

## Assignment #4 [25pts]

Segmentation of individual cells or nuclei is an important and challenging job for biomedical image analysis. Analysis of cell morphology (shape, structure, color, texture), cell distribution, cell motility and behavior, or cell-to-cell interactions, *ALL* heavily rely on identification of individual cells. For this assignment, we will explore different steps involved in segmentation of individual cells, nuclei, or particles.

The goals of this assignment are to understand:

- (i) A basic cell/nuclei/particle segmentation pipeline;
- (ii) Clustering-based segmentation (K-means and Meanshift clustering method);
- (iii) And watershed-based segmentation of overlapping/touching cells.**
- (iv) Quantification of object properties**

Steps (i) and (ii) have already been implemented during HW2 and HW3. This assignment's goal is to implement the following tasks for steps (iii) and (iv) and report the results for each of the given test images.

### Assignment: Segmentation of overlapping/touching cells

HW2 and HW3 segmented cells/nuclei/particles from the background but will failed to identify individual cells/nuclei/particles within a touching group. In this task you will use marker-controlled watershed method to segment overlapping/touching objects. You do NOT need to implement watershed transform, you will use Matlab watershed and imimposemin functions. See slide #8 in [2] and Matlab example in [4] for guidelines.

1. Identify internal and external markers for marker-controlled watershed segmentation.
  - a) use segmentation mask and local minima/maxima of the distance transform of the mask to determine markers (check Matlab functions `bwdist`, `imregionalmin`, `imregionalmax` or `bwulterode`).
  - b) use blob detection to determine markers (check scale-space Laplacian of Gaussian filter from Lecture slides 12-13, Matlab functions `fspecial` and `imfilter`).
2. Apply marker-controlled watershed to segment individual cells/nuclei/objects (use Matlab `watershed` and `imimposemin` functions).
3. Apply connected component labeling (Matlab function `bwlabel`), compute area for each cell ( you can use Matlab function `regionprops`), display number of cells/nuclei/objects and histogram of cell areas.
4. [Bonus] Explain how you can use this information to further improve segmentation results.

**Note:** You can use the `imoverlay` function (included in HW4supplement.zip) to mark markers on the original image. Usage:

```
color=[1 0 0]; % color of the overlay
```

```
mix=[0.5 0.5]; % how colors are mixed contribution of image & mask  
[im_marked]=imoverlay(im,mask_markers,color,mix)
```

### Test Images:

- Prostatecancer\_grade4\_cropped and malaria\_cropped.tif

### Required Output:

For each of the test images listed above, show

1. **Original image**,
2. Output of **K-means/Meanshift clustering**: This will be a multi-class output (HW2-3)
3. **Nuclei/cell mask**: Binary mask derived from K-means/Meanshift clustering (i.e. darkest class or combination of two darkest classes etc.)
4. Output of blob filter
5. **Markers** overlayed on image (both methods, 1a & 1b displayed separately)
6. **Watershed boundaries** (for both set of markers 1a & 1b displayed separately)
7. **Final segmentation**
8. Area histograms

### Submission instructions:

1. You should submit an electronic version from Blackboard.
2. Your submission should include a report (including output images & your interpretation of the outputs) and associated programs.

### References:

- [1] Slides for Lectures 9 and 10 : Clustering-based segmentation.
- [2] Slides for Lectures 10 and 11: Grayscale morphology and Watershed segmentation
- [3] Pages 7-13 of Jean Serra course on morphology chapter 10  
<http://cmm.ensmp.fr/~serra/cours/pdf/en/ch10en.pdf>
- [4] <http://www.mathworks.com/help/images/examples/marker-controlled-watershed-segmentation.html?prodcode=IP&language=en>
- [5] Slides for Lectures 12 and 13: Scale-space blob detection using Laplacian of Gaussian.