

```
1 function linkTrajSegments(image_label,start_frame,end_frame,spot_results,data_set_label)
2 %
3 % Created by Isabel Llorente-Garcia, Sept. 2011.
4 % If you use this code please acknowledge Isabel Llorente-Garcia in your
5 % publications.
6 %
7 % This function links and groups all found trajectory segments (of bright fluorescent spots)
8 % and overlays fully connected trajectories on image sequence in a video.
9 % Trajectory data results are exported to an .xls file.
10 %
11 % INPUTS:
12 % image_label: string such as '513', '490', etc... which corresponds to a certain label of image sequence.
13 % start_frame and end_frames are the frames through which the loop runs to plot the result trajectories.
14 % They should be the same as previously used to produce spot_results using FindTrajects.m.
15 % spot_results: parameters (spot_results{1}) and calculated trajectory segments (spot_results{2}). It is the output of
function FindTrajects.m
16 % (it is a cell array with two elements. The second one is a structure array containing all segment results).
17 % data_set_label: is a user-defined label that will form part of the name of the output excel file. E.g.: 'ATPase-GFP'.
18 %
19 % Example of how to call this function:
20 % linkTrajSegments('490',100,110,s1,'ATPase-GFP'),
21 % where s1 =FindTrajects('490',100,110) has been previously calculated;
22 % Takes image sequence 490 within the current folder,
23 % and plots the full trajectories that result from linking the trajectory segments in s1,
24 % overlaid on the image sequence, for frames 100 to 110.
25 % -----
26 % Note: for reading .sif files you need 'read_sif_data_direct.m',
27 % 'GetAndorSifSize', etc. which are currently in path:
28 % 'C:\Isabel\myMatlabFiles\IO_Input\'.
29 %
30 % NOTE: before running this function you should move into the directory which
31 % contains the image sequence data (labelled by 'image_label').
32
33
34 %% DEFINITIONS and PARAMETERS:
35 %
36 % define data (.sif image) directory:
```

```
37 % dir_data = 'Z:\Leake\Heiko Data\';
38 % dir_data = 'C:\Isabel\ExperimData\HeikoData\';
39 % print data directory on command window to guide user:
40 disp(' ') % empty line
41 disp(['The data directory (image sequences) is: ',cd])
42
43 disp(' ') % empty line
44 disp(['The start frame for plotting trajectories is ',num2str(start_frame)]) % start_frame is an input.
45 disp(['The end frame for plotting trajectories is ',num2str(end_frame)]) % end_frame is an input.
46
47
48 % I have saved all parameter values in the file "paramsForLinkTrajSegments.m"
49 % in the current directory. Just calling the name of that file loads the
50 % parameter values into the workspace:
51 paramsForLinkTrajSegments
52 % In this way, we can save different parameter sets for different data sets
53 % in an easy manner and run several Matlabs at the same time working with
54 % different parameter sets.
55
56
57 % % Use a very large number (larger than image size in pixels) for rejected asignments:
58 % rej = 10000;
59 % params.rej = rej; % Save parameters to results as structure "params".
60 %
61 % % PARAMETERS for linking trajectory segments:
62 % % For linking end spot in one trajectory with start spot in another trajectory:
63 % d_01_max = 5; % max distance in pixels between spot centres.(5)
64 % Iratio_01_min = 0.2; % min ratio of total spot intensities (after bgnd subtraction).(0.2)
65 % Iratio_01_max = 4; % max ratio of total spot intensities (after bgnd subtraction). (Large enough to account for blinking,
note that Iratio is for frame k-1/frame k.)(3)
66 % SigmaRatio_01_min = 0.5; % min ratio of spot widths (sigma of Gaussian fit).(0.5)
67 % SigmaRatio_01_max = 2; % max ratio of spot width (sigma of Gaussian fit). (2)
68 % Frames_away_max = 2; % max separation in frames (i.e., prop to time) for trajectory segments to be linked.(default = 2).
Write 1 if you want no jumps (no frame jumps) in the segment linking.
69 % % At most we skip one frame when Frames_away_max = 2.
70 % % Save parameters to results as structure "params":
71 % params.d_01_max = d_01_max;
```

```
72 % params.Iratio_01_min = Iratio_01_min;
73 % params.Iratio_01_max = Iratio_01_max;
74 % params.SigmaRatio_01_min = SigmaRatio_01_min;
75 % params.SigmaRatio_01_max = SigmaRatio_01_max;
76 % params.Frames_away_max = Frames_away_max;
77 %
78
79 %% Extract segment (small trajectory) results from "spot_results" input:
80
81 params_for_FindTrajects = spot_results{1}; % parameters used in function FindTrajects.m to obtain segments.
82 traj_results = spot_results{2}; % Results of segments, obtained by function FindTrajects.m.
83
84
85 %% list of colours for plotting trajectories:
86 % colors are in this order: red, green, blue, yellow, pink, lighter blue,
87 % darker red, darker green, darker blue, mustard, darker pink, light blue,
88 % light orange, light greenish, darker green, salmon, another light blue, very light green,
89 % lighter grey, light gray, light yellow, light pink, light blue, orange
90 % the length of this list determines the total no. of trajectories we can plot (7200 now).
91 color_list_0 = [1 0 0;    0 1 0;    0 0 1;    1 1 0;    1 0 1;    0 1 1;...
92    0.8 0 0; 0 0.8 0; 0 0 0.8; 0.8 0.8 0; 0.8 0 0.8; 0 0.8 0.8;...
93    0.95 0.72 0.4; 0.86 0.95 0.6; 0.4 0.8 0.4; 0.9 0.6 0.5; 0.4 0.8 0.7; 0.85 0.92 0.99;...
94    0.9 0.9 0.9; 0.7 0.7 0.7; 1 1 0.5; 1 0.5 1; 0.5 1 1; 1 0.6 0.2];
95 % Make of class single first to save memory:
96 color_list = repmat(single(color_list_0),300,1); % replicate copies of the matrix color_list_0.
97
98 % -----
99 %% Error control: if the number of trajectories to be plotted is larger than
100 % the number of colours in color_list, exit function:
101 if max([traj_results.TrajNumber])> length(color_list)
102     disp('') % empty line.
103     disp('!!!')
104     disp('ATTENTION!!!  number of trajectories to be plotted exceeds length of list of colours to plot...')
105     disp('')
106     disp('Exiting function.')
107     disp('No excel file of trajectory results was saved.')
108     disp('!!!')
```

```
109     disp('')
110     return
111 end
112 % -----
113
114 %% Read in the .sif image data:
115 %
116 % Read image-sequence file:
117 [numFrames frame_Ysize frame_Xsize image_data image_path] = extract_image_sequence_data(image_label);
118 % See "extract_image_sequence_data.m".
119 % numFrames is the number of frames in the image sequence.
120 % To get frame number "p" do: image_data(p).frame_data.
121 % Frame dimensions are frame_Ysize and frame_Xsize.
122 % -----
123
124
125 %% SORT and ARRANGE TRAJECTORY DATA, step 1:
126 % Convert structure resulting from function FindTrajects.m into useful
127 % data arranged in a large matrix (spot after spot).
128
129 max_no_spots = size(traj_results,2); % max. number of found spot centres in each frame.
130
131 data_to_export_labels = fieldnames(traj_results)'; % Row vector with the fieldnames of all found spots.
132 % ⚡
133 ('CentreX','CentreY','IspTot','rsqFit','SigmaFit','IOFit','BgNoiseStd','IbgAvg','IbgTot','SNR','IinnerTot','ClipFlag','TrajNumber',
134 '','FrameNumber','SpotNumber').
135
136 % Make of class single to save memory space:
137 all_data_to_export = single([]); % empty, to be filled in (original data, all spots).
138 data_to_export = single([]); % empty, to be filled in (only spots that have been linked into trajectories will be saved to
139 this variable).
140
141 traj_column = strmatch('TrajNumber',data_to_export_labels); % number of column containing trajectory numbers (used later to
142 sort traj data by TrajNumber).
143
144 x_column = strmatch('CentreX',data_to_export_labels); % number of column containing CentreX position (used later).
145 y_column = strmatch('CentreY',data_to_export_labels); % number of column containing CentreY position (used later).
146 IspTot_column = strmatch('IspTot',data_to_export_labels); % number of column containing IspTot (used later).
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142 Sigma_column = strmatch('SigmaFit',data_to_export_labels); % number of column containing SigmaFit (used later).
143 Frame_column = strmatch('FrameNumber',data_to_export_labels); % number of column containing Frame number (used later).
144
145 % Loop through selected frames:
146 for k = start_frame:end_frame
147
148     frame = image_data(k).frame_data; % extract frame data which is stored in field 'frame_data'.
149     frame = double(frame);
150     imshow(frame,[],'Border','tight','InitialMagnification',150); % show image scaled between its min and max values ([]).
151     hold on;
152     pause(0.5);
153
154     disp(['frame number: ',num2str(k)]) % print frame number to Command Window.
155
156     % Plot result trajectories:
157     for q = 1:max_no_spots % loop through found spots on each frame:
158
159         % traj_results(k,q) is a structure containing the found spot
160         % characteristics, including fields 'CentreX', 'CentreY' and
161         % 'TrajNumber'.
162         tr = traj_results(k,q).TrajNumber; % trajectory number corresponding to found spot q on frame k.
163
164         if ~isempty(traj_results(k,q).FrameNumber) % if the spot is not empty, save it to all_data_to_export.
165             if isempty(traj_results(k,q).TrajNumber) % if TrajNumber is empty fill it up with a 0 or dimensions in next step
will not match.
166                 traj_results(k,q).TrajNumber = 0;
167             end
168             all_data_to_export = [all_data_to_export; single(cell2mat(struct2cell(traj_results(k,q)))')]; % append row with
the spot data.
169         end
170
171         if tr > 0 % only plot trajectory numbers > 0 (TrajNumber=0 is for loose spots):
172             % Plot each trajectory in a different colour:
173             plot(traj_results(k,q).CentreX,traj_results(k,q).CentreY,'o','Color',color_list(tr,:), 'MarkerSize',10)
174             % Construct trajectory data to save (convert structure to matrix):
175             data_to_export = [data_to_export; single(cell2mat(struct2cell(traj_results(k,q)))')]; % append row with the spot
data.
```

```
176         end
177     end
178 %     pause(0.2);
179 %     hold off;
180
181 end % loop through selected frames.
182 % -----
183
184
185 %% SORT and ARRANGE TRAJECTORY DATA, step 2:
186 % Sort spot data by trajectory number and separate trajectories:
187
188 % % Unsorted trajectory data:
189 % data_to_export_unsorted = [data_to_export_labels; num2cell(data_to_export)]; % Add as a first row the fieldnames (labels
for each column).
190
191 % Sort spots data by trajectory number:
192 if ~isempty(data_to_export)
193     data_to_export_sorted = sortrows(data_to_export, traj_column); % sort data by trajectory number. This is of class single
by assignment.
194 else
195     disp('!!!')
196     disp('WARNING: spots found were not linked into trajectories. No trajectories of more than one point were found.')
197     disp('No excel file of trajectory results was saved.')
198     disp('!!!')
199     return
200 end
201
202 trajs_max = max([traj_results.TrajNumber]); % number of trajectories to be plotted/saved.
203
204 % trajs_max
205
206 % Make cell array in which each element is a matrix corresponding to a given trajectory number:
207 separated_trajs = cell(1, trajs_max); % make cell array (row) of empty matrices. (Pre-allocate size for speed.)
208 i = 1; % index for rows of sorted trajectory data.
209 for tr = 1:trajs_max % loop through all trajectory numbers:
210     j = 1; % index for rows of separated trajectory data.
```

```
211 while data_to_export_sorted(i, traj_column) == tr
212     separated_trajs{tr}(j,:) = data_to_export_sorted(i,:); % fill up row by row.
213     if i == size(data_to_export_sorted,1) % if we reach end row of data_to_export_sorted.
214         break
215     end
216     i = i+1;
217     j = j+1;
218 end
219 end
220 % The result separated_trajs is a cell array with as many matrices (of
221 % different sizes) as trajectories. E.g.: separated_trajs{tr} gives the
222 % data for trajectory "tr". E.g. separated_trajs{tr}(:,1) gives all the
223 % CentreX values (1st column) of trajectory number "tr".
224 % Note that the first row in each trajectory is reserved for adding column
225 % labels latter!!!
226 %-----
227
228
229 % Error control: if there is only one trajectory number (traj number 1 or 0 for loose spots):
230 if unique([traj_results.TrajNumber])<=1
231     disp(' ') % empty line.
232     disp('NOTE: there is only one trajectory in the input data. This trajectory has been sent to an excel file.')
233     % Export initial sorted trajectory data (before linking trajectory segments):
234     data_to_export_sorted = [data_to_export_labels; num2cell(data_to_export_sorted)]; % Add as a first row the fieldnames (labels for each column).
235     output_filename = strcat(data_set_label, '_', image_label, 'fullTrajs.xls'); % output .xls filename (before sorting data by trajectory number)
236     xlswrite(output_filename, data_to_export_sorted); % write data to excel file.
237     % the previous data contains only spots which have been linked into a
238     % trajectory.
239
240     % Export also all (non-empty) spots in the input (traj_results) structure, even if they have not been linked into trajectories, i.e.,
241     % export "all_data_to_export":
242     all_data_to_export_2 = [data_to_export_labels; num2cell(all_data_to_export)]; % Add as a first row the fieldnames (labels for each column).
243     output_filename = strcat(data_set_label, '_', image_label, 'allspots.xls'); % output .xls filename (before sorting data by
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trajectory number)
244     xlswrite(output_filename,all_data_to_export_2); % write data to excel file.
245
246     return % exit function
247 end
248
249
250 %% LINKING TRAJECTORY SEGMENTS:
251 % Compare end point of a given trajectory with starting point of another
252 % one. Create matrices with results of pair-wise spatial distances, intensity ratios, sigma ratios and distance in frames.
253
254 % Create cell arrays with empty elements to pre-assign sizes:
255 A = single(zeros(trajs_max,trajs_max)); % create matrix of the right size, with all zeros, single precission.
256
257 d01 = num2cell(A); % Note: d01 is a cell array (matrix) but d_01 below is a scalar.
258 Iratio01 = num2cell(A); % Note: Iratio01 is a cell array (matrix) but Iratio_01 below is a scalar.
259 SigmaRatio01 = num2cell(A); % Note: SigmaRatio01 is a cell array (matrix) but SigmaRatio_01 below is a scalar.
260 FramesAway = num2cell(A); % Note: FramesAway is a cell array (matrix) but Frames_away below is a scalar.
261 d01_best = num2cell(A); % Cell array which will contain only best assignment
262
263 clear A % remove variable from workspace
264
265 % d01 = cell(trajs_max,trajs_max); % Note: d01 is a cell array (matrix) but d_01 below is a scalar.
266 % Iratio01 = cell(trajs_max,trajs_max); % Note: Iratio01 is a cell array (matrix) but Iratio_01 below is a scalar.
267 % SigmaRatio01 = cell(trajs_max,trajs_max); % Note: SigmaRatio01 is a cell array (matrix) but SigmaRatio_01 below is a scalar.
268 % FramesAway = cell(trajs_max,trajs_max); % Note: FramesAway is a cell array (matrix) but Frames_away below is a scalar.
269 %
270 % d01_best = cell(trajs_max,trajs_max); % Cell array which will contain only best assignments
271 % of pairs of trajectory segments after solving all possible competitions
272 % first within row and then within column in d01.
273
274 for qend = 1:trajs_max % loop though end points of all trajectories.
275     % qend
276     for qstart = 1:trajs_max % loop though start points of all trajectories.
277         % [qend qstart]
278         d01_best{qend,qstart} = single(rej); % preliminarily fill up the best-assignment distance matrix with values rej.
```



```
279
280     x_end = separated_trajs{qend}(end,x_column); % CentreX position for last spot in trajectory qend.
281     y_end = separated_trajs{qend}(end,y_column); % CentreY position for last spot in trajectory qend.
282     IspTot_end = separated_trajs{qend}(end,IspTot_column); % IspTot for last spot in trajectory qend.
283     Sigma_end = separated_trajs{qend}(end,Sigma_column); % SigmaFit for last spot in trajectory qend.
284     Frame_end = separated_trajs{qend}(end,Frame_column); % frame number for last spot in trajectory qend.
285
286     x_start = separated_trajs{qstart}(1,x_column); % CentreX position for first spot in trajectory qstart.
287     y_start = separated_trajs{qstart}(1,y_column); % CentreY position for first spot in trajectory qstart.
288     IspTot_start = separated_trajs{qstart}(1,IspTot_column); % IspTot for first spot in trajectory qstart.
289     Sigma_start = separated_trajs{qstart}(1,Sigma_column); % SigmaFit for first spot in trajectory qstart.
290     Frame_start = separated_trajs{qstart}(1,Frame_column); % frame number for first spot in trajectory qstart.
291
292     % d_01: distance between end spot in trajectory qend and start point in trajectory qstart:
293     d_01 = sqrt((x_end-x_start)^2+(y_end-y_start)^2);
294     % Iratio_01: ratio of intensities of spot centre in previous and current frames:
295     Iratio_01 = IspTot_end/IspTot_start;
296     % SigmaRatio_01: ratio of widths of spots (Gaussian fits) in previous and current frames:
297     SigmaRatio_01 = Sigma_end/Sigma_start;
298     % Distance in frames (proportional to time) between end spot in trajectory qend and start point in trajectory qstart:
299     Frames_away = Frame_start-Frame_end; % should be >0 for linking segments (see later).
300
301     %           d_01
302     %           Iratio_01
303     %           SigmaRatio_01
304     %           Frames_away
305
306     % Accept assignment (link trajectory segments) and save values only if start and end spots in
307     % respective trajectories fulfill the following conditions:
308     if d_01 < d_01_max && ... % see PARAMETERS at start of this function.
309         Iratio_01_min <= Iratio_01 && Iratio_01 <= Iratio_01_max && ...
310         SigmaRatio_01_min <= SigmaRatio_01 && SigmaRatio_01 <= SigmaRatio_01_max && ...
311         Frames_away > 0 && Frames_away <= Frames_away_max
312         % Assign accepted values to cell array elements to store them:
313         d01{qend,qstart} = d_01; % use {} for cell arrays.
314         % Note that, by construction, cell array d01 has all elements in
315         % and below its diagonal equal to rej. this is because
```

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316         % trajectories appear in the algorithm in order frame by frame,
317         % so the end of a trajectory qend can never happen before the
318         % beginning of a trajectory q start, for elements
319         % d01{qend,qstart} with qend>=qstart, and therefore these
320         % elements are all equal to rej.
321         Iratio01{qend,qstart} = Iratio_01;
322         SigmaRatio01{qend,qstart} = SigmaRatio_01;
323         FramesAway{qend,qstart} = Frames_away;
324     else % rejected assignments:
325         d01{qend,qstart} = single(rej); % Use rej for asignments not accepted (images usually 512x512arrays, so rej pix
326         Iratio01{qend,qstart} = single(rej); % Use rej for asignments not accepted.
327         SigmaRatio01{qend,qstart} = single(rej); % Use rej for rejected assignments.
328         FramesAway{qend,qstart} = single(rej); % Use rej for rejected assignments.
329     end
330 end
331
332 %             d01
333 %             [d01{qend,:}]
334
335 % Note that [d01{qend,:}] gives only non-empty elements of row qend
336 % in the cell array d01 as a row vector, that's why we had to
337 % give a numeric value rej to non-accepted assignments.
338
339 % Note that if all asignments in previous step are rejected,
340 % [d01{qend,:}] will be a list of rej values, and its minimum will
341 % be rej.
342 % If list of "linkable" segments, [d01{qend,:}] (row vector), has at least one accepted
343 % assignment (at least one value smaller than rej):
344 if min([d01{qend,:}]) ~= rej
345     % if there is at least one accepted asignment in that row for trajectory segments:
346
347     % Decide of all possible asignments of starting trajectory segments (qstart) which one is the best:
348     % We take the best as the closest (minimum distance d01):
349
350     % sort non-empty elements in row qend of d01 from smaller to larger distance:
351     sorted_row = sort([d01{qend,:}]);
```

```
352     % For each, check column-wise:
353
354     % for the minimum distance (if we accept it, we do nothing else):
355     if sorted_row(1) < rej
356         qstart_chosen = find( [d01{qend,:}] == sorted_row(1) ); % find position of pair-wise distance 1 in the row (find
which column).
357
358         %             qstart_chosen
359
360         % Check column-wise in that column in d01 to avoid asigning a traj segment start qstart to a traj segment end
qend that had already
361         % had a traj segment assigned to it which might be at a shorter distance than the current one.
362
363         %             [d01{:,qstart_chosen}] % chosen column of d01 matrix of distances.
364
365         % If the found distance in that column is not the minimum one we don't proceed with the asignment, and we move on
to the next smaller distance in sorted_row:
366         if qend ~= find([d01{:,qstart_chosen}] == min([d01{:,qstart_chosen}]));
367             d01_best{qend,qstart_chosen} = single(rej+0.1); % we re-set that distance to rej+0.1 (in case we need to
distinguish these cases from cases rej later on).
368             % if rejected, it moves on to next element in sorted_row
369             % (go inside loop ii):
370
371             if length(sorted_row)>=2
372                 for ii = 2:length(sorted_row)
373                     if sorted_row(ii) < rej
374                         qstart_chosen = find( [d01{qend,:}] == sorted_row(ii) ); % find position of pair-wise distance ii
in the row (find which column).
375
376                         %             qstart_chosen
377
378                         % Check column-wise in that column in d01 to avoid asigning a traj segment start qstart to a traj
segment end qend that had already
379                         % had a traj segment assigned to it which might be at a shorter distance than the current one.
380
381                         %             [d01{:,qstart_chosen}] % chosen column of d01 matrix of
distances.
```

```
382
383         % If the found distance in that column is not the minimum one we don't proceed with the asignment:
384         if qend ~= find([d01{:,qstart_chosen}] == min([d01{:,qstart_chosen}]));
385             d01_best{qend,qstart_chosen} = single(rej+0.1); % we re-set that distance to rej+0.1 (in case
we need to distinguish these cases from cases rej later on).
386             % if rejected, it moves on to next element in sorted_row (loop ii).
387
388         else % if qend really corresponds to the minimum distance (best assignment column-wise):
389
390             d01_best(qend,qstart_chosen) = d01(qend,qstart_chosen); % Pass value on to the "best-
assignments" distance cell array d01_best.
391             % d01_best must have only one value different from rej per row and per column. All elements
initially have a pre-assigned value of rej.
392
393             % Re-set values of previous assignments which were not optimum:
394             for q0 = 1:(qend-1) % loop through previously filled-up elements in that chosen column
qstart_chosen:
395                 if d01_best{q0,qstart_chosen} < rej % for all elements in that column of d01_best except
for the best asignment q0:
396                     d01_best{q0,qstart_chosen} = single(rej+0.2); % re-set larger (not best) distance
values in that column.
397                 end
398             end
399
400         end
401
402     end
403
404     end
405
406
407     else % if qend really corresponds to the minimum distance (best assignment column-wise):
408
409         d01_best(qend,qstart_chosen) = d01(qend,qstart_chosen); % Pass value on to the "best-asignments" distance
cell array d01_best.
410         % d01_best must have only one value different from rej per row and per column. All elements initially have a
pre-assigned value of rej.
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```
411
412     % Re-set values of previous assignments which were not optimum:
413     for q0 = 1:(qend-1) % loop through previously filled-up elements in that chosen column qstart_chosen:
414         if d01_best{q0,qstart_chosen} < rej % for all elements in that column of d01_best except for the best ↵
assignment q0:
415             d01_best{q0,qstart_chosen} = single(rej+0.2); % re-set larger (not best) distance values in that ↵
column.
416         end
417     end
418
419     end
420
421     end
422
423     end
424
425     %         d01_best
426
427 end
428
429
430 % List of best assignments of linked trajectory segments:
431 [u v] = find(cell2mat(d01_best)<rej); % find positions of best assignments (elements in d01_best < rej). u is row, v is ↵
column.
432 best_assignmts = sortrows([u v],1); % sort the list of best assignments of trajectory segments by the first column (end of ↵
traj segment).
433
434 % [u v]
435
436
437 % -----
438 % GROUP BEST ASSIGNMENTS WHICH CORRESPOND TO THE SAME SPOT:
439
440 % Eg. Example with 16 trajectories in total for which d01_best only has 6 elements < rej,
441 % which are saved into best_assignmts: [5 16; 7 11; 8 13; 10 12; 11 15; 12 14].
442 % At the end of the following loop to group assignments, we will have the cell array best_assignmt_groups:
443 % {5 16; 7 11 15; 8 13; 10 12 14}, for which trajectory segments linked into full trajectories are: 5-16,
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```
444 % 7-11-15, 8-13 and 10-12-14.
445
446 best_assignmt_groups = num2cell(best_assignmts,2);
447 % preliminary copy of best_assignments from which we will delete the ones that we have already included into another
assignment as a group.
448 % convert matrix to cell array of as many 1x2 matrices as assignments, in order to be able to modify elements to store
matrices of different sizes later on.
449
450 jj = 1; %jj is index for accepted best assignments.
451 while jj < size(best_assignmt_groups,1)
452
453 %     jj
454
455     first_column = []; % make list of elements in first column of assignments.
456     for kk = 1:size(best_assignmt_groups,1)
457         first_column = [first_column; best_assignmt_groups{kk}(1)]; % append first element of other assignemnts.
458     end
459
460 %     first_column
461
462     row_link = find( first_column == best_assignmt_groups{jj}(end) ); % row corresponding to another found assignment which
links to the present one.
463     % note that in best_assignmt_groups all competing assignments have been solved already.
464
465 %     row_link
466
467     if isempty(row_link) % if there is not another assignment which links to the present one:
468         jj = jj+1; % we leave best_assignmt_groups unchanged and advance index jj to move on to next assignment in
best_assignmt_groups.
469     else % if present assingment jj can be grouped (linked) to another assignment:
470         best_assignmt_groups{jj,:} = [best_assignmt_groups{jj,:} best_assignmt_groups{row_link}(2)]; % group the linkable
assignments into one and save to assignment jj.
471         best_assignmt_groups(row_link) = []; % delete from best_assignmt_groups the row corresponding to assignment grouped
to the current one (use () instead of {} to delete!).
472
473         % do not advance jj index, try to find a possible following link
474         % for current grouped assignment.
```

```
475
476 %         best_assignmt_groups
477 %         best_assignmt_groups{jj}
478
479     end
480
481
482 end
483 % the final list of grouped assignments is best_assignmt_groups.
484 % -----
485
486
487
488 % -----
489 % CREATE FULL TRAJECTORIES AFTER LINKING TRAJECTORY SEGMENTS:
490
491 % Create fully linked trajectories:
492 % Modify Traj Number in separated_trajs data to give same Traj number to trajectories
493 % which have been grouped and linked as given by best_assignmt_groups.
494 % Then sort again by Traj Number again.
495 % In the previous example, after linkin and grouping trajectories, we would
496 % end up with only 10 trajectory numbers 1 to 10 instead of the previous 16.
497
498 % Reminder: the result separated_trajs is a cell array with as many matrices (of
499 % different sizes) as trajectories. E.g.: separated_trajs{tr} gives the
500 % data for trajectory "tr". E.g. separated_trajs{tr}(:,1) gives all the
501 % CentreX values (1st column) of trajectory number "tr".
502 % Note that the first row in each trajectory is reserved for adding column
503 % labels latter!!!
504 % Reminder: traj_column is the number of column containing trajectory numbers.
505
506 separated_trajs_linked = separated_trajs; % This will be the final result of linked trajectories (we assign it a preliminary
value to start with).
507 old_new_traj_numbers = []; % Matrix to be filled up. Each row will have two elements: the old traj number and the new traj
number we will change the old one into.
508
509 for m = 1:size(best_assignmt_groups,1) % loop through groups of linked trajectories.
```

```
510
511     first_traj_no = best_assignmt_groups{m}(1); % Traj. Number for first trajectory in group m. We assign that Traj number to
all trajectories in group m.
512
513     for n = 2:length(best_assignmt_groups{m}) % loop through all but first traj in the group:
514         next_traj_no = best_assignmt_groups{m}(n); % next Traj. Number in group m.
515         old_new_traj_numbers = [old_new_traj_numbers; [next_traj_no first_traj_no]]; % List of traj numbers that need to be
changed (first column) and assigned new traj numbers (second column). To be used later.
516         % Replace all Traj. Numbers in the other trajectory segments in the
517         % group by the Traj. Number (first_traj_no) of the first traj
518         % segment in group m:
519         for p = 1:length(separated_trajs_linked{next_traj_no}(:,traj_column))
520             separated_trajs_linked{next_traj_no}(p,traj_column) = first_traj_no;
521         end
522     end
523 end
524
525 end
526
527 % old_new_traj_numbers
528
529 % -----
530 % GENERATE FINAL DATA TO EXPORT:
531
532 data_to_export_unsorted_2 = [];
533 for tr = 1:trajs_max % loop through all former trajectory numbers (all elements in cell array separed_trajs_linked):
534     data_to_export_unsorted_2 = [data_to_export_unsorted_2; separed_trajs_linked{tr}];
535 end
536
537 data_to_export_sorted_2 = num2cell(sortrows(data_to_export_unsorted_2,traj_column)); % sort by Traj number and convert to
cell to be able to add labels.
538 % -----
539
540
541
542 % -----
543 % PLOT FINAL FULL TRAJECTORY RESULTS:
```



```
544
545 full_traj_results = traj_results;
546 % This will be the same full trajectory result (final result after linking and grouping traj segments),
547 % as a structure in order of image frames, so that we can overlay final
548 % trajectories on the original image sequence.
549
550 % Loop through selected frames:
551 for k = start_frame:end_frame
552
553     frame = image_data(k).frame_data; % extract frame data which is stored in field 'frame_data'.
554     frame = double(frame);
555
556     disp(['frame number: ', num2str(k)]) % print frame number to Command Window.
557
558     imshow(frame, [], 'Border', 'tight', 'InitialMagnification', 150); % show image scaled between its min and max values ([ ]).
559     hold on;
560     pause(0.5);
561
562 %     k
563
564     % Plot final result of full connected trajectories:
565     for q = 1:max_no_spots % loop through found spots on each frame:
566
567         %         q
568
569         % full_traj_results(k,q) is a structure containing the found spot
570         % characteristics, including fields 'CentreX', 'CentreY' and
571         % 'TrajNumber'.
572         tr = traj_results(k,q).TrajNumber; % trajectory number corresponding to found spot q on frame k (in old traj
573 results).
574
575         %         tr
576
577         if isempty(old_new_traj_numbers)==0 % if there are traj numbers to be changed:
578             if isempty(tr) == 0 % if that spot is not empty (due to padding of structure array):
579                 pos_to_change = find(old_new_traj_numbers(:,1)==tr); % check if traj number tr for this spot is contained in
580 the list of traj numbers that need to be changed (old_new_traj_numbers).
```

```
579
580             %             pos_to_change
581
582             if isempty(pos_to_change) == 0 % if the traj number tr for this spot is contained in the list of traj numbers
that need to be changed:
583                 full_traj_results(k,q).TrajNumber = old_new_traj_numbers(pos_to_change,2); % change that traj number.
584             end
585         end
586     end
587
588     if tr > 0 % only plot trajectory numbers > 0 (TrajNumber = 0 is for loose spots):
589         % Plot each trajectory in a different colour:
590         plot(full_traj_results(k,q).CentreX,full_traj_results(k,q).CentreY,'o','Color',color_list(full_traj_results(k,q).
TrajNumber,:), 'MarkerSize',10)
591         %             % Construct trajectory data to save (convert structure to matrix):
592         %             data_to_export = [data_to_export; cell2mat(struct2cell(traj_results(k,q)))']; % append row with the
spot data.
593     end
594 end
595     pause(0.2);
596     hold off;
597
598 end % loop through selected frames.
599 % -----
600
601
602
603 %% EXPORT TRAJECTORY DATA:
604
605 % Note: the file gets saved into the same directory where the .sif image is
606 % (sifImagePath).
607
608 % % Export unsorted data ("unsrc"):
609 % output_filename0 = strcat(data_set_label,'_',image_label,'unsrc0.xls'); % output .xls filename (before sorting data by
trajectory number)
610 % xlswrite(output_filename0,data_to_export_unsorted); % write data to excel file.
611
```

```
612 % % Export initial sorted trajectory data (before linking trajectory segments):
613 % data_to_export_sorted = [data_to_export_labels; num2cell(data_to_export_sorted)]; % Add as a first row the fieldnames (labels for each column).
614 % output_filename = strcat(data_set_label, '_', image_label, 'sorted0.xls'); % output .xls filename (before sorting data by trajectory number)
615 % xlswrite(output_filename, data_to_export_sorted); % write data to excel file.
616
617 % -----
618 % Export final sorted full trajectory data (after linking and grouping
619 % trajectory segments):
620 output_filename = strcat(data_set_label, '_', image_label, 'fullTrajs.xls'); % output .xls filename (before sorting data by trajectory number)
621 data_to_export_sorted_2 = [data_to_export_labels; data_to_export_sorted_2]; % Add as a first row the fieldnames (labels for each column).
622 % Export parameters used for function "FindTrajects.m" and
623 % "linkTrajSegments.m" to two other sheets in excel file:
624 warning off MATLAB:xlswrite:AddSheet % turn warning off when new sheet added to excel file.
625 % Params for "FindTrajects.m":
626 dataForSheet1 = [fieldnames(params_for_FindTrajects) struct2cell(params_for_FindTrajects)];
627 % Params for "linkTrajSegments.m":
628 dataForSheet2 = [fieldnames(params) struct2cell(params)];
629
630 xlswrite(output_filename, dataForSheet1, 'params FindTrajects'); % write data with parameters for "FindTrajects.m" to sheet 'params FindTrajects' in excel file.
631 xlswrite(output_filename, dataForSheet2, 'params linkTrajSegments'); % write data with parameters for "linkTrajSegments.m" to sheet 'params linkTrajSegments' in excel file.
632 xlswrite(output_filename, data_to_export_sorted_2, 'Track results'); % write trajectory data to sheet 'Track results' in excel file.
633 % Note: the file gets saved into the same directory where the .sif image is
634 % (sifImagePath).
635
636 % -----
637
638 % % Export also all (non-empty) spots in the input (traj_results) structure,
639 % % even if they have not been linked into trajectories, i.e.,
640 % % export "all_data_to_export":
641 % all_data_to_export_2 = [data_to_export_labels; num2cell(all_data_to_export)]; % Add as a first row the fieldnames (labels for each column).
```

```
for each column).  
642 % output_filename = strcat(data_set_label,'_',image_label,'allspots.xls'); % output .xls filename (before sorting data by  
trajectory number)  
643 % xlswrite(output_filename,all_data_to_export_2); % write data to excel  
644 % file.  
645  
646  
647  
648
```