  from ipykernel import kernelapp as app
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:15: RuntimeWarning: invalid value encountered in true_divide
  from ipykernel import kernelapp as app
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:16: RuntimeWarning: overflow encountered in square
  app.launch_new_instance()
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:44: RuntimeWarning: invalid value encountered in greater
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:45: RuntimeWarning: invalid value encountered in less_equal
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:14: RuntimeWarning: overflow encountered in multiply

C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:14: RuntimeWarning: invalid value encountered in subtract

C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:15: RuntimeWarning: invalid value encountered in add
  from ipykernel import kernelapp as app
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:16: RuntimeWarning: overflow encountered in multiply
  app.launch_new_instance()
C:\Users\John\Anaconda3\lib\site-packages\numpy\lib\function_base.py:1768: RuntimeWarning: invalid value encountered in subtract
  out[slice1] = (f[slice4] - f[slice2]) / (2. * dx[i])
C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:15: RuntimeWarning: overflow encountered in multiply
  from ipykernel import kernelapp as app

[[ nan  nan  nan ...,  nan  nan  nan]
 [ nan  nan  nan ...,  nan  nan  nan]
 [ nan  nan  nan ...,  nan  nan  nan]
 ...,
 [ nan  nan  nan ...,  nan  nan  nan]
 [ nan  nan  nan ...,  nan  nan  nan]
 [ nan  nan  nan ...,  nan  nan  nan]]

C:\Users\John\Anaconda3\lib\site-packages\ipykernel_launcher.py:52: RuntimeWarning: invalid value encountered in true_divide
```

```
In [94]: from sklearn.ensemble import RandomForestRegressor
         from sklearn.ensemble import RandomForestClassifier

         # Not rigorous but I picked a few images to train on - it went pretty well

         n_samples = [0, 19, 50, 100, 149, 150, 175, 177, 200, 250, 300, 350, 400, 51
0, 559]
         #n_samples = range(0,560)
```

```

x_train = np.zeros(8).reshape(1,8) # predicted segmentation using Otsu's thresholding

y_train = np.zeros(1) # "correct" segmentation from sum of masks

max_len = 0
conm = []
acc = []
for i in n_samples:
    (img, masks) = load_zipped_img(path+'/stage1_train.zip', i) # Loads image and associated masks

    print(i)
    (img_guess, markers, sure_bg, sure_fg, uncertain) = watershed(img)

    intensity=grayscale(img)
    #img_guess = otsu(intensity)
    img_guess=grayscale(img_guess)
    #img_guess = np.dot(0,intensity)
    laplac = laplace(grayscale(img))[uncertain==255].reshape(-1,1)
    fil = sobel(grayscale(img))[uncertain==255].reshape(-1,1)
    #fil = sobel(grayscale(img)).reshape(-1,1)
    intensity_raw = intensity[uncertain==255].reshape(-1,1)
    #intensity_raw = intensity.reshape(-1,1)
    watershed_raw = img_guess[uncertain==255].reshape(-1,1)
    #watershed_raw = img_guess.reshape(-1,1)
    #print(np.ones(len(watershed_raw)).shape)
    img_type = df['cflabel'][i]*np.ones(len(watershed_raw)).reshape(-1,1)
    img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
    ch1,ch2,ch3 = cv2.split(img3)
    #ch1 = ch1.reshape(-1,1)
    #ch2 = ch2.reshape(-1,1)
    #ch3 = ch3.reshape(-1,1)

    ch1 = ch1[uncertain==255].reshape(-1,1)
    ch2 = ch2[uncertain==255].reshape(-1,1)
    ch3 = ch3[uncertain==255].reshape(-1,1)

    feature = np.concatenate((intensity_raw,watershed_raw, fil, laplac, ch1,
    ch2, ch3, img_type), axis = 1) # Features include grayscale intensity, watershed, sobel, rgb channels

    y_raw = sum(masks)
    y_raw = y_raw[uncertain==255].reshape(-1,1)

    x_train = np.append(x_train,feature).reshape(-1,8)
    y_train = np.append(y_train,y_raw).reshape(-1,1)

Xtrain, Xtest, ytrain, ytest = train_test_split(x_train,y_train)
# Kept a split training set around but not needed

#clf = svm.SVC(C = 1.0)
clf = RandomForestClassifier(max_depth=6, random_state=0)
%time clf.fit(Xtrain, ytrain[:,0]) #599 ms for a training set of this size!

```

```

#y_pred = clf.predict(Xtest)
#cm = confusion_matrix(ytest, y_pred)
#print(cm)
#conm.append(cm)
#accuracy = sklearn.metrics.accuracy_score(ytest, y_pred)
#print(accuracy)
#acc.append(accuracy)

#(img_guess, markers, sure_bg, sure_fg, unknown, reduced_area) = watershed(img)

#fig, ax = plt.subplots(1,4, figsize = (10,10))

#ax[0].imshow(sum(masks), origin='lower')
#ax[1].imshow( grayscale(img), origin='lower')
#ax[2].imshow(output, origin='lower')
#ax[3].imshow(img_guess, origin='lower')
#plt.show()

#A = np.array(acc)
#Avg_acc = np.mean(A)
#print(Avg_acc)

```

```

0
19
50
100
149
150
175
177
200
250
300
350
400
510
559

```

Wall time: 12.7 s

```

Out[94]: RandomForestClassifier(bootstrap=True, class_weight=None, criterion='gini',
                                max_depth=6, max_features='auto', max_leaf_nodes=None,
                                min_impurity_decrease=0.0, min_impurity_split=None,
                                min_samples_leaf=1, min_samples_split=2,
                                min_weight_fraction_leaf=0.0, n_estimators=10, n_jobs=1,
                                oob_score=False, random_state=0, verbose=0, warm_start=False)

```



```

In [95]: # Testing the classifier on the remaining images
acc = []
for i in range(0,560):
    (img, masks) = load_zipped_img(path+'/stage1_train.zip', i)

    (img_guess, markers, sure_bg, sure_fg, unknown) = watershed(img)
    intensity = grayscale(img)
    shap = intensity.shape
    intensity_raw = intensity.reshape(-1,1)
    #watershed_raw = img_guess[unknown==255].reshape(-1,1)
    watershed_raw = grayscale(img_guess).reshape(-1,1)
    fil = sobel(grayscale(img)).reshape(-1,1)
    laplac = laplace(grayscale(img)).reshape(-1,1)
    img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
    #hsv = cv2.cvtColor(img, cv2.COLOR_BGR2HSV)
    img_type = df['cflabel'][i]*np.ones(len(watershed_raw)).reshape(-1,1)
    img3 = cv2.cvtColor(img, cv2.COLOR_RGB2BGR)
    #ch1,ch2,ch3 = cv2.split(hsv)

    ch1,ch2,ch3 = cv2.split(img3)
    ch1 = ch1.reshape(-1,1)
    ch2 = ch2.reshape(-1,1)
    ch3 = ch3.reshape(-1,1)
    #print(ch1.shape)
    #print(intensity_raw.shape)
    feature = np.concatenate((intensity_raw,watershed_raw, fil, laplac, ch1, c
h2, ch3, img_type), axis = 1)

    yp = clf.predict(feature)
    y_real = sum(masks).reshape(-1,1)
    accuracy = sklearn.metrics.accuracy_score(y_real, yp)
    precision = sklearn.metrics.precision_score(y_real, yp)
    f1 = sklearn.metrics.f1_score(y_real, yp)
    print(i, accuracy, precision, f1)
    acc.append([accuracy, precision, f1])

    #output = yp.reshape(shap[0], shap[1])

precision = np.mean(np.array(acc)[:,:1])
accuracy = np.mean(np.array(acc)[:,:0])
f1 = np.mean(np.array(acc)[:,:2])
print('The average precision of the model is {}'.format(precision*100))
print('The average accuracy of the model is {}'.format(accuracy*100))
print('The average f1-score of the model is {}'.format(f1*100))

```

0 0.969528198242 0.768273716952 0.83185989728
1 0.974746704102 0.740964840556 0.845572454978
2 0.892297363281 0.947755211117 0.828282828283
3 0.949694824219 0.824115876599 0.86447199658
4 0.980419921875 0.872095220102 0.851974898487
5 0.459716796875 0.0773522433976 0.143534420202
6 0.871292114258 0.689623740315 0.763533402484
7 0.976666666667 0.942311809868 0.953216374269
8 0.440200805664 0.0274388619532 0.053165406354
9 0.969714506173 0.913987645848 0.944333347516
10 0.953032407407 0.855305989583 0.911970150549
11 0.841542895145 0.546974185358 0.684526436579
12 0.894958496094 0.618857142857 0.758828475336
13 0.498123168945 0.131054131054 0.23034983035
14 0.966597222222 0.933867373366 0.934754103302
15 0.459533691406 0.0551354842182 0.103699579938
16 0.967849731445 0.683611924866 0.790160342595
17 0.970231481481 0.945651341485 0.950504195212
18 0.990325927734 0.608860759494 0.752150117279
19 0.431427001953 0.00837110103972 0.016574294009
20 0.992889404297 0.655325443787 0.791778373548
21 0.868520667551 0.815531584522 0.769230023133
22 0.953240740741 0.850072825312 0.900528544696
23 0.968495368958 0.422118094959 0.582675374878
24 0.435562133789 0.0227290747152 0.0444318152463
25 0.982411384583 0.392988929889 0.509664211842
26 0.468139648438 0.0884088542362 0.159650899272
27 0.94352722168 0.748766488773 0.800732245733
28 0.462295532227 0.0484602917342 0.0924099209313
29 0.996084770115 0.652748037116 0.72078817734
30 0.443344116211 0.0190191805881 0.0373136297665
31 0.465560913086 0.0865250098984 0.157668165749
32 0.955004882812 0.936489093337 0.773975962718
33 0.921493530273 0.827044834509 0.861986641273
34 0.92880859375 0.923701698253 0.865504358655
35 0.482131958008 0.13321737351 0.234832600609
36 0.968927469136 0.922659276645 0.941876073496
37 0.868005371094 0.865649877828 0.802477029027
38 0.947235107422 0.913841080078 0.836825217063
39 0.962072753906 0.949818181818 0.770785687938
40 0.922717285156 0.923751315064 0.853479599158
41 0.534957885742 0.181533899447 0.306474001593
42 0.839378868258 0.779763615462 0.749188872014
43 0.971195987654 0.935302104549 0.956791480988
44 0.505966186523 0.107053001189 0.193016126218
45 0.988315097259 0.960608639071 0.947788189686
46 0.9892578125 0.70141101502 0.814051769678
47 0.912201591512 0.620338596736 0.755109590308
48 0.507583618164 0.136539524599 0.23439538801
49 0.9619140625 0.984181141439 0.802681507716
50 0.989318847656 0.630843632456 0.770341207349
51 0.96598815918 0.486700215672 0.64568431092
52 0.473059082031 0.60203133709 0.310530434921
53 0.962713623047 0.878815228093 0.778104681995
54 0.965864197531 0.918872688768 0.938649285813
55 0.9650390625 0.856514503091 0.834201690402
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57 0.977145061728 0.920021056326 0.946528504892
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64 0.91103968078 0.715336231616 0.793993839836
65 0.980066299438 0.333876719307 0.484715511291
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72 0.942399691358 0.84689222152 0.909470160928
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74 0.523788452148 0.141937830503 0.245339136743
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116 0.985610961914 0.612278533169 0.759131545338
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128 0.944665527344 0.863162705667 0.892849544971
129 0.888549804688 0.87796670209 0.844463373083
130 0.500198364258 0.097869377524 0.176658371666
131 0.990295410156 0.712925170068 0.831746031746
132 0.51220703125 0.125598052657 0.219264397011
133 0.986022949219 0.607388316151 0.755341880342
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138 0.9357421875 0.818193855932 0.824416277518
139 0.903381347656 0.733561185824 0.833041533951
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191 0.991302490234 0.68838992333 0.815175097276
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225 0.904310344828 0.887758827518 0.830933109421
226 0.958726851852 0.905023190326 0.942339409488
227 0.469573974609 0.093192718408 0.168293616614

228 0.97998046875 0.743756003842 0.825206501465
229 0.930798339844 0.934079299441 0.575323994307
230 0.961074829102 0.655398203137 0.771395286316
231 0.502517700195 0.124179610588 0.21787213626
232 0.952484567901 0.8747858473 0.919631437446
233 0.930648803711 0.58140655106 0.726452001204
234 0.991485595703 0.780729401171 0.861400894188
235 0.981959876543 0.956570311409 0.966995115616
236 0.823571777344 0.410940447319 0.513416153251
237 0.938659667969 0.903769705278 0.867728349566
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239 0.964001464844 0.894068877551 0.904831058186
240 0.421752929688 0.00823887489505 0.0163015263213
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244 0.895106653404 0.876460583126 0.82104320362
245 0.951226851852 0.880725790717 0.927821866971
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247 0.985397338867 0.704827136334 0.818715665846
248 0.918121337891 0.674560629708 0.787468314322
249 0.958263888889 0.910116355876 0.929805209131
250 0.977561339523 0.910038506313 0.926001184564
251 0.956782407407 0.865915261729 0.907352576296
252 0.961134259259 0.890118047075 0.936549726019
253 0.929760742188 0.943057722309 0.863078241005
254 0.951477050781 0.671013713364 0.758395380641
255 0.91907043457 0.895739462727 0.703164351515
256 0.967163085938 0.727903181942 0.832633379997
257 0.985030174255 0.591875724263 0.706559736788
258 0.931237792969 0.842074074074 0.875982475067
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559 0.964992283951 0.925048097478 0.938502202643
The average precision of the model is 64.76129213178552%
The average accuracy of the model is 85.67608686011847%
The average f1-score of the model is 68.71381375457058%

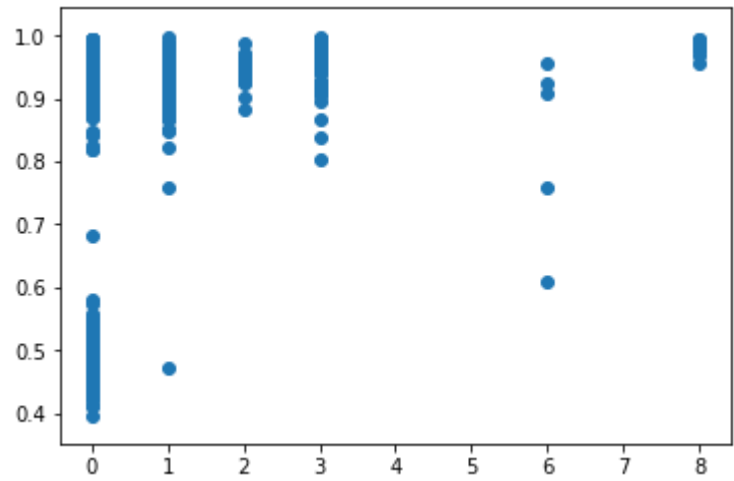
```
In [114]: fig, ax = plt.subplots()
zz = df['cflabel'].values
acc_vec = np.array(acc)

ax.scatter(zz, acc_vec[:,0])
plt.show()

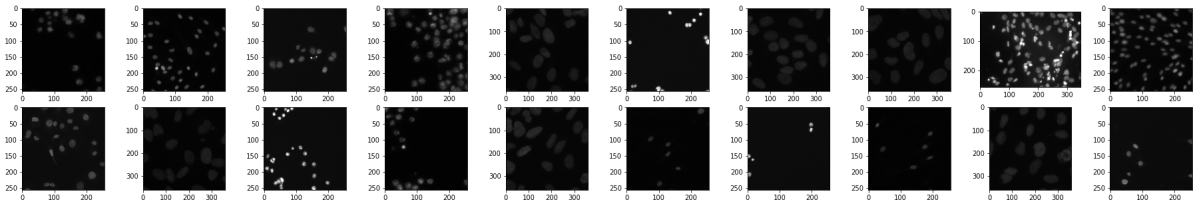
FDIV = 3
CDIV = 3
for t in range(FDIV*CDIV):
    print('for type=>' + str(t))
    fig, ax = plt.subplots(2, 10, figsize=(32, 5))

    n = 0
    for i in range(2):
        for j in range(10):
            if n < len(df[df['cflabel'] == t].index):
                sn = df[df['cflabel'] == t].index[n]
                (img, masks) = load_zipped_img(path + '/stage1_train.zip', sn)
                ax[i, j].imshow(img)
                n = n + 1

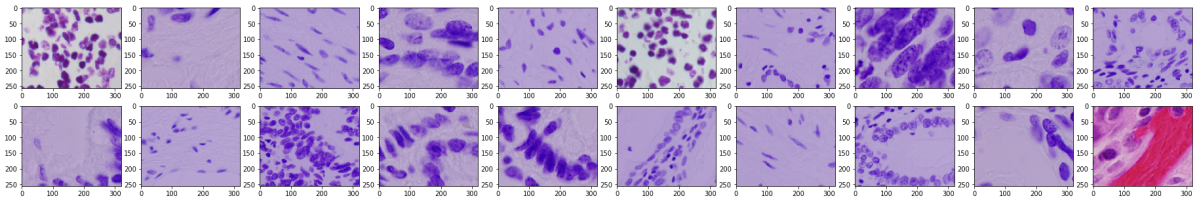
    plt.show()
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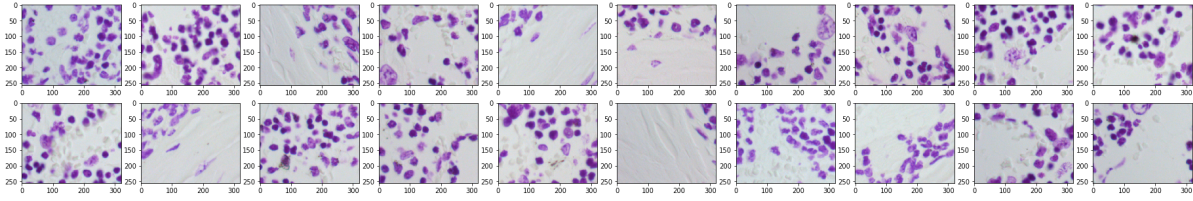
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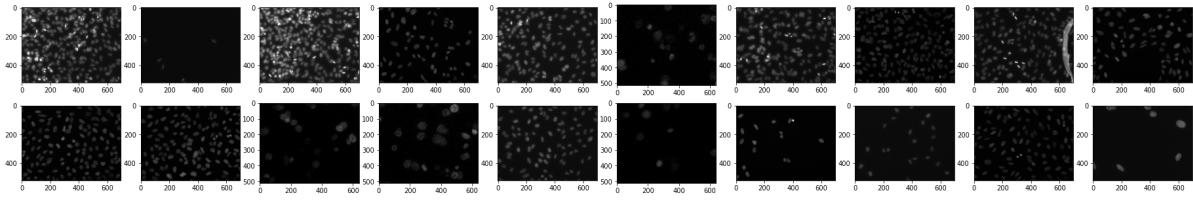
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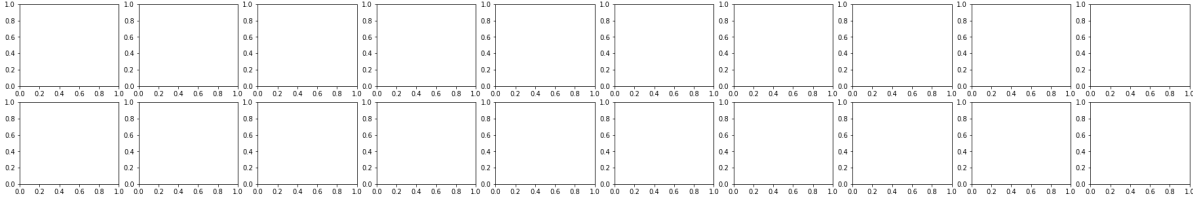
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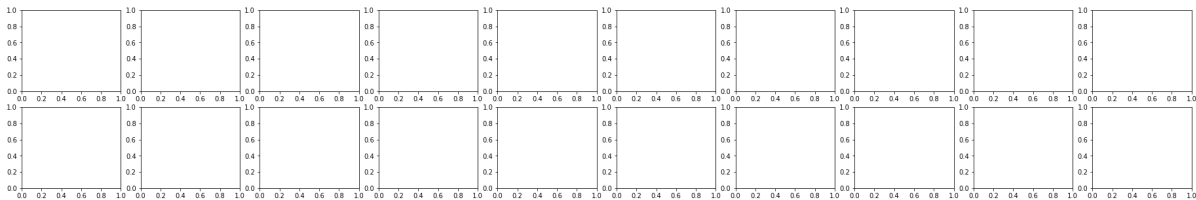
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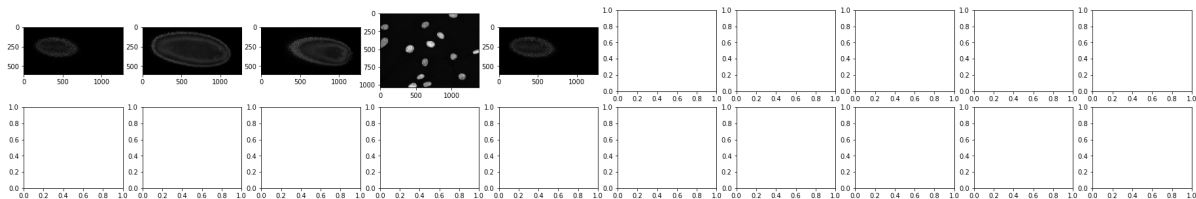
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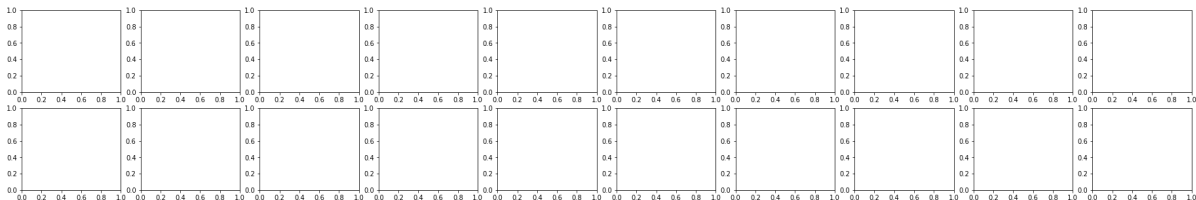
for type=>5



for type=>6



for type=>7



for type=>8

