LAB 5: Segmentation of cells in microscopy images

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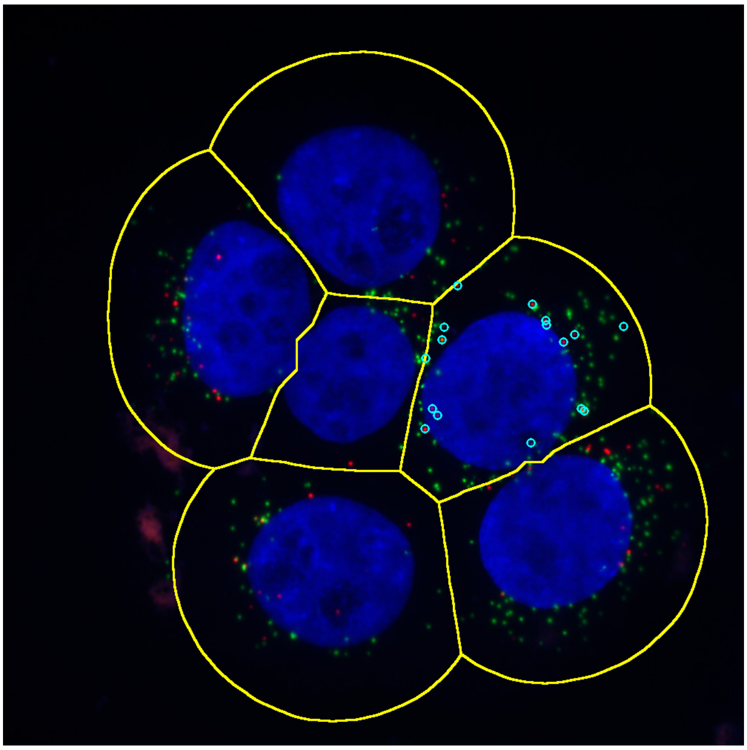

Figure 1: Desired result image.

1 Preparations, introduction and start of the lab

In this laboratory assignment, there is supposed that you work more independently than in the others. It is entirely based on MATLAB. You will probably not be ready in four hours, but have to finish the computer exercise of your own. You should demonstrate your results before the main task to the teacher, however. The main task is examined with a written report, one per group.

Before the session, read the article below, reference [1], so that you understand it approximately. You will get the article via an e-mail from the examiner. Also read the instructions here and repeat necessary theory from the lectures and the book.
[1] *Segmentation of Cytoplasms of cultured cell by Amin Allalou et al.*

Copy all the files on
`/site/edu/bb/DBgrk/segmentering/`
to your home directory and start MATLAB.

2 Watershed segmentation 1

Execute the program `MyWatershedNew.m` in MATLAB. It starts by making a simplified version of Figure 4, a binary image with three cell kernels. One of the resulting images is Figure 2. It shows a segmentation of the three cell kernels and

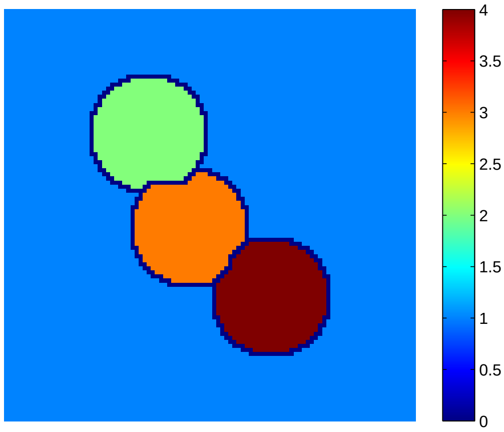

Figure 2: Segmentation result 1.

it corresponds to Fig 1B in reference [1] before thresholding.
In reference [1], the 5-7-11 chamfer distance transform is used. It provides a distance map containing only integers. It is fast and it gives only small errors. Here

we will use the Euclidean distance transform, instead. This distance map contains floating point numbers instead of integers and it is error free. It will be a bit slower, but that does not matter here. Try to understand each step in the MATLAB code. If you are uncertain regarding one function, for example bwlabel, you can write `help bwlabel` in the MATLAB window. You can also check the section “Equivalent MIPS and MATLAB functions” in the Introduction.

2.1 MyWatershedNew.m

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Watershed exemple, partly from Matlab
% Written  by Maria Magnusson,    2007-05-08
% Last update by Maria Magnusson, 2013-09-28
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Make a binary test image (you do not need to understand this code)
%--------------------------------------------------------------------
lims = [-50 50];
[x,y] = meshgrid(lims(1):lims(2));
center1x = -15;
center1y = -20;
center2x = -5;
center2y = 3;
center3x = 15;
center3y = 20;
radius = 15;
bw1 = sqrt((x-center1x).^2 + (y-center1y).^2) <= radius;
bw2 = sqrt((x-center2x).^2 + (y-center2y).^2) <= radius;
bw3 = sqrt((x-center3x).^2 + (y-center3y).^2) <= radius;
bw = bw1 | bw2 | bw3;
figure(1), imshow(bw,'InitialMagnification','fit'), title('bw');
colormap(gray), colorbar;

% Compute the distance transform inside the objects
%--------------------------------------------------
D = bwdist(~bw);
figure(2), imshow(D,[],'InitialMagnification','fit');
title('Distance transform of ~bw');
colormap(jet), colorbar;

% Change the sign of the distance transform and
% set pixels outside the object to the minimum value
%---------------------------------------------------
Dinv = -D;
Dinv(~bw) = min(min(Dinv));
figure(3), imshow(Dinv,[],'InitialMagnification','fit');
title('Complement distance transform of ~bw');
colormap(jet), colorbar;

% Search for regional min
%------------------------
RegMin = imregionalmin(Dinv,8);
```

```
figure(4), imshow(RegMin,[],'InitialMagnification','fit');
colormap(jet), colorbar;
title('regional min of Dinv');

% Perform labeling
%-----------------
labelstruct = bwconncomp(RegMin,8);

% Make a labelimage to look at
%----------------------------
NumObj = labelstruct.NumObjects;
labelim = zeros(labelstruct.ImageSize);
for no = 1:NumObj
  labelim(labelstruct.PixelIdxList{no}) = no;
end
figure(5), imshow(labelim,[],'InitialMagnification','fit');
colormap(jet), colorbar;
title('labeling of regional min');

% Compute the watershed transform
%-------------------------------
W1 = watershed_meyer(Dinv,8,labelstruct);
figure(6), imshow(W1,[],'InitialMagnification','fit');
colormap(jet), colorbar;
title('Watershed of Dinv');

W2 = W1;
loc = find(W1==1);
W2(loc) = 0;
figure(7), imshow(W2,[],'InitialMagnification','fit');
colormap(jet), colorbar;
title('Fixed Watershed of Dinv')

W2T = W2>=1;
figure(8), imshow(W2T,[],'InitialMagnification','fit');
colormap(gray), colorbar;
title('Final segmentation result')
```

3 Watershed segmentation 2

The cytoplasm is located around the cell kernels. The hypothesis is that a pixel rather close to, but outside, the cell kernels contains cytoplasm and belongs to the closest cell kernel. Complete the program MyWatershedNew.m so that you receive a segmentation of the cellkernels+cytoplasm, also, see Figure 3. The result corresponds to segmentation of the cytoplasms in Fig. 2A in reference [1].
Suggestion for implementation:

- Start by doing a distance map outside the cell kernels.
- Limit the distance map to a suitable value e.g. 15.

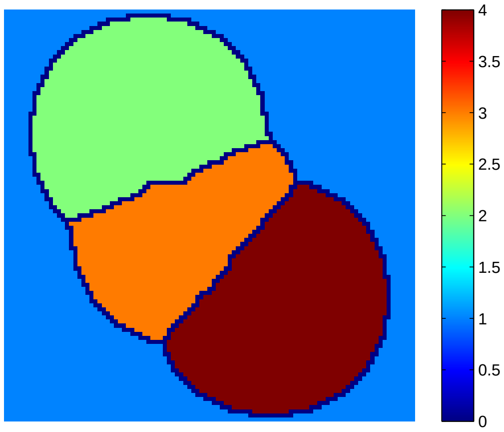

Figure 3: Segmentation result 2.

- Modify Figure 2 slightly so that it can be used as "water holes" for the watershed algorithm. Don't forget to insert a water hole in the big "sea" outside the cell kernels.

Why is it necessary to insert a water hole in the big "sea" outside the cell kernels?

4 Morphological image processing

Execute the program `Binarymanip.m` in MATLAB. Try to understand each step in the MATLAB code. One of the resulting images shows how to set parts of the image to zero and how to overlay the letter M on the input image. That trick you may need later. The MATLAB fig. 7 is not as nice as Figure 4 below. Change the threshold value and the number of erosions and dilations so that the MATLAB figure will be just as nice as shown below. Amin Allalou uses Otsu's automatically thresholding. For you, it is enough to just look at the histogram and make a manual thresholding.

4.1 Binarymanip.m

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% An example about how to read images,
% how to do binary manipulation
```

```
% and how to overlay a pattern.
% Written by Maria Magnusson, 2007-05-08
% Updated by Maria Magnusson, 2008-08-11
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Read a colour image
%--------------------
im1 = double(imread('C9minpeps2.bmp'));
figure(1), imshow(im1/255);

% Look at the three colour components RGB
%----------------------------------------
im1r=im1(:,:,1); im1g=im1(:,:,2); im1b=im1(:,:,3);
figure(2), imshow(im1r,[0 255]), colormap(gray), colorbar;
figure(3), imshow(im1g,[0 255]), colormap(gray), colorbar;
figure(4), imshow(im1b,[0 255]), colormap(gray), colorbar;

% Compute the histogram of the blue image and do threshholding
%-------------------------------------------------------------
histo = hist(im1b(:),[0:255]);
figure(5), stem(histo);
im1bT = im1b>80;
figure(6), imshow(im1bT,[0 1]), colormap(gray), colorbar;

% Perfom opening
%---------------
tmp = bwmorph(im1bT,'erode',1);
tmp = bwmorph(tmp,'dilate',1);

% Perform closing
%----------------
tmp = bwmorph(tmp,'dilate',1);
im1bTmorph = bwmorph(tmp,'erode',1);

figure(7), imshow(im1bTmorph,[0 1]), colormap(gray), colorbar;

% Overlay a pattern
%------------------
immask1 = zeros(1000,1000);
immask1(200:800,200:800) = 1;
immask2 = zeros(1000,1000);
M = [ 1 1 0 0 0 0 0 0 0 0 1 1;
      1 1 1 0 0 0 0 0 0 1 1 1;
      1 1 1 1 0 0 0 0 1 1 1 1;
      1 1 0 1 1 0 0 1 1 0 1 1;
      1 1 0 0 1 1 1 1 0 0 1 1;
      1 1 0 0 0 1 1 0 0 0 1 1;
      1 1 0 0 0 0 0 0 0 0 1 1;
      1 1 0 0 0 0 0 0 0 0 1 1;
      1 1 0 0 0 0 0 0 0 0 1 1;
      1 1 0 0 0 0 0 0 0 0 1 1;
      1 1 0 0 0 0 0 0 0 0 1 1;
      1 1 0 0 0 0 0 0 0 0 1 1];
immask2(500:511,500:511) = 255*M;
```

```
imny = zeros(1000,1000,3);
imny(:,:,1) = max(im1r, immask2);
imny(:,:,2) = max(im1g, immask2);
imny(:,:,3) = max(im1b, immask2);
imny(:,:,1) = imny(:,:,1) .* immask1;
imny(:,:,2) = imny(:,:,2) .* immask1;
imny(:,:,3) = imny(:,:,3) .* immask1;

figure(8), imshow(imny/255);
```

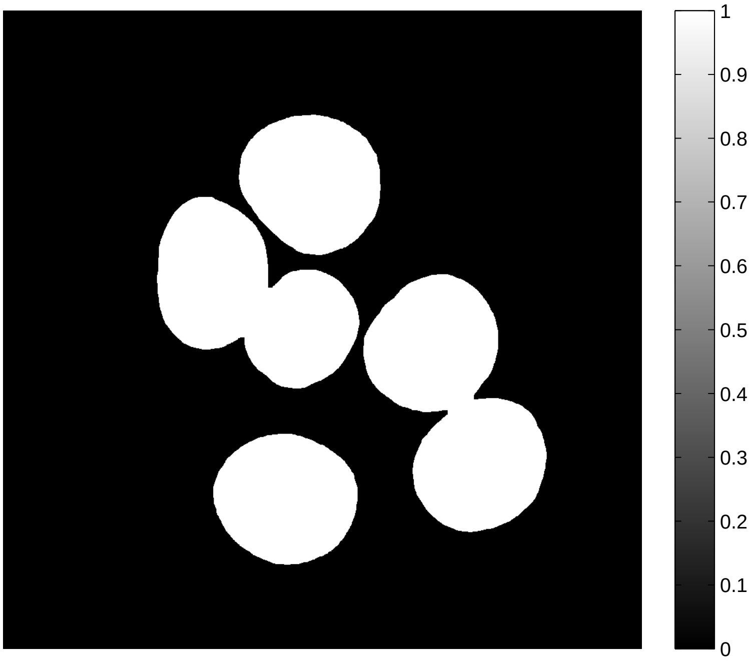

Figure 4: Enhanced binary image of the cells.

5 Localization of padlock-signals

Reference [1] talks about a 5×5 convolution filter which can be used to enhance local maxima. Such a filter might be the negative Laplacian filter,

$$-\Delta = -\frac{\partial^2}{\partial x^2} - \frac{\partial^2}{\partial y^2}.$$

Construct such a filter based on the Sobel-operators. Two-dimensional convolution can be perfomed with the the `conv2`-command.
Sketch your negative Laplacian filter below!

Your negative Laplacian filter can also be thought of as a matching filter for small circular objects, blobs. (More about this during the next laboratory assignment!) Motivate this by describing your filter above!

One metod to detect the padlock signals is shown in Figure 5 below. The negative Laplacian filter acts as a padlock cell detector. After filtering, the image contains smooth humps (or hills) located at the padlock signals. To get rid of weak responses originated from noise, a thresholding and multiplication is performed. The function `imreginalmax` locates the maxima, which is one for each padlock signal. Write a program that counts the number of red padlock signals in Figure 6!

How many red padlock signals did you find?

Now, before continuing with the main exercise, demonstrate all your results to the teacher, i.e.
- the result corresponding to Figure 3
- the result corresponding to Figure 4
- the padlock signal detection

Make sure the that teacher register you in the lab list! Okey?

6 Main exercise

Given Figure 6, create the image on the front page, Figure 1. Use your modified code in `MyWatershedNew.m` and `BinaryManip.m`. You need to make some

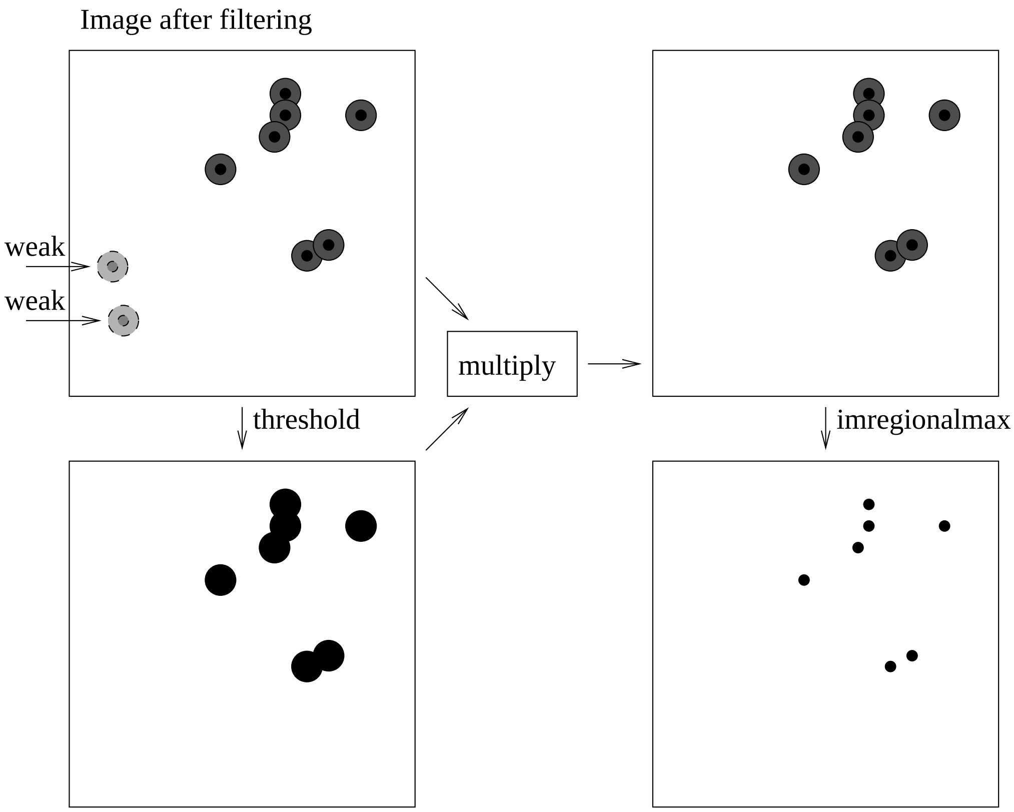

Figure 5: Padlock signal detection.

small adjustments, see reference [1]; Section 2.2, paragraph 2.

Let the color of the cytoplasm borders be *yellow* and let the color of the rings around the padlock signals be *cyan*.

Also, create Figure 7, an isolated cell with its corresponding red padlock cells. The program should also print out the number of red padlock signals belonging to the selected cell.

7 Report

Present the results of the *main exercise*, step by step. Illustrate the steps with your own calculated images, also use the images produced in between. Write explanatory text to the images. Put the program code in the appendix. One report per group is sufficient!

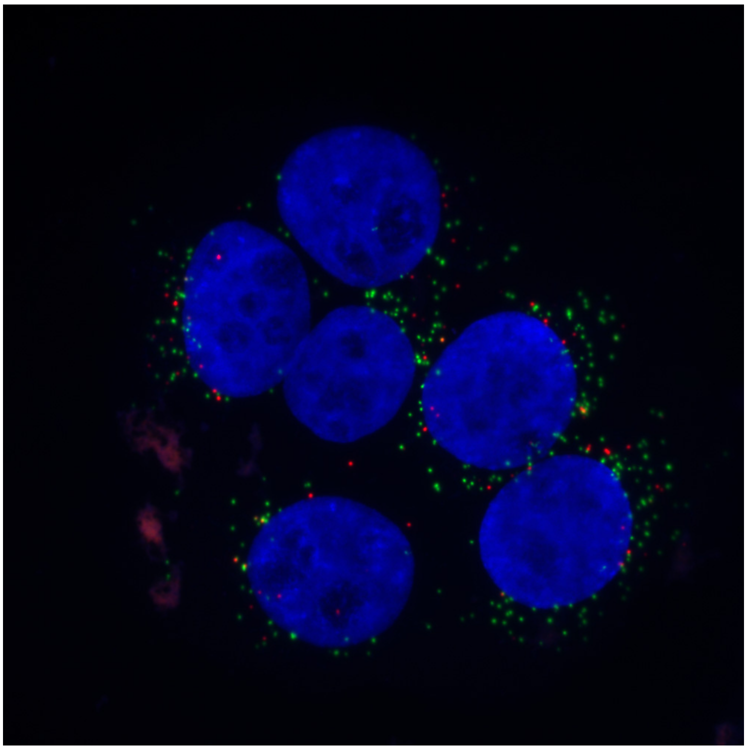

Figure 6: Original image.

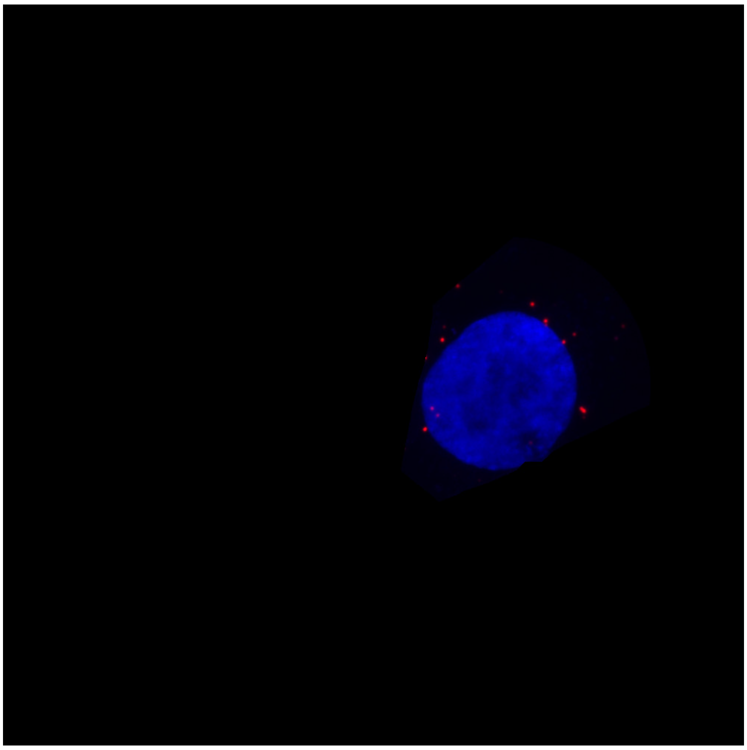

Figure 7: A cell and its padlock signals.