Cell Counting Based on Local Intensity Maxima Grouping for In-Situ Microscopy

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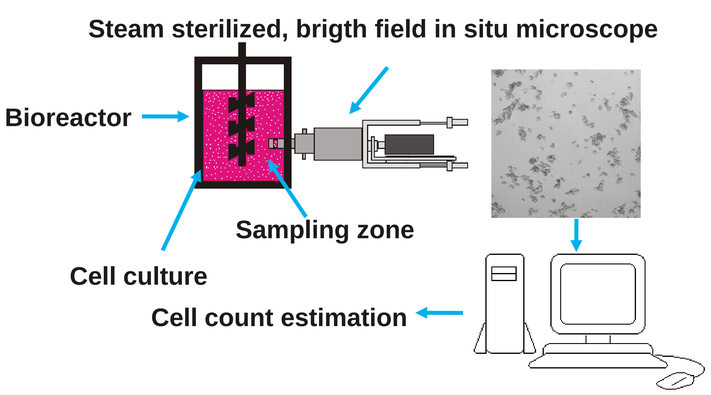
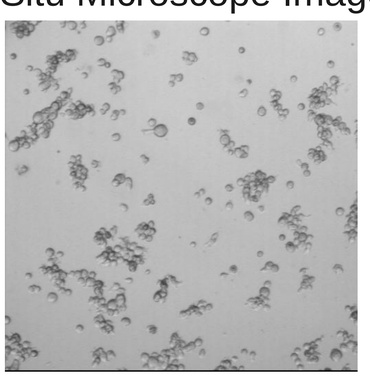
In Situ Microscopy



Cell count in the bioreactor can be estimated from an intensity image, through image processing algorithms.

In Situ Microscope Image

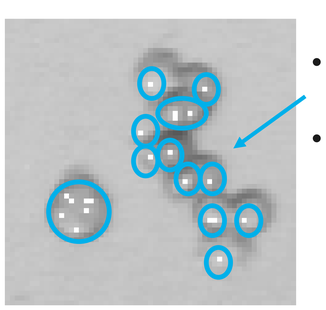


Three important characteristics are to be noted:

1. Cell borders are always darker tan cell interiors.
2. At least one local intensity maxima inside each cell.
3. Local maxima inside a given cell are close to each other, but far from local maxima inside neighbor cells.

Cell Counting Algorithm

1. Segment cell cluster regions.
2. Detect local maxima inside each cell cluster region.
3. Group local maxima inside each cell cluster region, so that each group contains only those local maxima that are inside the same cell.
4. Count the number of local maxima groups. Number of local maxima groups = number of cells on the image.



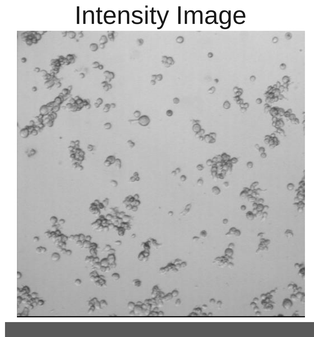
- Each white pixel represents a local maxima.
- Each light blue circle represents a local maxima group.

1. Cell Cluster Segmentation

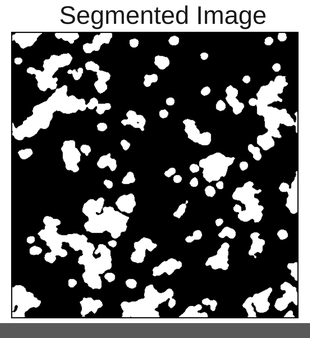
Martinez's cell segmentation algorithm is used (ICASSP, 2005):

1. Estimate local variance for each pixel on the image.
2. Classify pixels in two clases: background and cell clusters.
3. Generate a binary image, where black pixels are background and white pixels are cell clusters.
4. Apply a median filter, and remove small regions. Let *K* be the number of segmented clusters, and *k* an arbitrary cluster.

Intensity Image



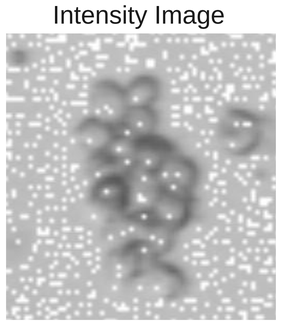
Segmented Image



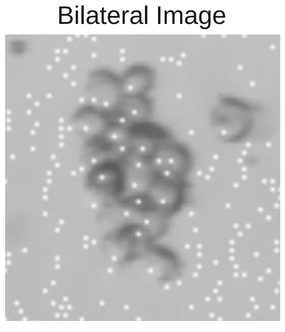
2. Local Maxima Detection

1. Bilateral filter is applied to intensity image, to remove undesired local máxima. Result is known as bilateral image.

Intensity Image

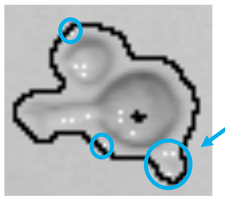


Bilateral Image



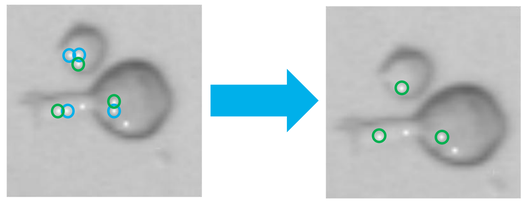
2. Detect all local maxima present in bilateral image.
3. Remove all local máxima present in bilateral image, that are not inside a segmented cell cluster region.

4. Remove all local maxima close to the segmented cell cluster border.



Local maxima close to the cell border are likely to be part of the image's background (over-segmentation).

5. Merge local máxima that are very close to each other.

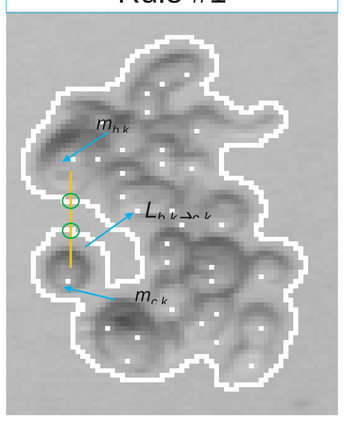


Local maxima very close to each other is likely to be on the inside of the same cell (redundant).

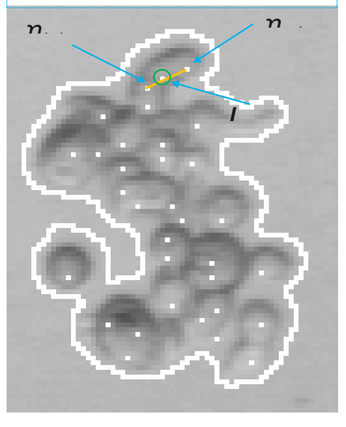
3. Local Maxima Grouping

- Local maxima are first grouped in pairs. Let $m_{b,k}$ and $m_{c,k}$ be two local maxima from the k-eth cluster, $L_{b,k \to c,k}$ be the line profile between $m_{b,k}$ and $m_{c,k}$, I($m_{b,k}$) and I($m_{c,k}$) their respective intensity values, and p($m_{b,k}$) and p($m_{c,k}$) their respective positions.
- Local maxima $m_{b,k}$ and $m_{c,k}$ are grouped into the pair ($m_{b,k}$,$m_{c,k}$) if 5 rules are met:
  1. $L_{b,k \to c,k}$ does not intersect any segmented cell cluster region border.
  2. $L_{b,k \to c,k}$ does not contain any local maxima other than $m_{b,k}$ and $m_{c,k}$.
  3. $L_{b,k \to c,k}$ does not intersect any cell border. Cell borders are detected via valleys in $L_{b,k \to c,k}$.
  4. $m_{b,k}$ and $m_{c,k}$ have similar intensity values, as measured by the relative intensity difference between I($m_{b,k}$) and I($m_{c,k}$) being smaller than a given threshold.
  5. p($m_{b,k}$) and p($m_{c,k}$) are close to each other, as measured by the 2D euclidean distance between p($m_{b,k}$) and p($m_{c,k}$) being smaller than a given threshold.

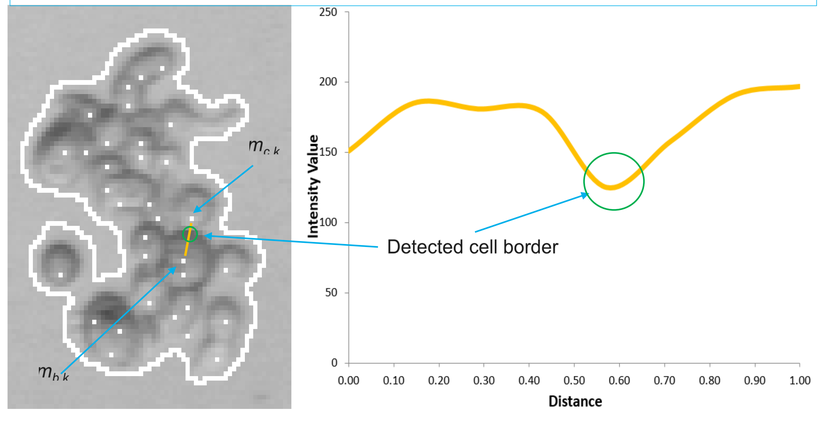
Rule #1



Rule #2



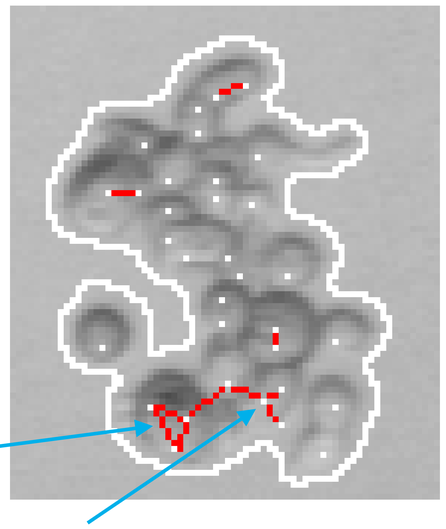
Rule #3



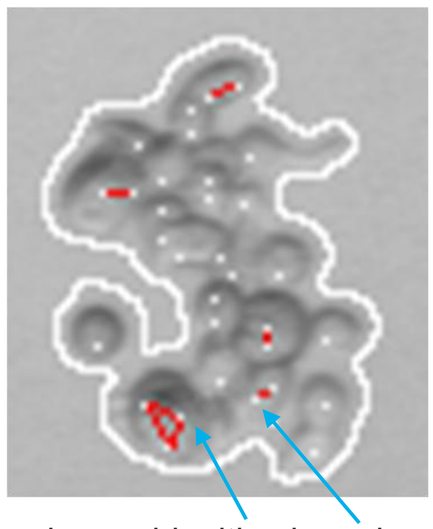
3. Local Maxima Grouping

- Now, local maxima pairs are now merged into larger groups.
- Two or more local maxima are merged into a larger group if they share a common local maximum, and the previously mentioned set of 5 rules are satisfied for all the pairs that can be formed out of the individual local maxima to be grouped.

Three local maxima pairs that qualify to be grouped into a single group.



Local maxima can be grouped to more than one group. Such ambguities are solved by assigning such shared local maximum to the nearest group.



Grouping ambiguities have been solved. Every local maximum now belongs only to one group.

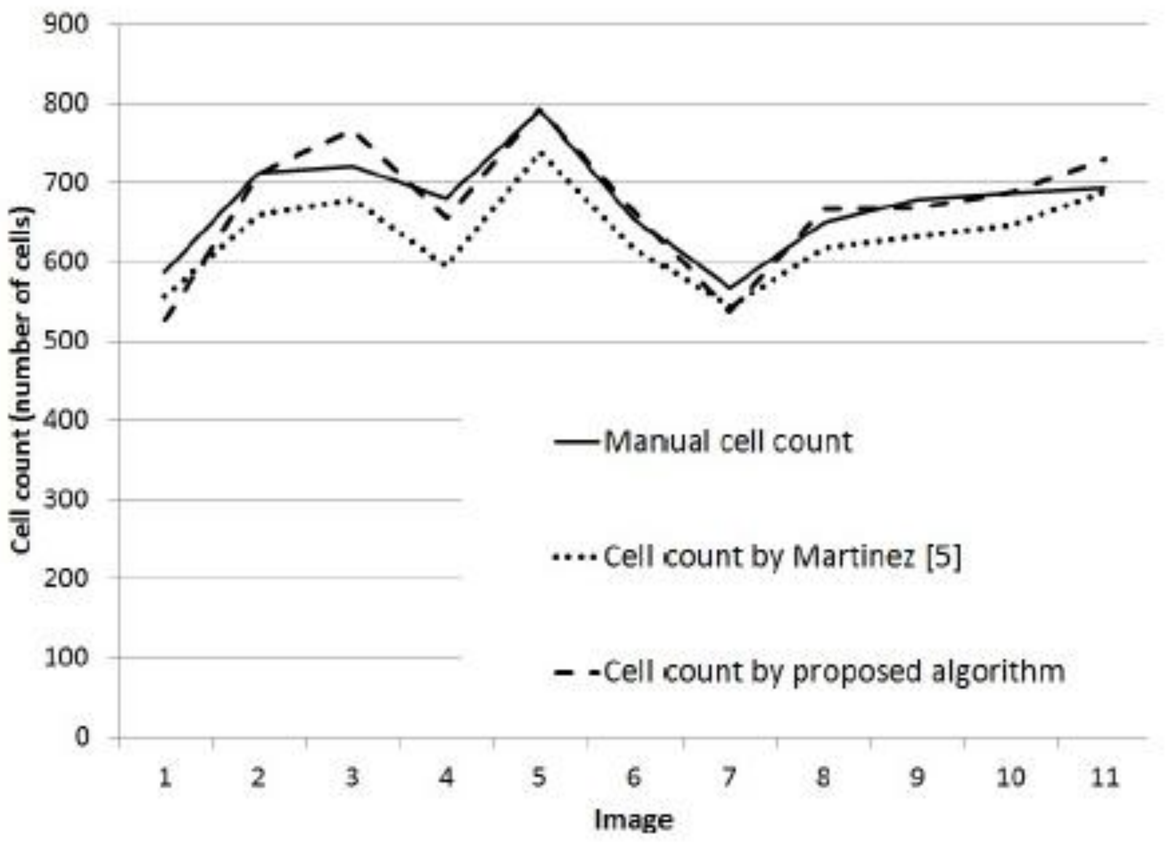
4. Cell Counting

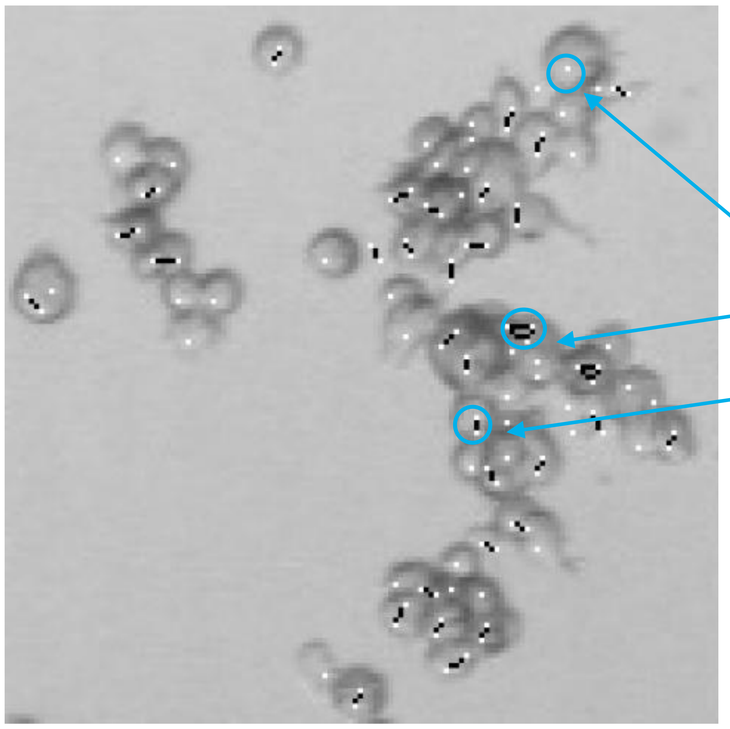
- Let $D_{I,k}$ be the amount of local maxima groups found on the *k*-eth segmented cell cluster belonging to image I.
- The total number of cells (D_I) is estimated as the sum of all the amounts of local maxima groups found on all segmented cell clusters:

$$D_I = \sum_{k=0}^{K-1} D_{I,k}$$

Expermental Results

- Proposed algorithm has been tested on a set of 11 still intensity images captured by the in-situ microscope.
- To facilitate the comparison process, same set of images used by Martinez (ICASSP, 2005).
- Obtained results are compared against Martinez's, as well as to manual counting results.
- Average absolute error was of 3.35% (Martinez yielded 6.01%) - **79% improvement on cell count accuracy**.
- Average processing time per image was of 3.00 sec (Martinez yielded 5.15 sec) – **42% improvement on image processing time**.





Light blue circles highlight resulting local maxima groups. Some groups are conformed of one local maxima, others contain two local maxima, and a few contain three or more local maxima.

Conclusions

- A new cell count algorithm for in-situ microscopy is presented. Local maxima present inside each cell cluster are grouped by evaluating their relative intensity values and position. The number of local maxima groups equals to the number of cells present in the image.
- Experimental results show that cell count accuracy improved by 79%, and processing time per image improved by 42%.
- Algorithm is highly relying on clear cell border edges, as well as precise cell cluster segmentation. Future work will include edge enhancement algorithms to highlight cell edges, as well as improved cell cluster segmentation algorithms, to avoid local maxima belonging to the backgrouned to be wrongly grouped as part of a cell cluster (over-segmentation).