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

Contents

1	Intr	roduction DRAFT I	7
	1.1	Motivation DRAFT I	7
	1.2	Objectives DRAFT I	8
	1.3	Contributions DRAFT I	8
	1.4	Report structure DRAFT I	9
2	\mathbf{Rel}	ated work DRAFT I	10
	2.1	Cell detection DRAFT I	10
		2.1.1 Cell segmentation using the Watershed technique DRAFT I	10
		2.1.2 Cell segmentation using level sets DRAFT I	11
		2.1.3 Cell detection by model learning DRAFT I	11
		2.1.4 Cell detection by image restoration DRAFT I	12
	2.2	Cell tracking DRAFT I	12
		2.2.1 Tracking by model evolution DRAFT I	13
		2.2.2 Tracking by frame-by-frame data association DRAFT I	13
		2.2.3 Tracking with a dynamics filter DRAFT I	14
		2.2.4 Cell tracking by global data association DRAFT I	14
	2.3	Conclusion DRAFT I	15
3	Det	section of cells DRAFT I	17
3	Det 3.1	$egin{array}{cccc} egin{array}{cccc} egin{array}{cccc} egin{array}{ccccc} egin{array}{cccccc} egin{array}{cccccc} egin{array}{cccccc} egin{array}{cccccc} egin{array}{cccccc} egin{array}{ccccccccc} egin{array}{ccccccccc} egin{array}{ccccccccc} egin{array}{ccccccccc} egin{array}{ccccccccc} egin{array}{cccccccccc} egin{array}{cccccccccc} egin{array}{ccccccccccc} egin{array}{cccccccccccccccccccccccccccccccccccc$	17 17
3			
3	3.1	Method overview DRAFT I	17
3	3.1 3.2	Method overview DRAFT I	17 18
3	3.1 3.2 3.3	Method overview DRAFT I	17 18 19
3	3.1 3.2 3.3 3.4	Method overview DRAFT I	17 18 19 20
3 4	3.1 3.2 3.3 3.4 3.5 3.6	Method overview DRAFT I	17 18 19 20 20
	3.1 3.2 3.3 3.4 3.5 3.6	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I	17 18 19 20 20 21
	3.1 3.2 3.3 3.4 3.5 3.6	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I	177 188 199 200 200 211
	3.1 3.2 3.3 3.4 3.5 3.6 Tra 4.1	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I Method overview DRAFT I	177 188 199 200 210 244 244
	3.1 3.2 3.3 3.4 3.5 3.6 Tra 4.1 4.2	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I Method overview DRAFT I Joining cell detections into robust tracklets DRAFT I	177 188 199 200 200 211 244 246
	3.1 3.2 3.3 3.4 3.5 3.6 Tra 4.1 4.2 4.3	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I Method overview DRAFT I Joining cell detections into robust tracklets DRAFT I Global data association DRAFT I	177 188 199 200 211 244 246 277
	3.1 3.2 3.3 3.4 3.5 3.6 Tra 4.1 4.2 4.3 4.4	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I Method overview DRAFT I Joining cell detections into robust tracklets DRAFT I Global data association DRAFT I Implementation using linear programming DRAFT I	177 188 199 200 210 244 244 266 277 299
	3.1 3.2 3.3 3.4 3.5 3.6 Tra 4.1 4.2 4.3 4.4	Method overview DRAFT I Detection of candidate regions DRAFT I. Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I Method overview DRAFT I Joining cell detections into robust tracklets DRAFT I Global data association DRAFT I Implementation using linear programming DRAFT I Hypotheses likelihood definitions DRAFT I	177 188 199 200 210 244 244 266 277 299 300
	3.1 3.2 3.3 3.4 3.5 3.6 Tra 4.1 4.2 4.3 4.4 4.5	Method overview DRAFT I Detection of candidate regions DRAFT I Inference under the non-overlap constraint DRAFT I Learning the classifier DRAFT I Feature selection DRAFT I Performance improvements DRAFT I cking of cells DRAFT I Method overview DRAFT I Joining cell detections into robust tracklets DRAFT I Global data association DRAFT I Implementation using linear programming DRAFT I Hypotheses likelihood definitions DRAFT I Computing the likelihoods DRAFT I	177 188 199 200 211 244 246 277 299 300 311

		4.7.3 Best feature selection NEW	35								
	4.8	Implementation details NEW	35								
5	Data acquisition and annotation DRAFT I										
	5.1	Data acquisition and example datasets DRAFT II	36								
		5.1.1 Datasets DRAFT II	37								
		5.1.2 Image analysis challenges DRAFT II	40								
		5.1.3 Manual data annotation DRAFT I	42								
	5.2	The annotation tool DRAFT I	42								
6	Exp	perimental results IN PROGRESS	45								
	6.1	Cell detector IN PROGRESS	45								
		6.1.1 Performance metrics NEW	46								
		6.1.2 Detection accuracy NEW	46								
		6.1.3 Computations time NEW	49								
	6.2	Cell tracker NEW	49								
		6.2.1 Performance metrics NEW	49								
		6.2.2 Tracking accuracy NEW	50								
		6.2.3 Computation time NEW	50								
	6.3	Limitations and areas of improvement NEW	50								
	6.4	Summary NEW	50								
7	Con	nclusions and future work DRAFT I	49								
	7.1	Conclusion DRAFT I	49								
	7.2	Future work DRAFT I	50								
Aı	Appendices 52										
\mathbf{A}	Use	er Guide for the Annotation Tool	53								
В	B User Guide for the Interactive Annotation Viewer 5										
\mathbf{C}	C Cell detection results 55										
Bi	bliog	graphy	59								

6 Experimental results IN PROGRESS

In this chapter we quantitatively and qualitatively analyse the performance of the automatic cell detector and tracker. Although some evaluation of the performance of the detection method is performed by the original authors in [4] it is useful to see how the method performs on the studied datasets in order to understand how much of the tracking accuracy is lost due to cells missed by the detection module. First, in section 6.1 we evaluate the performance and computation time of the cell detector and in section 6.2 those of the cell tracker. Finally, in section 6.3, we explore the limitations of the methods and in section 6.4 summarize the results.

See the cell population tracking and linear construction with spation temporal ocntet by Kang et al for a good results section

6.1 Cell detector IN PROGRESS

In this section we evaluated the performance of the automatic cell detection module. First, we introduce the performance metrics used to evaluate the accuracy of the cell detector. Then we present detection accuracy results. To evaluate the accuracy and generalizability of the detection module we perform two sets of experiments. First, we train the cell detector on a number of frames from each individual dataset, and measure the accuracy on the same dataset. Second, we train the detector on combinations of datasets in order to judge the performance degradation due to the learning on the more types of cells. Because of the varying size of the cells in the datasets, and the varying brightness of the cells, we expect that such a trained detector will perform poorer than when trained and tested on individual datasets, sometimes mistakenly detecting small artefacts in the background as cells. Finally, we compute the average detection time per frame for each dataset.

The aim of this research was to develop an automatic cell detection and tracking pipeline that would require as little manual work as possible. This implies that a balance between accuracy and amount of manual work had to be established. There is also an direct relationship between accuracy and computation time. In order to reduce the amount of manual work we aimed to configure the cell detection module such that it would perform well on all the tested datasets without any manual adjustments of parameters. The consequences of this decision are twofold:

1. The features computed on the candidate cell regions are the same for all datasets and have been presented in section 3.5. Although some datasets could be analysed faster or more accurately with a different subset of features, using the same features for all dataset eliminates the complicated feature selection process for the user and makes the system generalizable to a

large number of different cell types.

2. The parameters of the MSER detector should be adequately set to perform well on all datasets. This means that the MSER detector should be able to detect cells of varying size and contrast in the different datasets. The consequence of this limitation for datasets with large cells and some background noise is that a potentially much larger number of candidate regions will be detected than necessary. Since each candidate region has to be evaluated this results in an increased computation time.

We were able to identify features that compute in an acceptable time for all these datasets (see section 3.5). However, it should be noted that in the case of testing the detector on very large datasets with thousands of frames, some adjustments of the parameters could result in a significant reduction in computation time and increased accuracy.

6.1.1 Performance metrics NEW

We measure the performance of the cell detector in terms of precision and recall. The metrics are defined in terms of:

True Positive instances (TP) are candidate cell regions that are manually annotated as cells and the detector successfully classified as cells.

False Positive instances (FP) are candidate cell regions that are not manually annotated as cells, but the detector incorrectly classified them as cells.

False Negative instances (FN) are candidate cell regions that are manually annotated as cells, but the detector incorrectly classified.

We then define precision as:

$$\mathrm{PRE} = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FP}},$$

and recall (also known as sensitivity) as:

$$REC = \frac{TP}{TP + FN} \ .$$

6.1.2 Detection accuracy NEW

As mentioned previously, we performed two different experiments to measure the performance of the cell detection module. The first experiment consisted of training and testing of the algorithm on the same dataset. The training set was 70% and the testing set 30% of the entire dataset. The training

and testing datasets were created from images in randomized order from the entire dataset. This allowed us to measure, for each dataset, the maximum precision and recall values we could expect from the algorithm. In the second experiment the training was performed on combined datasets. The goal of this experiment was to observe how well the algorithm is able to generalize, and still return acceptable results.

Training and testing on single dataset

The training was performed on 70% of the manually dot-annotated images from each dataset, and tested on the remaining 30%. Table 6.1 displays the computed precision and recall values, together with the total number of cells that were annotated in each dataset.

Repeat the measurements with the best feature selection

Dataset	Precision	Recall
A	25.0	22.5
В	90.1	89.1
\mathbf{C}	76.5	84.2
D	86.1	85.3
E	93.4	78.6

Table 6.1: Precision and recall values for the cell detector trained on each dataset individually.

The detector tested on datasets B to E achieved precision and recall values above 75. A manual comparison of the annotation confirms that the results are good, with few bad detections. Most of the differences in detection were caused by minor inconsistencies in annotations. The importance of consistent annotations was stressed in section 5.1.3.

Dataset A is an outlier with extremely low precision and recall values. This is likely caused by the specific characteristics of this image sequence, which we described in section 5.1.1. Briefly, between frame 17 and 18 there is an abrupt change of image clarity. The background from the threshold frame onwards have a texture that is very similar to that of the cells in the first 17 frames. This means that the negative candidate regions from frame 18 and onward clash with the positive candidate regions from the previous frames. The result is that the detector is unable to learn to discriminate cells from background.

These results show us the detector can correctly detect most of the (annotated) cells. Further manual reviews of the annotations would likely improve the performance, but this is not done here as it should have been tested on a separate validation dataset. Additionally, it is unlikely that future users of this detection method will always have perfect annotations available. In the next experiment, we will measured the performance of the detector when trained on a composite dataset. This will tell us whether it would be possible to learn a single, general detector and use it to test on a new, possibly unforeseen datasets.

Figures C.1 to C.5 in appendix C display a temporal view of the detected results in each datasets. The vertical axis represents the consecutive frame number of the image sequence. The figures show that "cell tracks" are discernible, even if the number of outliers is significant. The detectors used to detect the cells on the entire dataset were trained on all the annotated frames.

Repeat the training+testing using all frames for training

Training on composite dataset

In this second experiment we were interested in measuring how well the algorithm is able to generalize when it is trained on a larger, combined dataset. For this purpose, several of the datasets were grouped, and the detector was trained to recognize cells of all types. The detector was trained on a random 70% of all annotated images in the combined dataset. It was then tested on a random 30% of annotated frames from each individual dataset separately. This means that sometimes the same frames could be used for training and testing. However, we also run combinations of datasets where one dataset is left out of training at each time. Testing on that dataset should then reveal if the algorithm generalizes well.

Table 6.2 summarizes the precision and recall values for all tested combined datasets. The column denoted γ contains testing performance as tested on 30% of the combined dataset. The values shown in bold correspond to the performance of testing on the dataset that was left out from the combined training dataset.

Repeat the measurements with the best feature selection

Try different dataset combinations based on the observations... similar datasets

Try testing the model on an unforseen dataset

each time train on all others, but 1, test it on that one.

Combined dataset	Precision			Recall								
	γ	A	В	\mathbf{C}	D	E	γ	A	В	\mathbf{C}	D	E
A+B+C+D+E	74.5	49.2	76.0	85.2	69.2	94.5	79.9	58.3	90.8	57.1	98.9	79.7
$A{+}B{+}C{+}D$												
$A{+}B{+}C{+}E$												
$A{+}B{+}D{+}E$												
$A {+} C {+} D {+} E$												
B + C + D + E												

Table 6.2: Precision and recall values for the cell detector trained on combined datasets.

Add discussion: generally how much precision/recall we loose, which dataset seems to suffer less/more, why do they suffer less/more (have fewer cells to be trained on, like like noise in another dataset)

6.1.3 Computations time NEW

In order to positionally track cells in image sequence the computation time of extracting the cell position from each frame is just as important as the accurate identification of cells. Table 6.3 displays the average detection times per frame for each dataset. The detection time is dependent on the number of candidate cell regions extracted and their dimensions.

Explain hardware and software, say 1 core... can use more

It might be useful to show the comparison with the old detector if I have the time

Measure the speed of detection in images of different sizes, and different number of cells

Explain what the candidate-annoation ration is, and what it tells. Also the ocmputations timer per candidate vary from 0.0086-0.0226

Dataset	Time per frame $[s]$	Time per annotated cell $[s]$	Candidate-Annotation ratio
A	0.9224	0.5854	26:1
В	1.5899	0.2015	16:1
\mathbf{C}	1.1214	0.0586	7:1
D	1.5215	0.1454	18:1
E	0.6016	0.0853	9:1

Table 6.3: Average computation times per frame and annotated cell using a single worker.

Discuss which is faster, try to explain why-less noise - less candidate cells...

6.2 Cell tracker NEW

Define the different measures of accuracy

6.2.1 Performance metrics NEW

TODO: test detector on full dataset, but train it on ALL (notjust70%) of the annotation TODO: train tracker on 70% of the trajectories, test on rest

great Metrics: Research Article, Evaluating Multiple Object Tracking Performance: The CLEAR

MOT Metrics

- Explain how the testing data was generation. from annotation to mapped detection to genreated tracklets

6.2.2 Tracking accuracy NEW

- trained on single dataset
- for each dataset explain how the parameters were setup
- trained on combined dataset

6.2.3 Computation time NEW

Meause the speed of generating tracks, as a measure of per 1, 100, 1000 frames, depending on the number of tracks

6.3 Limitations and areas of improvement NEW

Answer: what, why, how to improve in future

- display examples where the tracker did not perform well, and anlyse why. Suggest possible improvement. - detection training: only first few frames of datasets, not random – expect to detect later frames worse - testing on only long datasets: no data on short datasets. diffucult to train (what to link?), difficult to annotate - speed of detector. Reduce number of hypothesis

6.4 Summary NEW

Brief review of accuracy... whether it is comparable to other methods in literuature review Whether is could be improved in the future... how much

Appendices

Bibliography

- [1] P. K. Elzbieta Kolaczkowska, "Neutrophil recruitment and function in health and inflammation," 2013. 7
- [2] J. Pillay, I. den Braber, N. Vrisekoop, L. M. Kwast, R. J. de Boer, J. A. M. Borghans, K. Tesselaar, and L. Koenderman, "In vivo labeling with 2h2o reveals a human neutrophil lifespan of 5.4 days," *Blood*, vol. 116, no. 4, pp. 625–627, 2010.
- [3] P. S. Tofts, T. Chevassut, M. Cutajar, N. G. Dowell, and A. M. Peters, "Doubts concerning the recently reported human neutrophil lifespan of 5.4 days," *Blood*, vol. 117, no. 22, pp. 6050–6052, 2011. 7
- [4] 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 Im*age Computing and Computer-Assisted Intervention - Volume Part I, MICCAI'12, (Berlin, Heidelberg), pp. 348–356, Springer-Verlag, 2012. 8, 9, 11, 17, 18, 19, 20, 21, 22, 45, 49
- [5] 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 Meetring of the Biomedical Engineering Society] BMES/EMBS Conference, 1999. Proceedings of the First Joint, vol. 2, pp. 819 vol.2—, Oct 1999. 10
- [6] 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. 10, 13
- [7] L. Vincent, "Morphological grayscale reconstruction in image analysis: applications and efficient algorithms," *Image Processing, IEEE Transactions on*, vol. 2, pp. 176–201, Apr 1993. 10
- [8] J. Serra, Image Analysis and Mathematical Morphology. Orlando, FL, USA: Academic Press, Inc., 1983. 10
- [9] 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. 11, 13
- [10] 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." 11, 14
- [11] D. Xu and L. Ma., "Segmentation of image sequences of neuron stem cells based on level-set

Bibliography

algorithm combined with local gray threshold.," Master's thesis, Harbin Engineering University, 2010. 11

- [12] C. Arteta, V. S. Lempitsky, J. A. Noble, and A. Zisserman, "Learning to detect partially overlapping instances.," in *CVPR*, pp. 3230–3237, IEEE, 2013. 11, 12, 19
- [13] 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. 11
- [14] T. Joachims, T. Finley, and C.-N. J. Yu, "Cutting-plane training of structural syms," *Mach. Learn.*, vol. 77, pp. 27–59, Oct. 2009. 11
- [15] 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. 12, 25
- [16] 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. 12, 13
- [17] 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. 13
- [18] 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. 14
- [19] 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. 14
- [20] 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.
- [21] 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. 15, 27
- [22] 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,

Bibliography 61

- eds.), vol. 5303 of *Lecture Notes in Computer Science*, pp. 788–801, Springer Berlin Heidelberg, 2008. 15, 27
- [23] R. Bise, Z. Yin, and T. Kanade, "Reliable cell tracking by global data association.," in ISBI, pp. 1004–1010, IEEE, 2011. 15, 25, 27, 30, 49
- [24] H. Kuhn, "The hungarian method for the assignment problem," Naval Research Logistics Quarterly, vol. 2, pp. 83–97, 1955. 15
- [25] 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. 18
- [26] 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. 20
- [27] K. Li, E. D. Miller, M. Chen, T. Kanade, L. E. Weiss, and P. G. Campbell, "Cell population tracking and lineage construction with spatiotemporal context," *Medical Image Analysis*, vol. 12, no. 5, pp. 546 566, 2008. Special issue on the 10th international conference on medical imaging and computer assisted intervention {MICCAI} 2007. 25, 51
- [28] M. Looney, E. Thornton, D. Sen, W. Lamm, R. Glenny, and M. Krummel, "Stabilized imaging of immune surveillance in the mouse lung.," *Nature Methods*, vol. 8, no. 5, pp. 91–6, 2011-01-01 00:00:00.0. 36