Three-Dimensional Cell Counting for In-Situ Microscopy

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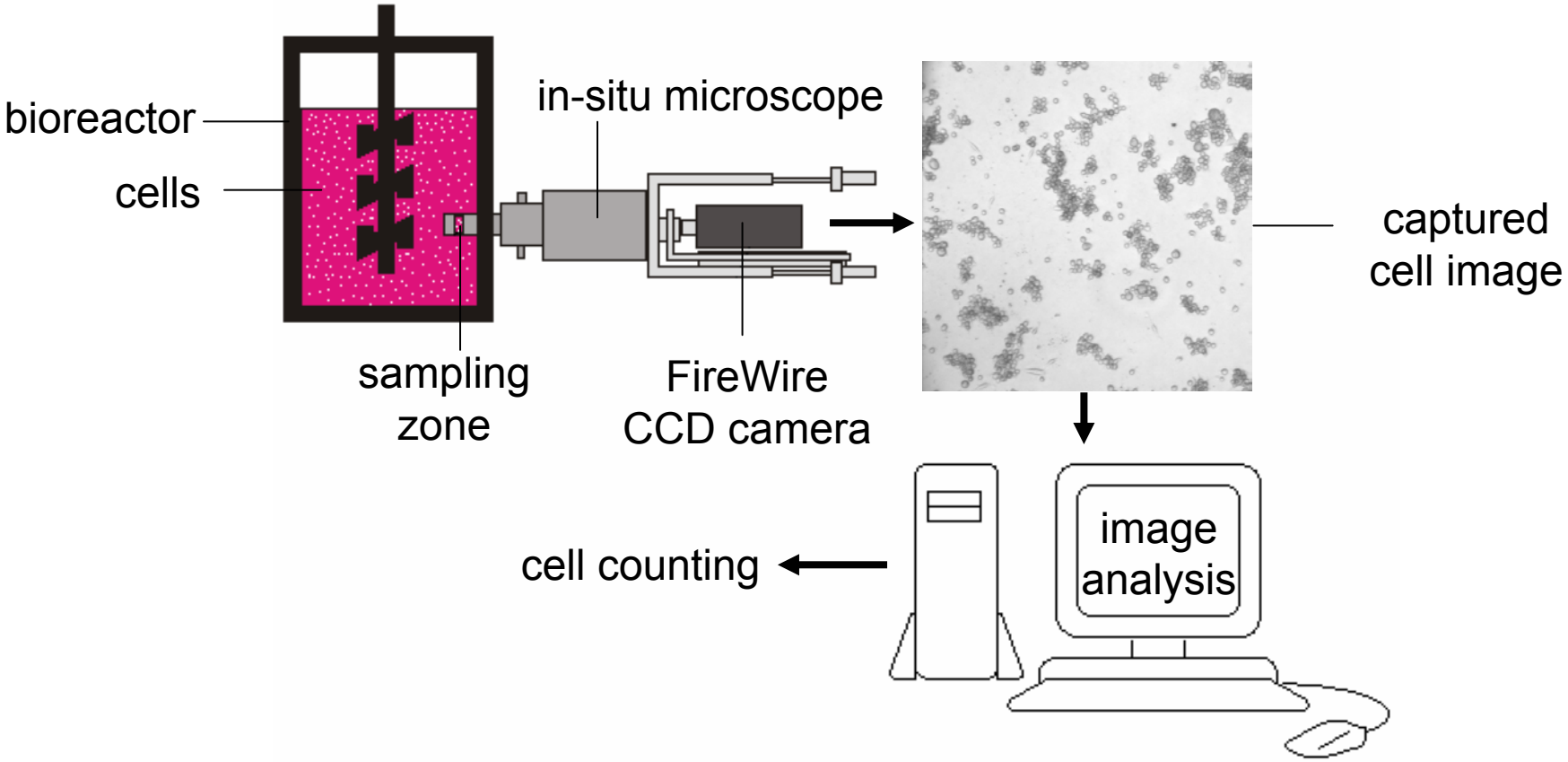
Introduction

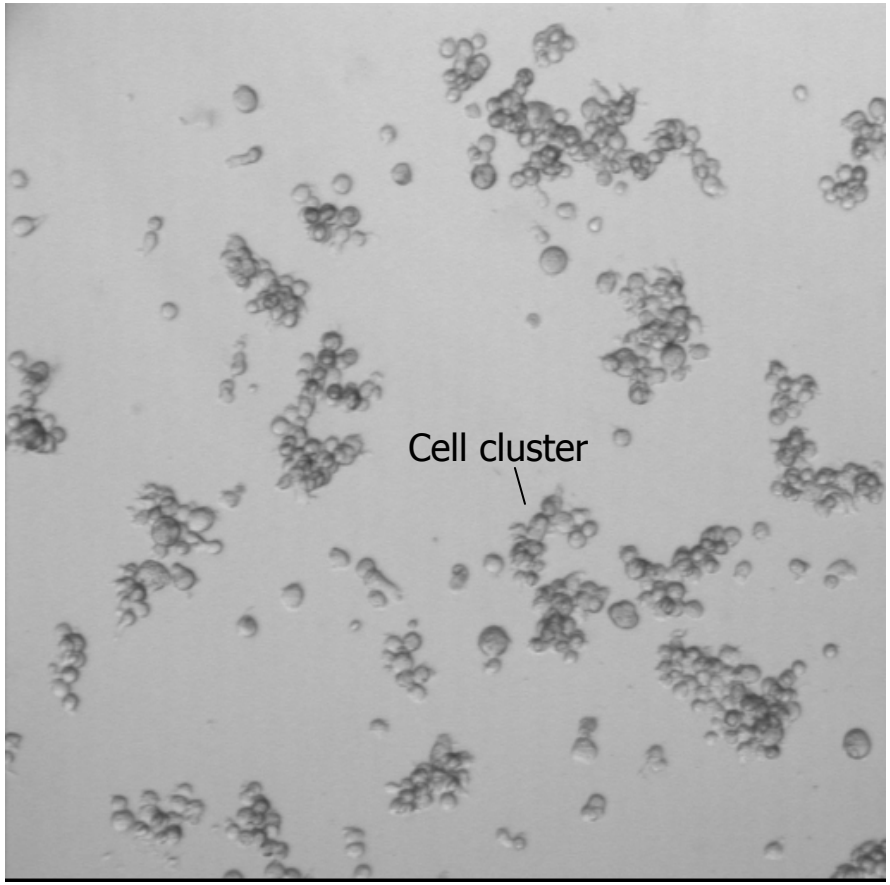
On-line automatic cell counting with no risk of culture contamination



Assumptions: the cells are round and they aggregate forming symmetrical 3D clusters up to three-layers high

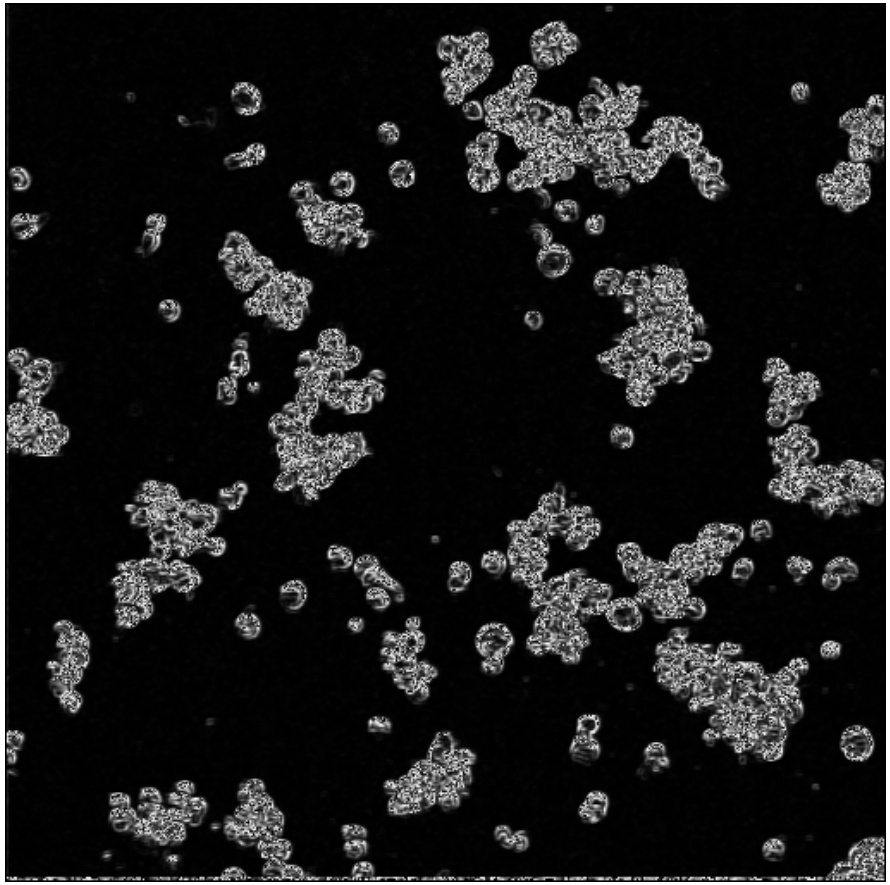
3D Cell Counting

1) Capture an intensity image I



3D Cell Counting

2) Estimate the local intensity variance at each pixel position



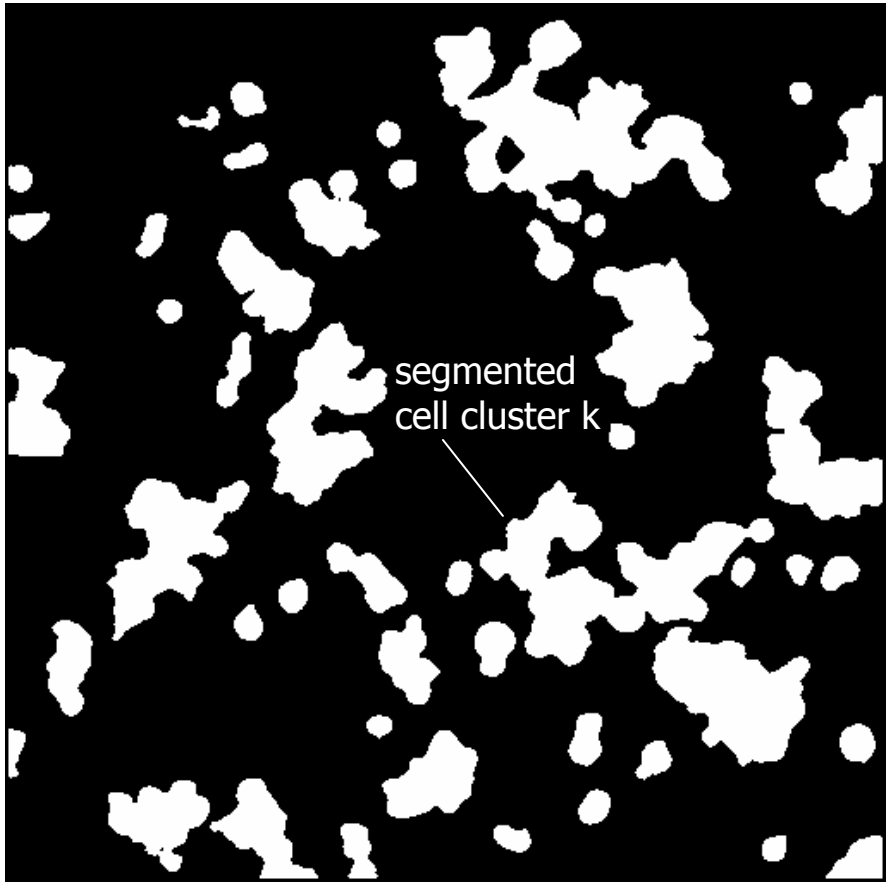
Variance image V

3D Cell Counting

3) Segment the image regions of the cell clusters by applying a Maximum-Likelihood thresholding algorithm to the variance image V

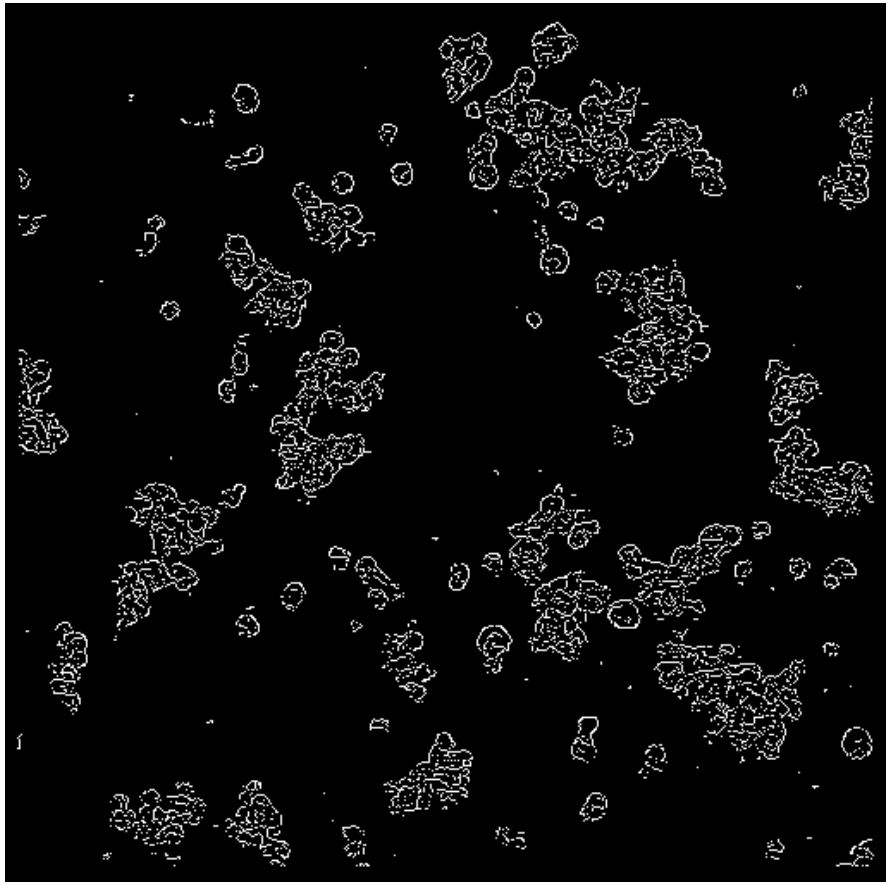
   Eliminate isolated white pixels by applying a 5x5 median filter

   Eliminate black holes inside white regions and also those white regions whose image area is less than 0.05% of the total image area



3D Cell Counting

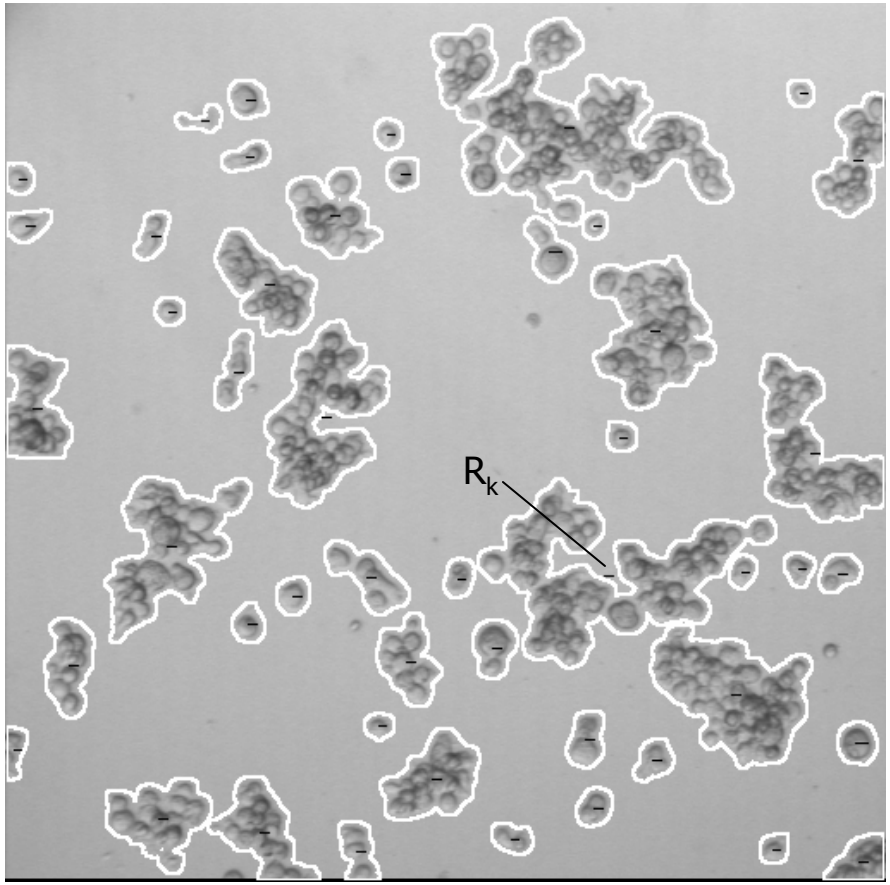
4) Estimate the edges of cell clusters by applying the Smallest Univalue Segment Assimilating Nucleus algorithm (SUSAN algorithm) to the intensity image I



3D Cell Counting

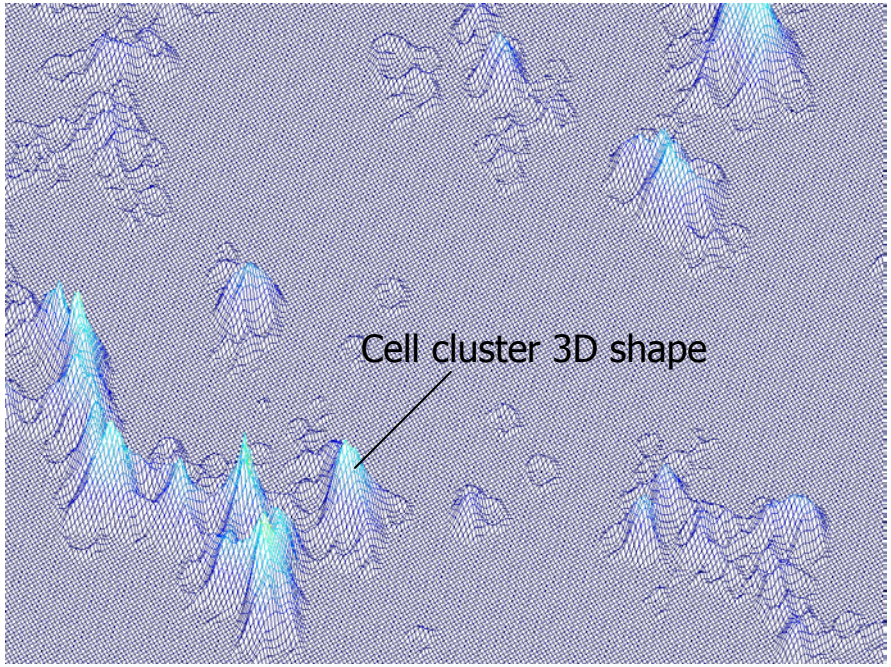
5) Estimate the average cell radius R_k of each segmented cell cluster k by maximizing the variance of the circular Hough transform of the edges inside the cluster

$$\sigma(H_{R_k,k})^2 \geq \sigma(H_{r,k})^2 \ \ \forall \ \ r = 1,2,3,..$$



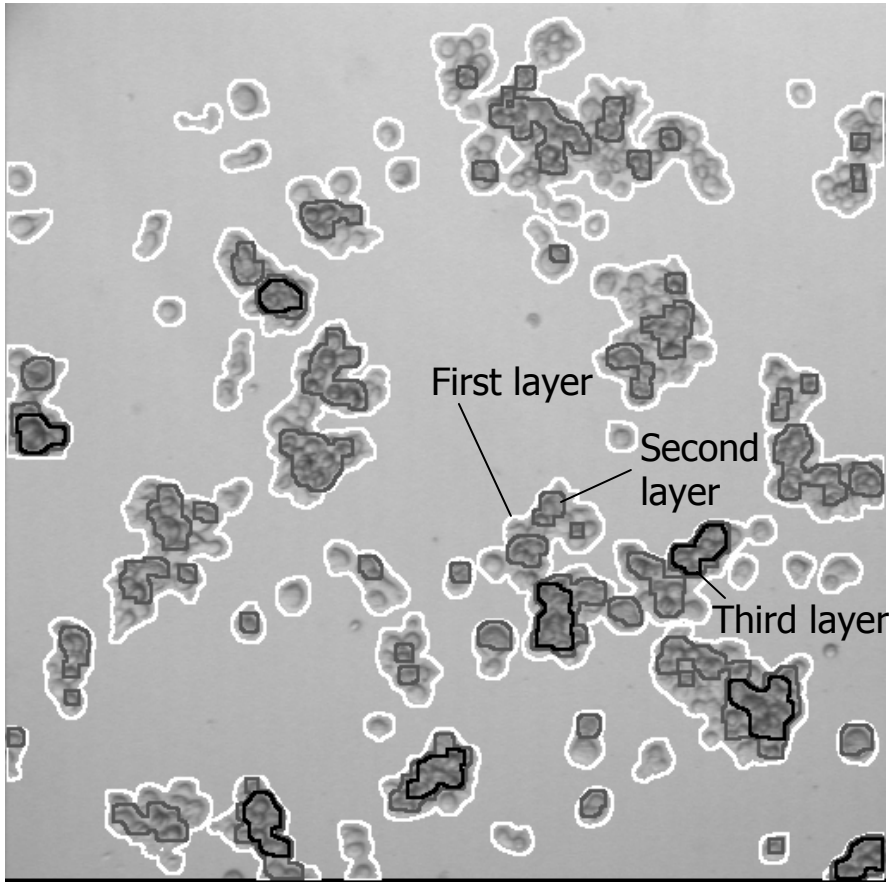
3D Cell Counting

6) Estimate the 3D shape of the cell clusters by applying the Bichsel and Pentland's Shape from Shading algorithm to the intensity image I



3D Cell Counting

7) Segment the image regions of the parallel projections of the first-, second- and third-layers of the cell clusters into the image plane by applying a Maximum-Likelihood multi-thresholding algorithm to the estimated 3D shape of the cell clusters

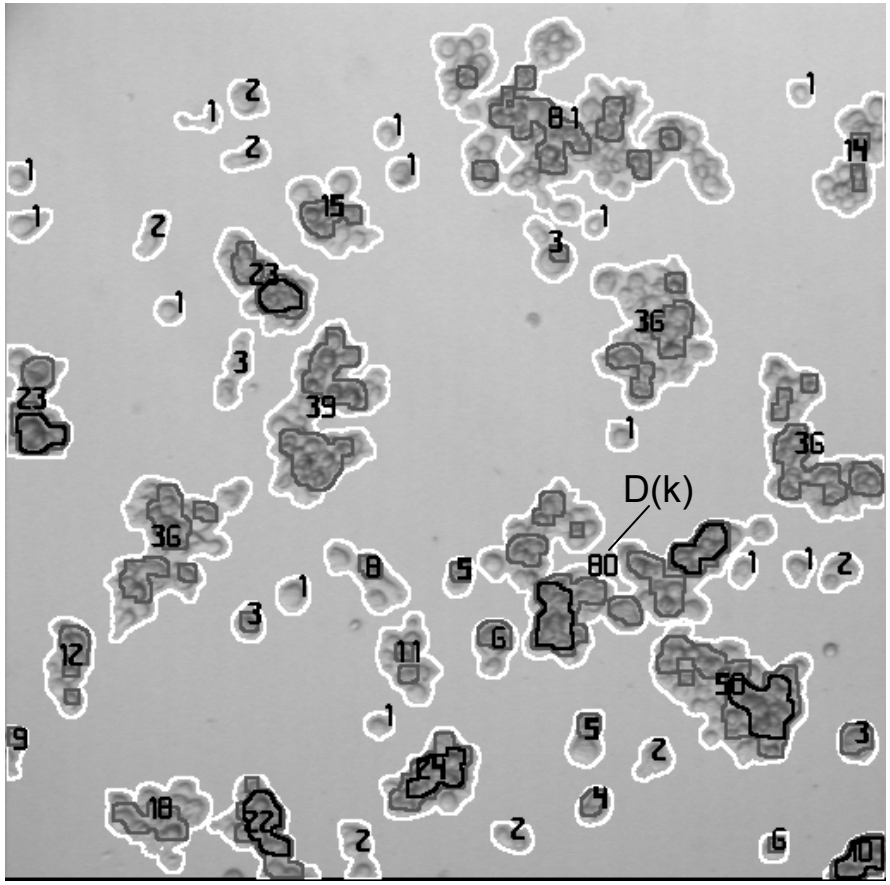


3D Cell Counting

8) Compute the number of cells D(k) in each segmented cell cluster k as follows:

$$D(k) = \frac{F \cdot A_1(k) + 2 \cdot A_2(k) + 2 \cdot A_3(k)}{\pi \cdot R_k^2},$$

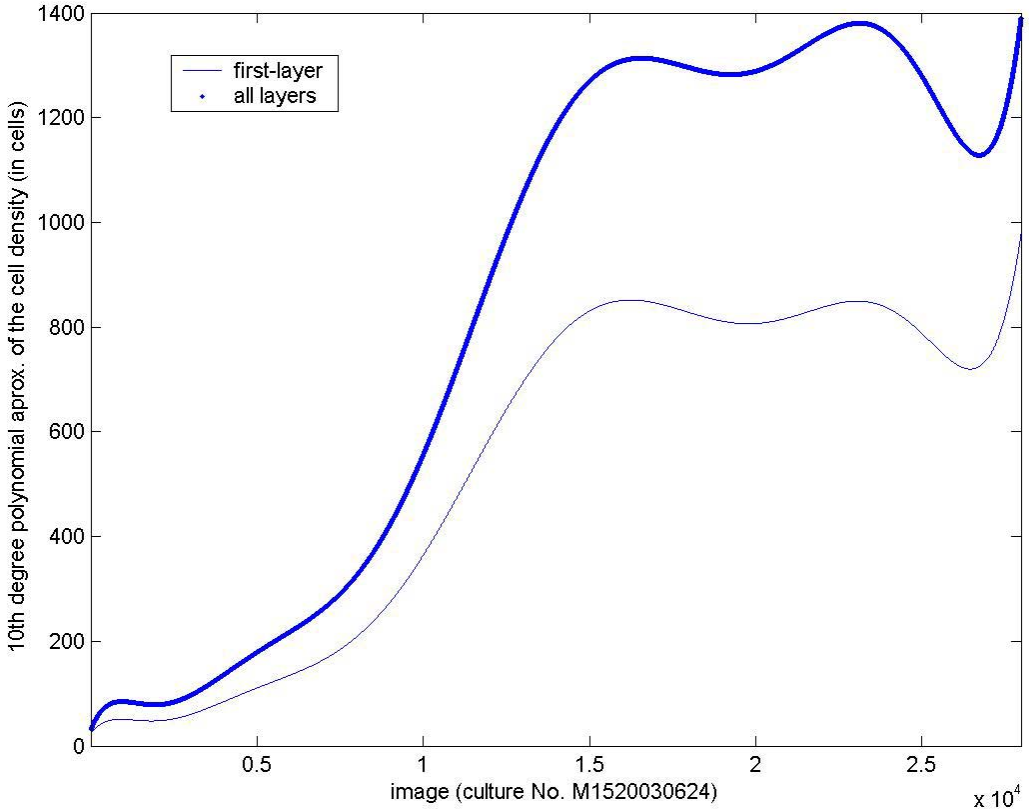
where $A_1(k)$, $A_2(k)$ and $A_3(k)$ are the areas of the segmented image regions of the parallel projections of the first-, second- and third-layers of the cell cluster k into the image plane and F is a segmentation error correction factor



Experimental Results

9) Compute the total number of cells D of the image I

$$D = \sum_{k=1}^{K} D(k)$$



Total number of cells D per image obtained by applying the proposed algorithm to 27980 images of a real culture of mammalian Baby Hamster Kidney cells (BHK cells)

Conclusions

- An algorithm is presented for counting cells in the first-, second- and third-layers of three-dimensional three-layer cell clusters from an intensity image captured by an in-situ microscope
- The number of cells in an arbitrary cell cluster is estimated as the ratio between the sum of the areas of the image regions of the parallel projections of the first-, second- and third-layers of the cluster into the image plane and the image area of a circle with a radius equal to the average cell radius of the cluster
- The experimental results revealed that counting the cells also in the second- and third-layers of the clusters improved the cell count in 56% in high cell concentrations