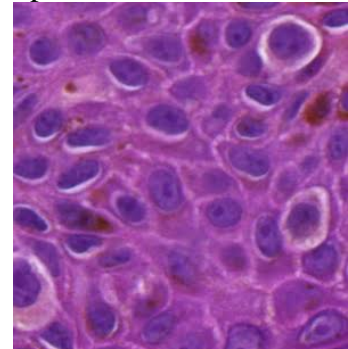


**Assignment 2 (Programming)****Due: Friday ~~February 26~~ March 4, 2016 at 2.00 pm****(deliver it to Room ICE 11-233 (Dr. Mandal))****Topics: Image Segmentation and Image Analysis**

During a biopsy test, a pathologist looks at a specimen under a microscope. With the advances in digital imaging, these microscopic images can be magnified, digitized, and the digital images can be analyzed using image processing algorithm. In this assignment, you will analyze a histopathological image obtained from a stained skin tissue sample (this is a real image obtained from UofA Hospital). The image looks pink/red due to the staining. Your task is to segment the nuclei present in the image and calculate nuclei size, centroid, perimeter and ellipticity. Use the MATLAB environment to do the programming and obtain the solution. The programming can be written according to the following steps.



- (a) Download the image **histopath.jpg** from the EClass website. It is a color image, which has three channels. Display the **Red channel** (only) of the image, which should look like a gray level image.
- (b) Calculate a global threshold (using Otsu's method) for the Red channel image by using MATLAB function `graythresh`. Denote the global threshold value as  $t$ .
- (c) Using the global threshold  $t$ , generate a segmented binary image BW. Display the binary image BW using `imshow`. Note that the nuclei will appear as dark areas.
- (d) Generate a binary image BWC, which is complement of the binary image BW, and display it using `imshow`. Note that in BWC, the nuclei pixels will be brighter (foreground) and the background pixels will be darker.
- (e) In order to remove small noise components, perform **morphological opening operation** on BWC using a disk shape structuring element with a radius of 5 pixels. To learn about morphological opening operation, please see the appendix at the end of the assignment.
- (f) Remove small noisy regions with the area smaller than 200 pixels.
- (g) Compute the **Area, Centroid and Perimeters** of connected nuclei regions.
- (h) Compute the best fitted ellipses of connected nuclei regions, and then calculate the **ellipticity** (as defined in the following equation) of each nuclei region.

$$ellipticity = \frac{|A_{ellipse} \cap A_{nucleus}|}{|A_{ellipse}|}$$

where  $||$  is the cardinality operation,  $A_{ellipse}$  denotes the set of pixels in the best fitted ellipse,  $A_{nucleus}$  denotes the set of pixels in the nucleus and  $\cap$  is the intersection operation.

(i) Note down the centroid, area, perimeter and ellipticity corresponding to each nucleus in the following table.

**Table: Parameters of segmented nuclei**

Nuclei#	Area	Centroid	Perimeter	Ellipticity
<b>1</b>				
<b>2</b>				
<b>3</b>				
..				
..				

**What is to be submitted:**

1. The assignment is due by **2 pm, ~~February 26~~ March 4, 2016** (Friday).
2. The submitted assignment shall include (i) answer to questions asked in the assignment with appropriate explanation, (ii) all intermediate figures/images generated during this assignment, and (iii) MATLAB code used to obtain the results. Note that the **main solution should not be mixed with the MATLAB code**, and the MATLAB code should be provided only as an appendix.
3. This assignment is to be done individually. You may collaborate and discuss friends while doing the assignment. But, the submitted assignment must be in your own words.

## Appendix:

### MATLAB Functions

The following MATLAB functions may be helpful in doing this assignment.

#### Built-in MATLAB functions:

- `graythresh`: Image threshold calculation using Otsu's method
- `im2bw`: Convert image to binary image, based on threshold
- `strel`: Create morphological structuring element
- `imopen`: Morphologically open image
- `bwconncomp`: Find connected components in binary image
- `regionprops`: Measure properties of image regions
- `find`: Find indices and values of nonzero elements
- `ismember`: Array elements that are members of set
- `labelmatrix`: Create label matrix from `bwconncomp` structure

#### User Written MATLAB functions (Download from EClass)

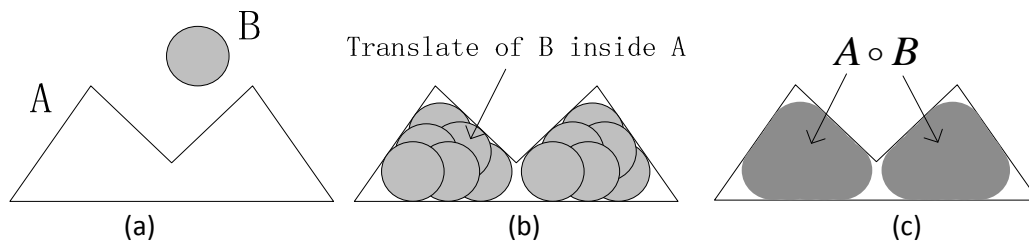
- `LshowMaskContouronIM(mask,im)`: show the object contour defined in binary image named 'mask' onto the image named 'im'.

### Morphological Opening

The morphological opening of  $A$  by  $B$ , denoted by  $A \circ B$ , is simply erosion of  $A$  by  $B$ , followed by dilation of the result by  $B$ . A mathematical formulation of opening is:

$$A \circ B = \cup \left\{ (B)_z \mid (B)_z \subseteq A \right\}$$

where  $\cup \{ \cdot \}$  denotes the union of all sets inside the braces, the notation  $C \subseteq D$  means that  $C$  is a subset of  $D$ , and  $(B)_z$  denotes the translation of set  $B$  by point  $z$ . The morphological opening operation can be intuitively understood by a simple geometric interpretation:  $A \circ B$  is the union of all translations of  $B$  that fit entirely within  $A$ . The following figure (a) shows a set  $A$  and a disk-shaped structuring element  $B$ . Figure (b) shows some of the translations of  $B$  that fit entirely within  $A$ . The union of all such translations is the shaded region in figure (c); this region is the complete opening. As observed from following images, morphological opening removes completely regions of an object that cannot contain the structuring element, smoothes object contours, and breaks thin connections.



Let us see another example. Assume we have the following image  $A$ , and the disk-shaped structuring element  $B$ . The figure (B) shows the output of image opening operation. Note that the narrow bridge and bulges are cleaned up by the operation (as they may be considered as noise).

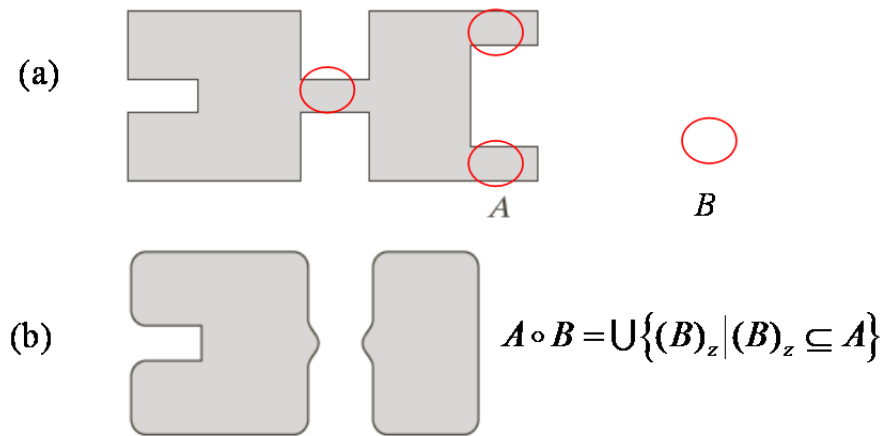


Figure: Morphological Opening. (a) An object  $A$ , and structuring element (SE)  $B$  (shown as a red circle). The structuring element is shown in various positions in  $A$ . (b) The output of image opening operation. Note that the IMOPEN operation broke the narrow isthmus (in the middle) and eliminated two thin protrusions in the right side of  $A$ .

In Matlab, it already has built-in functions to perform morphological opening operations: **imopen**, and **strel**. For anyone who is not familiar with these two functions, please using help in Matlab to learn how to use them.