Legends for the Code and test images

Attached are four Matlab code files ".m" :

1. "letsgo.m" This file contains the code for the menu and the initial parameters
2. "mcount.m" This file contains the code for counting all the images that are placed in folder.
3. "test.m" This file contains the code for counting the input images and mark the cells on the images with circles.
4. "lm.m" This file contains the code for detecting the local maximum in the images

And, six test images (image1.tif, image2.tif, image3.tif, image4.tif, image5.tif, and image6.tif).

How to run the software:

- Create a folder that contains all four ".m" files listed above and the test images.
- Make sure that no other files are in that folder
- Run Matlab.
- Change the directory in Matlab to the new folder (created above) that contains the ".m" files and the test images.

- In Matlab, type: 'letsgo ↵'

- A new window will appear that contains three buttons ('Test', 'Start Counting', and 'Quit'), and Three parameters ("Image dimensions", 'Cell Radius' and 'Threshold')
- The "Image Dimensions" or image size is determined by the resolution the image was obtained under (Default: 600×800).
- The "Cell Radius" can be change using the scroll bar or textbox.
- The "Threshold" for the three images ("blue", "green", and "red" colored images) can be changed separately from the corresponding text boxes.
- Click "Test", then type the image numbers of consecutive images one at a time.
- The code should return the number cells in the Blue, Green, and Red images, consecutively, along with the cells marked in circles in each of the three images.

- If you are not satisfied with the accuracy. Type "Q ↵" to quit

- Re-run the program by typing "letsgo ↵", and adjust the parameters (cell radius and threshold) and click test, until you are satisfied with the accuracy.
- Click "start counting"

- The software will count all the images in your folder, and return the numbers of the blue, red, and green in three columns in Matlab, along with the total number of cells in each color: Blue, green, and Red, which corresponds to DAPI, CD4+, and CD3+, respectively.
- The code will also create an excel file, “The final count.xls”, in the same folder, which contains three columns that corresponds to number of cells in each blue, green, and red images, respectively.

MATLAB M-file scripts:

1. letsgo.m

```
close all;
clear;

H = figure;

Start = uicontrol(H, 'Style', 'PushButton','string', 'start
counting','FontSize', 10, 'Position', [10 70 100 25],'Callback',
'jjj=1;uiresume(H)');
Test = uicontrol(H, 'Style', 'PushButton','string', 'Test','FontSize',
10, 'Position', [400 70 70 25],'Callback', 'jjj=2;uiresume(H)');

Quit = uicontrol(H, 'Style', 'PushButton','string', 'Quit', 'FontSize',
10,'Position', [10 30 100 25], 'Callback', 'jjj=0;uiresume(H)');

csizem = uicontrol(H, 'Style', 'Text','string', 'Cell Radius:',
'FontSize', 11, 'Position', [20 360 80 20]);
csizev = uicontrol(H, 'Style', 'slider','Min',1, 'Max', 21,
'SliderStep',[0.05 0.1],'Position', [140 360 200 20],'value', 8,
'Callback', 'set(csizedis,''string'',num2str(get(csizev,''value'')))');
csizedis = uicontrol(H, 'Style', 'Edit','string', get(csizev,'value'),
'FontSize', 11, 'Position', [400 360 80
20],'Callback','set(csizev,''value'',str2double(get(csizedis,''string''
)))');

Trdm = uicontrol(H, 'Style', 'Text','string', 'Blue
Threshold:','FontSize', 11,'Position', [20 250 140 20]);
Trdv = uicontrol(H, 'Style', 'Edit','String','45','FontSize', 11
,'Position', [200 250 80 20]);

Trdm1 = uicontrol(H, 'Style', 'Text','string', 'Green
Threshold:','FontSize', 11,'Position', [20 220 140 20]);
Trdv1 = uicontrol(H, 'Style', 'Edit','String','10','FontSize', 11
,'Position', [200 220 80 20]);


Trdm2 = uicontrol(H, 'Style', 'Text','string', 'Red
Threshold:','FontSize', 11,'Position', [20 190 140 20]);
Trdv2 = uicontrol(H, 'Style', 'Edit','String','20','FontSize', 11
,'Position', [200 190 80 20]);


typeimagesize = uicontrol(H, 'Style', 'Text','string', 'Image
Dimensions','FontSize', 8,'Position', [395 300 90 15]);
typex = uicontrol(H, 'Style', 'Text','string', 'X','FontSize',
9,'Position', [430 285 20 15]);
ils = uicontrol(H, 'Style', 'Edit','String','600','FontSize', 9
,'Position', [395 285 40 15]);
iws = uicontrol(H, 'Style', 'Edit','String','800','FontSize', 9
,'Position', [445 285 40 15]);
```

```
copyright = uicontrol(H, 'Style', 'Text','string', 'CopyRights (C) -
Mohamad A.K - 2008','FontSize', 11,'Position', [1 1 560 20]);


uiwait(H)

thresholdb = str2double(get(Trdv,'String'));
thresholdg = str2double(get(Trdv1,'String'));
thresholdr= str2double(get(Trdv2,'String'));
ilngth = str2double(get(ils,'String'));
iwdth = str2double(get(iws,'String'));


cs = get(csizev,'value')

close(H)
if jjj==1
    mcount
elseif jjj==0
    close all
elseif jjj==2
    test
end
```

2. mcount.m

```
%calibrated


%clear; close all;
clc;
clear c1; clear c2; clear c3;
clear chkg; clear chkb;
j=1;
tcntb= []; tcntg=[]; tcntr=[];
nb=[]; ng=[]; nr=[];

% deltab= 57;
deltab= thresholdb;
deltag= thresholdg;
deltar= thresholdr;

db=2*cs; dg=2*cs; dr=2*cs;

csb=cs; csg=10; csr= 20;

% image pixel length and width
imgl = ilngth;
imgw = iwdth;


% note for full device chng 680 instead of 800

%showing image (don't use for automatic)
showb=0; showg=0;showr=0;


enablenumber = 2; % (0 -- type name,  1  -- type no. , 2 -- automatic,
3 -- quit)

if enablenumber ==1
    nameImage='Image'; nametif='.tif';

    imgnob = input('BLUE image number:  ','s');
    imgnog = input('GREEN image number:  ','s');
    imgnor = input('RED image number:  ','s');

    imgb = [nameImage,imgnob,nametif];
    imgg = [nameImage,imgnog,nametif];
    imgr = [nameImage,imgnor,nametif];
elseif enablenumber==0
        imgb = input('Please type the BLUE image:  ','s');
        imgg = input('Please type the GREEN image:  ','s');
        imgr = input('Please type the RED image:  ','s');
end

while j==1
```

```
    if enablenumber==2 %automatic image reader (in counting folder) %%
        xz = dir;
        no_files = size(xz);
        for z= 3:3:(no_files(1,1)-6) %
            imgb = xz(z).name;
            imgg = xz(z+1).name;
            imgr = xz(z+2).name;

            ibr = double(imread(imgb));
            igr = double(imread(imgg));
            irr = double(imread(imgr));

            ib = ibr(:, 1:imgw);
            ig = igr(:, 1:imgw);
            ir = irr(:, 1:imgw);

            [x y]= meshgrid(1:imgw, 1:imgl);
            c1=[];  c2=[]; c3=[];
            close all;
        % ------------------------------------------------------------
start blue


            lmaxb = lm(ib, [csb csb], true);
        %        figure; mesh(x, y, ib);hold on;

            c1=[];
            for i = 1 : size(lmaxb)
                if (lmaxb(i)-db) > 1 && (lmaxb(i)+db) < imgw &&
(x(lmaxb(i))-db) > 1 && (x(lmaxb(i))+db) < imgl %condition for edges
                    if ib(y(lmaxb(i)),x(lmaxb(i))) >
(ib(y(lmaxb(i)),x(lmaxb(i))-db) + deltab) ||
ib(y(lmaxb(i)),x(lmaxb(i))) > (ib(y(lmaxb(i)),x(lmaxb(i))+db) + deltab)
|| ib(lmaxb(i)) > (ib(lmaxb(i)-db )+ deltab) || ib(lmaxb(i)) >
(ib(lmaxb(i)+ db)+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (lmaxb(i)-db) > 1 && (lmaxb(i)+db) < imgw
                    if ib(lmaxb(i)) > (ib(lmaxb(i)-db )+ deltab) ||
ib(lmaxb(i)) > (ib(lmaxb(i)+ db)+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (x(lmaxb(i))-db) > 1 && (x(lmaxb(i))+db) < imgl
                    if ib(y(lmaxb(i)),x(lmaxb(i))) >
(ib(y(lmaxb(i)),x(lmaxb(i))-db) + deltab) ||
ib(y(lmaxb(i)),x(lmaxb(i))) > (ib(y(lmaxb(i)),x(lmaxb(i))+db) + deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (lmaxb(i)-db) > 1
                    if ib(lmaxb(i)) > (ib(lmaxb(i)-db )+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (lmaxb(i)+ db) < imgw
                    if ib(lmaxb(i)) > (ib(lmaxb(i)+ db )+ deltab)
```

```
                        c1 = [c1 ; lmaxb(i)];
                    end
                end
            end

            if showb
                imshow(imgb); hold on;
                plot3(x(c1), y(c1), ib(c1), 'y*', 'markersize', 10,
'linewidth', 1.5);
            end

            [cntb, z]= size(c1);
        %    fprintf('total number of cells = %g  \n',cntb);
            nb = [nb; imgb];
            tcntb = [tcntb ; cntb];

        % ------------------------------------------------------------
start green


            lmaxg = lm(ig, [csg csg], true);
        %        figure; mesh(x, y, ig); hold on;

            c2=[];
            for i = 1 : size(lmaxg)
                if (lmaxg(i)-dg) > 1 && (lmaxg(i)+dg) < imgw &&
(x(lmaxg(i))-dg) > 1 && (x(lmaxg(i))+dg) < imgl %condition for edges
                    if ig(y(lmaxg(i)),x(lmaxg(i))) >
(ig(y(lmaxg(i)),x(lmaxg(i))-dg) + deltag) ||
ig(y(lmaxg(i)),x(lmaxg(i))) > (ig(y(lmaxg(i)),x(lmaxg(i))+dg) + deltag)
|| ig(lmaxg(i)) > (ig(lmaxg(i)-dg )+ deltag) || ig(lmaxg(i)) >
(ig(lmaxg(i)+ dg)+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (lmaxg(i)-dg) > 1 && (lmaxg(i)+dg) < imgw
                    if ig(lmaxg(i)) > (ig(lmaxg(i)-dg )+ deltag) ||
ig(lmaxg(i)) > (ig(lmaxg(i)+ dg)+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (x(lmaxg(i))-dg) > 1 && (x(lmaxg(i))+dg) < imgl
                    if ig(y(lmaxg(i)),x(lmaxg(i))) >
(ig(y(lmaxg(i)),x(lmaxg(i))-dg) + deltag) ||
ig(y(lmaxg(i)),x(lmaxg(i))) > (ig(y(lmaxg(i)),x(lmaxg(i))+dg) + deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (lmaxg(i)-dg) > 1
                    if ig(lmaxg(i)) > (ig(lmaxg(i)-dg )+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (lmaxg(i)+ dg) < imgw
                    if ig(lmaxg(i)) > (ig(lmaxg(i)+ dg )+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                end
            end
```

```
        %        imshow(imgg); hold on;
        %        plot3(x(c2), y(c2), ig(c2), 'y*', 'markersize', 10,
'linewidth', 1.5);

            [cntg, z]= size(c2);
        %        fprintf('number of total green stuff in the green
image = %g  \n',cntg);

            chkg = [];
            for i = 1 : cntg
                if  (c2(i)-10) > 1 && (c2(i)+10) < imgw     %condition
for edges
                    if ib(c2(i)) > (ib(c2(i)-10 )+ deltab) || ib(c2(i))
> (ib(c2(i)+ 1)+ deltab)
                        chkg = [chkg; c2(i)];
                    end
                elseif (c2(i)- 10) > 1
                    if ib(c2(i)) > (ib(c2(i)- 10 )+ deltab)
                        chkg = [chkg; c2(i)];
                    end
                elseif (c2(i)+ 10) < imgw
                    if ib(c2(i)) > (ib(c2(i)+ 10 )+ deltab)
                        chkg = [chkg; c2(i)];
                    end
                end
            end
            [cntcg, z]= size(chkg);

            if showg
                figure; imshow(imgg); hold on;
                plot3(x(chkg), y(chkg), ig(chkg), 'r*', 'markersize',
10, 'linewidth', 1.5);
            end

        %    fprintf('total number of "CD4+" cells = %g  \n',cntcg);
        %        ng = [ng; imgg];
            tcntg = [tcntg ; cntcg];

        % ------------------------------------------------------------
start red

           lmaxr = lm(ir, [csr csr], true);
        %        figure; mesh(x, y, ir); hold on;

            c3=[];
            for i = 1 : size(lmaxr)
                if (lmaxr(i)-dr) > 1 && (lmaxr(i)+dr) < imgw &&
(x(lmaxr(i))-dr) > 1 && (x(lmaxr(i))+dr) < imgl %condition for edges
                    if ir(y(lmaxr(i)),x(lmaxr(i))) >
(ir(y(lmaxr(i)),x(lmaxr(i))-dr) + deltar) ||
ir(y(lmaxr(i)),x(lmaxr(i))) > (ir(y(lmaxr(i)),x(lmaxr(i))+dr) + deltar)
|| ir(lmaxr(i)) > (ir(lmaxr(i)-dr )+ deltar) || ir(lmaxr(i)) >
(ir(lmaxr(i)+ dr)+ deltar)
                        c3 = [c3 ; lmaxr(i)];
```

```
                    end
                elseif (lmaxr(i)-dr) > 1 && (lmaxr(i)+dr) < imgw
                    if ir(lmaxr(i)) > (ir(lmaxr(i)-dr )+ deltar) ||
ir(lmaxr(i)) > (ir(lmaxr(i)+ dr)+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (x(lmaxr(i))-dr) > 1 && (x(lmaxr(i))+dr) < imgl
                    if ir(y(lmaxr(i)),x(lmaxr(i))) >
(ir(y(lmaxr(i)),x(lmaxr(i))-dr) + deltar) ||
ir(y(lmaxr(i)),x(lmaxr(i))) > (ir(y(lmaxr(i)),x(lmaxr(i))+dr) + deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (lmaxr(i)-dr) > 1
                    if ir(lmaxr(i)) > (ir(lmaxr(i)-dr )+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (lmaxr(i)+ dr) < imgw
                    if ir(lmaxr(i)) > (ir(lmaxr(i)+ dr )+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                end
            end

        %        imshow(imgr); hold on;
        %        plot3(x(c3), y(c3), ir(c3), 'y*', 'markersize', 10,
'linewidth', 1.5);

            [cntr, z]= size(c3);
        %        fprintf('number of total red stuff in the red image =
%g  \n',cntr);

            chkr = [];
            for i = 1 : cntr
                if  (c3(i)-10) > 1 && (c3(i)+10) < imgw     %condition
for edges
                    if ib(c3(i)) > (ib(c3(i)-10 )+ deltab) || ib(c3(i))
> (ib(c3(i)+ 1)+ deltab)
                        chkr = [chkr; c3(i)];
                    end
                elseif (c3(i)- 10) > 1
                    if ib(c3(i)) > (ib(c3(i)- 10 )+ deltab)
                        chkr = [chkr; c3(i)];
                    end
                elseif (c3(i)+ 10) < imgw
                    if ib(c3(i)) > (ib(c3(i)+ 10 )+ deltab)
                        chkr = [chkr; c3(i)];
                    end
                end
            end
            [cntcr, z]= size(chkr);

            if showr
                figure; imshow(imgr);hold on;
                plot3(x(chkr), y(chkr), ir(chkr), 'b*', 'markersize',
10, 'linewidth', 1.5);
            end
```

```
        %     fprintf('total number of "CD3+" cells = %g  \n',cntcr);
        %        nr = [nr; imgr];
            tcntr = [tcntr ; cntcr];



        % ------------------------------------------------------ end
image analysis

        end
        if enablenumber==2
            enablenumber=3; j=0;
        end
    end


     if enablenumber==1
        imgnob = input('BLUE image number:  ','s');
        if imgnob == 'q'
            j=0;
        end
        if j==1
        imgnog = input('GREEN image number:  ','s');
        end
        if imgnog == 'q'
            j=0;
        end
        if j==1
        imgnor = input('RED image number:  ','s');
        end
        if imgnor == 'q'
            j=0;
        end
        imgb = [nameImage,imgnob,nametif];
        imgg = [nameImage,imgnog,nametif];
        imgr = [nameImage,imgnor,nametif];
     elseif enablenumber==0
        imgb = input('Please type the BLUE image:  ','s');
        if imgb == 'q'
            j=0;
        end
        if j==1
            imgg = input('Please type the GREEN image:  ','s');
        end
        if imgg == 'q'
            j=0;
        end

        if j==1
            imgr = input('Please type the RED image:  ','s');
        end
        if imgr == 'q'
            j=0;
        end
```

```
        end

    end

    % name = [nb, ng]
    final = [tcntb, tcntg, tcntr]
    xlswrite('The final count.xls', final);
    fprintf('Total no. of DAPI = %1.0f\n',sum(final(:,1)));
    fprintf('Total no. of CD4+ = %1.0f\n',sum(final(:,2)));
    fprintf('Total no. of CD3+ = %1.0f\n',sum(final(:,3)));


    % fopen   fprintf    \t    \n   fclose
```

3. test.m

```
close all; clc;
clear c1; clear c2; clear c3;
clear chkg; clear chkb;
j=1;
tcntb= []; tcntg=[]; tcntr=[];
nb=[]; ng=[]; nr=[];
%deltab= 55;
deltab= thresholdb;
deltag= thresholdg;
deltar= thresholdr;

db=2*cs; dg=2*cs; dr=2*cs;

csb=cs; csg=10; csr= 20;

% image pixel length and width
imgl = ilngth;
imgw = iwdth;

%showing images
showb=1; showg=1;showr=1;


enablenumber = 1;

%automaticimageopener=1;
%x = dir;
%no_files = size(x);
%for i= 3: no_files(1,1)
%imgname = x(i).name;
%end




if enablenumber
    nameImage='Image'; nametif='.tif';

    imgnob = input('BLUE image number:  ','s');
    imgnog = input('GREEN image number:  ','s');
    imgnor = input('RED image number:  ','s');

    imgb = [nameImage,imgnob,nametif];
    imgg = [nameImage,imgnog,nametif];
    imgr = [nameImage,imgnor,nametif];
else
        imgb = input('Please type the BLUE image:  ','s');
        imgg = input('Please type the GREEN image:  ','s');
        imgr = input('Please type the RED image:  ','s');
end
```

```
while j==1
        ibr = double(imread(imgb));
        igr = double(imread(imgg));
        irr = double(imread(imgr));


        ib = ibr; ig = igr; ir = irr;

%        ib = ibr(:, 1:680);
%        ig = igr(:, 1:680);
%        ir = irr(:, 1:680);

        [x y]= meshgrid(1:imgw, 1:imgl);
%        [x y]= meshgrid(1:680, 1:600);

        c1=[];  c2=[]; c3=[];
        close all;
% ------------------------------------------------------------ start
blue

            [I,map] = imread(imgb);
            %figure; imshow(I,map)
            bkgrnd = imopen(I,strel('disk',14));
            %figure, imshow(bkgrnd)
            I2 = imsubtract(I,bkgrnd);
            ibnb = imadjust(I2, [0 .65], [0 1], 1);
            %figure, imshow(I3,map);
            % level = graythresh(I3);
            % bw = im2bw(I3,level);
            % figure, imshow(bw)

            lmaxb = lm(ib, [csb csb], true);
        %        figure; mesh(x, y, ib);hold on;

            c1=[];
            for i = 1 : size(lmaxb)
                if (lmaxb(i)-db) > 1 && (lmaxb(i)+db) < imgw &&
(x(lmaxb(i))-db) > 1 && (x(lmaxb(i))+db) < imgl %condition for edges
                    if ib(y(lmaxb(i)),x(lmaxb(i))) >
(ib(y(lmaxb(i)),x(lmaxb(i))-db) + deltab) ||
ib(y(lmaxb(i)),x(lmaxb(i))) > (ib(y(lmaxb(i)),x(lmaxb(i))+db) + deltab)
|| ib(lmaxb(i)) > (ib(lmaxb(i)-db )+ deltab) || ib(lmaxb(i)) >
(ib(lmaxb(i)+ db)+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (lmaxb(i)-db) > 1 && (lmaxb(i)+db) < imgw
                    if ib(lmaxb(i)) > (ib(lmaxb(i)-db )+ deltab) ||
ib(lmaxb(i)) > (ib(lmaxb(i)+ db)+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (x(lmaxb(i))-db) > 1 && (x(lmaxb(i))+db) < imgl
                    if ib(y(lmaxb(i)),x(lmaxb(i))) >
(ib(y(lmaxb(i)),x(lmaxb(i))-db) + deltab) ||
ib(y(lmaxb(i)),x(lmaxb(i))) > (ib(y(lmaxb(i)),x(lmaxb(i))+db) + deltab)
```

```
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (lmaxb(i)-db) > 1
                    if ib(lmaxb(i)) > (ib(lmaxb(i)-db )+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                elseif (lmaxb(i)+ db) < imgw
                    if ib(lmaxb(i)) > (ib(lmaxb(i)+ db )+ deltab)
                        c1 = [c1 ; lmaxb(i)];
                    end
                end
            end


            if showb
                imshow(imgb); hold on;
                plot3(x(c1), y(c1), ib(c1), 'yo', 'markersize', 10,
'linewidth', 1.5);
            end

            [cntb, z]= size(c1);
        %    fprintf('total number of cells = %g  \n',cntb);
            nb = [nb; imgb];
            tcntb = [tcntb ; cntb];

        % ------------------------------------------------------------
start green

            [I,map] = imread(imgg);
            %figure; imshow(I,map)
            bkgrnd = imopen(I,strel('disk',14));
            %figure, imshow(bkgrnd)
            I2 = imsubtract(I,bkgrnd);
            ignb = imadjust(I2, [0 .65], [0 1], 1);
            %figure, imshow(I3,map);
            % level = graythresh(I3);
            % bw = im2bw(I3,level);
            % figure, imshow(bw)

            lmaxg = lm(ig, [csg csg], true);
        %        figure; mesh(x, y, ig); hold on;

            c2=[];
            for i = 1 : size(lmaxg)
                if (lmaxg(i)-dg) > 1 && (lmaxg(i)+dg) < imgw &&
(x(lmaxg(i))-dg) > 1 && (x(lmaxg(i))+dg) < imgl %condition for edges
                    if ig(y(lmaxg(i)),x(lmaxg(i))) >
(ig(y(lmaxg(i)),x(lmaxg(i))-dg) + deltag) ||
ig(y(lmaxg(i)),x(lmaxg(i))) > (ig(y(lmaxg(i)),x(lmaxg(i))+dg) + deltag)
|| ig(lmaxg(i)) > (ig(lmaxg(i)-dg )+ deltag) || ig(lmaxg(i)) >
(ig(lmaxg(i)+ dg)+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (lmaxg(i)-dg) > 1 && (lmaxg(i)+dg) < imgw
```

```
                    if ig(lmaxg(i)) > (ig(lmaxg(i)-dg )+ deltag) ||
ig(lmaxg(i)) > (ig(lmaxg(i)+ dg)+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (x(lmaxg(i))-dg) > 1 && (x(lmaxg(i))+dg) < imgl
                    if ig(y(lmaxg(i)),x(lmaxg(i))) >
(ig(y(lmaxg(i)),x(lmaxg(i))-dg) + deltag) ||
ig(y(lmaxg(i)),x(lmaxg(i))) > (ig(y(lmaxg(i)),x(lmaxg(i))+dg) + deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (lmaxg(i)-dg) > 1
                    if ig(lmaxg(i)) > (ig(lmaxg(i)-dg )+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                elseif (lmaxg(i)+ dg) < imgw
                    if ig(lmaxg(i)) > (ig(lmaxg(i)+ dg )+ deltag)
                        c2 = [c2 ; lmaxg(i)];
                    end
                end
            end

        %        imshow(imgg); hold on;
        %        plot3(x(c2), y(c2), ig(c2), 'y*', 'markersize', 10,
'linewidth', 1.5);

            [cntg, z]= size(c2);
        %       fprintf('number of total green stuff in the green
image = %g  \n',cntg);

            chkg = [];
            for i = 1 : cntg
                if  (c2(i)-10) > 1 && (c2(i)+10) < imgw     %condition
for edges
                    if ib(c2(i)) > (ib(c2(i)-10 )+ deltab) || ib(c2(i))
> (ib(c2(i)+ 1)+ deltab)
                        chkg = [chkg; c2(i)];
                    end
                elseif (c2(i)- 10) > 1
                    if ib(c2(i)) > (ib(c2(i)- 10 )+ deltab)
                        chkg = [chkg; c2(i)];
                    end
                elseif (c2(i)+ 10) < imgw
                    if ib(c2(i)) > (ib(c2(i)+ 10 )+ deltab)
                        chkg = [chkg; c2(i)];
                    end
                end
            end
            [cntcg, z]= size(chkg);

            if showg
                figure; imshow(imgg); hold on;
                plot3(x(chkg), y(chkg), ig(chkg), 'yo', 'markersize',
10, 'linewidth', 1.5);
            end

        %    fprintf('total number of "CD4+" cells = %g  \n',cntcg);
```

```
        %        ng = [ng; imgg];
            tcntg = [tcntg ; cntcg];

        % ------------------------------------------------------------
start red
            [I,map] = imread(imgr);
            %figure; imshow(I,map)
            bkgrnd = imopen(I,strel('disk',14));
            %figure, imshow(bkgrnd)
            I2 = imsubtract(I,bkgrnd);
            irnb = imadjust(I2, [0 .65], [0 1], 1);
            %figure, imshow(I3,map);
            % level = graythresh(I3);
            % bw = im2bw(I3,level);
            % figure, imshow(bw)

            lmaxr = lm(ir, [csr csr], true);
        %        figure; mesh(x, y, ir); hold on;

            c3=[];
            for i = 1 : size(lmaxr)
                if (lmaxr(i)-dr) > 1 && (lmaxr(i)+dr) < imgw &&
(x(lmaxr(i))-dr) > 1 && (x(lmaxr(i))+dr) < imgl %condition for edges
                    if ir(y(lmaxr(i)),x(lmaxr(i))) >
(ir(y(lmaxr(i)),x(lmaxr(i))-dr) + deltar) ||
ir(y(lmaxr(i)),x(lmaxr(i))) > (ir(y(lmaxr(i)),x(lmaxr(i))+dr) + deltar)
|| ir(lmaxr(i)) > (ir(lmaxr(i)-dr )+ deltar) || ir(lmaxr(i)) >
(ir(lmaxr(i)+ dr)+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (lmaxr(i)-dr) > 1 && (lmaxr(i)+dr) < imgw
                    if ir(lmaxr(i)) > (ir(lmaxr(i)-dr )+ deltar) ||
ir(lmaxr(i)) > (ir(lmaxr(i)+ dr)+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (x(lmaxr(i))-dr) > 1 && (x(lmaxr(i))+dr) < imgl
                    if ir(y(lmaxr(i)),x(lmaxr(i))) >
(ir(y(lmaxr(i)),x(lmaxr(i))-dr) + deltar) ||
ir(y(lmaxr(i)),x(lmaxr(i))) > (ir(y(lmaxr(i)),x(lmaxr(i))+dr) + deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (lmaxr(i)-dr) > 1
                    if ir(lmaxr(i)) > (ir(lmaxr(i)-dr )+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                elseif (lmaxr(i)+ dr) < imgw
                    if ir(lmaxr(i)) > (ir(lmaxr(i)+ dr )+ deltar)
                        c3 = [c3 ; lmaxr(i)];
                    end
                end
            end



        %        imshow(imgr); hold on;
```

```
        %        plot3(x(c3), y(c3), ir(c3), 'y*', 'markersize', 10,
'linewidth', 1.5);

            [cntr, z]= size(c3);
        %        fprintf('number of total red stuff in the red image =
%g  \n',cntr);

            chkr = [];
            for i = 1 : cntr
                if  (c3(i)-10) > 1 && (c3(i)+10) < imgw     %condition
for edges
                    if ib(c3(i)) > (ib(c3(i)-10 )+ deltab) || ib(c3(i))
> (ib(c3(i)+ 1)+ deltab)
                        chkr = [chkr; c3(i)];
                    end
                elseif (c3(i)- 10) > 1
                    if ib(c3(i)) > (ib(c3(i)- 10 )+ deltab)
                        chkr = [chkr; c3(i)];
                    end
                elseif (c3(i)+ 10) < imgw
                    if ib(c3(i)) > (ib(c3(i)+ 10 )+ deltab)
                        chkr = [chkr; c3(i)];
                    end
                end
            end
            [cntcr, z]= size(chkr);

            if showr
                figure; imshow(imgr);hold on;
                plot3(x(chkr), y(chkr), ir(chkr), 'yo', 'markersize',
10, 'linewidth', 1.5);
            end

        %     fprintf('total number of "CD3+" cells = %g  \n',cntcr);
        %        nr = [nr; imgr];
            tcntr = [tcntr ; cntcr];

 % ------------------------------------------------------ end image
analysis


     if enablenumber
        imgnob = input('BLUE image number:  ','s');
        if imgnob == 'q'
            j=0;
        end
        if j==1
        imgnog = input('GREEN image number:  ','s');
        end
        if imgnog == 'q'
            j=0;
        end
        if j==1
        imgnor = input('RED image number:  ','s');
        end
```

```
            if imgnor == 'q'
                j=0;
            end
            imgb = [nameImage,imgnob,nametif];
            imgg = [nameImage,imgnog,nametif];
            imgr = [nameImage,imgnor,nametif];
        else
            imgb = input('Please type the BLUE image:  ','s');
            if imgb == 'q'
                j=0;
            end
            if j==1
                imgg = input('Please type the GREEN image:  ','s');
            end
            if imgg == 'q'
                j=0;
            end

            if j==1
                imgr = input('Please type the RED image:  ','s');
            end
            if imgr == 'q'
                j=0;
            end
        end

    end

    % name = [nb, ng]
    final = [tcntb, tcntg, tcntr]
```

4. lm.m

```
function varargout = lm(x,minDist, exculdeEqualPoints)


    if nargin < 3
        exculdeEqualPoints = false;
        if nargin < 2
            minDist = size(x)/10;
        end
    end

    if isempty(minDist)
        minDist = size(x)/10;
    end

    dimX = length ( size(x) );
    if length(minDist) ~= dimX
        minDist = minDist( ones(dimX,1) );
    end

    % validity checks
    minDist = ceil(minDist);
    minDist = max( [minDist(:)' ; ones(1,length(minDist))] );
    minDist = min( [minDist ; size(x)] );

    % ------------------------------------------------------------------
----
    if exculdeEqualPoints
        y = sort(x(:));
        dY = diff(y);
        minimumDiff = min( dY(dY ~= 0) );
        x = x + rand(size(x))*minimumDiff;
    end
    % ------------------------------------------------------------------
----

    se = strel('disk',minDist(1),0);
    % se = ones(minDist);
    X = imdilate(x,se);
    f = find(x == X);


    if nargout
        [varargout{1:nargout}] = ind2sub( size(x), f );
    else
        varargout{1} = f;
    end
```

These figures are also supplied as separate files

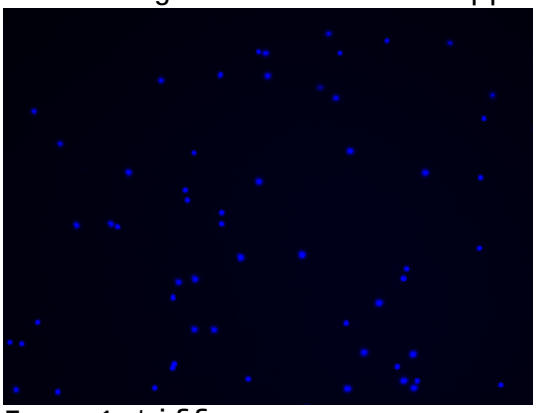

Image1.tiff

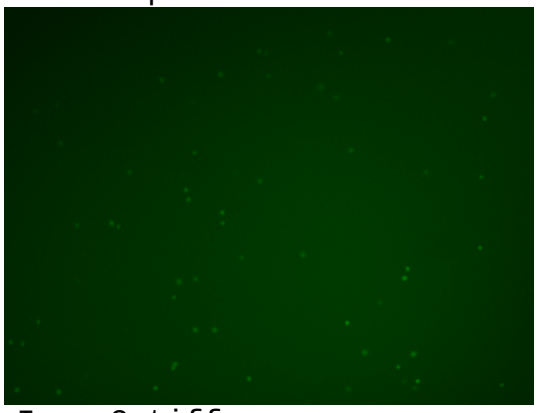

Image2.tiff

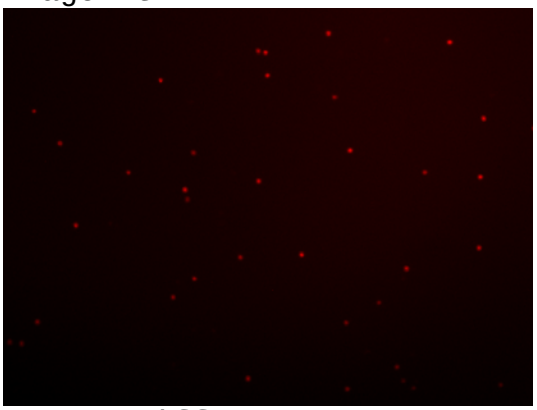

Image3.tiff

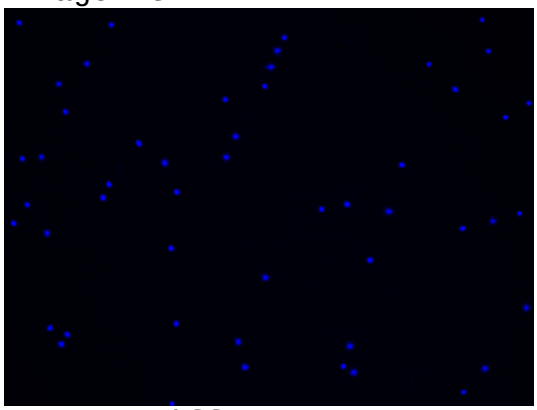

Image4.tiff

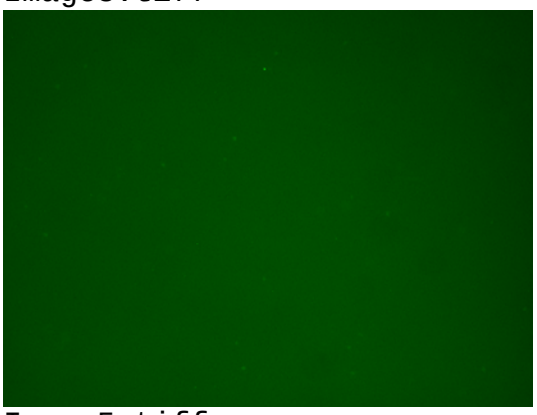

Image5.tiff

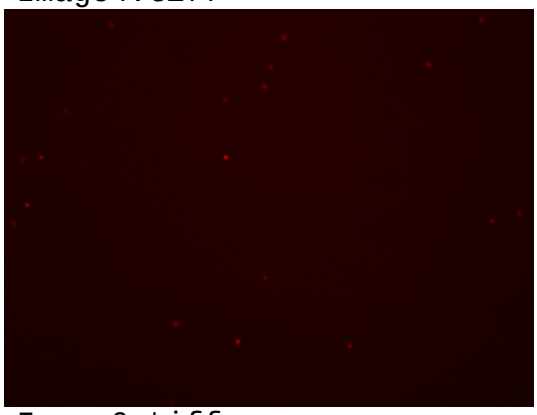

Image6.tiff