



Habilitation Thesis Reviewer's Report

Masaryk University	
Faculty	Faculty of Informatics
Procedure field	Informatics
Applicant	RNDr. Martin Maška, Ph.D.
Applicant's home unit, institution	Faculty informatics, Masaryk University
Habilitation thesis	Object Tracking in Bioimage Data
Reviewer	Prof Janne Heikkilä
Reviewer's home unit, institution	CMVS and Faculty of Information Technology and Electrical Engineering, University of Oulu, Finland

This report is based on a manuscript submitted for review on August 31, 2020. It is an article thesis that consists of a separate commentary part of 26 pages including the references, followed by 12 research papers. From those, 9 papers have been published in international journals with an impact factor and 3 papers in international peer-reviewed conferences.

1. Research problem

Dr. Maška's habilitation thesis addresses the problem of object tracking from time-lapse microscopy images. While image-based object tracking is a research problem that has been widely studied within the computer vision community, this work focuses on biological domain, where the objects of interest have usually a blob-like structure that makes the problem more constrained. These objects can be living cells or other types of microscopic particles exhibiting motion patterns that reveal some important insight to the scientists. Object tracking aims to determine the location of individual objects along with the time, and it is often a prerequisite for motion analysis. It can be divided into two subproblems: detection and linking. Detection involves localizing the objects from the image while linking associates them into spatiotemporal tracks. Both of these steps are prone to errors that can accumulate over time, which makes objective comparison of tracking methods challenging. This is a major issue since development of new tracking algorithms requires some standard ways of assessing their performance, and due to the special characteristics of biological images it may not be feasible to employ the metrics that have been used in other fields of application. Thus, developing evaluation protocols that are widely accepted within the bioimaging community is of high importance. This is the main research problem studied in the thesis. Besides algorithm evaluation, the thesis also contributed to development of new cell and filopodium tracking methods both from 2D and 3D time-lapse series.

2. Review of contributions

The thesis groups the contributions into three categories: particle tracking, cell tracking, and filopodium tracking. In the next discussion, the same grouping is used.

2.1. Particle tracking

Dr. Maška has been one of the organizers of the ISBI Particle Tracking Challenge 2012 (PTC) in collaboration with researchers from two other universities. The challenge provided a benchmark for comparison of different particle tracking algorithms. This was implemented by defining common performance evaluation criteria and using completely annotated sets of computer-generated image sequences in different scenarios. This challenge can be seen as an important step towards open science and reproducible research in a field where proprietary datasets and methods have been extensively used in prior research works. The results of the challenge were published in *Nature Methods* that is the highest-ranked biochemical research methods journal based on 2019 ISI impact factor (30.822). This clearly demonstrates the significance of the contribution. From among 35 co-authors listed in the paper, Dr. Maška is the fourth author being responsible for preparation of the data and evaluation of the results. Inspired by the analysis of the PTC evaluation results, Maška and Matula also proposed a new evaluation protocol based on Linear Oriented Forests Matching (LOFM) measure published in *ICCV 2017 Workshop*. It is claimed that the new protocol can assess the detection and linking performance in an isolated and unbiased manner. Using the new protocol instead of the original PTC protocol leads to significant changes in the ranking order of the methods. However, because there is obviously no single right way of assessing the tracking performance, it is still an open question if this is the optimal or the fairest way to perform the evaluation.

2.2. Cell tracking

Dr. Maška has also contributed to organizing Cell Tracking Challenge (CTC) that appears to be the follow-up of PTC. It has been held five times in 2013-2020 as an open competition in conjunction with the ISBI conference offering an opportunity for researchers to benchmark their cell tracking methods based on annotated bioimage data consisting of real and synthetic time-lapse image sequences of various modalities. The results of these challenges have been presented in two journal papers. The first one was published in *Bioinformatics* (IF 5.610), and it described the findings of the 2013 challenge. The second one summarized the results of the 2013, 2014 and 2015 challenges, and it was published in *Nature Methods*. In those papers, Dr. Maška has been the first and the second author, respectively, reflecting his central role in organizing the challenges. Besides the organizing responsibilities, he has contributed to development of a new Acyclic Oriented Graphs Matching (AOGM) measure for assessing the performance of cell tracking methods that seems to be an extension of the LOFM measure to take into account different cellular events. The AOGM measure was published in *PLoS ONE* journal (IF 2.740) along with the corresponding results of the methods participated in the first CTC. Interestingly, this paper appeared already in 2015 that is two years before the paper presenting the LOFM measure.

The thesis includes also two other journal papers from the cell tracking area. The first one proposes a cell tracking method for multidimensional fluorescence microscopy images. The cell boundaries are detected by minimizing the Chan-Vese model in the fast level set-like (FLS) and graph cut (GC) frameworks. The paper where Dr. Maška is the first author was published in *IEEE Transactions on Medical Imaging* (IF 9.710). The FLS variant of the method has been occupying the top position in the CTC segmentation benchmark for one of the datasets since 2014, which is quite impressive. Moreover, the GC variant of the method was used in the second paper published in *PLoS ONE* to characterize cancer cell migration in mixed collagen-Matrigel scaffolds using a microfluidic device, which nicely demonstrates the applicability of the method in a real biological study.

2.3. Filopodium tracking

Besides cell tracking, Dr. Maška has focused on tracking and analysis of filopodial protrusions from 3D time-lapse fluorescence microscopy data. This work that started already in 2012 has resulted in many publications, and five of those have been included in the thesis. The first one proposes an automatic approach for quantifying the filopodia using some relatively simple image processing operations. Dr. Maška is the first author of this paper that has been published in *ISBI 2013*. A more advance method for filopodium tracking and analysis was presented in a recent paper from 2019 published in *IEEE Transactions on Medical Imaging*, where he is the second author. It uses two convolutional neural networks (CNN), one for segmenting the cell volumes, and another for detecting the individual filopodial protrusions. Interestingly, the paper still relies on simple nearest-neighbor particle tracker for temporal linking of the filopodial tip locations.

One of the main results in Dr. Maška's research work has been obviously the development of image synthesis pipelines for creating realistic microscopy data with ground-truth annotations. Such data is highly important when evaluating particle and cell tracking algorithms, but it can also provide a practical way of training machine learning algorithms, and particularly, deep neural networks that require a huge amount of annotated data. For this purpose, FiloGen, which is a model-based generator capable of synthesizing 3D time-lapse sequences of single motile cells with filopodial protrusions, is an ideal tool. The data synthesis method has been described in detail in another *IEEE Transactions on Medical Imaging* paper from 2018, where Dr. Maška is the last author suggesting that he has been the main supervisor of the work.

The fourth paper, published in *Bioinformatics*, presents a work co-supervised by Dr. Maška that is a web-interface for generating synthetic benchmark datasets, called CytoPacq. It combines different simulation modules, including FiloGen, and makes them easy to use with a graphical interface that allows configuring the necessary parameters and downloading the data. Maintaining such a free online simulation tool that also requires computational resources is certainly a notable service to the research community. The last paper has been published in *ICIP 2019* conference. It presents a new benchmark dataset called FiloData3D for performance evaluation of 3D filopodium segmentation and tracking algorithms generated with FiloGen. The paper, where Dr. Maška is the first author, shows that quantification using 2D protocols can lead to underestimated filopodial properties suggesting that 3D analysis pipelines are needed but does not propose any practical guidelines how to implement them.

3. Questions

- Both in Particle Tracking Challenge and in Cell Tracking Challenge the evaluation is based on objective criteria computed by comparing the output of the algorithm with the ground-truth. However, there is no single right way of assessing the performance, and different aspects can be weighted in the result. Has there been any studies from the biological point of view, what properties are important when evaluating the algorithm performance?
- Various algorithms have been developed for linking the detected cells when using the tracking by detection approach. In your previous work, (IEEE-TMI 2019) you use a nearest-neighbor particle tracker for this purpose. Please elaborate the reasons why this approach was chosen instead of more advanced ones.

- Machine learning, and especially deep learning, has enabled huge progress in the computer vision field. What are its prospects in cell tracking?

4. Conclusions

The thesis presents several new contributions to performance evaluation as well as development of object tracking methods. It also clearly demonstrates the author's ability to conduct independent scientific research. Thus, it is my pleasure to conclude that the habilitation thesis entitled "Object Tracking in Bioimage Data" by Martin Maška **fulfils** the requirements expected of a habilitation thesis in the field of Informatics.

Oulu, 21.10.2020

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