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Comparing human and algorithmic tracking of subviral particles in fluorescence microscopic image sequences

Abstract: Tracking of subviral particles with automated methods enables the analysis of intracellular processes exhibited by viruses. A linear assignment problem solver and a Kalman-filter have been added to an existing particle tracking algorithm. First results produced with simulated image sequences showed that the improved algorithm is able to improve tracking results by closing gaps in the particle's trajectories. Here we report on the evaluation of the LAP-Kalman algorithm using real fluorescence-microscopic images. The results from the original and improved algorithm have been compared to the results of manual tracking. Evaluation results indicate that the improved algorithm is capable to reconstruct missing parts of particle tracks in difficult conditions. However, the evaluation of the algorithms and the manual tracking is a complex task because of the low image contrast and high object density with intersecting tracks in the live-cell images.

Keywords: Fluorescence microscopy, Subviral particles, Tracking, Detection, Evaluation.

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1 Introduction

Particle tracking is a very useful tool for the analysis of intracellular processes exhibited by viruses. Image sequences of fluorescence labelled subviral particles, also called nucleocapsids (NCs), often contain a large amount of data,

therefore a manual tracking is very time-consuming. If many image sequences have to be analysed, automated methods are recommended. However, the development of a tracking algorithm which delivers high-quality results is a challenging task. Low contrast of the images, auto fluorescent background, high object density, complex motion patterns and other aspects complicate the tracking task [4].

A particle tracking algorithm ("Kienzle-algorithm") based on expert-knowledge has been developed in cooperation with the Institute of Virology at the Philipps-University Marburg, Germany [1]. Although this algorithm can achieve good detection results with a low false-positive and a high true-positive rate quite often, some tracks were divided by gaps into multiple tracklets. A combination of a linear assignment problem (LAP) solver and a Kalman-filter has been added to the algorithm to improve the tracking performance by closing gaps. Early evaluation results of the improved algorithm using two simulated image sequences showed that the LAP-Kalman algorithm is able to reduce the number of gaps in detected tracks by approximately 75 %. The number of true positives remained the same and no additional false positives were produced compared to the original Kienzle-algorithm [3].

Here we report on the evaluation of the LAP-Kalman algorithm using real fluorescence microscopic image sequences. All investigated sequences contain cells with a large amount of NCs from the Marburg-virus. The tracking results are compared regarding the total amount of data produced by the different algorithms and the completeness of the particle's trajectories. Additionally, the accuracy of position determination of the different tracking methods is investigated.

2 Methods

To visualize intracellular movements of subviral particles of Marburg virus, green fluorescent-protein tagged nucleocapsids were used [5]. This method enables the recording of image sequences under biosafety-level 4

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conditions. Three different image sequences have been chosen for evaluation. In each sequence, ten particle trajectories in different regions of the images have been selected and tracked manually by an expert. Most of the manually tracked NCs were easy to determine by a human observer, however, some trajectories were difficult to capture correctly because of severe image noise and intersecting tracks of other NCs. Length of particle tracks has been measured with Leica LAS AF Software and varies between 2 and 42 μm [9]. Both algorithms processed the same data set. Image and track characteristics can be seen in table 1.

Table 1: Length of tracks and image sequences.

sequence Nr.	number of frames	average tracklength / μm
1	600	17,0
2	600	13,1
3	120	5,1

Detection and tracking of subviral particles is divided into three steps by the Kienzle-algorithm:

- Detection of possible candidates
- Characterization of detected objects
- Comparison of characteristics to templates and linking of objects

The Kienzle-algorithm uses a nearest-neighbour approach with restricted freedom of movement to connect the objects in subsequent frames. Evaluation of the Kienzle-algorithm resulted in a sensitivity of 22 % and a specificity > 98% using a threshold value of 0.9 for object similarity [1]. Further investigations showed, that not all tracks were captured completely, some tracks were separated by gaps or the beginning or end was missing [3]. Therefore, a LAP-solver and a Kalman-filter have been added to the algorithm to improve tracking results. To provide as many subviral particle candidates as possible to the improved algorithm, we are working with the unfiltered raw data from the second step of the Kienzle-algorithm. The following data are provided by the Kienzle-algorithm: spatial and time distance, size, velocity and orientation of objects. These parameters are used to create a cost matrix which is solved by the linear assignment problem solver. Links of objects in subsequent images are determined by the LAP-Kalman algorithm which chooses the lowest global costs. Different types of LAP-Kalman-algorithms were tested and compared [6], the auction algorithm was chosen due to the good performance and short processing time. Implementation of the LAP-solver

enables a closing of gaps in the particle tracks. However, some longer gaps often resulting from intersecting cell structures can not be closed by the LAP-solver, as no data are available for these track sections.

One approach to estimate the position of an object in the future or the past is the Kalman-filter. The quality of results is dependent from the available amount of data. All tracks from the LAP-solver are processed in forward- and backward direction by the Kalman-filter to increase the accuracy of position estimation. In this step, a closing of gaps and an interpolation of the tracks resulting in smoothed trajectories is achieved. The resulting data set is processed again by the LAP-solver and the Kalman-filter which enables the closing of large gaps in the particle tracks.

Different methods have been used for the evaluation. Manual and algorithmic determined tracks have been compared visually by different human observers to investigate the completeness of tracks and correctness of links. The total amount of detected tracks, tracklets and connections from the algorithms in each image sequence has been calculated. Additionally, the spatial accuracy of the different methods has been examined. Manual tracks have been painted as an overlay over a maximum-intensity-projection of the image sequences. For a better visualization, the trajectories of the tracks have been drawn next to the real trajectories. These drawn lines have been extracted by the means of image processing to determine the coordinates of tracks. To examine the deviation between the algorithmic and manual tracks, the tracks from manual tracking and the LAP-Kalman filter are drawn to the same logical image. Gaps between the coordinates of the two tracks are closed using a linear interpolation. Algorithmic determined coordinates in every frame are assigned to the corresponding NC position of the manual tracking results. The area spanned by these two tracks is minimized by the use of optimization, this operation also minimizes the spatial deviation due to the lines which were drawn next to the original tracks. Mean deviation, standard deviation, variance and maximum deviation are used for evaluation purposes.

The Kienzle-algorithm was written with Scilab 5.4.1 [7]. Comparison of tracking accuracy has been performed using Matlab Version 2016 a [8].

3 Results

We analysed 30 tracks from three different fluorescent image sequences of Marburg virus infected cells. Comparison of results from LAP-Kalman algorithm and the Kienzle-algorithm revealed a significant reduction of tracklets and an

increased number of connections between objects in subsequent frames. On average, the number of tracklets in all three analysed sequences could be reduced by 77 % and simultaneously, the number of connections was increased by about 50 % by the LAP-Kalman algorithm. The results are shown in figures 1 and 2.

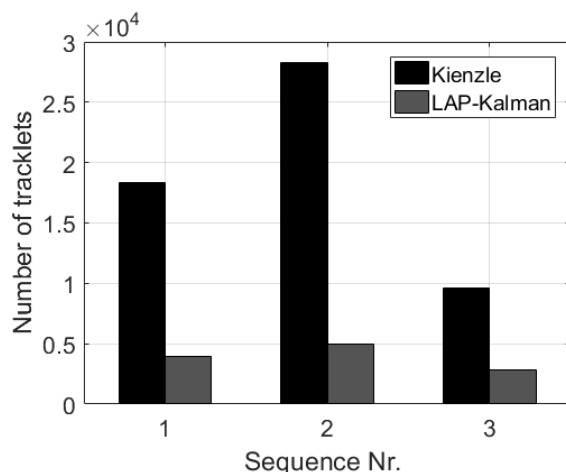


Figure 1: Number of tracklets

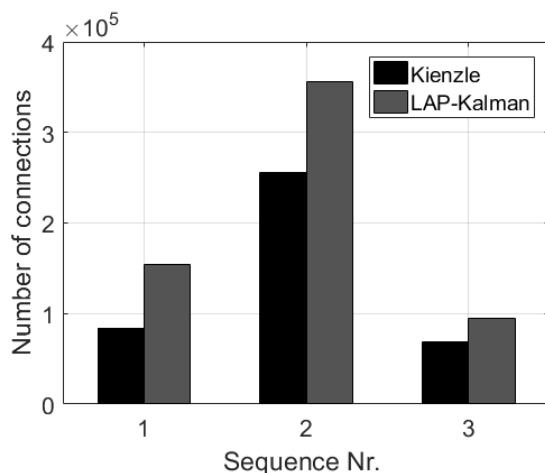


Figure 2: Number of connections

These results concern all tracks detected by the two different algorithms. Further evaluation steps deal with the manually tracked particles in the image sequences. Regarding the manual tracks, not all NCs have been tracked from the beginning to the end of their visible trajectories. Comparison of manual and algorithmic tracks has been performed from the first frame until the last frame of the manual tracks. For each algorithm, the number of gaps in the tracks and the percentage of NC-positions captured has been determined. Investigations show that the number of gaps after the application of the LAP-Kalman algorithm is lower compared to the Kienzle-algorithm. In the three analysed image sequences, the number of gaps was reduced from 8, 16 and

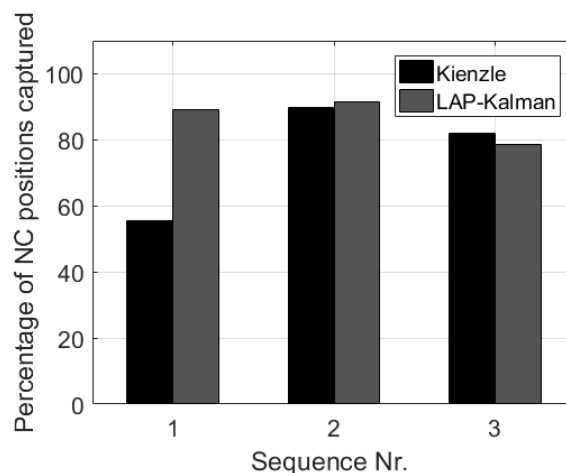


Figure 3: percentage of detected NC positions

15 to 3, 0 and 0. However, both algorithms missed some beginnings and ends of the tracks, these missing detections were not counted as gaps. Length of the gaps varied between 1 and 15 frames.

Additionally, the percentage of detected NC positions for both algorithms was determined. The raw data from the Kienzle algorithm contained 85, 88 and 96 % of all NC positions in the different image sequences with a standard deviation of 16, 17 and 7 %. On average, the LAP-Kalman captured 7 % more NC positions compared to the Kienzle-algorithm. Figure 3 shows the difference of the percentage of detected NC positions between the Kienzle-algorithm with filtering of tracks and the LAP-Kalman algorithm. Regarding the results of image sequences 2 and 3, the percentage of NC positions captured by the LAP-Kalman algorithm should be higher than for the Kienzle-algorithm. However, not all tracks in these sequences could be assigned to a detected track from the LAP-Kalman algorithm, therefore not all tracks from the LAP-Kalman algorithm were included and the amount of detected NC positions may be smaller compared to the raw data from the Kienzle-algorithm.

For the computation of tracking accuracy, the algorithmic tracks have been cut to the same length as the manual tracks which have been extracted from the processed images. Results show a good match between the human and algorithmic subviral particle trackers. Every track has been examined separately regarding the mean deviation, maximum deviation, standard deviation and variance. We compare the ratio of standard and mean deviation to track length which delivers values from 0,6 % up to 2,6 % and 0,8 % up to 3 %. An example of a manual and the corresponding algorithmic track can be seen in figure 4. Unsteady course of the manual tracks is due to the width of a few pixels of drawn lines which are processed to determine the track coordinates.

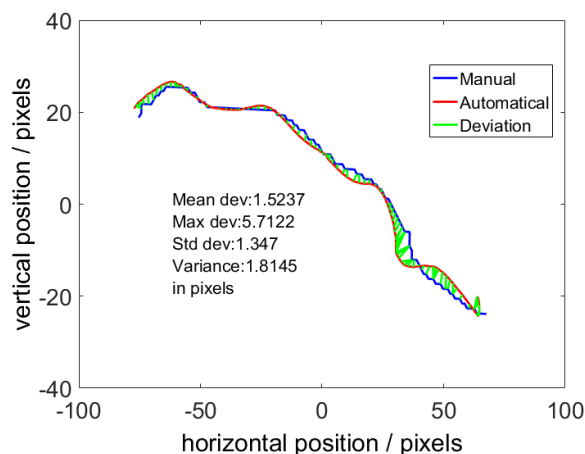


Figure 4: Comparison of manual and algorithmic track from LAP-Kalman algorithm

However, not all tracks could be compared due to the missing corresponding algorithmic tracks. Out of 30 particle tracks, 27 have been used to investigate the tracking accuracy.

4 Discussion

A data set consisting of three different image sequences has been chosen for the evaluation of an improved particle tracking algorithm. In each sequence, ten NCs have been tracked manually by an expert. The tracking results have been compared with the results from the Kienzle-algorithm and the LAP-Kalman algorithm, which is based on the raw-data from the Kienzle-algorithm but enables closing of gaps in the particle tracks. It was shown that the Kalman algorithm reduces the number of gaps in the tracks by connecting separated tracklets into complete tracks. This aspect was analysed regarding the whole data set of detected tracks and the manual tracked subviral particles. The accuracy of the different tracking methods has been analysed and compared. A slight advantage for the tracking algorithms must be considered because the tracking accuracy is in subpixel resolution while a human observer can only track the NCs with pixel resolution. Although the manual tracks have been drawn next to the true particle's trajectories, the deviation between human and algorithmic tracks after algorithmic correction is in a range of a few pixels. Calculated deviations show a good agreement with the visual observations of the tracks from the different methods.

To conclude, the LAP-Kalman algorithm can improve the tracking results from the Kienzle-algorithm by closing gaps and smoothing trajectories. The accuracy of algorithmic and human tracking methods show a good match. However, the evaluation of tracking results is difficult because of severe image noise and proximity of NCs. In some situations, a correct assignment can not be guaranteed. Future investigations could deal with the influence of an iterative approach of the LAP-Kalman algorithm on the quality of tracking results and computational effort.

The current version of the LAP-Kalman algorithm is working with the raw-data from the Kienzle-algorithm. These data contain false-positives resulting from image noise or intracellular objects. A possible extension of the algorithm could be the implementation of a filtering step to remove these false-positive hits.

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