

```

function [filteredtrackslist,
tracks]=particletracker3D_astigmatism_trackplotter(pixelsize,calibrati
on,resultstable,max_linking_distance,max_gap_closing,t,minimumtracklen
gth,Channel1,currentfolder)

Results=importdata([resultstable]);
n_dim = 3;
debug = true;

%sorting xyz coordinates from QuickPALM Results.txt and putting into
new
%array

FN=(Results.data(:,end));
x=(Results.data(:,3));
y=(Results.data(:,4));
wmh=(Results.data(:,14));
I=(Results.data(:,2));

%convert pixels to μm
x=(x*pixelsize);
y=(y*pixelsize);
z=interp1(calibration(:,2),calibration(:,1),wmh,'linear');%, 'extrap');

[FN_sorted,sortIdx] = sort(FN);
x_sorted = x(sortIdx);
y_sorted = y(sortIdx);
z_sorted = z(sortIdx);
I_sorted= I(sortIdx);

FN_x_y_z_I=[FN_sorted x_sorted y_sorted z_sorted I_sorted];

%generating "points" cell with each frame having its own array

clear points
points{1,max(FN_x_y_z_I(:,1))}=[];
points=points';
for i=1:length(FN_x_y_z_I)
    points{FN_x_y_z_I(i,1)}=[points{FN_x_y_z_I(i,1)}; FN_x_y_z_I(i,
2:4)];
end

%Fill empty arrays with zeros. (As QuickPALM sometimes cannot localize
any
%particle in a frame) but Simpletracker needs values in each frame)

for j=1:length(points)
    if (isempty(points{j,1}))==1
        points{j,1}=[0,0,0];
    end

```

```

end

%generate pointst
pointst{1,max(FN_x_y_z_I(:,1))}=[];
pointst=pointst';
for i=1:length(FN_x_y_z_I)
    pointst{FN_x_y_z_I(i,1)}=[pointst{FN_x_y_z_I(i,1)}; FN_x_y_z_I(i,
1:4)];
end

%Fill empty arrays with zeros. (As QuickPALm sometimes cannot localize
any
%particle in a frame) but Simpletracker needs values in each frame)

for j=1:length(pointst)
    if (isempty(pointst{j,1}))==1
        pointst{j,1}=[0,0,0,0];
        display('zero added')
    end
end

%track linking

[ tracks adjacency_tracks ] = simpletracker(points, ...
    'MaxLinkingDistance', max_linking_distance, ...
    'MaxGapClosing', max_gap_closing, ...
    'Debug', debug);

%lookup the point numbers from "adjacency_tracks" in "allpoints" and
%combine them in a cell array "tracks"
allpoints=vertcat(pointst{:});

clear tracks
tracks=cell(length(adjacency_tracks),1);

for i=1:length(adjacency_tracks)
    trackpointlist=adjacency_tracks{i,1};

    for k=1:length(trackpointlist)
        tracks{i,1}=[tracks{i,1}; (allpoints((trackpointlist(k,1)),
1))*t, (allpoints((trackpointlist(k,1)),2:4))];
    end
end

```

```

%filter out tracks made of empty frames (zero-tracks)

trackslist={};
counter=0;
for i=1:length(tracks)

    if sum( sum(tracks{i,1}))==0;
    display('found and deleted zero track')
    else
        counter=counter+1;
        trackslist{counter,1}=tracks{i,1};
    end

end

%filter tracks shorter than minimumtracklength

clear i
filteredtrackslist=[];

for i=1:length(trackslist)
    if length(trackslist{i,1})>= minimumtracklength
        filteredtrackslist=[filteredtrackslist; trackslist(i,
1)];
    end
end

%% track plotting

%% get metadata for channel 1
InfoImageCh1=imfinfo(Channel1);
mImageCh1=InfoImageCh1(1).Width;
nImageCh1=InfoImageCh1(1).Height;
NumberImagesCh1=length(InfoImageCh1);

framenumber=length(InfoImageCh1);

%% import channel 1

clear i
FinalImageCh1=zeros(nImageCh1,mImageCh1,NumberImagesCh1,'uint16');
for i=1:NumberImagesCh1
    FinalImageCh1(:,:,:)=imread(Channel1,'Index',i);
end

%% prepare tracks to be plotted into image

%concatanate filteredtrackslist

```

```

filteredtracksperframe2 = vertcat( filteredtrackslist{:} );

framet = filteredtracksperframe2(:,1);
x = filteredtracksperframe2(:,2);
y = filteredtracksperframe2(:,3);

%convert x,y from pixels to µm and round to full pixels
x=round(x/pixelsize);
y=round(y/pixelsize);

%sort by t
[t_sorted,sortIdx] = sort(framet);
x_sorted = x(sortIdx);
y_sorted = y(sortIdx);

%convert t to FN
FN_sorted = round(t_sorted/t); %round is needed here to make sure
integers are the result

trackperframesorted=[FN_sorted, x_sorted, y_sorted];
trackperframesorted=uint16(trackperframesorted);

%generate new cell structure with each frame having its own arrray

Ch1points{(framenumber),1}=[];

for i=1:length(trackperframesorted)
    Ch1points{trackperframesorted(i,1)}
=[Ch1points{trackperframesorted(i,1)}; trackperframesorted(i,1:3)];
end

%Fill empty arrays with zeros.
for j=1:length(Ch1points)
    if (isempty(Ch1points{j,1}))==1
        Ch1points{j,1}=[0,0,0];
    end
end

%% plot tracks into file

clear i
clear k
Modimage=zeros((mImageCh1+8),(nImageCh1+8),framenumber);
Modimage=uint16(Modimage);
trackimage=zeros((mImageCh1+8),(nImageCh1+8));

```

```

for      i=1:length(Ch1points)

    if  sum(Ch1points{i,1})>0
        Image=zeros((nImageCh1+8),(mImageCh1+8)); %make image of
size prelimage + 8 in each dimension so that white square fits

        %annotate next two lines out if you want tracks on black
background

%
%         prelimImage=(FinalImageCh1(:,:,i));%extract picture i
%         Image(5:(nImageCh1+4),5:(mImageCh1+4))=prelimImage; %put
prelimimage into Image

        Image=imrotate(Image, 270); %rotate array 90 degrees
clockwise
        Image=flipdim(Image,2); %flip array horizontally as
Quickpalm flips the coordinates

for k=1:(length (Ch1points{i,1}(:,1)))

    xpix=(Ch1points{i,1}(k,2))+4;
    ypix=(Ch1points{i,1}(k,3))+4;

    trackimage(xpix,ypix)=65536;%makes white dot at
particle position

    %draw white square around particle position
    Image((xpix-4):(xpix+4),ypix-4)=65536;
    Image((xpix-4):(xpix+4),ypix+4)=65536;
    Image(xpix-4,(ypix-4):(ypix+4))=65536;
    Image(xpix+4,(ypix-4):(ypix+4))=65536;

    Image=Image+trackimage;

    %draw white pixel at particle position
    Image(xpix,ypix-4)=65536;

end

Modimage(:,:,i)=[Image];

else

    %if tracks on black background are ok:
    Modimage(:,:,i)=zeros((mImageCh1+8),(nImageCh1+8));

    %if plotting tarcks into image then code is needed here

```

```

that           %takes ith image, flips and turns it and inserts it into
bigger        %image wit dimension+8

end

end

%restore orientation of image

Modimage=imrotate(Modimage, 90); %rotate array 90 degrees
counterclockwise
Modimage=flipdim(Modimage,1); %flip array vertically

%crop back Modimage to original size

Modimage=Modimage(5:(nImageCh1+4),5:(mImageCh1+4),1:framenumber);

%write final image stack

t = Tiff([currentfolder, '/tracks.tif'],'w');

for i=1:size(Modimage,3)

t = Tiff([currentfolder, '//tracks.tif'],'a');

tagstruct.ImageLength = nImageCh1;
tagstruct.ImageWidth = mImageCh1;
tagstruct.Photometric = Tiff.Photometric.MinIsBlack;
tagstruct.SampleFormat = Tiff.SampleFormat.UInt;
tagstruct.Compression = Tiff.Compression.None;
tagstruct.BitsPerSample = 16;
tagstruct.SamplesPerPixel = 1;
tagstruct.RowsPerStrip = 1;
tagstruct.PlanarConfiguration = Tiff.PlanarConfiguration.Chunky;
tagstruct.Software = 'MATLAB';
t.setTag(tagstruct);

t.write(Modimage(:, :, i));

t.close();

end

end

```

