%% set file path: manually select folder

fp = uigetdir;

cd(fp)

%% load in parameters for graphical representation

cm=1/25:1/25:1;cm=cm';

pe=[0 0 0];

pe=cat(1, pe, [cm cm/2 zeros(25, 1)]);

pe=cat(1, pe, [ones(25, 1) 0.5+cm/2 zeros(25, 1)]);

pe=cat(1, pe, [ones(25, 1) ones(25, 1) cm]);

colormap(pe)

%% create list of.HIS files in the selected folder

list=dir('\*.HIS');

list={list.name};

h = waitbar(0, 'Finding points...');

%% for all .HIS files in the list, localize single molecules using the Localizer function with PSF standard deviation factor 1.8 and intensity selection sigma factor 25

for fn=1:size(list, 2)

clear 'pts' 'im'

file=list{fn};

if isfile([file(1:strfind(file, '.HIS')-1) '\_LocRes.mat'])

rep = questdlg('File already analysed.', 'File exists', 'Repeat Analysis', 'Use existing file', 'Use existing file');

if strcmp(rep,'Repeat Analysis')

try

im = Localizer('readccdimages', 0, -1, file); %from frame 0 to all frames (-1)

pts = Localizer('localize', 1.8, 'glrt', 25, '2DGauss', im); %Localizer function fits 2D Gaussian with PSF standard deviation factor 1.8 and intensity selection sigma factor 25

save([file(1:strfind(file, '.HIS')-1) '\_LocRes.mat'], 'pts') %save information of localized points (pts). Filename = core name of the .HIS file followed by \_LocRes.mat

catch

disp([file ' was not analysed!'])

end

else

load([file(1:strfind(file, '.HIS')-1) '\_LocRes.mat']);

end

else

try

im = Localizer('readccdimages', 0, -1, file);

pts = Localizer('localize', 1.8, 'glrt', 25, '2DGauss', im);

save([file(1:strfind(file, '.HIS')-1) '\_LocRes.mat'], 'pts')

catch

disp([file ' was not analysed!'])

end

end

% Plot reconstructed image in a 4x enlarged figure of 2048x2048 pixels (512 pixels/0.25). In the 512x512 image, 1 pixel = 80.8 nm. In the 4x enlarged image, 1 pixel = 20.2 nm)

if exist('pts', 'var')

mx = 512/0.25;

cod = floor(pts(:,4:5)/0.25)+1;

cod(cod(:,1)<1, :)=[];

cod(cod(:,2)<1, :)=[];

hst = accumarray(cod(:, [2, 1]), 1, [mx, mx]);

hst=single(hst);

hsts=imgaussfilt(hst,1);

imagesc(hsts, [0 7]); colormap(pe); %for adjusting LUT scale, for visual purposes only

axis xy equal tight

line([1752.5,2000],[50,50],'Color','w','LineWidth',2) % put scalebar of 5µm. 1 pixel = 20.2 nm, so 5 µm = 247.5 pixels.

title(file(1:strfind(file, '.HIS')-1))

print([file(1:strfind(file, '.HIS')-1) '.png'], '-dpng'); % export reconstructed image as png

end

waitbar(fn/size(list, 2), h);

end

%% combine 2 images with the same core name

list=dir('\*LocRes.mat');

list={list.name};

while ~isempty(list)

file = list{1};

core = file(1: strfind(file, '\_mov')-1);

index = find(contains(list, core));

fr = 0; pts\_total =[];

for i=1:length(index)

load(list{i});

pts(:,1) = pts(:,1) + fr;

pts\_total = [pts\_total; pts];

fr = pts(end, 1) + 1;

end

pts = pts\_total;

save([core '\_Res\_all\_movies.mat'], 'pts'); % %save combined information of localized points (pts). Filename = core name of the .HIS file followed by \_Res\_all\_movies.mat

mx = 512/0.25;

figure

cod = floor(pts(:,4:5)/0.25)+1;

cod(cod(:,1)<1, :)=[];

cod(cod(:,2)<1, :)=[];

hst = accumarray(cod(:, [2, 1]), 1, [mx, mx]);

hst=single(hst);

hsts=imgaussfilt(hst,1);

imagesc(hsts, [0 5]); colormap(pe);

axis xy equal tight

line([1752.5,2000],[50,50],'Color','w','LineWidth',2)

title(core)

print([core 'all.png'], '-dpng');

list(index)=[];

end