Medical Image Analysis 24 (2015) 163–189

Contents lists available at ScienceDirect

Medical Image Analysis

journal homepage: www.elsevier.com/locate/media

Quantitative comparison of multiframe data association techniques for particle tracking in time-lapse fluorescence microscopy

Ihor Smal*, Erik Meijering

Biomedical Imaging Group Rotterdam, Erasmus MC–University Medical Center Rotterdam, Departments of Medical Informatics and Radiology, P.O. Box 2040, Rotterdam 3000 CA, The Netherlands

ARTICLE INFO

Article history:
Received 5 November 2014
Revised 29 April 2015
Accepted 17 June 2015
Available online 27 June 2015

Keywords:
Object tracking
Correspondence problem
Multiframe data association
Lagrangian relaxation
Fluorescence microscopy

ABSTRACT

Biological studies of intracellular dynamic processes commonly require motion analysis of large numbers of particles in live-cell time-lapse fluorescence microscopy imaging data. Many particle tracking methods have been developed in the past years as a first step toward fully automating this task and enabling high-throughput data processing. Two crucial aspects of any particle tracking method are the detection of relevant particles in the image frames and their linking or association from frame to frame to reconstruct the trajectories. The performance of detection techniques as well as specific combinations of detection and linking techniques for particle tracking have been extensively evaluated in recent studies. Comprehensive evaluations of linking techniques per se, on the other hand, are lacking in the literature. Here we present the results of a quantitative comparison of data association techniques for solving the linking problem in biological particle tracking applications. Nine multiframe and two more traditional two-frame techniques are evaluated as a function of the level of missing and spurious detections in various scenarios. The results indicate that linking techniques are generally more negatively affected by missing detections than by spurious detections. If misdetections can be avoided, there appears to be no need to use sophisticated multiframe linking techniques. However, in the practically likely case of imperfect detections, the latter are a safer choice. Our study provides users and developers with novel information to select the right linking technique for their applications, given a detection technique of known quality.

1. Introduction

Proper understanding of intracellular dynamic processes and how to manipulate them in a controlled way is a prerequisite to combat a wide variety of diseases and improve human health care. The past decades have witnessed the development of groundbreaking techniques for imaging these processes with high spatial and temporal resolution (Ji et al., 2008; Kanchanawong and Waterman, 2012; Lakadamyali, 2014; Patterson, 2009; Pawley, 2006; Schermelleh et al., 2010; Stephens and Allan, 2003; Vonesch et al., 2006; Weisshart et al., 2013). Of these, time-lapse fluorescence microscopy is currently the most often used live-cell imaging technique by biologists. It consists in the labeling of structures of interest within cells using fluorescent probes and imaging them over time using an advanced light microscope system of choice (see Fig. 1 for example images). The dynamics of the labeled structures can subsequently be studied quantitatively by reconstructing their observed trajectories, from which biophysical parameters such as velocity, acceleration, and mean-squared displacement can be easily estimated.

Current biological studies using time-lapse fluorescence microscopy imaging typically produce image data sets containing very large numbers of moving particles and require automatic tracking tools to allow for a complete analysis of all available information (Dorn et al., 2008; Jaqaman and Danuser, 2009; Kalaidzidis, 2007; 2009; Meijering et al., 2012; 2009; 2006; Rohr et al., 2010; Saxton, 2008). In addition to having high accuracy and precision, the used tracking methods should be robust to varying experimental conditions, as well as computationally efficient in order to process the high volumes of image data within reasonable time. The majority of tracking methods described in the literature use a two-step approach: (i) *detecting* the objects of interest independently in each frame of the time series and (ii) creating trajectories by *linking* the sets of detected objects in successive frames. For both processing steps, many different techniques have been proposed over the years.

Recently, the performances of 14 different particle tracking methods were compared in an international challenge (Chenouard et al., 2014). Each tracking method consisted of a specific combination of detection and linking techniques, as chosen by the corresponding

* Corresponding author. Tel: +31 10 7043050.
E-mail address: i.smal@erasmusmc.nl (I. Smal).

http://dx.doi.org/10.1016/j.media.2015.06.006
1361-8415/

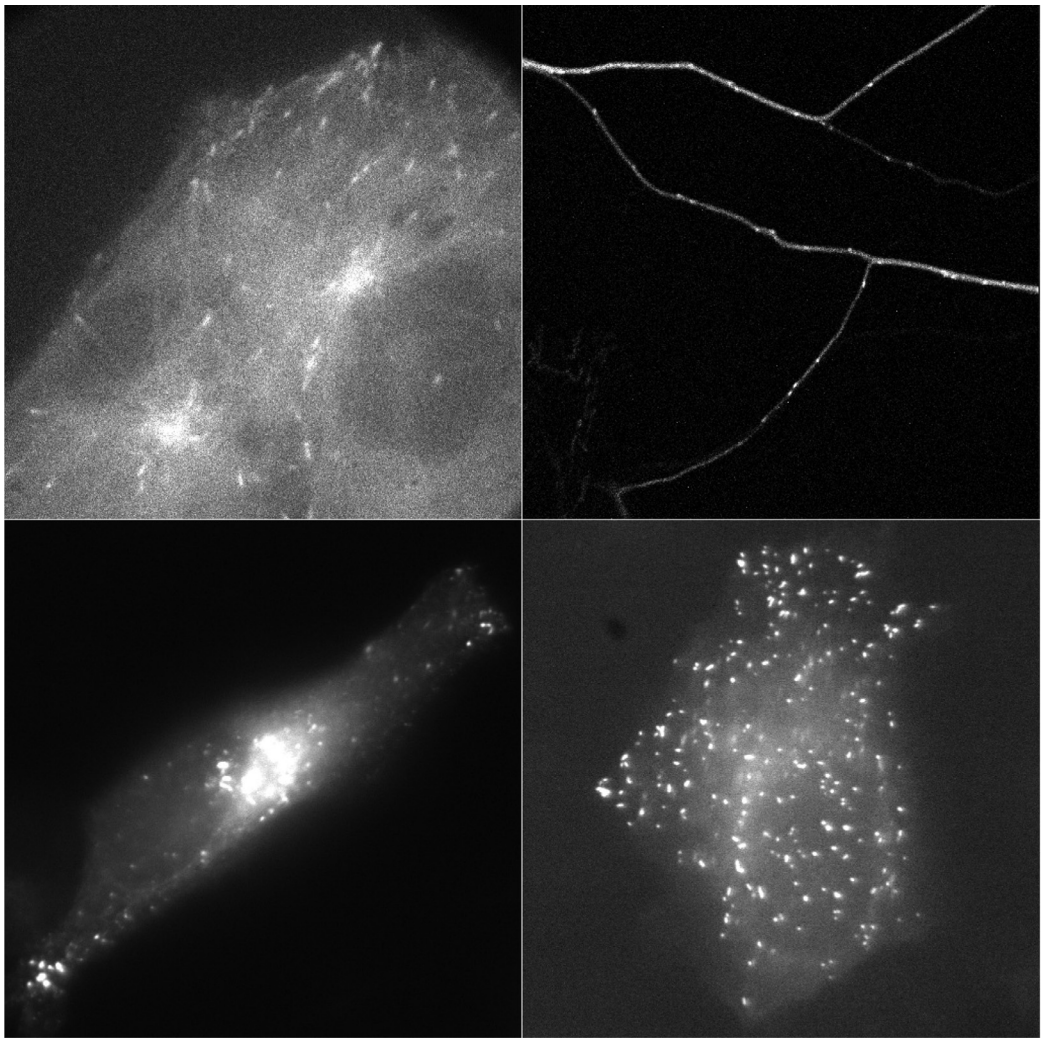

Fig. 1. Examples of images acquired for different biological studies based on GFP labeling and fluorescence microscopy. The images are single frames from 2D time-lapse studies of activity of microtubule plus-ends (top left), microtubule plus-ends in neurons (top right), Rab5 proteins (bottom left), and peroxisomes (bottom right). In all cases the objects of interest appear as bright (compared to the local background) blurred spots.

participants, and hence the results of the study revealed their combined performance. That is, even if any two methods would have used the exact same linking technique but a different detection technique (or vice versa), their scores for all performance measures used in the study would likely be different, as the measures were computed from the final output of the tracking methods. The sec performance of particle detection (including localization) techniques has been evaluated comprehensively in various earlier studies (Carter et al., 2005; Cheezum et al., 2001; Ruusuvuori et al., 2010; Smal et al., 2010b). However, to the best of our knowledge, the sec performance of commonly used linking techniques for particle tracking applications has not yet been evaluated in the literature.

The goal of this paper is to complement previous studies by presenting a quantitative comparison of data association techniques for the linking step in particle tracking. The contributions of our study are fourfold. First, in contrast with the mentioned particle tracking challenge, we completely separate the two steps of the tracking problem and evaluate all considered data association techniques based on the same set of detections, thus providing a direct comparison. Second, we go one step further and evaluate the techniques based solely on positional information. Although it is conceptually possible for most data association techniques to incorporate information about, for example, object size, shape, and intensity, in many particle tracking applications this information is highly ambiguous, since all particles appear as diffraction-limited spots with high levels of noise. Therefore, we evaluate the degree to which the techniques can reconstruct true trajectories reliably when only minimal information (sampled coordinates) is available, thus providing a lower bound on performance. Although minimal, information about position (and velocity) seems more essential for object tracking than form and appearance, at least in human vision (Dawson, 1991). However, if reliable and discriminative information about object appearance is available from the detection stage, the ranking of the resulting trackers may change depending on how the linking techniques exploit that information. Third, we evaluate the robustness of the techniques in a controlled fashion, by varying the levels of false-negative (missing) and false-positive (spurious) detections. In practice, due to ambiguity in the data and/or the use of nonoptimal detection techniques or parameter settings, detections are rarely perfect, and it is important to know how the different linking techniques perform relative to one another given the same set of imperfect detections. And fourth, we focus on linking techniques that are able to exploit multiple frames in solving the correspondence problem at each time point, and we study their strengths and weaknesses in dealing with missing and spurious detections compared to simpler techniques that use only two consecutive frames at a time.