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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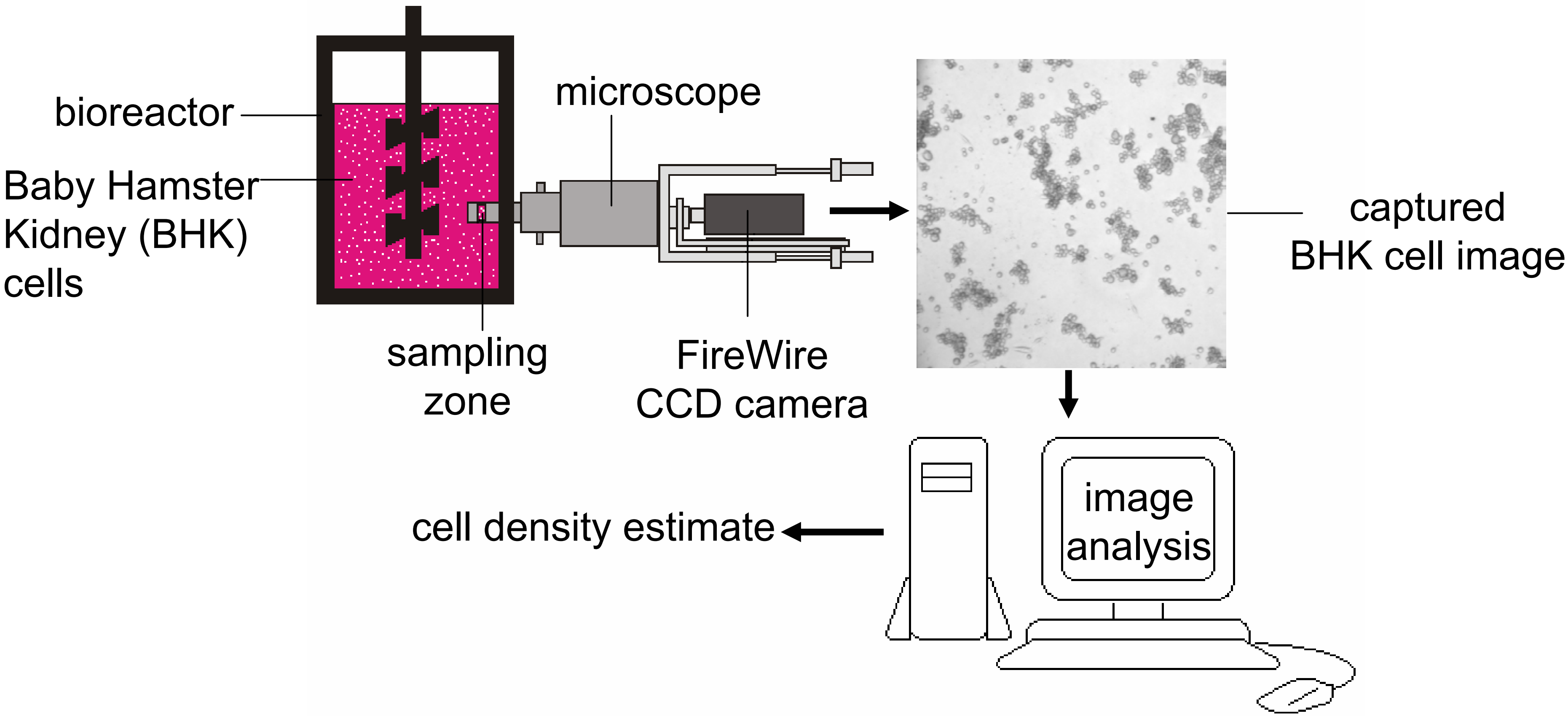
In-Situ Microscopy

On-line automatic cell density estimation (cell count) with no risk of culture contamination

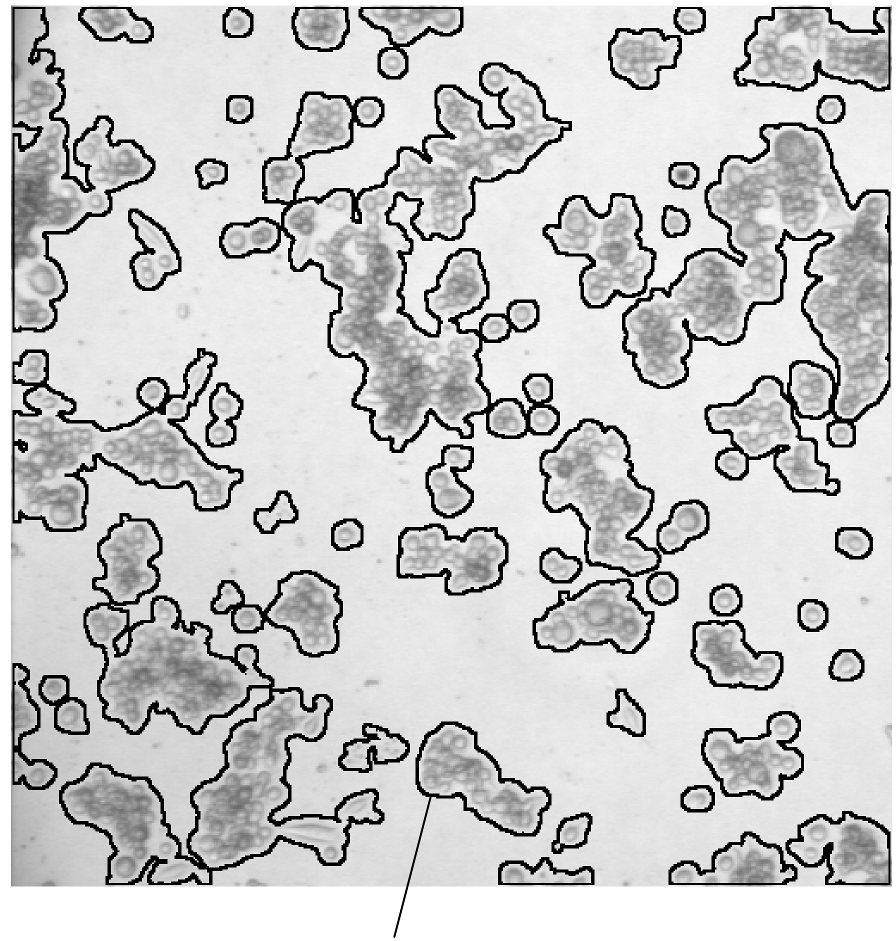


Cell density estimation algorithm

1) Capture a BHK cell image I

2) Segment all K cell clusters

3) Estimate the average cell radius R_k of each segmented cluster k, $k=1...K$, by maximizing the variance of the circular Hough transform of the edges inside the cluster

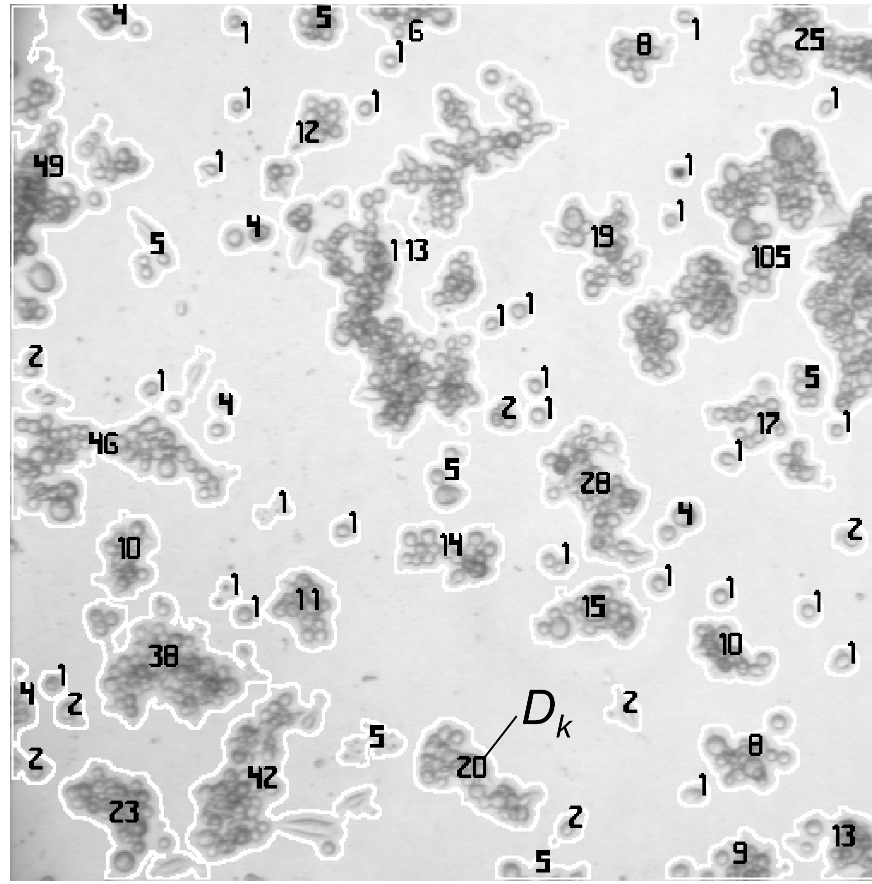


Cell density estimation algorithm

4) Compute the number of cells D_k of each segmented cluster k, $k=1\ldots K$, as the quotient between the area of the cluster A_k and the area of a circle of radius R_k

5) Compute the total cell density D of the image I

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



D = 732 cells

Problem

In high cell concentrations (>$5x10^6$ cell/mL) the cell count is lower than that obtained with established off-line methods

Causes

This difference is primarily because in high cell concentrations the shape of the cell clusters is no longer two dimensional parallel to the image plane (as it is supposed in the previously explained cell density estimation algorithm) but rather three dimensional (3D). Thus, the cells that aggregate perpendicular to the image plane are missing in the cell count

Approach

To estimate the 3D shape of the cell clusters and then to take it also into account during cell density estimation

- The 3D shape is estimated from gradual variations of shading in the captured cell image (Shape From Shading)

- Instead of developing a new Shape From Shading (SFS) algorithm, three of the most popular SFS algorithms are implemented and tested with real cultivation data

- The one with the best performance is selected

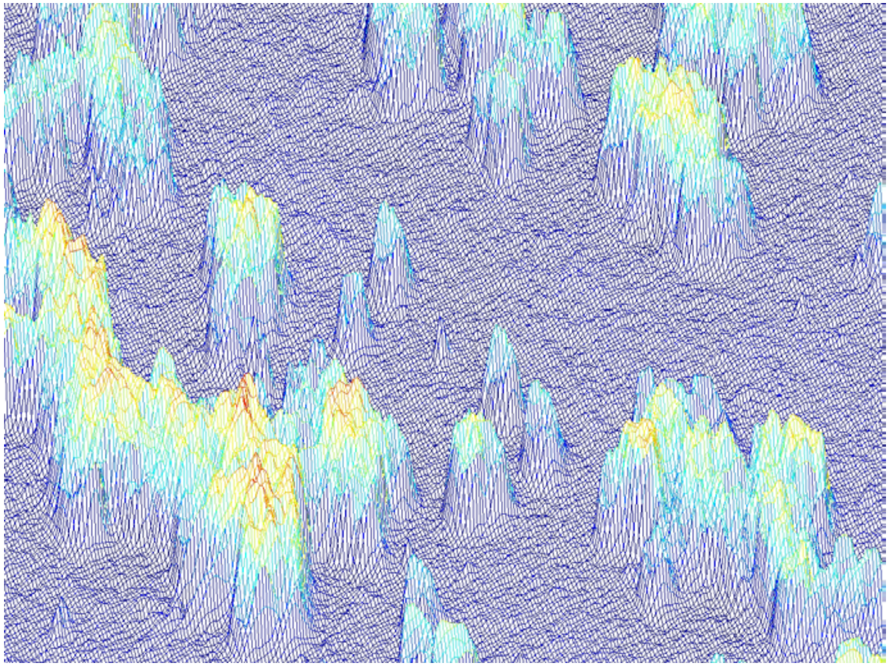
Assumptions

- A Lambertian image formation is assumed
- The light source direction is supposed to be opposite to the viewing direction

3D shape estimation (1)

Lee and Rosenfeld's **local** Shape From Shading algorithm

- The surface is supposed to be **locally** spherical at each point

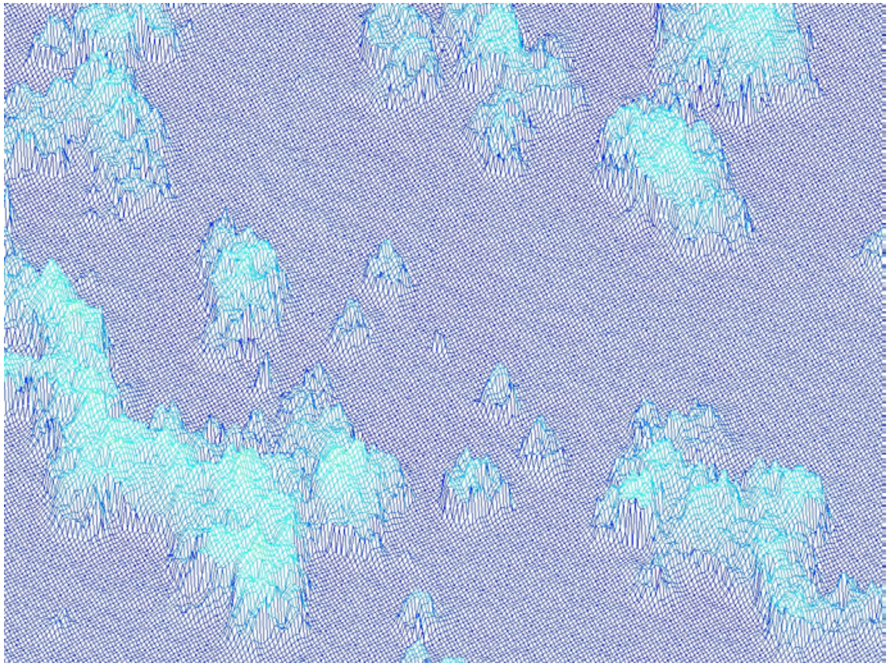


Zoom of a rectangular region of the slightly rotated estimated shape

3D shape estimation (2)

Tsai and Shah's **linear** Shape From Shading algorithm

- The reflectance map is **linear** approximated in terms of depth using the Taylor series of the reflectance up to the first order term

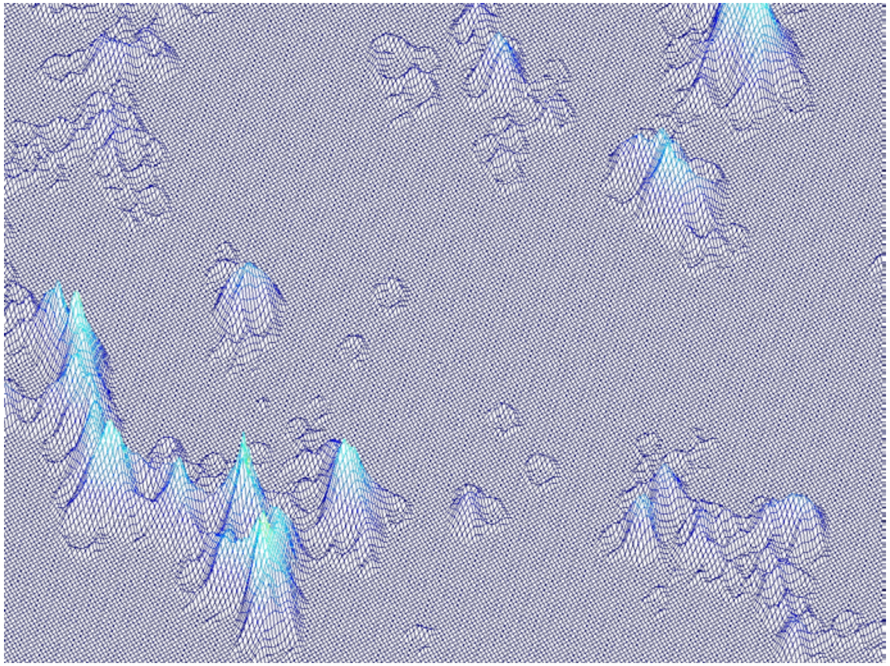


Zoom of a rectangular region of the slightly rotated estimated shape

3D shape estimation (3)

Bichsel and Pentland's **propagation** Shape From Shading algorithm

- The shape is iteratively **propagated** from the pixels of maximum brightness with a Gauss-Seidel scheme



Zoom of a rectangular region of the slightly rotated estimated shape

Performance evaluation

Algorithm	Processing time	Subjective evaluation of the estimated shape
Local SFS (Lee and Rosenfeld)	0.525 s	Very noise
Linear SFS (Tsai and Shah)	1.816 s	Noise and flat
Propagation SFS (Bichsel and Pentland)	1.552 s	Less noise and more detailed

Conclusions

The Bichsel and Pentland's SFS algorithm is the most suitable algorithm for 3D shape estimation of BHK cell clusters because it is fast and provides less noise and more detailed shapes

Future work

The estimated 3D shape of the cell clusters will be also taken into account during cell density estimation