PROCEEDINGS

55. IWK

Internationales Wissenschaftliches Kolloquium
International Scientific Colloquium

13 - 17 September 2010

Crossing Borders within the ABC

Automation,

Biomedical Engineering and

Computer Science

Faculty of
Computer Science and Automation

www.tu-ilmenau.de

TECHNISCHE UNIVERSITÄT
ILMENAU

Impressum
Published by

Publisher:	Rector of the Ilmenau University of Technology Univ.-Prof. Dr. rer. nat. habil. Dr. h. c. Prof. h. c. Peter Scharff
Editor:	Marketing Department (Phone: +49 3677 69-2520) Andrea Schneider (conferences@tu-ilmenau.de) Faculty of Computer Science and Automation (Phone: +49 3677 69-2860) Univ.-Prof. Dr.-Ing. habil. Jens Haueisen
Editorial Deadline:	20. August 2010
Implementation:	Ilmenau University of Technology Felix Böckelmann Philipp Schmidt

USB-Flash-Version.

Publishing House:	Verlag ISLE, Betriebsstätte des ISLE e.V. Werner-von-Siemens-Str. 16 98693 Ilmenau
Production:	CDA Datenträger Albrechts GmbH, 98529 Suhl/Albrechts
Order trough:	Marketing Department (+49 3677 69-2520) Andrea Schneider (conferences@tu-ilmenau.de)

ISBN: 978-3-938843-53-6 (USB-Flash Version)

Online-Version:

Publisher:	Universitätsbibliothek Ilmenau ilmedia Postfach 10 05 65 98684 Ilmenau

Home / Index:
http://www.db-thueringen.de/servlets/DocumentServlet?id=16739

SEGMENTATION OF CYTOLOGICAL STAINED CELL AREAS AND GENERATION OF CELL BOUNDARIES IN COMPLEX SHADED CELL CLUSTERS

Sven Buhl, Burkhard Neumann, Eva Eisenbarth

Institute for Computer Science, Vision and Computational Intelligence,
South Westphalia University of Applied Sciences, Frauenstuhlweg 31, 58644 Iserlohn, Germany
Tel. (+492371) 566-214 Fax (+492371) 566-251
E-Mail: {Buhl, Neumann.B,Eisenbarth}@fh-swf.de

ABSTRACT

In this paper a method is introduced to segment cytological stained cells in microscopic images with the histogram backprojection algorithm (HB). The segmented cell areas are classified in cell clusters and individual cells by a function based on iterative erosion. The cell cores are also segmented with the HB algorithm and the result is modified by image processing functions to get connected areas. The detected cell cores help with the separation of the cells in the clusters. The locations of the waists of the cell clusters are determined by means of so called dominant contour points [5]. At these positions a cell separation makes sense from a biological point of view. The creation of separation lines is carried out by opposing contour points. To find opposing contour points the shortest path between two neighboring cell cores in a cell cluster is calculated by the A*-algorithm [6] and utilizing additional suitable rules.

1. INTRODUCTION

The segmentation of non fluorescent stained cells in microscopic images is a great challenge. At first glance it appears convenient to use fluorescent staining for analyzing the biocompatibility of materials because of the high contrast. But there are several reasons not to use fluorescent staining:

- The fluorescent stain is located only at specific cell areas which is embarrassing for our purpose
- Fluorescent staining is very complex for usual cell laboratories
- Fluoresce staining is bleaching out after several hours, so a permanent cell preparation is impossible, etc.

A lot of scientific papers are dealing with the segmentation and analysis of cells [1][2][3].
In our case the cells under investigation show complex geometries (fig. 1) which is quite different to the cells in most publications.

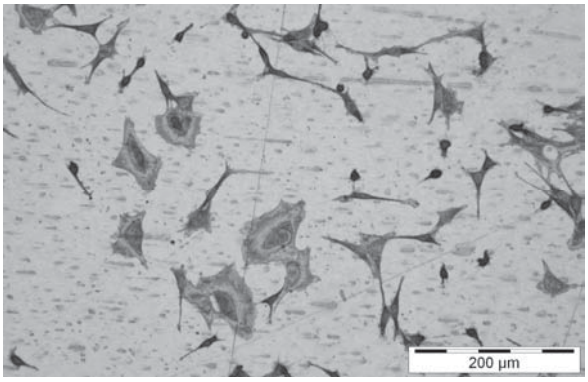

Figure 1: Cytological stained cells on a titanium substrate

2. CELLSEGMENTATION WITH HISTOGRAM BACKPROJECTION

In our case the segmentation of the cell area is done with the histogram backprojection algorithm which is described in [4]. This algorithm uses color templates to find objects in images.
The cells color differs from the color of the substrate and thus the HB algorithm is the method of choice.
Other segmentation algorithms such as watershed transformation or the use of different edge filters are less successful. For example edge filters emphasizes scratches which disturbs the segmentation results.
The creation of the color templates is supported by a specially developed software tool. This software tool supports zoom in and zoom out function and copies preselected areas of the image.

3. DETECTION OF CELL CLUSTERS WITH ITERATIVE EROSION

Before separating the cells in the clusters they have to be distinguished from single cells. The cells we are concerned with have very variable shapes, so morphological features e.g. cell area or compactness cannot be used to distinguish between single cells and cell clusters. As turned out during our investigation the iterative erosion function of the cell areas is an

effective method for classifying single cells and cell clusters. The erosion is carried out with a circular structure element with a radius of one pixel. After each iteration the algorithm checks whether the region is separated. If so, the area is classified as a cell cluster (fig. 3). As long as the separation does not accure the iteration will be continued up to a given maximum number of iterations.

Figure 2: Erosion of a blob without separation after 0, 10 and 20 steps, indicating a single cell

Figure 3: Erosion of a blob. After two iterations the blob separation will accure indicating that it must be a cell cluster

If the blob (binary large object) vanishes within the given number of iterations the blob is expected to be a single cell (fig. 2).

4. DETECTION OF CELL CORES WITH HISTOGRAM BACKPROJECTION

The segmentation of the cell cores within the cell areas is also carried out by the HB algorithm. For this purpose we first generate a color template of the cell cores. In our cell material the color composition of the cell cores are different in large and small cells. In large cells the cores used to be brighter than the cores in the small cells. Because of that reason we have to use two different color templates, one for the cell cores of the large cells and one for those of the small cells. In fig. 4 the result of the HB algorithm applied on a typical cell image is shown.

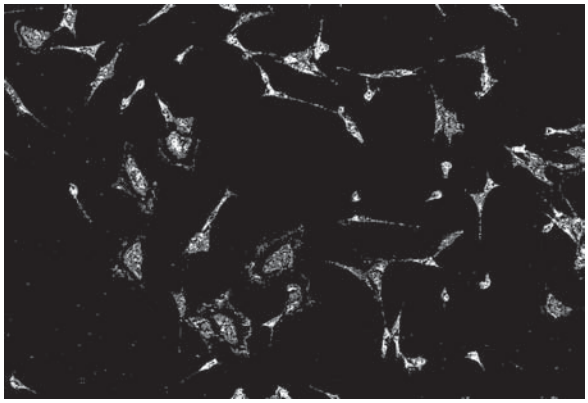

Figure 4: Result Image of cell core detection with HB

The bright pixels indicate the area of the cell cores in the small cells whereas the dark pixels indicate the area of the cell cores in the large cells. The HB algorithm produces a cloud of separate points which have to be connected to an area indicating the whole cell core. This is achieved by a closing operation.

5. EVALUATION OF DOMINANT CONTOUR POINTS OF THE CELL AREA

As is evident in our cell samples the cells forming cell clusters showing waist like contours at their contacts, indicated by arrows in fig. 5.

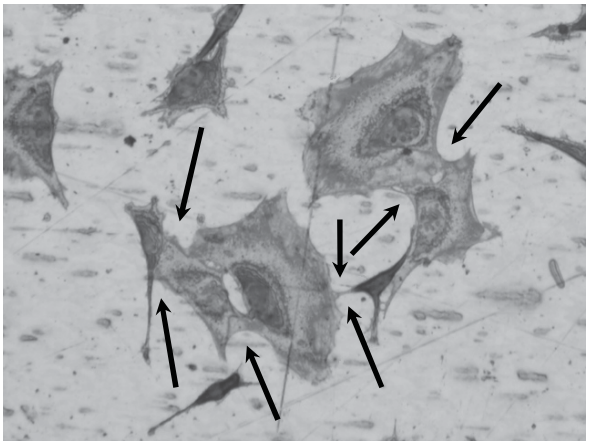

Figure 5: Waist shaded areas where cells are typically connected to each other indicated by arrows

The cell segmentation in a cluster is carried out with the help of dominant contour points (DCP). The calculation of the DCP is mainly adapted from the method described in [5]. In a first step the bounding box of the cluster is calculated and the distances from each contour point to the four sides of the bounding box are determined. As an example the distance function for the right direction to the bounding box is shown in fig. 6.

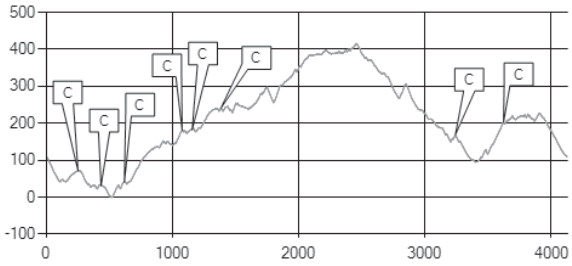

Figure 6: Distance function for right hand direction to the bounding box of the cell cluster. The Cs indicate the location of the DCPs

Only that contour points are DCPs which are local extrema of the distance function and satisfy additional conditions. They are explained in [5].

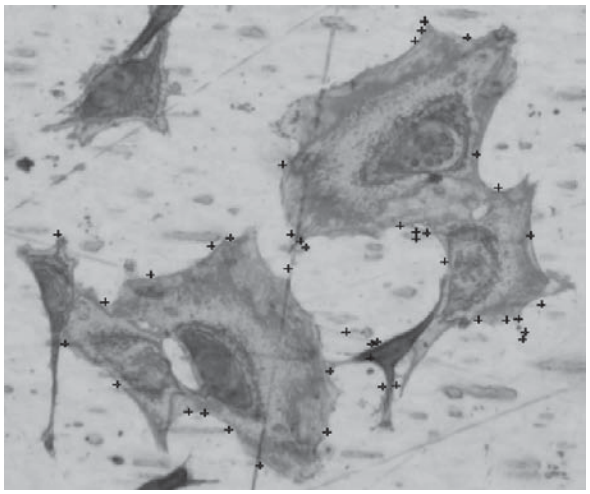

Figure 7: Cell cluster with calculated DCPs

The calculated DCPs of the cell cluster in fig.7 are indicated by black crosses. Our determining of the DCPs differs in some details from the method described in [5].
We define only that contour points as local maxima whose distances of the previous contour points increases monotonously in n steps and whose successive contour points decreases in also n steps. In our case we choose n=5. By this approach we avoid digital differentiation which often leads to unusable results in this context. The parameter n influences the number of DCPs. With increasing n the number of DCPs decreases.

6. CALCULATION OF THE SHORTEST PATHS BETWEEN NEIGHBORING CELL CORES

It is very challenging to separate the individual cells within a cell cluster obvious from biological reasons. To achieve this, we first calculate the center of gravity for each cell core in the cluster. Then we determine the shortest path beginning from the most upper left center of gravity to the nearest next center and so on (fig. 8). For the calculation of the shortest path we use the A*-algorithm, as described in [6]. This algorithm is often used in game development. When calculating the shortest paths the algorithm has to take care of holes within the cell cluster. Possible obstacles, in our case holes must be avoided by the path. The basic idea of the algorithm is a cost function which is calculated for all neighboring pixels. The calculated cost from the intermediate point at position (x,y) to the starting point and the estimated cost from the same point to the endpoint are calculated by g(x,y) and h(x,y) respectively. The sum of the two cost functions is the total cost function f(x,y). That set of points {(x,y)} belong to the shortest path which minimizes f(x,y). We use the city block distance in order to reduce computing time.

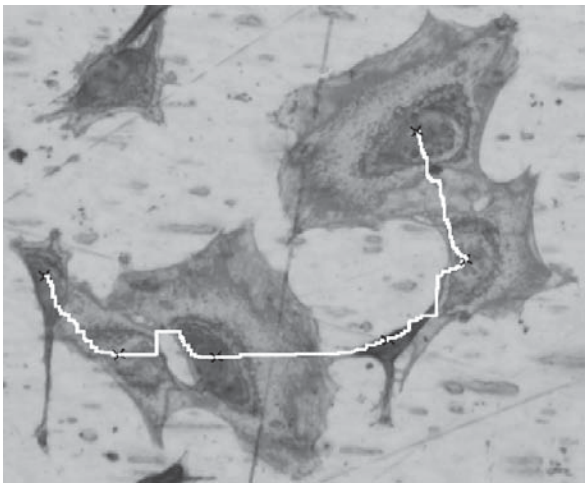

Figure 8: Shortest path through all cell cores calculated by the A*-algorithm

Each found path pixel gets a pointer which points to the previous path pixel. The reason is that we can recover the path after finishing the calculation.
All path pixels are invalid which lie within a cell core because a cell boundary cannot pass through a cell core.

7. CREATING CELL SEPARATORS IN THE CELL CLUSTER

We carry out the cell separation by calculating the distances of all path points between two cell cores to the DCPs. With a recursive function that pair of path point and DCP is accepted, whose distance is shortest with the restriction that this connection line does not pass a cell core. As an example a calculated separation line is shown in fig. 9.

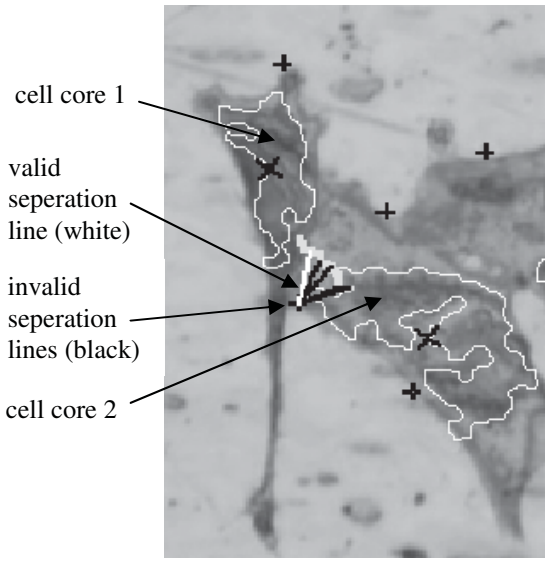

Figure 9: Accepted pair of DCP and path point creating the valid separation line point (white line) and several invalid separation lines (black lines). The black crosses indicate the locations of the DCPs

After the first DCP is found, the second DCP on the opposite side of the path has to be detected. For that reason we divide the cell cluster into two regions. This is carried out by connecting the endpoints of the whole path to the nearest points of the cell contour. The two contour points create the starting and endpoint of the remaining contour (fig. 10).

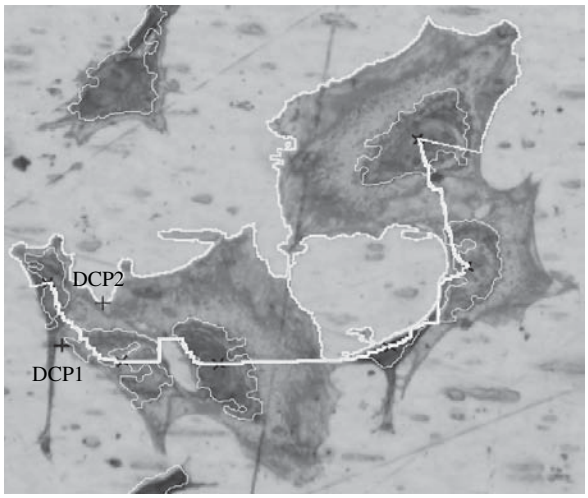

Figure 10: Created Region (white border). First DCP is outside so the second DCP must be inside

Now we can calculate the opposite DCP in the same manner but in the complementary region of the cell cluster.
If a valid connection line cannot be created (all connection lines are crossing cell cores) an alternative function is used. In this case the path point at the middle of two neighboring cores is selected and then connected to the nearest two opposite contour points.

8. RESULTS AND OUTLOOK

The segmented cell areas are classified as cell clusters with an accuracy of 89% (47 from 53 cell clusters). If two or more cells are connected to each other without forming waists the classifier based on iterative erosion will fail. For that reason we are working on new classifiers which use more features and with the help of the AdaBoost [7] algorithm we hope to get better results. As shown in fig. 11 the cell separation method as presented here is working well for relative complex cell clusters. At the moment the algorithm is very time consuming. The cell separation within a large cell cluster with about 60.000 Pixels takes 15s. One intention of our future work is to calculate time intensive algorithms on the graphic board.

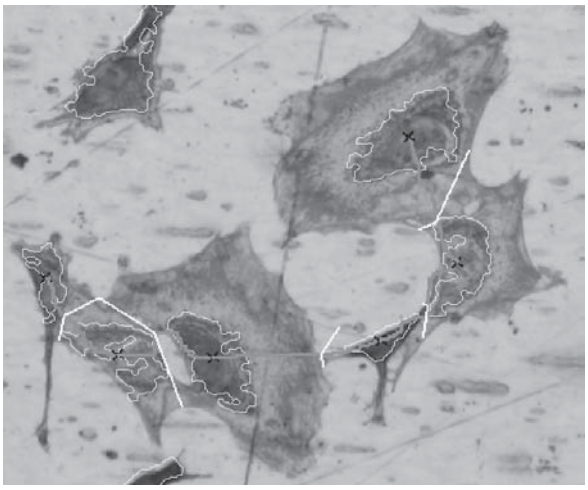
Figure 11: Divided cells within the cell cluster indicated by white lines

9. REFERENCES

[1] M. Tscherepanow, F.Zöllner and F.Kummert, „Aktive Konturen für die robuste Lokalisation von Zellen“, Springer Verlag; Berlin Heidelberg; 2005
[2] L. Dornheim, J. Dornheim, „Automatische Detektion von Lymphknoten in CT-Datensätzen des Halses“, Springer Verlag, Berlin Heidelberg; 2008
[3] G. Ramella, G. Sanniti di Baja, “Image Segmentation by Non-Topological Erosion and Topological Expansion”, Advances in Mass Data Analysis of Signals and Images in Medicine Biotechnology and Chemistry – International Converences MDA 2006/2007
[4] M. Swain, J. Ballard, D., H., “Indexing via Color Histograms”, *Proc. ICCV*, pp. 390-393, 1990
[5] U. Pal, K. Rodenacker and B.B. Chaudhuri, “Automatic cell segmentation in cyto- and histometry using dominant contour feature points”, Analytical cellular pathology, European Society for Analytical Cellular Pathology, pp.243-250, Amsterdam, 1998
[6] P. E. Hart, N. J. Nilsson and B. Raphael. “Correction to A Formal Basis for the Heuristic Determination of Minimum Cost Paths“, SIGART Newsletter, 37, pp. 28–29, 1972
[7] Y.Freund, R.E. Schapire, „A Short Introduction to Boosting”, Journal of Japanese Society for Artificial Intelligence, 14(5), pp. 771-780, September, 1999

10. ACKNOWLEDGMENT

This work was supported by the Bundesministerium für Bildung und Forschung.