```
clc
clear all
close all
addpath('D:\Program Files\MATLAB\R2013a\work\')

FolderNames={'ben test'};
fileFolder = ['D:\Program Files\MATLAB\R2013a\work\'
sprintf('%s',FolderNames{1})];
cd(fileFolder)
cluster=3;

Dapictrl=imread(['dapi control.tif']);
Dapiexp=imread(['dapi exp.tif']);
redctrl=imread(['red control.tif']);
redexp=imread(['red exp.tif']);

[a b]=size(Dapictrl);
allstage=zeros(a, b, 2);
allstagee=zeros(a, b, 2);

allstage(:,:,1)=Dapictrl;
allstage(:,:,2)=Dapiexp;
allstagee(:,:,1)=redctrl;
allstagee(:,:,2)=redexp;


[v3 , c3, breakbin] = kmeanssubbreak(allstagee(:,:,2),
cluster,floor(numel(Dapictrl)/10));
Thresher=breakbin(length(breakbin));
for i=1:2;

    [v1 , c1] = kmeanssub(allstage(:,:,i),
cluster,floor(numel(Dapictrl)/10));
    noback=c1>2;
    noback=bwareaopen(noback,4);
    toobig=bwareaopen(noback,200);
    noback=noback-toobig;
    figure(1)
    imshow(noback)
    nobackred=allstagee(:,:,i)>Thresher;
    nobackred=bwareaopen(nobackred,4);
    figure(2)
    imshow(nobackred)
    ldapi=bwlabel(noback);
    overlapim=ldapi.*double(ldapi&nobackred);
    % figure
    %imagesc(overlapim)
    idxon=nonzeros(unique(overlapim));
    max(max(ldapi))
    numel(idxon)
```

```
    numel(idxon)/max(max(ldapi))
    figure(4)
    imshow(noback)
    hold on
    [yy xx]=find(nobackred);
    plot(xx,yy,'.r')
    hold off
    pause
    % figure(3)
    % imshow(allstagee(:,:,1)>90)
    % stop
end
```