CELL DENSITY ESTIMATION FROM A STILL IMAGE FOR IN-SITU MICROSCOPY

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ABSTRACT

In this contribution an algorithm to estimate the cell density (cell count) from a still intensity image captured by an in-situ microscope directly from inside of a bioreactor is investigated. In comparison with other algorithms, ours has the advantage that it allows a reliable cell density estimation even though the cells build clusters in the scene. First, image regions containing at least one cell are segmented by applying a Maximum-Likelihood Thresholding technique. Then, the cell density inside of each segmented region is estimated by maximizing the variance of the circular Hough transform of the edges inside of it. The edges are extracted by applying the Smallest Univalue Segment Assimilating Nucleus Algorithm (SUSAN). The total cell density is the sum of the cell densities estimated inside of the segmented regions. The proposed algorithm has been implemented and applied to thousands of real images of cultures of mammalian Baby Hamster Kidney cells (BHK cells) captured by an in-situ microscope. The average of the percentage of the absolute cell density estimation error was 6.27%. The estimates are similar to those obtained with current off-the-shelf cell density monitoring instruments for cultures up to cell densities of $5x10^6$ cells/mL

1. INTRODUCTION

The online estimation of the cell density (cell count) inside of a bioreactor is an important issue for reliable control of a bioprocess. By the current off-the-shelf cell density monitoring instruments, the cell density is estimated from turbidity, conductivity, optical density or fluorescense measurements [1, 2]. However, a continuous re-calibration of these instruments is required due to sensors drift, changes in the physical or chemical environment, etc. For re-calibration, a comparison of the instruments results with those obtained by offline techniques, usually by counting the cells using a hemocytometer and a microscope, is required. Thus, the operator intervention and the risk of contaminating the culture are very high. In order to avoid those problems an in-situ microscope is being developed [3, 4, 5, 6].

An in-situ microscope delivers online intensity images of cells in a defined volume inside of a bioreactor. The cell density is estimated from the intensity video signal by image processing algorithms with minimal operator intervention and without the risk of contaminating the culture. For cell density estimation, first, the local intensity variance at each pixel position of the current intensity image is computed. Then, the resulted variance image is segmented into background and regions containing just one cell. For segmentation a Maximum-Likelihood thresholding algorithm is applied [7]. The cell density is estimated as the quotient between the number of segmented regions and the volume of the scene. The volume of the scene is supposed to be known.

The cell density estimation algorithm described above fails when the cells build clusters in the scene because the Maximum-Likelihood thresholding algorithm fails finding regions with just one cell when the cells get close together. In this contribution, a new approach for cell density estimation is presented that allows a reliable cell density estimation even though there are clusters in the scene. The cell density estimation will be based on a maximization of the variance of the circle hough transform of the edges of the intensity image assuming that the cells build clusters only along a plane parallel to the camera plane.

The proposed cell density estimation algorithm will be implemented and a number of experiments on thousands of real intensity images of cultures of mammalian Baby Hamster Kidney cells (BHK cells) captured by an in-situ microscope will be performed to assess its accuracy, reliability and limitations for cell density estimation.

This paper is organized as follows. In section 2, the in-situ microscope is described. In section 3, the proposed cell density estimation algorithm is described. In section 4, some experimental results with real images are presented. In section 5, a brief summary and the conclusions are given.

2. IN-SITU MICROSCOPE

An in-situ microscope allows in-line capture of intensity images of cells directly inside of a bioreactor during a biopro-

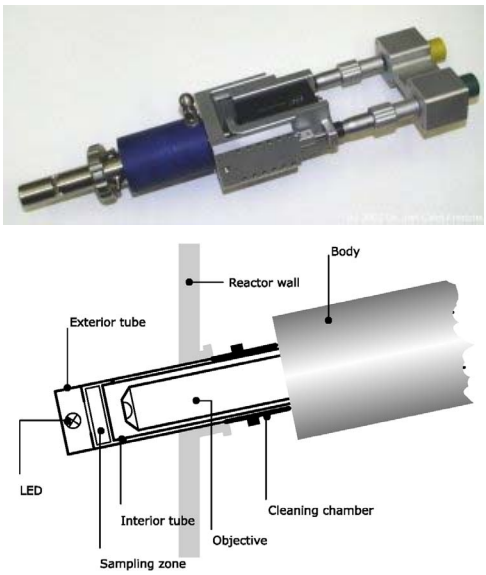

Fig. 1. In-situ microscope.

cess with minimal operator intervention (see Fig. 1). The in-situ microscope fits into a bioreactor's standard 25mm side port and once installed it can be retracted for cleaning the sample zone inside the reactor without interruption of the process or risking contamination. The sampling zone encloses a defined volume of culture that represents the scene that will be captured. It consists of two sapphire windows that work as slide and cover slip of a standard light microscope. The sampling zone stays inside the bioreactor during the entire cultivation process and does not enclose a sample completely, but allows a continuous flow of cells through it. The height of the sampling zone is set by the control programm and can be adjusted during the cultivation to accommodate a wide range of change in cell density.

For image capture a progressive scan CCD video camera with an exposure time of 0.5 ms is used. The analog video signal is digitalized by a frame grabber. Image processing algorithms estimate process relevant information like cell density from each digitalized intensity image.

For yeast cultivations achromatic objectives with a 20-fold magnification (overall magnification 400-fold) and numerical aperture of 0.4 are used. For mammalian cell cultivations, achromatic objectives with 4-fold magnification (overall magnification 80-fold) and a numerical aperture of 0.1 are used.

3. CELL DENSITY ESTIMATION ALGORITHM

In some cultivation processes the cells get close together and build clusters (see Fig. 2). The current cell density estimation algorithms found in the professional literature for in-situ microscopy fails counting cells inside of clusters. In

Fig. 2. Intensity image I of BHK-cells.

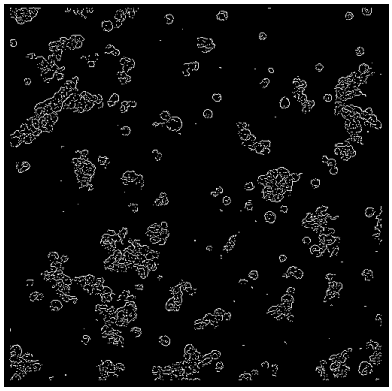

Fig. 3. Edge image E.

following, a new cell density estimation algorithm will be described that allows reliable estimates even though there are clusters in the scene.

First, an intensity image I of the scene is captured (see Fig. 2). Then, the edges of the intensity image are computed by applying the Smallest Univalue Segment Assimilating Nucleus Algorithm (SUSAN) [8]. The resulted image is called edge image E (see Fig. 3). In the next step, the local intensity variance at each pixel position of the intensity image is computed. The resulted image is called variance image V (see Fig. 4). Then, the variance image is segmented into background and clusters. A cluster is defined here as an image region with one or more cells. For segmentation a Maximum-Likelihood thresholding algorithm is applied [7]. The resulted image is called binary image B (see Fig. 5). The binary image is black on the background and white on the clusters. In the next step, isolated white points are eliminated from the binary image by applying a 5X5 median filter. Then, the remaining K clusters are numbered by applying a recursive labeling algorithm. Clusters with small areas are also eliminated. The resulted image is called labeled image L (see Fig. 6).

In the next step, the cells inside each one of the remain-

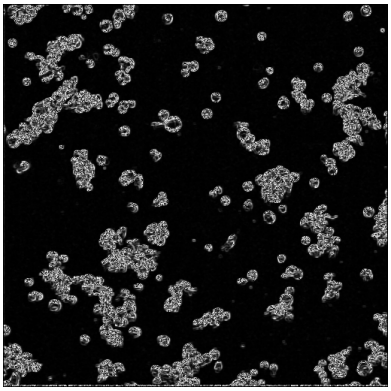

Fig. 4. Variance image V.

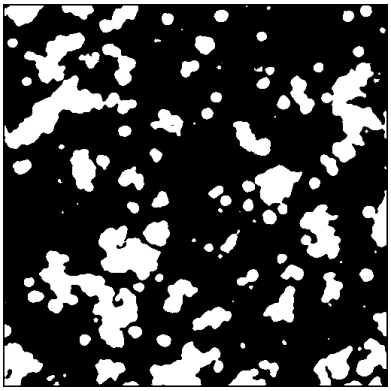

Fig. 5. Binary image B.

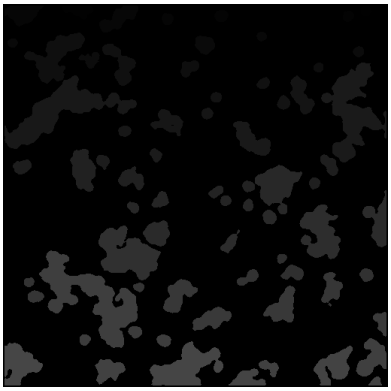

Fig. 6. Labeled image L.

ing K clusters, $k : 1..K$, are counted. To this end, the Hough transform for circles $H(r)$ of known radius r is applied to the edges E_k inside of each cluster k. The edges are taken from the previously computed edge image E. Assuming that the cells inside of the cluster k are round, the radius R_k which maximizes the variance of the Hough transform $\sigma(H(r))^2$ is supposed to be the average cell radius inside of the cluster k:

$$\sigma(H(R_k))^2 \geq \sigma(H(r))^2 \ \forall \ r = 1, 2, ... \tag{1}$$

Assuming that the cells build clusters only along a plane parallel to the camera plane, the cell density in the cluster k is estimated as the quotient between the area of the cluster k and the area of a circle with radius R_k:

$$D_k = \frac{A_k}{\pi {R_k}^2} * F, \tag{2}$$

where D_k is the cell density in the cluster k, $\pi = 3.14...$ and F is a calibration factor that is used to compensate the influence of the segmentation errors. This because the segmented clusters are always bigger than the real ones. Here, the value of F is experimentally set to 0.765 in all experiments. Fig. 7 depicts on each cluster the estimated cell density overlapped to the original image. Finally, the cell density of the intensity image I is estimated as follows:

$$D = \sum_{k=1}^{K} D_k. \tag{3}$$

4. EXPERIMENTAL RESULTS

We have implemented the cell density estimation algorithm described in this contribution and performed a number of experiments on real cultivation data to assess its estimation error, reliability and limitations for cell density estimation. The data represents thousands of real intensity images (512x510 pel^2) captured by an in-situ microscope depicting clusters of mammalian Baby Hamster Kidney cells (BHK-cells). The experiment was performed on a Pentium IV (3.06Gz) laptop with 0.5 GB RAM. The average of the processing time per image was 15.88 s.

The Fig. 2 depicts one of the intensity images used in the experiments. Fig. 7 depicts the estimated cell density on each cluster overlapped to the original intensity image. The total cell density was 402 cells.

To assess the estimation error, the cell density of 11 randomly selected images was determined by counting manually. Then, the estimated cell density of each image was compared with that obtained counting manually (see Fig. 8). The resulted absolute error was 31.36 ± 7.36 cells and the average of the percentage absolute error was 6.27%.

To assess the reliability and limitations of the algorithm, it has been applied to complete cultures of BHK-cells (approximated 28 thousand images each) and the cell density

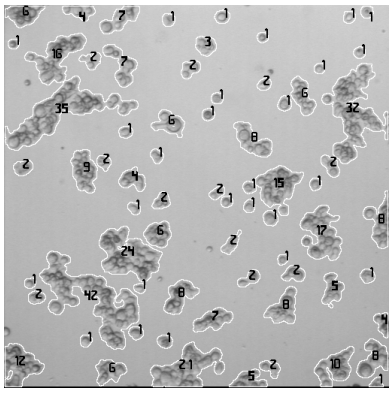

Fig. 7. Cell density results overlapped to the original image. Each number represents the estimated cell density (number of cells) in each cluster. The total cell density was for this image 402 cells.

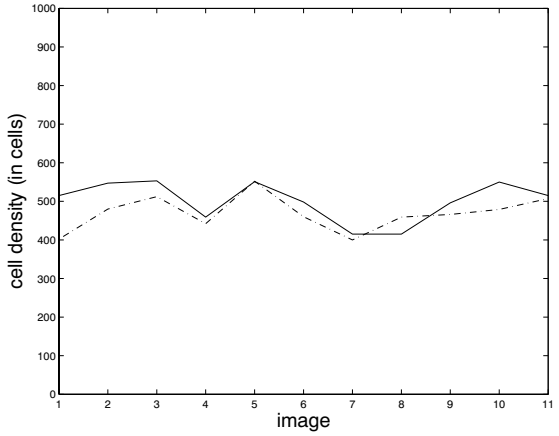

Fig. 8. Manually determined cell density (solid line) and automatically estimated cell density (dashdot line).

estimates compared with those obtained with current off-the-shelf cell density monitoring instruments. The comparison shows similar results for cultures up to cell densities of $5x10^6$ cells/mL. For higher cell concentrations the estimated cell density is less than that obtained with current off-the-shelf cell density monitoring instruments. We believe that this difference is because in the real world the cells build clusters in the three-dimensional space and not only along a plane parallel to the camera plane as we suppose in our current approach. For higher cell concentrations the three-dimensional shape of the cell clusters must also be estimated and taking into account for cell density estimation.

5. SUMMARY AND CONCLUSIONS

In this contribution a new algorithm for cell density estimation for in-situ microscopy is presented. In comparison with other algorithms our has the advantage that it allows a reliable cell density estimation even though the cells build clusters in the scene. The average of the percentage of the absolute cell density estimation error was 6.27%. The estimates are similar to those obtained with current off-the-shelf cell density monitoring instruments for cultures up to cell densities of $5x10^6$ cells/mL. For higher cell concentrations the three-dimensional shape of the cell clusters must also be estimated and taking into account for cell density estimation.

6. ACKNOWLEDGMENTS

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7. REFERENCES

[1] T. Scheper and K. F. Reardon, "Sensors in biotechnology," *Sensors, Wiley-VCH, Weinheim*, vol. 2, no. 2, pp. 1024–1046, 1991.

[2] K. F. Reardon and T. Scheper, *Bioprocess Analysis*, Encyclopedia of Analytical Science, London, Academic Press, 1995.

[3] K. Joeris, J.-G. Frerichs, K. Konstantinov, and T. Scheper, "In-situ microscopy: Online process monitoring of mammalian cell cultures," *Cytotechnology*, vol. 38, pp. 129 – 134, 2002.

[4] C. Bittner, G. Wehnert, and T. Scheper, "In-situ microscopy for online determination of biomass," *Biotechnology & Bioeng.*, vol. 60, pp. 24 – 35, 1998.

[5] J.-G. Frerichs, *Entwicklung eines In-situ Mikroscops zur Bildgestuetzen Online-Ueberwachung von Bioprozessen*, Ph.D. thesis, University of Hannover, Hannover, Germany, 2000.

[6] H. Suhr, G. Wehnert, K. Schneider, C. Bittner, T. Scholtz, P. Geissler, B. Jaehne, and T. Scheper, "In-situ microscopy for online characterisation of cell-populations in bioreactors, including cell concentration measurements by depth from focus," *Biotechnology & Bioeng.*, vol. 47, pp. 106–117, 1995.

[7] T. Kurita, N. Otsu, and N. Abdelmalek, "Maximum-likelihood thresholding based on population mixture models," *Pattern Recognition*, vol. 25, no. 10, pp. 1231 – 1240, 1992.

[8] S. Smith and J. Brady, "Susan - a new approach to low level image processing," *International Journal of Computer Vision*, vol. 23, no. 1, pp. 45 – 78, 1997.