I. AIM

This project aims to segment out five tissue layers on ten consecutive 'axial' cross-sections of publicly available MRI of a single human subject so that it can be further analyzed. So we are required to develop 2D as well as 3D segmentation algorithm that will segment out the following regions: Air (class 0), Skin/Scalp (class 1), Skull (class 2), CSF (class 3), Gray Matter (class 4), White Matter (class 5). Also, we need to evaluate the results obtained from 2D and 3D algorithms and conclude with our observation based on the results.

II. METHODOLOGY

A. 2D Segmentation

To solve the 2D segmentation problem, we first need to analyze and identify broadly different regions present in the image. Once this is done, we can go ahead to generate masks to extract these regions. In our problem, we can see three regions that stand out: skin/scalp, skull, and brain matter region. So now we can plan to generate masks for these regions. We have experimented with three methods to get the masks for these regions.

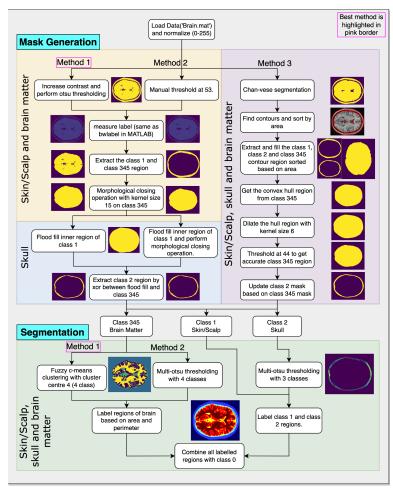


Figure 1: Flowchart of the 2D segmentation algorithm.

Method 1 and method 2 are similar and rely on thresholding and then labeling connected regions of the image (measure label). Method 3 is different as we use the chan-vese algorithm to get the initial binary mask for the regions and find contours to generate the required masks.

Figure 1 'Mask Generation' section gives more details in this regard. Once the masks are generated, we can go to the next stage to segment the regions within the masks. For this, we have used two methods. Method 1 uses Fuzzy c-means clustering, and method 2 uses Multi-otsu thresholding as shown in figure 1 'Segmentation' section. 2D segmentation algorithm runs slice by slice at a time. For simplicity, class 1 indicates skin/scalp mask, class 2 indicates skull mask and class 345 indicates brain matter and CSF mask.

➤ Mask Generation

Method 1 (Best accuracy)

- At first, we increase the image contrast by a factor of 2.8 and perform otsu thresholding. By increasing the contrast, we get well-defined edges of the region. We also perform the morphological opening operation to remove small bright regions using a disk-shaped kernel of size 1.
- Then we label all the connected regions using the measure label function. Measure label checks neighbor pixels to tell if the current pixel is connected. If they are connected, they are assigned to the same label; otherwise, a different label is assigned.
- Extract the class 345 and class 1 region and perform morphological closing operation on the class 345 mask using a disk-shaped kernel of size 2.
- Next, we take the class 1 mask and flood-fill the region inside the skin/scalp to get the filled mask. Flood-fill function will fill the region defined by their boundaries with the same values essentially filling the hole. Next, take out the class 2 mask by performing xor operation on the fully filled mask and class 345 mask.

O Method 2

- Method 2 is very similar to method 1, except we use manual thresholding in the first step based on the histogram of pixel intensities. In this work, we have used 53 as the thresholding value after experimentation.
- Similar to method 1, we do a flood fill operation, but soon after that, we do an additional closing operation with a disk-shaped kernel of size 4 to the fully-filled mask and class 1 mask to plug in small holes in the masks.

○ Method 3

- We first perform the chan-vese algorithm to get the class 1 mask and class 345 mask.
- Once we get masks, we find contours to the generated mask and sort them based on the area. We will have three contours that we will need class 1 outer and inner contours and class 345 contour. From these contours, extract class 1, class 2, and class 345 region and create individual masks by filling in between contours.
- For the class 345 (brain matter region) we run into some problems.
 - 1. The outer edges are not smooth and round.
 - 2. We seem to miss out on important regions related to CSF since our initial chan-vese couldn't identify those regions due to those regions being on the darker scale. If we tune it to identify those regions, then we risk detecting unwanted portions which might ruin the separateness of the brain matter region.

To remedy this we have first find the convex hull points of the region which will smoothen out points, thus creating a smooth and almost circular-shaped outer region. Second, we dilate the generated convex hull mask and threshold manually at 44 to perfectly get the class 345 mask.

■ We then update the class 2 mask based on the updated class 345 mask, by identifying the intersection of class 2 and class 345 masks and subtracting it from class 2 mask.

By this stage we will have successfully generated all the masks for all the unique regions in the image as shown in Figure 2.

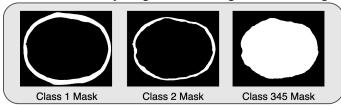


Figure 2: Masks generated for class 1, 2 and 345 region

➤ Segmentation

Method 1 (Best accuracy)

- We will use the masks generated for class 345 to get the brain matter region of the image and then run fuzzy c-means clustering algorithm with number of centres as four representing three different classes within brain matter region and background class.
- Once we get all the segmented regions in the brain matter region, we then identify and label all the different regions of the class 345 based on the area and perimeter.
- For class 2, we use multi-otsu thresholding with three classes to get the skin/scalp region inside class 2.
- Finally, by combining all the different labelled regions we get the segmented image of the brain as shown in Figure 3.

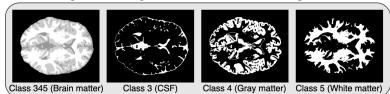


Figure 3: Segmentation result of the brain matter region (class 345)

o Method 2

■ Every step in method 2 is the same as method 1, but instead fuzzy c-means clustering algorithm we use thresholding multi-otsu with number of classes as four.

B. 3D Segmentation

Some major disadvantages of 2D segmentation algorithms are that they do not make utilize the depth aspect of the images, and since they operate slice by slice, sometimes they might be slower. For this reason we have designed a 3D segmentation algorithm which operates on all the slices simultaneously. Figure 4 illustrates the steps in the 3D segmentation algorithm. 3D segmentation algorithm flow is very much similar to the 2D segmentation algorithm.

➤ Mask Generation

O Method 1

- Increase the contrast of the 3D image by a factor of 2.7 and then perform otsu thresholding to separate the background mask and foreground mask.
- Then perform morphological opening operation to remove small bright marks from the 3D image. For this, we use a custom-built ball-shaped 3D kernel of size 1 where we take the normal ball shaped 3D kernel and elongate it to cover the depth. This will clear most of the bright marks and improve the accuracy. Then

we call the measure label to label all the connected regions (in 3D it will consider the depth aspect while checking for neighbour pixel connectivity).

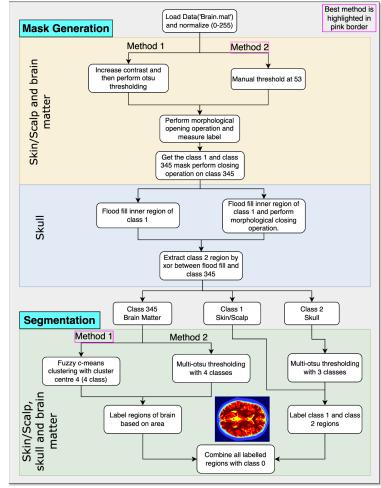


Figure 4: Flowchart of the 2D segmentation algorithm.

- Get the class 1 and class 345 regions of the image and perform morphological closing operation on class 345 with ball shaped 3D kernel of size 13 to cover all the holes and cracks.
- Flood fill the inner region of the class 1 mask which is same as 2D segmentation algorithm except that filling will include depth.
- Extract the class 2 region by just performing xor operation of flood fill mask and class 345 mask.

Method 2 (Best accuracy)

- This is same as method 1 except in the first step we use manual thresholding at 53 to get the foreground/background
- Also after flood fill operation, we do closing operation with 3D ball-shaped kernel of size 5 on class 1 mask.

> Segmentation

o Method 1 (Best accuracy)

■ This is same as what we did in 2D segmentation algorithm (i.e use fuzzy c-means clustering with 4 centers) except this is done on all the slices simultaneously. Labelling the region is done only by sorting based on the area for class 345.

o Method 2

■ This is same as what we did in 2D segmentation algorithm (i.e use multi-otsu thresholding with 4 class) except this is done on all the slices simultaneously. Labelling the region is done only by sorting based on the area for class 345.

III. EVALUATION AND COMPARISON

A. Evaluation for 2D and 3D segmentation algorithms

For evaluation we have used three types of metic namely:

- <u>Pixel wise accuracy</u>: It is one of the easiest metric to understand. It indicates the percentage of pixels that are correctly classified. But if there is class imbalance then it is not at all accurate in giving the accuracy for the segmentation. So this metric is ignored in this work.
- Intersection-over-union(IOU): This is one of the most commonly used evaluation criteria for the segmentation tasks. IOU is the area of the overlap between predicted and ground truth segmentation divided by the union of the predicted and ground truth segmentation. The reason why it's so effective is that it tells us how well the two masks are overlapping with each other. Also handles class imbalance problem.
- <u>Structural similarity(SSIM)</u>: This metric takes into account the structural as well as perceptual phenomena which include luminance and contrast. In our work, we only use it to get the overall structural accuracy.

Figure 5 and 6 shows the scores for various combination of methodologies. From the table, it is apparent that the fuzzy c-means (segmentation) with otsu thresholding (mask) has the best overall accuracy. In the case of 3D, it is clear that both fuzzy c-means (segmentation) with otsu/manual thresholding (mask) have the best overall accuracy.

		C0	C1	C2	C3	C4	C5	Mean
Mask Method 1 + Segmentation Method 1	Avg. IOU	0.99	0.939	0.944	0.764	0.91	0.967	0.92
	Avg. SSIM	0.984	0.933	0.985	0.912	0.903	0.964	0.947
Mask Method 2 + Segmentation Method 1	Avg. IOU	0.985	0.923	0.943	0.741	0.907	0.967	0.911
	Avg. SSIM	0.977	0.924	0.985	0.905	0.901	0.964	0.943
Mask Method 3 + Segmentation Method 1	Avg. IOU	0.973	0.92	0.928	0.775	0.898	0.957	0.909
	Avg. SSIM	0.963	0.939	0.982	0.915	0.898	0.954	0.941
Mask Method 1 + Segmentation Method 2	Avg. IOU	0.99	0.939	0.944	0.731	0.907	0.981	0.915
	Avg. SSIM	0.984	0.933	0.985	0.898	0.904	0.979	0.947
Mask Method 2 + Segmentation Method 2	Avg. IOU	0.985	0.923	0.943	0.708	0.904	0.981	0.907
	Avg. SSIM	0.977	0.924	0.985	0.89	0.901	0.98	0.943
Mask Method 3 + Segmentation Method 2	Avg. IOU	0.973	0.92	0.928	0.789	0.872	0.922	0.901
	Avg. SSIM	0.963	0.939	0.982	0.921	0.868	0.92	0.932

Figure 5: Accuracy scores for various 2D segmentation algorithms

		CO	CI	C2	C3	C4	CS	Mean
Mask Method 1 + Segmentation Method 1	Avg. IOU	0.98	0.92	0.892	0.758	0.9	0.943	0.898
	Avg. SSIM	0.976	0.932	0.975	0.916	0.893	0.942	0.939
Mask Method 2 + Segmentation Method 1	Avg. IOU	0.984	0.929	0.916	0.759	0.894	0.943	0.904
	Avg. SSIM	0.976	0.933	0.98	0.912	0.887	0.942	0.938
Mask Method 1 + Segmentation Method 2	Avg. IOU	0.984	0.923	0.892	0.727	0.886	0.943	0.8925
	Avg. SSIM	0.976	0.932	0.975	0.901	0.88	0.942	0.934
Mask Method 2 + Segmentation Method 2	Avg. IOU	0.984	0.929	0.916	0.727	0.879	0.943	0.896
	Avg. SSIM	0.976	0.933	0.98	0.897	0.875	0.942	0.933

Figure 6: Accuracy scores for various 3D segmentation algorithms

We can observe overall class 3 accuracy is lower than all other accuracies. This can be attributed to the fact that the number of pixels are less. Furthermore, these pixels are spread out and are very thin in the outer ring region. So a small change in the structure will lead to big accuracy difference. Additionally, fuzzy c-means clustering performs better for class 4 and 5, whereas multi-otsu has better

accuracy for class 3. Overall for class 345, fuzzy c-means beats multi-otsu. This is due to the fact that fuzzy c-means assigns soft probability for the cluster memberships so it handles outliers well whereas multi-otsu thresholding might not handle the boundary cases that well. Moreover, one observation we could find with respect to class 3 near the back region of the skull the ground truth mask annotation seems to be little off which might also drastically reduces accuracy of our class 3.

B. Comparison of 2D and 3D segmentation algorithms

Between 2D and 3D segmentation results, we can observe that 2D segmentation outperforms 3D segmentation by a very small margin. This difference arise due to the fact that, in 3D segmentation algorithms, when measure label is called, the neighbour connectivity considers surrounding pixels in depth whereas in 2D it doesn't consider depth. Also we use 3D shaped kernel in morphological operations in case of 3D segmentation which results in slightly varied structure of the mask. But results are fairly comparable. Final segmentation output for both 3D and 2D segmentation is shown in Figure 7.

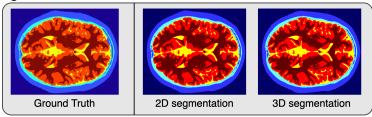


Figure 7: Final segmentation result of 2D and 3D algorithms (Slice0)

IV. CONCLUSION

In this work, we have experimented using a combination of various methodologies. From the best 2D and 3D segmentation algorithms, we can observe that for all the classes except class 3, average IOU scores over all the slices are above 90%. For class 3 however due to the fact that the number of pixels are less and also pixels are very thin in the outer ring region, the accuracy is in the range of 75% to 80%. Also, there is not much difference in the accuracies of 2D and 3D segmentation and if we manually inspect it the results are comparable. Hence we can conclude that the best performing algorithm for both 2D and 3D is otsu thresholding for getting mask and fuzzy c-means clustering for segmentation with average IOU accuracy of 92% and average SSIM score of 95% for 2D and 90% and 94% in case of 3D. In future work, we can try out other segmentation algorithms like watershed, region growing etc. We can also try out other evaluation metics like f1 score, precision score, and recall score, time taken by the algorithm.

V. APPENDIX

A. Threshold (multi-)otsu

Otsu thresholding is a type of automatic thresholding method where threshold is selected based on minimizing intra-class intensity variance i.e. maximizing inter-class intensity variance iteratively. The steps in calculating the threshold values in terms of class probabilities w, class mean μ and class variance σ are given below:

- Compute the histogram and probabilities at each intensity level.
- Initialize w(0) and u(0)

- Iterate over all possible threshold values i.e t = 1 to max intensity
 - \circ Update w and μ .
 - \circ Compute σ^2
- Choose the highest σ^2 as the threshold value

Multi-otsu is an extension of otsu thresholding where it can detect several threshold and this is given by the desired number of classes. Some related formulas and implementation can be found under this link.

B. Fuzzy c-means clustering

Fuzzy c-means clustering is a soft clustering technique where the datapoint in the dataset belongs to every cluster with some probability. If a data point is closer to the cluster center then it will have a higher membership grade to that cluster. For a far-away cluster, it will have a low membership value to that cluster.

Steps in fuzzy c-means clustering are as follows:

 Randomly initialise cluster center and assign each data point random membership grade for each cluster.

- Iteratively move the cluster center to the right spot and keep updating the membership grade for each data point
- Objective is to minimize the distance between any given data point and cluster center weighted by the membership value of the data point in that cluster.

Algorithm and the objective function is given in this <u>link</u>.

C. Chan-vese algorithm

Chan-vese for active contours is a powerful method to segment images and it is capable of segmenting difficult segmentation problems. It is based on Mumford-Shah functional for segmentation. It is basically an energy minimization problem that can be solved using level set formulation. So this energy function is given by the weighted values which corresponds to the sum of differences intensity from average value of intensity outside segmented region, the sum of differences from the average value inside segmented region and a term dependent on the perimeter of the boundary of segmented region. More information about the actual energy equation and additional information is given in this paper.

VI. CODE

Complete working code with implementation and running details is uploaded to github. Please find the complete code in this git link.

Segmentation Code - 2D and 3D

```
: 2D and 3D brain MRI segmentation (6 classes and 10 slices)
pplication
                  : Raghuveer Bhat R
ast Modified Date : 13/05/2022
ibraries required : skimage, numpy, matplotlib, scipy, skfuzzy, cv2(opencv), sklearn
                  : python3 main3d.py [3D/2D] [fuzzyc/multiotsu (brain segmentation method)] [default/otsu (foreground background segmentation)] [write/show]
mport matplotlib.pyplot as plt
mport scipy.io
mport numpy as np
rom skimage.filters import threshold_multiotsu
rom skimage import measure
mport skfuzzy as fuzz
rom skimage.morphology import (erosion, dilation, opening, closing, white_tophat, cube, octahedron, ball, disk)
rom brain regions import BrainRegions3I
rom evaluation import *
rom utils import *
 Load the "Brain.mat" file and normalize it in the range (0-255)
ef load dataset(file):
 mat = scipy.io.loadmat(file)
 image3d = mat['T1']
     image = image3d[:,:,i]
     im = cv2.normalize(image, None, alpha=0, beta=1, norm type=cv2.NORM MINMAX, dtype=cv2.CV 32F)
     im = np.uint8(im*255)
     image3d[:,:,i] = im
 labels = mat['label']
 return image3d, labels
     3D segmentation algorithm on all the slices simultaneuosly
ef segmentation 3D(image3d, labels, segmentation method="default", segment brain method="fuzzyc", write or show="show"):
 print("3D segmentation in progress ...")
 b = BrainRegions3D(image3d, method=segmentation method)
  # Class1 in class 2 region
```

```
chresholds_or2 = threshold_multiotsu(image3d,classes=3)
regions or2 = np.digitize(image3d, bins=thresholds or2)
t = b.or2 > 44
print("Brain region segmentation in progress ...")
 regions_brain = No
if segment_brain_method == "fuzzyc":
    vec br = b.br.reshape((1,-1))
    ncenters = 4
    vec_br, ncenters, 2.0, error=0.005, maxiter=1000, init=None)
    cluster membership = np.argmax(u, axis=0)
    regions_brain = cluster_membership.reshape(b.br.shape).astype('uint8')
    thresholds_or2 = threshold_multiotsu(b.br,classes=4)
    # Using the threshold values, we generate the three regions.
    regions_brain = np.digitize(b.br, bins=thresholds_or2)
region brain final = label regions brain 3d(regions brain)
final_segmentation = np.zeros(image3d.shape, dtype=np.uint8)
final_segmentation[np.where(b.or1_mask == 255)] = 1
 # Class 2
final segmentation[np.where(b.or2 mask == 255)] = 2
 final_segmentation[np.where(t == 1)] = 1
final segmentation = final segmentation | region brain final
iou_scores3d = getIOUScores(labels,final_segmentation)
 mssim_3d = getSSIMScores(labels,final_segmentation,channel_axis=2)
msqe_3d = getMeanSqError(labels,final_segmentation)
print("IOU SCORES: ",iou scores3d)
print("SSIM: ",mssim 3d)
 show_3D(final_segmentation)
Run 2D segmentation algorithm on each slice one by one.
f segmentation_2D(image3d, labels, segmentation_method="default", segment_brain_method="fuzzyc", write_or_show="show"):
iou_scores_2d = []
mssim 2d = []
msqe 2d = []
file_names = ["Slice1 ", "Slice2 ", "Slice3 ", "Slice4 ", "Slice5 ", "Slice6 ", "Slice7 ", "Slice8 ", "Slice9 ", "Slice9 ", "Slice9 "]
 file_names.append("Average ")
for idx in range(0,image3d.shape[2]):
    print(f'Processing {file_names[idx]} ...')
    b = BrainRegions3D(image3d[:,:,idx], type="2D", method=segmentation_method)
    vec_br = b.br.reshape((1,-1))
    regions_brain = None
    if segment_brain method == "multiotsu":
        b.br = b.increaseContrast(b.br, 0.8)
        thresholds_brain = threshold_multiotsu(b.br,classes=4)
        # Using the threshold values, we generate the three regions
        regions_brain = np.digitize(b.br, bins=thresholds_brain)
    if segment brain method == "fuzzyc":
        ncenters = 4
        cntr, u, u0, d, jm, p, fpc = fuzz.cluster.cmeans(
        vec br, ncenters, 2.0, error=0.005, maxiter=1000, init=None)
        cluster_membership = np.argmax(u, axis=0)
        regions_brain = cluster_membership.reshape(b.br.shape).astype('uint8')
     # Segmenting class 1 in class 2 region
    # Applying multi-Otsu threshold for the default value, generating three classes.
    b.or2 = b.increaseContrast(b.or2, 0.048)
    thresholds_or2 = threshold_multiotsu(b.or2,classes=3)
     # Using the threshold values, we generate the three regions.
    regions or2 = np.digitize(b.or2, bins=thresholds or2)
    brain_final_segement = label_regions_brain(regions_brain)
    or2_class1_segment = np.zeros(b.or2.shape, dtype=np.uint8)
    regions = measure.regionprops(regions_or2)
    regions = sorted(regions, key=sort by area, reverse=True)
     npc = np.array(regions[-1].coords)
    or2_class1_segment[(npc[:,0],npc[:,1])] = 1
    region or1 or2 = b.or1 mask
    region_or1_or2[np.where(region_or1_or2 == 255)] = 1
    region_or1_or2 = region_or1_or2 | b.or2_mask
    region_or1_or2[np.where(region_or1_or2 == 255)] = 2
    region_or1_or2[np.where(regions_or2 == 2)] = 1
```

```
final_segmentation = np.uint8(brain_final_segement) | np.uint8(region_or1_or2)
    # Calculate scores (metrics)
    score = getIOUScores(labels[:,:,idx],final_segmentation)
    iou_scores_2d.append(score.tolist())
    mssim = getSSIMScores(labels[:,:,idx],final_segmentation)
    mssim_2d.append(mssim.tolist())
    mse = getMeanSqError(labels[:,:,idx],final segmentation)
    msqe 2d.append(mse)
    if write_or_show == "write":
       write segmented image(final segmentation, file names[idx])
    elif write or show == "show":
        show_image(show_seg_regions(final_segmentation))
iou scores 2d = np.array(iou scores 2d)
mssim 2d = np.array(mssim 2d)
msqe_2d = np.array(msqe_2d)
iou_scores_2d = np.vstack((iou_scores_2d, np.average(iou_scores_2d,axis=0)))
iou scores 2d = np.hstack((iou scores 2d, np.array([np.average(iou scores 2d,axis=1)]).T))
mssim_2d = np.vstack((mssim_2d, np.average(mssim_2d,axis=0)))
mssim_2d = np.hstack((mssim_2d, np.array([np.average(mssim_2d,axis=1)]).T))
msqe_2d = np.append(msqe_2d, np.average(msqe_2d))
   header = np.array(['File names', ' Class0 ', ' Class1 ', ' Class2 ', ' Class3 ', ' Class4 ', ' Class5 ', 'Overall'])
   write_scores(iou_scores_2d,'Output1/iou_scores.txt',np.array(file_names), header)
    print("Average IOU scores:\n", iou_scores_2d[-1])
     print("Average SSIM: ", mssim 2d[-1])
__name__ == "__main__":
args = sys.argv[1:]
type = "2D"
segment_brain_method = "fuzzyc"
segmentation_method = "otsu
write_or_show = "show
       print("python3 main3d.py [3D/2D] [fuzzyc/multiotsu (brain segmentation method)] [default/otsu (foreground background segmentation)] [write/show]")
        exit()
   type = args[0]
    segment_brain_method = args[1]
elif arglen == 3:
    type = args[0]
    segment_brain_method = args[1]
    segmentation_method = args[2]
elif arglen == 4:
    segment_brain_method = args[1]
    segmentation_method = args[2]
   write_or_show = args[3]
    print("Running with defaults - 2D, fuzzyc, otsu and show options")
image3d, labels = load dataset('Brain.mat')
    segmentation_3D(image3d,labels,segmentation_method,segment_brain_method,write_or_show)
   {\tt segmentation\_2D\,(image3d,labels,segmentation\_method,segment\_brain\_method,write\_or\_show)}
```

Mask generation code 2D and 3D

```
File : brain_regions.py

Application : 2D and 3D brain MRI segmentation (6 classes and 10 slices)

Author : Raghuveer Bhat R

Last Modified Date : 13/05/2022

description : This file is used to create brain mask regions for further segmentation

**Create custom 3D kernel by elongating it.

def elongate_3d_structure(x):
```

```
idx = int(x.shape[2]/2)
first = x[:,:,0:idx+1]
second = x[:,:,idx+1:x.shape[2]]
f1 = x[:,:,idx]
stacked = np.dstack((f1,f1))
y = np.concatenate((first,stacked),axis=2)
y = np.concatenate((y,second),axis=2)
return y

...
This class is used for getting all the masks (regions) using method 3 i.e chan-vese algorithm and finding contours.
...
```

```
self.orlbr = self.imageFromMask(self.orlbr mask)
self.or2br = self.imageFromMask(self.or2br mask)
    init 1s = checkerboard level set(image.shape, 6)
```

Evaluation code

Utility code

---END----