

Imperial College London
Department of Computing

Automatic Cell Tracking in Noisy Images for Microscopic Image Analysis

by

Pedro Damian Kostelec

September 2014

Supervised by Ben Glocker

Submitted in part fulfilment of the requirements for the
MSc degree in Computer Science (Artificial Intelligence) of Imperial College London

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7 Conclusions and future work DRAFT I

add something

7.1 Conclusion DRAFT I

Improved microscopy imaging techniques allow us to gather large amounts of cell microscopy images. The manual analysis of these images would be an error prone and slow process, requiring days of manual work to review some hundreds of frames. The advances of computer vision algorithms for cell detection and tracking over the past decades magnified by the increased computational power of modern computers allow for efficient analysis of these datasets in the fraction of the time.

The increased throughput of these new methods improve the quality of cell research. They allow for new insights in drug development and understanding of the living body. Specifically, this research was focused on enabling the efficient analysis of neutrophils which have a crucial role in the clearance of infections. Their careful analysis could help explain their prominent presence in certain organs, such as the lung. It could also help discover any additional activities that these leukocytes perform, and clarify whether they develop from a single or several neutrophil predecessors.

In this three months project we have developed a pipeline of algorithms that enables the effective analysis of neutrophil behaviour in sometimes noisy images of varying contrast. This required identifying a robust algorithm to detect cells in these images, and develop a tracking method that would perform well with imperfect cell segmentation and a certain amount of missed detections.

We have upgraded a cell detector developed by Arteta et al [1]. The detector was able to learn to discriminate candidate cell regions as cell or no-cell. We have upgraded the method to recognize a better subset of features and significantly improved its speed to make it usable for detecting cells in hundreds of frames. The method was able to robustly detect cell detections (albeit with some false positive and false negatives) after being trained with a small number of dot-annotated images.

We have developed a tracking method inspired by Bise et al [20] that performs a global decision to join short robust tracklets into longer ones. We have modified the original method to heavily rely on the input data, thus eliminating the need for a large number of heuristics that would make the algorithm likely to perform worse when presented with a new dataset. The new approach only requires the algorithm to be retrained using a small number annotated trajectories. Although the method is automatic, the user is presented with four parameters that can be adjusted to improve the generated trajectories. The tracking module is able to generate tracklets of quality comparable

to that of a human.

As a side effect, we have developed efficient image annotation tools to annotate images with dots and links connecting them. These tools can be used for the annotation of any point-like objects and include features specifically designed to increase the clarity of noisy and low contrast images to facilitate the annotation.

I need one concluding paragraph

Rewrite: Maybe I am too optimistic? I need to present some of the drawbacks... I am sure I will get them when I do the experiments...

7.2 Future work DRAFT I

Organize: first detector, then tracker

The work developed in this thesis is promising in delivering autonomous cell detection and tracking, however the method can be further improved, and alternative methods researched. Below we present a list of possible improvements that would likely make the algorithm more robust.

The process of obtaining cell images *in vivo* is challenging especially in moving organs like the lung, where the motion of the tissue causes the images to jiggle or loose focus. The jiggling can be eliminated using a pre-processing step that would stabilize the images using the information hidden in the background of the images, such blood vessels. This would reduce the jiggling of cells, and would result in smoother trajectories. Furthermore, this would simplify the computations of spatio-temporal features to train the cell tracker module, as it would be easier to predict the velocity of the cells.

Second, it would be worth experimenting with some preprocessing steps to improve the clarity of the images. This includes de-noising, improving the contrast, etc. This could improve the accuracy of the cell detection module.

From the datasets we analysed, the original images for dataset D (described in section 5.1.1) include a large portion that are completely unusable for the tracker, because the cells were fully out of focus or invisible. These frames have been manually removed. It could be beneficial to automate this process by automatically detecting images that are of too low quality to be usable and automatically discarding them, while leaving a mark with the number of frames skipped. If this step could be performed quickly the total computation time would be reduced as they wouldn't need to be analysed by the cell detector.

The accuracy of the tracking module is heavily dependent on the quality of features that are computed for the tracklets. I would already be a major improvement if a Kalman filter were used to predict the future positions of the trajectories, instead of assuming that the tracklet's direction and speed would be linear with respect to the last few frames of a trajectory. To further improve

the prediction an interactive multiple models filter has been shown to better prediction future cells positions

Cite: .

The developed tracking method has been tested on hundreds of frames of microscopy images. However, the method is likely to reach a limit that would require more *Random Access Memory*. In order to improve the space requirements of the of the tracker and permit the tracking of thousands of image frames it would be beneficial to make the processing of tracklets in windows of a few hundred frames at a time. Thus the tracker would first generate tracklets in each window, and then link these tracklets between windows.

One of the main drawbacks of this method is the difficulty to use for an untrained user, because it requires changing a configuration file to load new datasets. A simple graphic user interface to load the image sequences and start the tracking process would greatly improve the approachability of the methods to a larger non-technical audience.

Finally, while the above were improvements to the software, it is expected that the imaging technique will improve as well. This could alleviate the problem of jiggling cells and out of focus images, thus reducing the need to overcome these hardware limitations in the software.

Appendices

Bibliography

- [1] C. Arteta, V. Lempitsky, J. A. Noble, and A. Zisserman, “Learning to detect cells using non-extremal regions,” in *Proceedings of the 15th International Conference on Medical Image Computing and Computer-Assisted Intervention - Volume Part I*, MICCAI’12, (Berlin, Heidelberg), pp. 348–356, Springer-Verlag, 2012. 8, 10, 15, 16, 17, 18, 19, 41
- [2] Y. Chen, K. Biddell, A. Sun, P. Relue, and J. Johnson, “An automatic cell counting method for optical images,” in *[Engineering in Medicine and Biology, 1999. 21st Annual Conference and the 1999 Annual Fall Meeting of the Biomedical Engineering Society] BMES/EMBS Conference, 1999. Proceedings of the First Joint*, vol. 2, pp. 819 vol.2–, Oct 1999. 9
- [3] X. Chen, X. Zhou, and S.-C. Wong, “Automated segmentation, classification, and tracking of cancer cell nuclei in time-lapse microscopy,” *Biomedical Engineering, IEEE Transactions on*, vol. 53, pp. 762–766, April 2006. 9, 12
- [4] L. Vincent, “Morphological grayscale reconstruction in image analysis: applications and efficient algorithms,” *Image Processing, IEEE Transactions on*, vol. 2, pp. 176–201, Apr 1993. 9
- [5] J. Serra, *Image Analysis and Mathematical Morphology*. Orlando, FL, USA: Academic Press, Inc., 1983. 9
- [6] D. Mukherjee, N. Ray, and S. Acton, “Level set analysis for leukocyte detection and tracking,” *Image Processing, IEEE Transactions on*, vol. 13, pp. 562–572, April 2004. 9, 11
- [7] C. Tang, Y. Wang, and Y. Cui, “Tracking of active cells based on kalman filter in time lapse of image sequences of neuron stem cells.” 10, 12
- [8] D. Xu and L. Ma., “Segmentation of image sequences of neuron stem cells based on level-set algorithm combined with local gray threshold.” Master’s thesis, Harbin Engineering University, 2010. 10
- [9] C. Arteta, V. S. Lempitsky, J. A. Noble, and A. Zisserman, “Learning to detect partially overlapping instances,” in *CVPR*, pp. 3230–3237, IEEE, 2013. 10, 17
- [10] J. Matas, O. Chum, M. Urban, and T. Pajdla, “Robust wide baseline stereo from maximally stable extremal regions,” in *Proceedings of the British Machine Vision Conference*, pp. 36.1–36.10, BMVA Press, 2002. doi:10.5244/C.16.36. 10
- [11] T. Joachims, T. Finley, and C.-N. J. Yu, “Cutting-plane training of structural svms,” *Mach.*

- Learn.*, vol. 77, pp. 27–59, Oct. 2009. 10
- [12] R. Bise, T. Kanade, Z. Yin, and S. il Huh, “Automatic cell tracking applied to analysis of cell migration in wound healing assay,” in *Engineering in Medicine and Biology Society, EMBC, 2011 Annual International Conference of the IEEE*, pp. 6174–6179, Aug 2011. 10, 22
 - [13] S. Huh, *Toward an Automated System for the Analysis of Cell Behavior: Cellular Event Detection and Cell Tracking in Time-lapse Live Cell Microscopy*. PhD thesis, Robotics Institute, Carnegie Mellon University, Pittsburgh, PA, March 2013. 10, 12
 - [14] D. House, M. Walker, Z. Wu, J. Wong, and M. Betke, “Tracking of cell populations to understand their spatio-temporal behavior in response to physical stimuli,” in *Computer Vision and Pattern Recognition Workshops, 2009. CVPR Workshops 2009. IEEE Computer Society Conference on*, pp. 186–193, June 2009. 12
 - [15] B. Xu, M. Lu, P. Zhu, Q. Chen, and X. Wang, “Multiple cell tracking using ant estimator,” in *Control, Automation and Information Sciences (ICCAIS), 2012 International Conference on*, pp. 13–17, Nov 2012. 12
 - [16] K. Li and T. Kanade, “Cell population tracking and lineage construction using multiple-model dynamics filters and spatiotemporal optimization,” in *Proceedings of the 2nd International Workshop on Microscopic Image Analysis with Applications in Biology (MIAAB)*, September 2007. 12
 - [17] A. Massoudi, D. Semenovich, and A. Sowmya, “Cell tracking and mitosis detection using splitting flow networks in phase-contrast imaging,” in *Engineering in Medicine and Biology Society (EMBC), 2012 Annual International Conference of the IEEE*, pp. 5310–5313, Aug 2012. 13
 - [18] L. Zhang, Y. Li, and R. Nevatia, “Global data association for multi-object tracking using network flows,” in *Computer Vision and Pattern Recognition, 2008. CVPR 2008. IEEE Conference on*, pp. 1–8, June 2008. 13, 23
 - [19] C. Huang, B. Wu, and R. Nevatia, “Robust object tracking by hierarchical association of detection responses,” in *Computer Vision - ECCV 2008* (D. Forsyth, P. Torr, and A. Zisserman, eds.), vol. 5303 of *Lecture Notes in Computer Science*, pp. 788–801, Springer Berlin Heidelberg, 2008. 13, 23
 - [20] R. Bise, Z. Yin, and T. Kanade, “Reliable cell tracking by global data association.,” in *ISBI*, pp. 1004–1010, IEEE, 2011. 13, 14, 23, 26, 41
 - [21] H. Kuhn, “The hungarian method for the assignment problem,” *Naval Research Logistics Quarterly*, vol. 2, pp. 83–97, 1955. 13
 - [22] J. Matas, O. Chum, M. Urban, and T. Pajdla, “Robust wide-baseline stereo from maximally stable extremal regions,” *Image and Vision Computing*, vol. 22, no. 10, pp. 761 – 767, 2004.

British Machine Vision Computing 2002. 15, 16

- [23] I. Tsochantaridis, T. Hofmann, T. Joachims, and Y. Altun, “Support vector machine learning for interdependent and structured output spaces,” in *Proceedings of the Twenty-first International Conference on Machine Learning*, ICML '04, (New York, NY, USA), pp. 104–, ACM, 2004. 17
- [24] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, and A. Cardona, “Fiji: an open-source platform for biological-image analysis,” *Nature Methods*, vol. 9(7), pp. 676–682, 2012.
- [25] S. F. I. o. T. L. Philippe Thévenaz, Biomedical Imaging Group, “Point picker: An interactive imagej plugin that allows storage and retrieval of a collection of landmarks,” May 2014.