THREE-DIMENSIONAL SHAPE ESTIMATION OF BHK CELL CLUSTERS FROM A STILL IMAGE BASED ON SHAPE FROM SHADING FOR IN-SITU MICROSCOPY

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ABSTRACT

In this contribution, the Lee and Rosenfeld's local Shape From Shading (SFS) algorithm, the Tsai and Shah's linear SFS algorithm and the Bichsel and Pentland's propagation SFS algorithm are investigated with the aim of selecting the most suitable for three-dimensional shape estimation of clusters of mammalian Baby Hamster Kidney cells (BHK cells) from an intensity image captured by an in-situ microscope in an industrial mammalian cell culture process. All three were implemented and tested using several thousand intensity images captured under varying experimental conditions. The Bichsel and Pentland's SFS algorithm was finally selected as the most suitable algorithm for three-dimensional shape estimation of BHK cell clusters. It is fast and provides less noise and more detailed depth estimates and therefore the best overall performance.

1. INTRODUCTION

An in-situ microscope is an instrument to capture and analyze intensity images of cells in a defined volume inside of a bioreactor with minimal operator intervention and without the risk of contaminating the culture [1, 2, 3]. The cell density inside of the bioreactor can be estimated by means of image analysis algorithms from these captured intensity images in near real-time [4]. So far, the image regions of the cell clusters are first segmented by applying a Maximum-Likelihood Thresholding technique [5, 6]. A cluster is a group of one or more cells that are very close to each other, almost overlapping (see Fig. 1). Then, assuming that the cells build clusters only along a plane parallel to the camera plane, the number of cells 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 each segmented region's estimated number of cells divided by the known volume.

Experimental studies on cultures of mammalian Baby Hamster Kidney cells (BHK-cells) have shown that the previously explained cell density estimation algorithm works well for cultures up to cell densities of $5x10^6$ cells/mL. For higher cell concentrations the estimated cell density is lower than that obtained with established off-line methods. This difference is primarily caused by aggregation of the BHK cells in clusters in the three-dimensional space and not only along a plane parallel to the camera plane as assumed in [4]. Thus, for higher cell concentrations the three-dimensional shape of the BHK cell clusters must also be estimated and taken into account for cell density estimation.

In this paper, an algorithm for three-dimensional shape estimation of the BHK cell clusters will be developed. The shape will be estimated from the gradual variations of shading in the intensity image (shape from shading). A lambertian image formation was assumed with a known light source direction opposite to the viewing direction. Instead of developing a new algorithm from scratch, first, three of the most popular Shape From Shading (SFS) algorithms described in the literature will be implemented. Then, a number of experiments on real cultivation data will be performed to assess their timing (CPU time) and ability to estimate the three-dimensional shape of cell clusters. Finally, the one that performs best will be selected as the most suitable SFS algorithm for estimating the three-dimensional shape of BHK cell clusters for in-situ microscopy.

This paper is organized as follows. In section 2, three of the most popular SFS algorithms from the professional literature are selected and briefly described. In section 3, the selected SFS algorithms are implemented and tested with real data. In section 4, a brief summary and the conclusions are given.

2. SHAPE FROM SHADING ALGORITHMS

The SFS algorithms found in the professional literature can be roughly subdivided into four groups: local algorithms [7, 8], linear algorithms [9, 10], propagation algorithms [11, 12] and the minimization algorithms [13, 14, 15, 16, 17, 18] (see [19] for a comprehensive review). Here, instead of evaluating all possible SFS algorithms only the most popu-

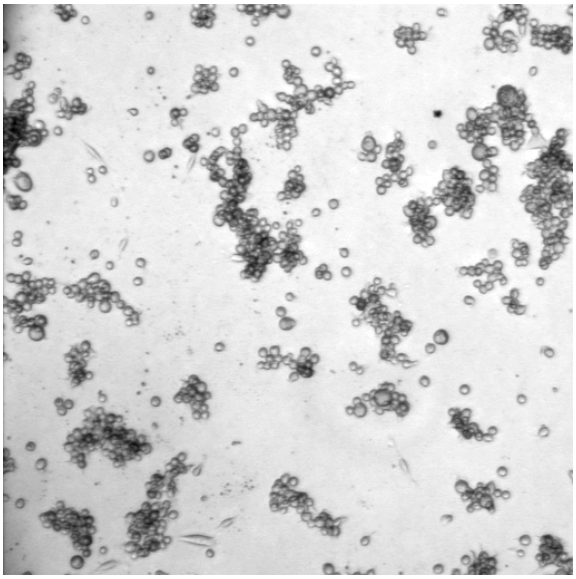

Fig. 1. Original intensity image of BHK cells captured by an in-situ microscope.

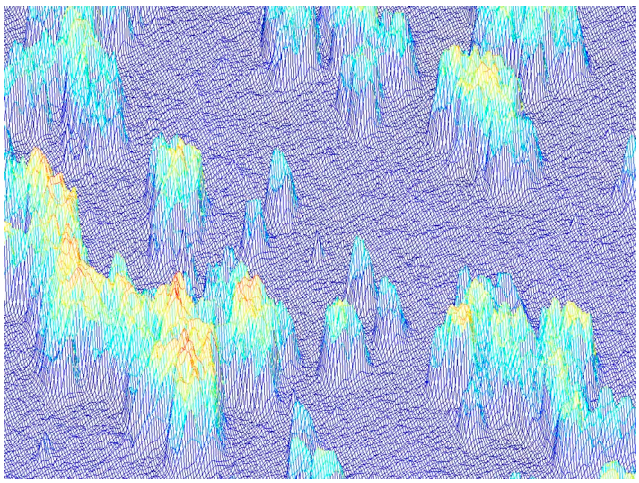

Fig. 2. Zoom of a rectangular region of the estimated depth obtained with the Lee and Rosenfeld's SFS algorithm.

lar SFS algorithm of each group will be evaluated: the local algorithm described by Lee and Rosenfeld in [8], the linear algorithm described by Tsai and Shah in [10] and the propagation algorithm described by Bichsel and Pentland in [12]. No minimization algorithm was considered because they are known to be very slow although slightly more robust [19].

In the Lee and Rosenfeld's SFS algorithm the object shape is described by the surface slant and tilt, where the tilt is defined as the angle between the projection of the surface normal on the image plane and the x-axis and the slant is defined as the angle between the surface normal and the direction toward the viewer. The surface slant and tilt are estimated with the aid of a coordinate system having one axis in the assumed direction of the light source and assuming that the surface is locally spherical at each point. In this coordinate system the surface tilt is related to the direction of the intensity gradient at the giving pixel. Assuming that the surface has Lambertian reflectivity and that one of the surface patches points toward the illumination direction, the surface slant at each pixel is related to the quotient between the intensity of the pixel and the intensity of the brightest pixel of the image.

In the Tsai and Shah's SFS algorithm the object shape is described by the rate of change of depth along the x- and y axis of the image plane, p and q, respectively (surface gradient). The surface gradient is estimated from shading by linear approximation of the reflectance map. First, the surface normal, p and q, are linear approximated using discrete finite differences. Then, the reflectance map is linear approximated in terms of depth using the Taylor series of the reflectance up to the first order term. Finally, p and q are recursively estimated by using a Kalman Filter.

In the Bichsel and Pentland's SFS algorithm the object shape is described by the surface height above the image plane. Giving initial values at the singular pixels of an image, i.e. the pixels of maximum brightness, the Bichsel and Pentland's SFS algorithm propagates the height iteratively with a Gauss-Seidel scheme as follows. First, the slopes along eight discrete directions are estimated at each pixel of the image. Each slope p is estimated by rotating the coordinate system of the image plane such as the x-axis is aligned with the discrete direction and then taking the derivative of the Lambertian reflectance map with respect to q, setting it to zero and then solving for p and q. For each discrete direction a value of the pixel height is estimated by adding the current height of the first neighboring pixel found along the discrete direction and the corresponding estimated slope. Finally, the maximal value is selected and considered to be the new updated pixel height for the next iteration. This propagates the height information always away from the light source to guarantee the convergence of the algorithm (minimum downhill propagation direction). The updated pixel height becomes also immediately available for estimation of the height of other pixels in the same iteration. The convergence of the algorithm is accelerated by altering the way the pixels are sequentially processed at each iteration. As in [19], the initial hight values for the singular points are assigned a fixed positive value of 55 and the hight values of the other points are initialized to the large negative value of -1.0e10. Since the surface gradients in low brightness regions are close to zero for most directions except the directions which form a very narrow angle with the illumination direction, the original image is rotated in order to align one of the discrete directions with the illumination direction so that the existence of a solution is not

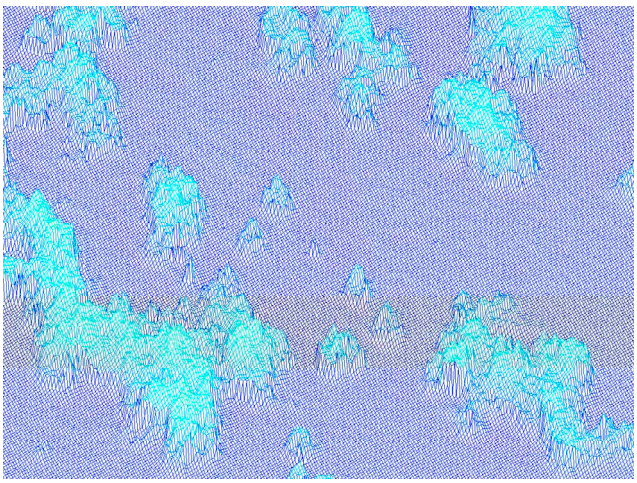

Fig. 3. Zoom of a rectangular region of the estimated depth obtained with the Tsai and Shah's SFS slgorithm.

further restricted. The inverse rotation is performed on the resulting depth map in order to get the original orientation back.

3. EXPERIMENTAL RESULTS, PERFORMANCE EVALUATION AND FINAL SFS ALGORITHM SELECTION

The three SFS algorithms described in section 2 were implemented in C under Windows XP and tested on real cultivation data to assess their timing (CPU time) and ability to estimate the three-dimensional shape of cell clusters. The intensity images of BHK-cell clusters (512x512 pixels square) were captured by an in-situ microscope in an industrial cell culture process. 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 0.525 s, 1.552 s and 1.816 s for the Lee and Rosenfeld's algorithm, the Bichsel and Pentland's algorithm and the Tsai and Shah's algorithm, respectively. Experimental results obtained from one typical real intensity image will be depicted as an example.

Figure 1 depicts the original intensity image. Figs. 2, 3 and 4 show a rectangular region of the slightly rotated depth estimates obtained with the Lee and Rosenfeld's, the Tsai and Shah's and the Bichsel and Pentland's SFS algorithms, respectively. Low surface height are shown blue and high surface height above the image red. It is possible to see the different heights clearly inside of each cluster. This is experimental evidence that the cells do build clusters in the three-dimensional space and not only along a plane.

The estimated depth obtained with the Lee and Rosenfeld's algorithm is very noisy (see Fig. 2). This is caused by the assumption that the real shape is locally spherical at each point on the cluster surface. This assumption fails at edge points between neighboring cells inside the clusters. The estimated depth with the Tsai and Shah's SFS algorithm is also noisy and particularly very flat, i.e. all the cells seem to be on the same plane (see Fig. 3). We believe that this is because the linearization of the reflectance map, using the Taylor series of the reflectance up to the first order term, is not accurate enough for describing the reflectance map of in-situ microscope BHK images. The estimated depth with the Bichsel and Pentland's SFS algorithm is less noisy and more detailed (see Fig. 4).

We concluded after comparing the performance of the three SFS algorithms that the Lee and Rosenfeld's SFS algorithm is the fastest but the estimated depth is very noisy. Although the Bichsel and Pentland's SFS algorithms is 2.89 times slower than the Rosenfeld and Pentland's SFS algorithm, the estimated depth is less noisy and much more detailed. The Tsai and Shah's SFS algorithm is the slowest and the estimated depth is noisy and very flat.

The Bichsel and Pentland's SFS algorithm is selected here as the most suitable algorithm for three-dimensional shape estimation of cell clusters. It is fast and provides less noise and more detailed depth estimates, However, if the processing time becomes an important issue, it is recommended to use the Lee and Rosenfeld's instead of the Bichsel and Roselfeld's SFS algorithm.

4. SUMMARY AND CONCLUSIONS

In this contribution, the local SFS algorithm described by Lee and Rosenfeld in [8], the linear SFS algorithm described by Tsai and Shah in [10] and the propagation SFS algorithm described by Bichsel and Pentland in [12] were implemented. They were applied to real intensity images captured by an in-situ microscope showing clusters of BHK-cells. The Bichsel and Pentland's SFS algorithm was selected as the most suitable algorithm for three-dimensional shape estimation of BHK cell clusters, because it is fast and provides less noise and more detailed depth maps than the one by Lee and Rosenfeld or Tsai and Shah.

In the future work, the selected Bichsel and Pentland's SFS algorithm will be incorporated in the cell density estimation algorithm described in [4]. The three-dimensional shape of the cell clusters will be estimated and used for the cell density estimation. We believe that this will improve the accuracy and reliability of the cell counting at high cell concentrations.

5. ACKNOWLEDGMENTS

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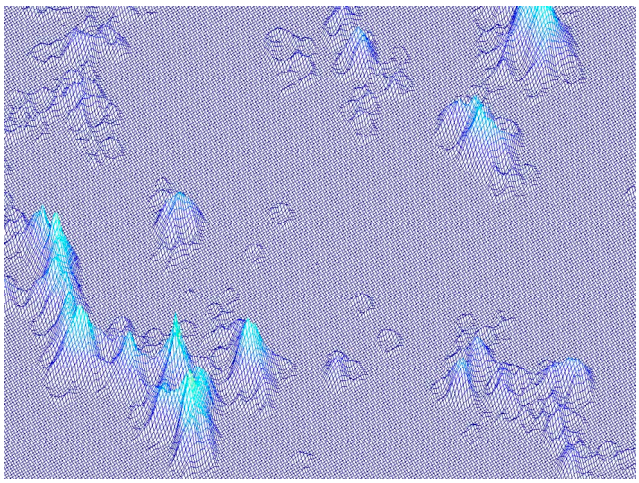

Fig. 4. Zoom of a rectangular region of the estimated depth obtained with the Bichsel and Pentland's SFS Algorithm.

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