Contents

From Voxels to Knowledge: A Practical Guide to the Segmentation of Complex Electron Microscopy 3D-Data *Wen-Ting Tsai (1), Ahmed Hassan (1), Purbasha Sarkar (2), Joaquin Correa (1) (3), Zoltan Metlagel (1), Danielle M. Jorgens (1), Manfred Auer (1) (2)*

1. Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94025, USA.
2. Joint Bioenergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA
3. National Energy Research Scientific Computing Center, Lawrence Berkeley National Laboratory, Berkeley, CA

Automated segmentation of bacteria This example shows a method to perform 2D segmentation of bacteria using VLFEAT and MATLAB's Image Processing Toolbox.

bact_seg.m The parameters used are example specific

in_dir Work directory rad Margin nbins Number of bins thr Background threshold Corr Correction factor (Corr) di Correction factor (dI) LS Correction factor (LS) bgd_area_th Background area for im opening cell_area_th_low Cell area low cell_area_th_high Cell area high rad_s Estimated cell radii thr_pb Theshold for prob map

```
function all_ims = bact_seg(in_dir, rad, nbins, thr, Corr, di, LS, bgd_area_th, cell_area_th_
low, cell_area_th_high, rad_s, thr_pb)
```

Step 1: VLFEAT

Add the VLFeat Toolbox to MATLAB path. For more information visit VLFEAT MATLAB API

```
addpath vlfeat-0.9.14/toolbox
vl_setup
```

Step 2: Set the work directory

The images are stored in the `JoVe/images` directory, in this example TIFF images will be processed.

```
% Clear workspace
% clear all; close all; clc

D = dir(fullfile(in_dir, '*.tif'));
```

Step 3: Script parameters

The user can specify a different background threshold value if needed, or multiple threshold values for independent images.

```
% For N images with independant background threshold |thr| should be:
% thr = [thr1 thr2 thr3 thrN]
% and |im = di*(Corr*or-thr)-LS| should be:
% im = di*(Corr*or-thr(i))-LS

% Margin
% rad = 3;

% Number of bins
% nbins = 4;

% Pre-image background threshold
% thr = 0;

% CValues
% Corr = 0.9;
% di = 1;
% LS = 0;
```

Step 4: Histogram collection and distance matrix with custom kernel

$K : 2 * sum(X. * Y)./(X + Y)$

```
all_ims = cell(numel(D), 1);
for i = 1 : numel(D),
```

```
    im = imread(fullfile(in_dir, D(i).name));

    %   im = imadjust(im);
    im = im2double(im);

    % for RGB images
    % im = im2double(rgb2gray(im));

    % if resize is required
    % im = imresize(im, 1);

    or = im;
```

Use Otsu's method if gives better result for I estimation thr = graythresh(or);

```
    im = di*(Corr*or-thr)-LS;
    im = max(min(1, im), 0);

    figure(1); title(D(i).name); imshow(imresize(im,0.5), 'Border', 'tight'); drawnow

    hh = histc(im(:), 0.001:0.001:1);
```

```
figure(2); bar(hh);
```

Collect histograms

```
hists = zeros(numel(im), nbins);
cnt = 0;
idx = zeros(size(im));

for x = rad+1 : size(im,1) - rad,
    for y = rad+1 : size(im,2) - rad,
        patch = im(x-rad:x+rad, y-rad:y+rad);
        cnt = cnt + 1;
        hists(cnt,:) = histc(patch(:), 0:1/nbins:1-1/nbins);
        idx(x, y) = cnt;
    end
end
hists = hists(1:cnt, :);
sm = sum(hists,2);
sm2 = repmat(sm, [1,size(hists,2)]);
hists = hists./(sm2 + (sm2==0));
```

Define exemplars

```
exemplars = eye(nbins);
dst_all = vl_alldist2(exemplars', hists', 'KCHI2');
```

Store results as a cell type array with multiple properties such as image (im), name, prob1, prob2 and prob3

```
all_ims{i}.im = im;
all_ims{i}.name = D(i).name(1:end-4);

cell_proba = dst_all(1, :);
idx2=idx;
idx2(idx==0)=numel(cell_proba)+1;
cell_proba(numel(cell_proba)+1)=0;
cell_proba=cell_proba(idx2);
all_ims{i}.proba1=cell_proba;

cell_proba = dst_all(2, :);
idx2=idx;
idx2(idx==0)=numel(cell_proba)+1;
cell_proba(numel(cell_proba)+1)=0;
cell_proba=cell_proba(idx2);
all_ims{i}.proba2=cell_proba;

cell_proba = dst_all(3, :);
idx2=idx;
idx2(idx==0)=numel(cell_proba)+1;
cell_proba(numel(cell_proba)+1)=0;
cell_proba=cell_proba(idx2);
all_ims{i}.proba3=cell_proba;
```

Probability maps

```
    figure;
    subplot(1,3,1); imshow(all_ims{i}.proba1); title('Prob. map 1'); colormap(jet);
    subplot(1,3,2); imshow(all_ims{i}.proba2); title('Prob. map 2'); colormap(jet);
    subplot(1,3,3); imshow(all_ims{i}.proba3); title('Prob. map 3'); colormap(jet);
    truesize
```

```
end
```

Step 5: Save results

Save all_ims

```
save hists_JoVe.mat all_ims
```

Step 6: Segmentation

Add the VLFeat Toolbox to MATLAB path. For more information visit VLFEAT MATLAB API

```
rad2 = rad_s + 1;

out_dir = 'JoVe/results';
mkdir(out_dir);

str  = strel(fspecial('disk', rad_s));
str2 = strel(fspecial('disk', rad2));

for i = 1 : numel(all_ims),
    disp(i);
    im = all_ims{i}.im;
    figure; imshow(im, 'Border', 'tight'); drawnow
    figure; imshow(all_ims{i}.proba1, 'Border', 'tight');colormap(jet); drawnow

    % Write initial cell probability map
    cell_proba = all_ims{i}.proba1;
    imwrite(cell_proba, fullfile(out_dir, [all_ims{i}.name '.png']));

    % Find max labeling and background
    probas = all_ims{i}.proba1;
    probas = cat(3, probas,cell_proba);
    probas = cat(3, probas,all_ims{i}.proba3);
    [vl labels]= max(probas,[],3);
    bgd = labels~=2;
    bgd = bwareaopen(bgd,bgd_area_th);

    % Find cells
    fg = ~bgd;
    op = imopen(fg, str);
    er = imerode(op, str2);
    lbl = bwlabel(er);
    dl = imdilate(lbl, str);
    R = regionprops(dl, 'Area');
    for r = 1:numel(R),
        if (R(r).Area<cell_area_th_low) || (R(r).Area>cell_area_th_high),
            dl(dl==r) = 0;
        end
    end
    dl = cmunique(dl);

    nb_cells = max(dl(:));
    bdry = seg2bdry(dl, 'imageSize');

    metal = (labels==1);
    lmet = bwlabel(metal);
    fr = false(size(metal));fr(1:5,:)=true; fr(:,1:5)=true; fr(end-4:end,:)=true;fr(:,end-4:e
nd)=true;
    for m =1:max(lmet(:)),
        bw = (lmet==m) &fr;
        if max(bw(:))==1,
            metal(lmet==m)=0;
        end
    end

    % Find other features
    dl( dl==0 & all_ims{i}.proba2>thr_pb) = nb_cells + 1;
    dl(metal) = nb_cells+2;

    % Display result
    mp = rand(nb_cells+3,3);mp(1,:)=[ 1 1 1];mp(end-1,:)=[0 0 0];mp(end,:)=[0 1 0];
    Lrgb=ind2rgb(uint8(dl),mp);
    figure;imshow(Lrgb,'Border','tight');

    % Display result overlaid on original image
    figure, clf; imshow(im.*~bdry,'Border','tight'), hold on
    himage = imshow(Lrgb,'Border','tight');
    set(himage, 'AlphaData', 0.5)
    drawnow;

end
```

```
end

function [ bdry ]  = seg2bdry(seg, fmt)
    if nargin<2, fmt = 'imageSize'; end;

    if ~strcmp(fmt,'imageSize') && ~strcmp(fmt,'doubleSize'),
        error('possible values for fmt are: imageSize and doubleSize');
    end

    [tx, ty, nch] = size(seg);

    if nch ~=1,
        error('seg must be a scalar image');
    end
    bdry = zeros(2*tx+1, 2*ty+1);

    edgels_v = ( seg(1:end-1, :) ~= seg(2:end, :) );
    edgels_v(end+1, :) = 0;
    edgels_h = ( seg(:, 1:end-1) ~= seg(:, 2:end) );
    edgels_h(:, end+1) = 0;

    bdry(3:2:end, 2:2:end) = edgels_v;
    bdry(2:2:end, 3:2:end) = edgels_h;
    bdry(3:2:end-1, 3:2:end-1)= max ( max(edgels_h(1:end-1, 1:end-1), edgels_h(2:end, 1:end-1
)), max(edgels_v(1:end-1,1:end-1), edgels_v(1:end-1,2:end)) );

    bdry(1, :) = bdry(2, :);
    bdry(:, 1) = bdry(:, 2);
    bdry(end, :) = bdry(end-1, :);
    bdry(:, end) = bdry(:, end-1);

    if strcmp(fmt,'imageSize'),
        bdry = bdry(3:2:end, 3:2:end);
    end
end
```

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