Cell Cluster Segmentation Based on Global and Local Thresholding for In-Situ Microscopy

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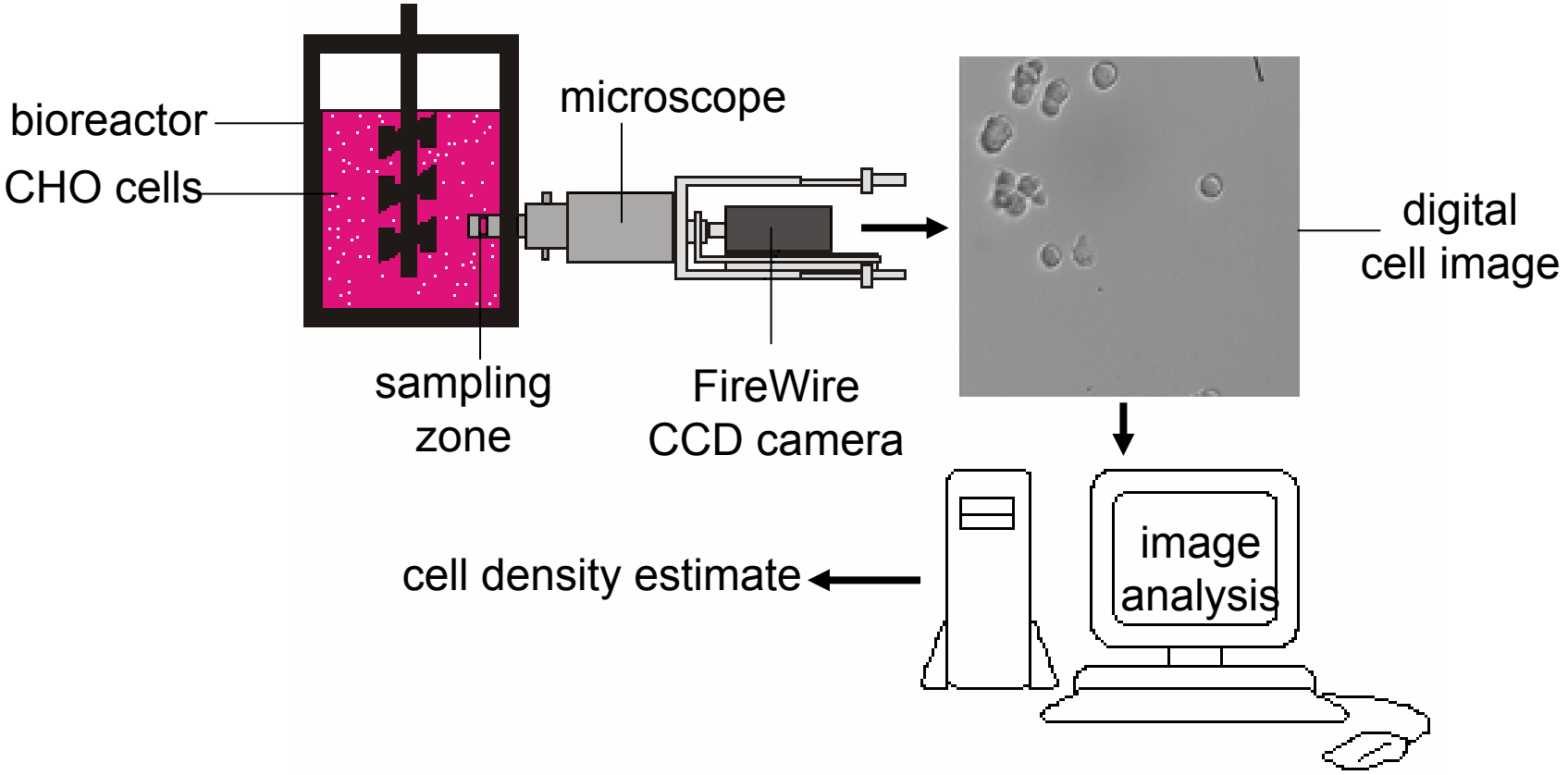
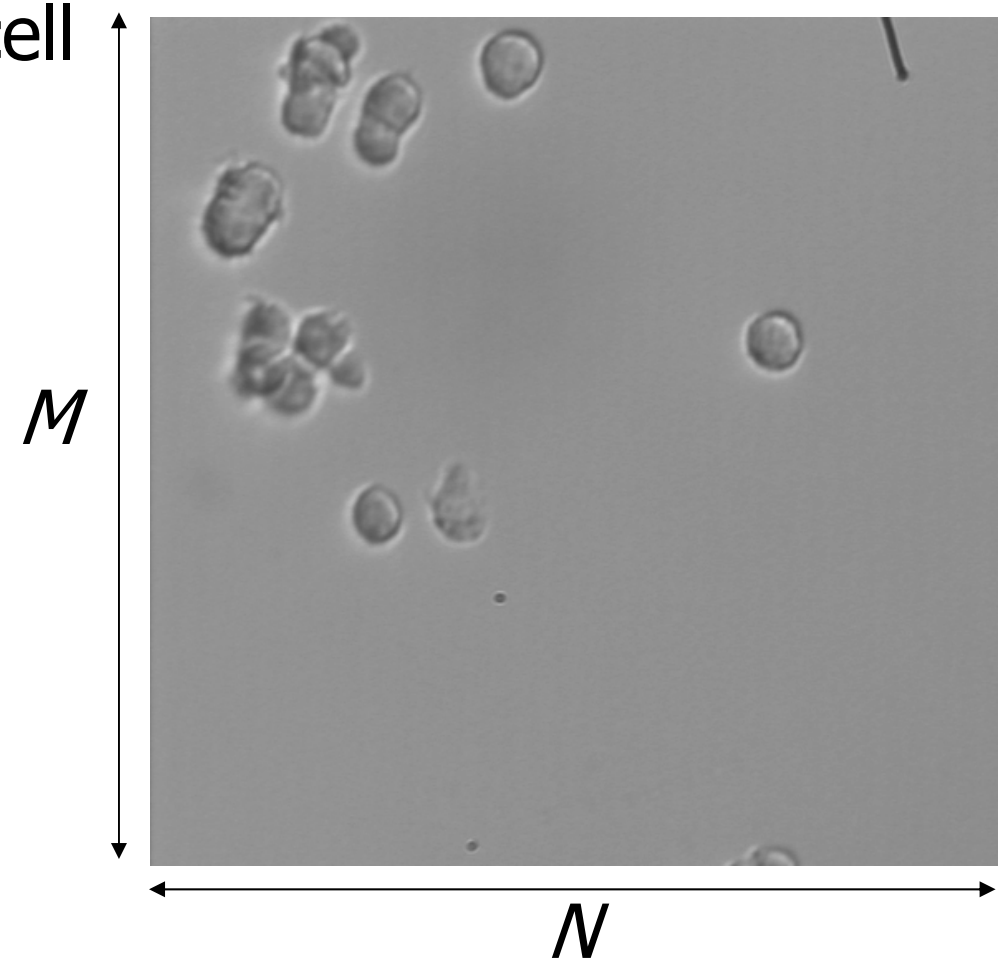
In-Situ Microscopy

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



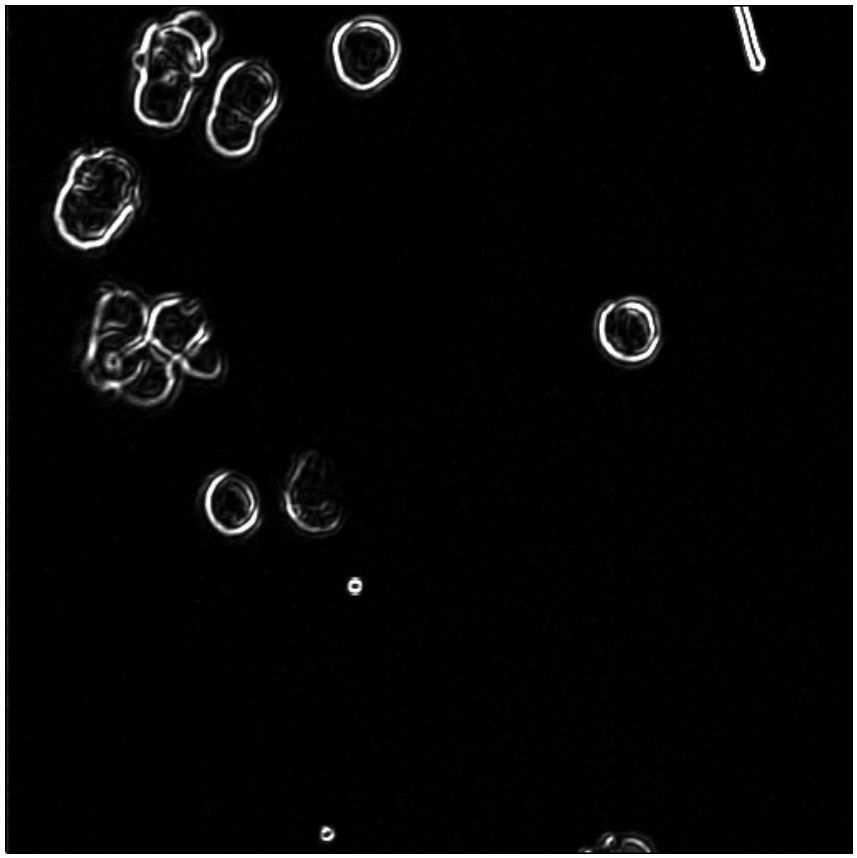
Global Threshold Estimation Algorithm

1) Capture a digital cell image I_i,
   i:0…(N-1)(M-1)*



Global Threshold Estimation Algorithm

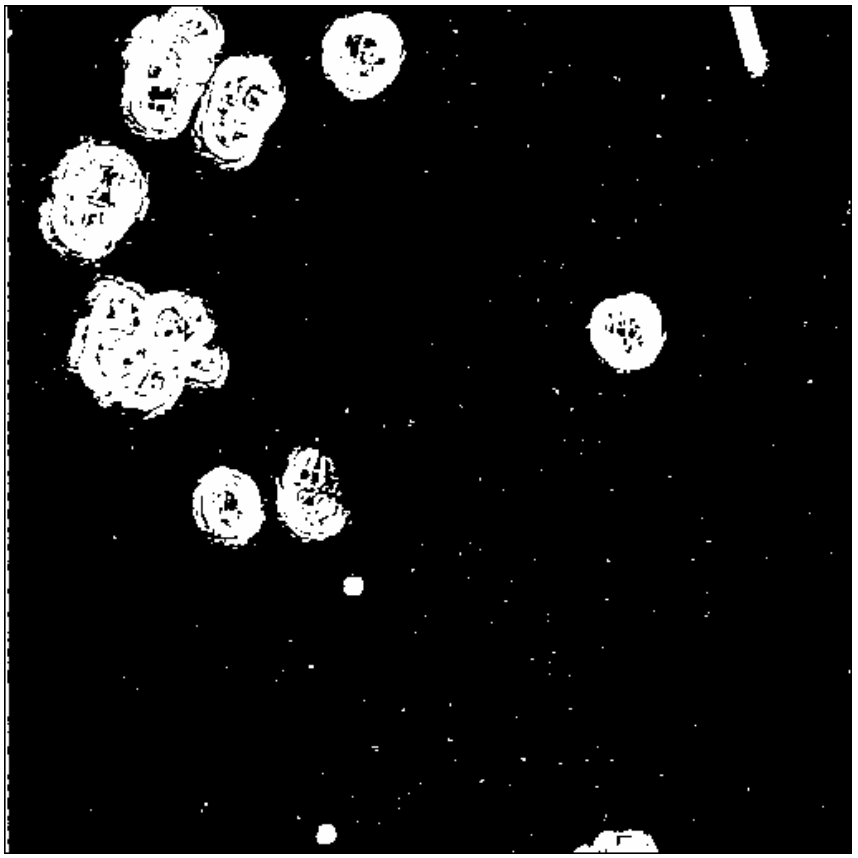
2) Compute the local variance image V_i



Global Threshold Estimation Algorithm

3) Classify all the pixels of the variance image V_i into pixels of the cell clusters and pixels of the background by using the global Maximum-Likelihood threshold th_g

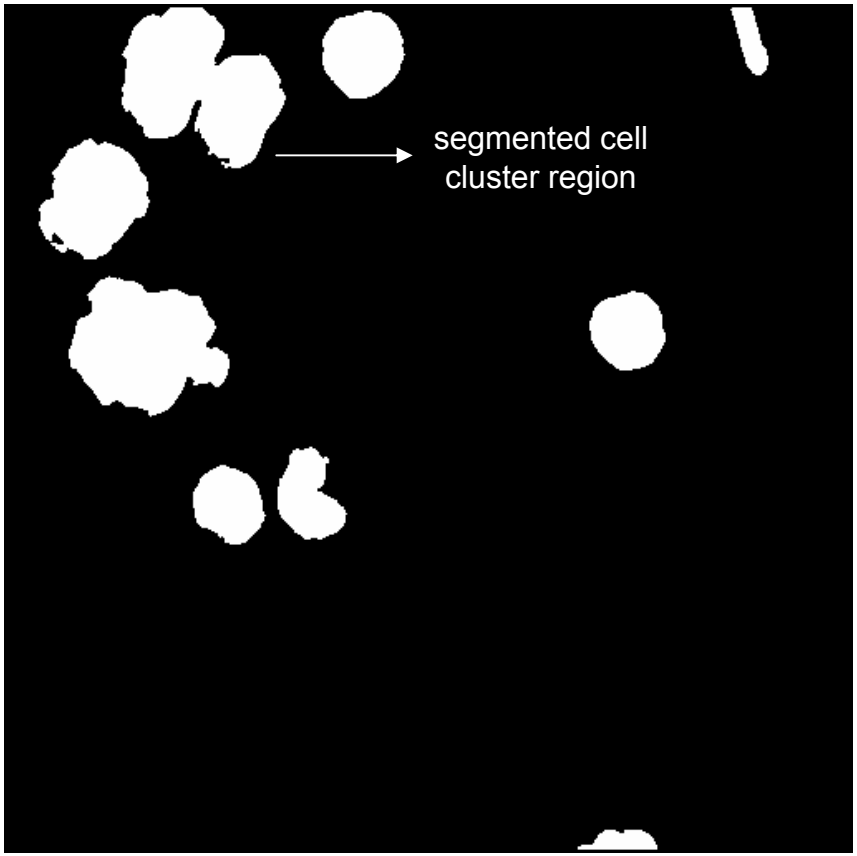
$V_i \leq th_g$: background
$V_i > th_g$: cell cluster



Global Threshold Estimation Algorithm

4) Eliminate isolated white pixels by applying a 5x5 median filter

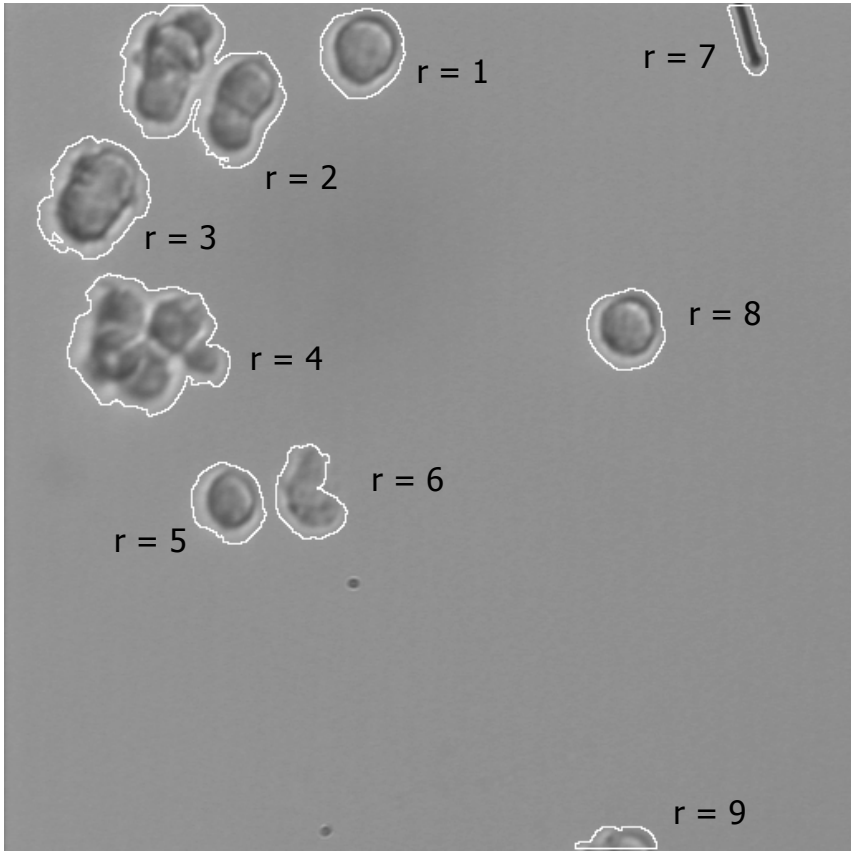
5) Eliminate black holes inside white regions and also those white regions whose image area is less than 0.05% of the total image area



cell cluster region (white)
background (black)

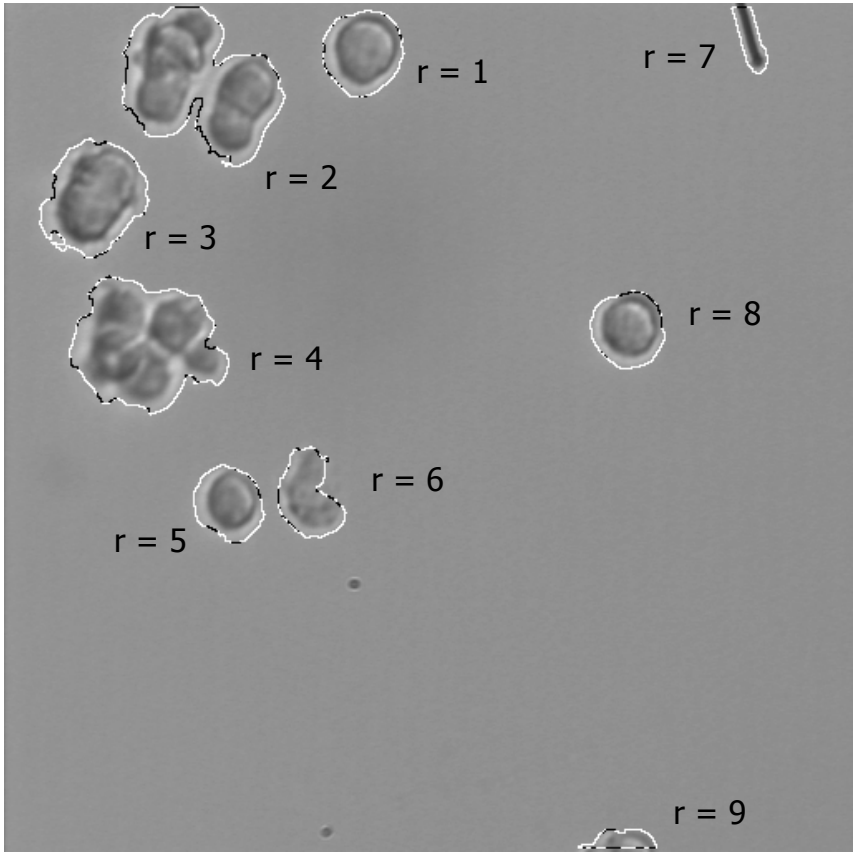
Local Threshold Estimation Algorithm

6) Select for each segmented region *r* all the border pixels $I_g^{(r)}$, $g:0\ldots G^{(r)}$



Local Threshold Estimation Algorithm

7) Choose for each segmented region *r* only those border pixels $I_q^{(r)}$ (inliers), $q:1\ldots Q^{(r)}$, $Q^{(r)}<G^{(r)}$, with an intensity value similar to the background intensity value by applying a RANSAC algorithm



inliers (white)
outliers (black)

Local Threshold Estimation Algorithm

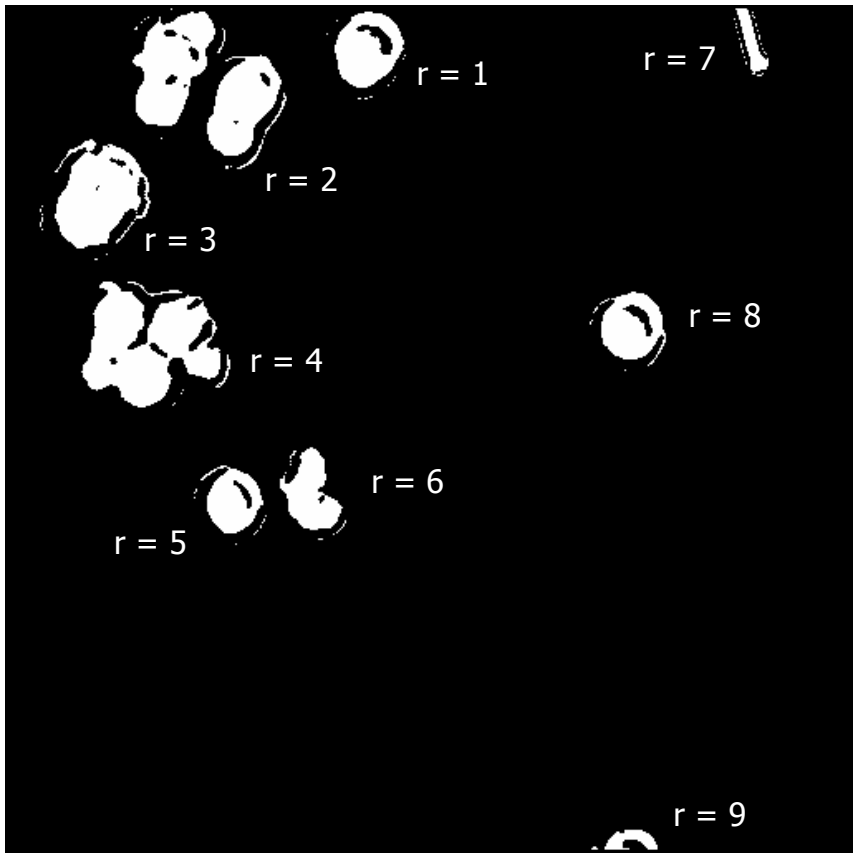
8) Estimate for each segmeted region *r* the local threshold $th_l^{(r)}$ as follows

$$th_l^{(r)} = \frac{1}{Q^{(r)}} \sum_{q=1}^{Q^{(r)}} I_q^{(r)}$$

Local Threshold Estimation Algorithm

9) Reclassify all the intensity pixels I_i inside of each segmented region r using the local threshold $th_i^{(r)}$

$I_i \leq th_i^{(r)}$: cell cluster
$I_i > th_i^{(r)}$: background

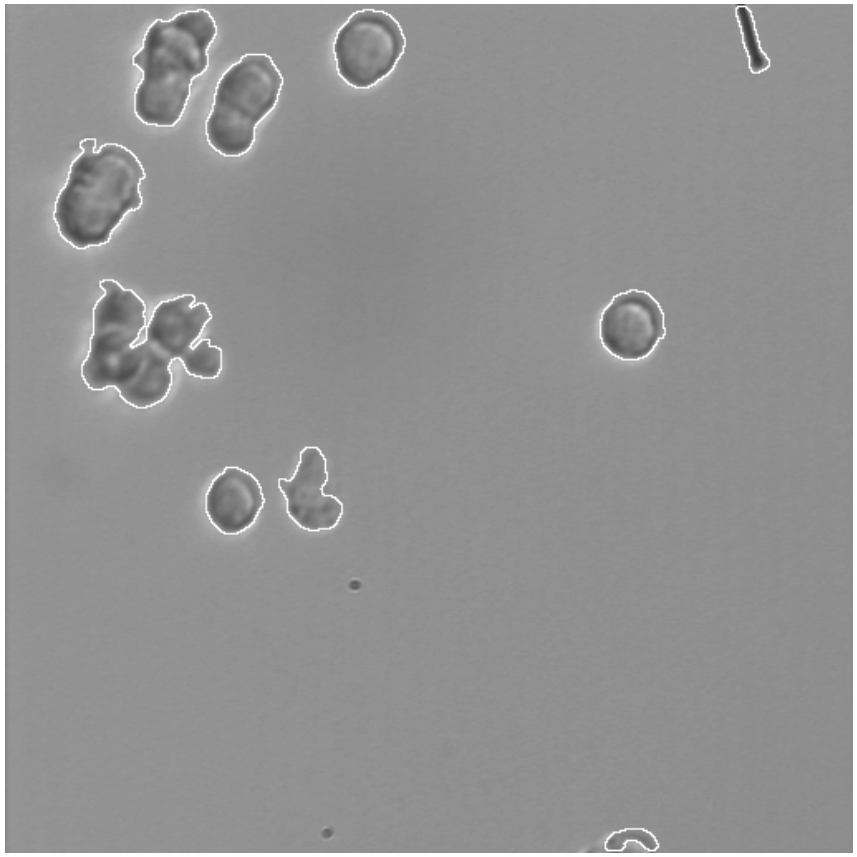


cells (white)
background (black)

Local Threshold Estimation Algorithm

10) Eliminate isolated white pixels by applying a 5x5 median filter

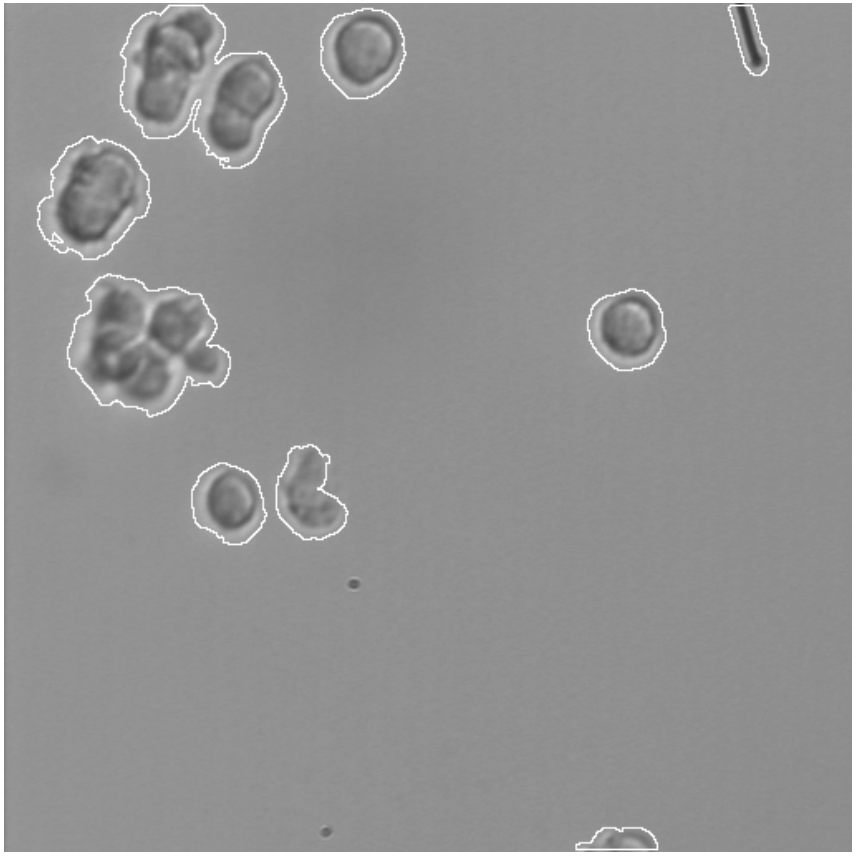
11) Eliminate black holes inside white regions and also those white regions whose image area is less than 0.05% of the total image area



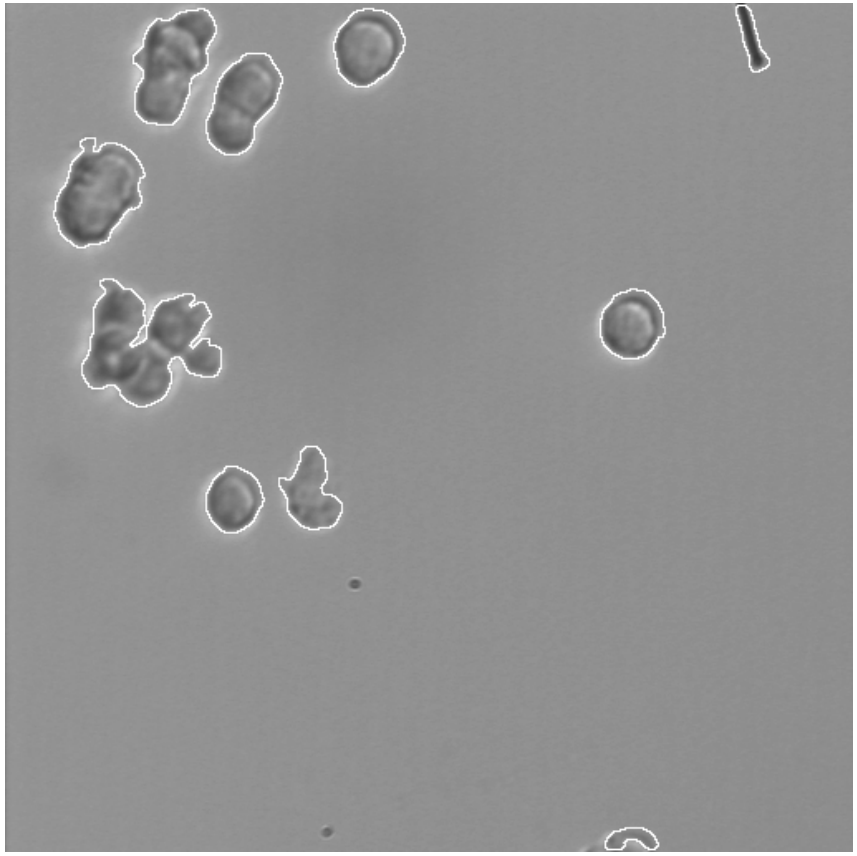
Results

- Experiments performed on real intensity images of CHO (Chinese Hamster Ovary)-cells
- Average processing time: **2.62 s**
- MSE used to measure segmentation accuracy against manually drawn segmentations

Results

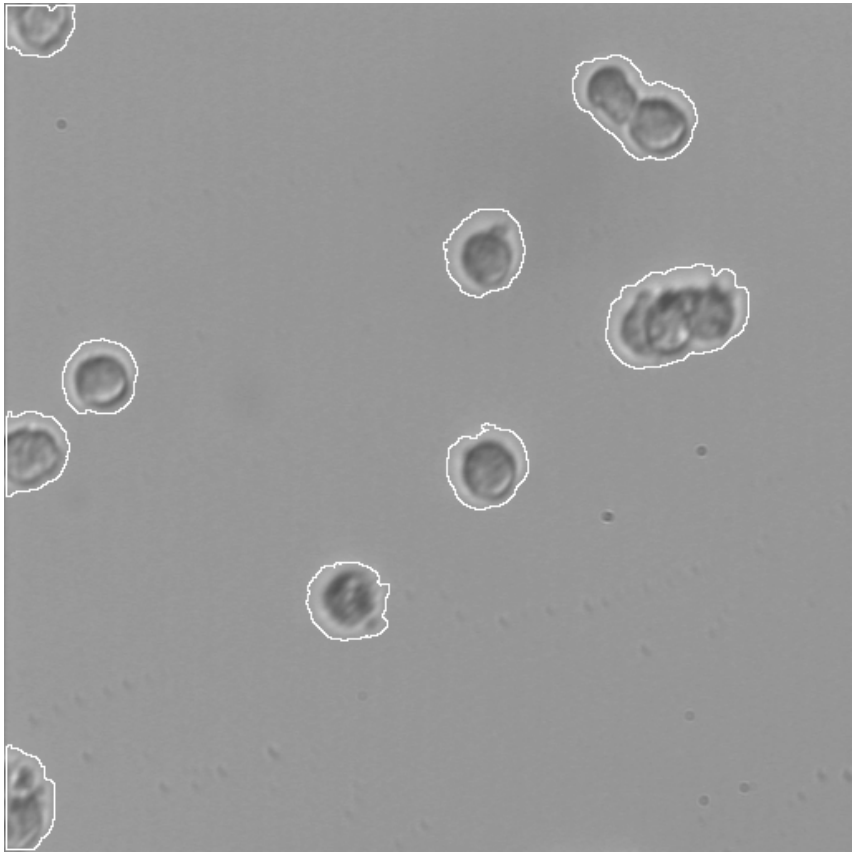


Global thresholding

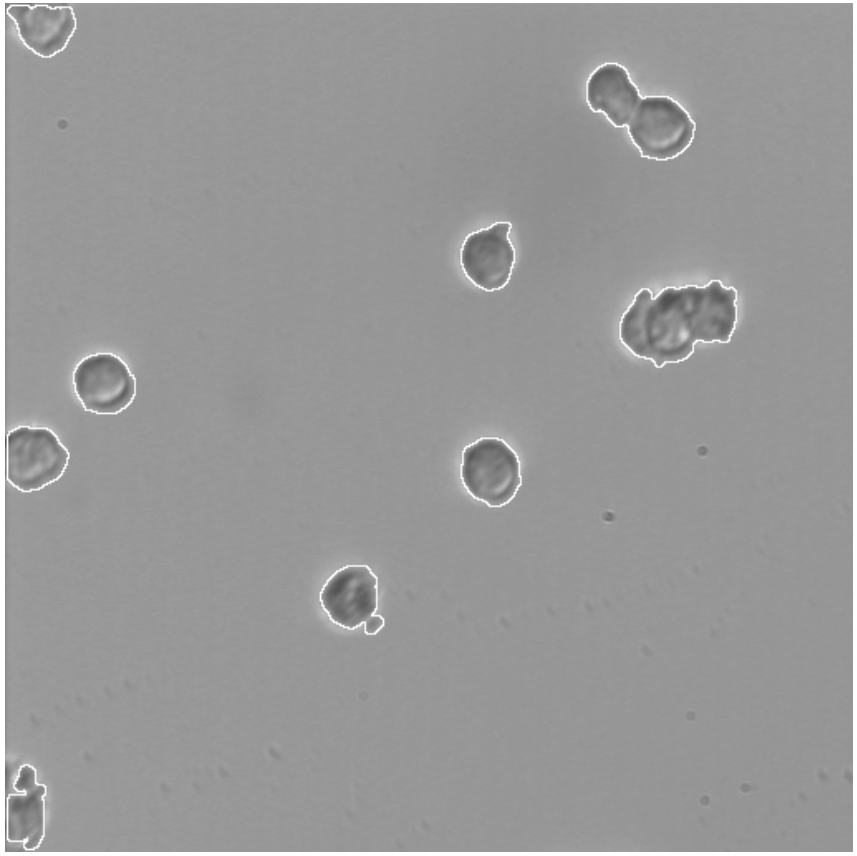


Global and local thresholding

Results

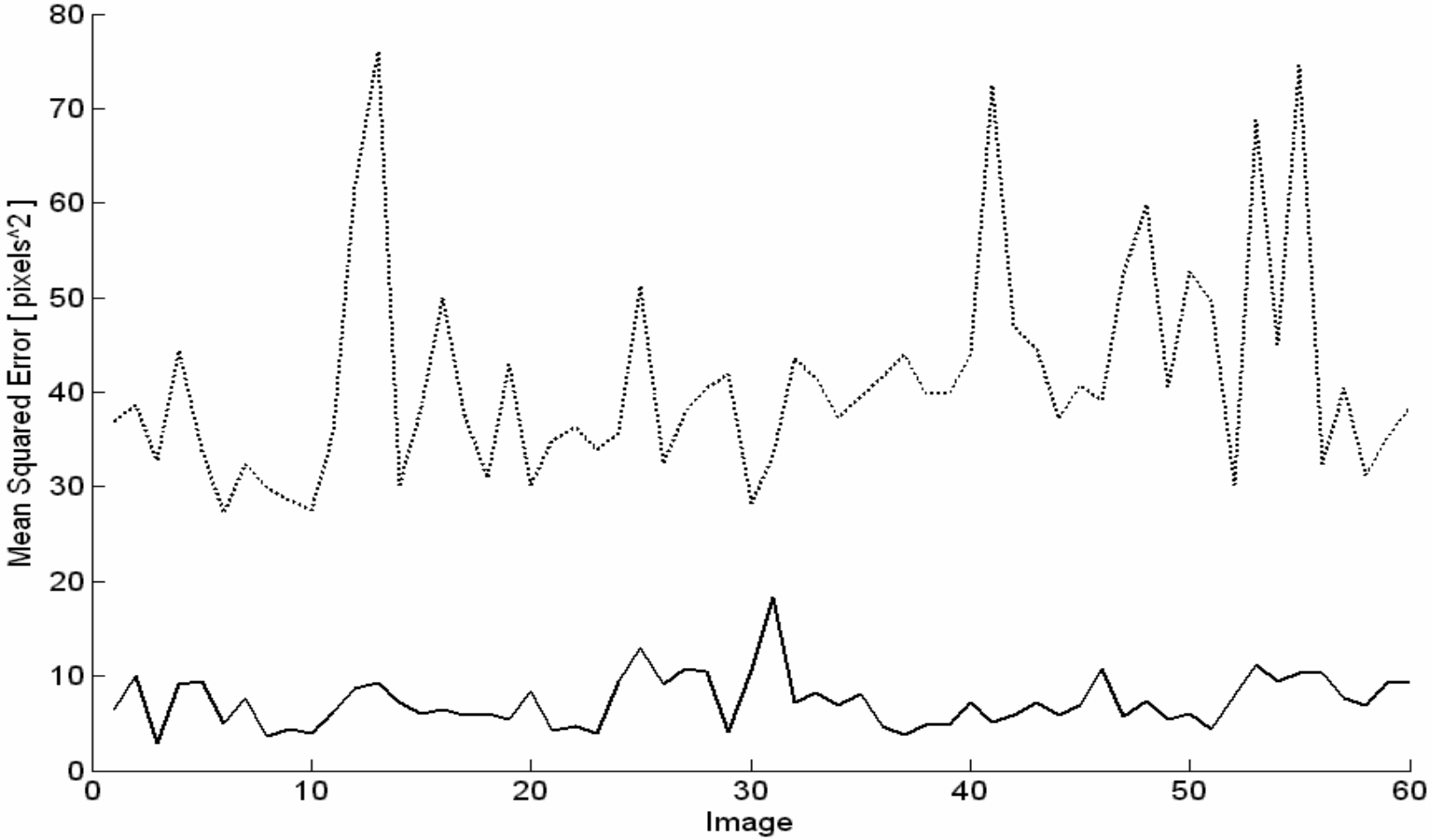


Global thresholding



Global and local thresholding

Results



MSE of the segmentation algorithm based only on global thresholding (dotted line)
MSE of the segmentation based on global and local thresholding (solid line)

Conclusions

- First all image pixels are classified into pixels of the background and pixels of the cell clusters using a global threshold
- Then the misclassifications are corrected by classifying again all the pixels inside of each segmented cell cluster using a local threshold
- Average processing time: **2.62 s**
- Segmentation accuracy improvement: **82.26%**