A Combined Watershed and Level Set Method for
Segmentation of Brightfield Cell Images

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ABSTRACT
Segmentation of brightfield cell images from microscopy is challenging in several ways. The contrast between
cells and the background is low. Cells are usually surrounded by “halo”, an optical artifact common in brightfield
images. Also, cell divisions occur frequently, which raises the issue of topological change to segmentation. In
this paper, we present a robust segmentation method based on the watershed and level set methods. Instead of
heuristically locate where the initial markers for watershed should be, we apply a multiphase level set marker
extraction to determine regions inside a cell. In contrast with the standard level set segmentation where only one
level set function is used, we apply multiple level set functions (usually 3) to capture the different intensity levels
in a cell image. This is particularly important to be able to distinguish regions of similar but different intensity
levels in low contrast images. All the pixels obtained will be used as an initial marker for watershed. The region
growing process of watershed will capture the rest of the cell until it hits the halo which serves as a “wall” to
stop the expansion. By using these relatively large number of points as markers together with watershed, we
show that the low contrast cell boundary can be captured correctly. Furthermore, we present a technique for
watershed and level set to detect cell division automatically with no special human attention. Finally, we present
segmentation results of C2C12 cells in brightfield images to illustrate the effectiveness of our method.

Keywords: Segmentation, watershed, level set method, brightfield, cell image, microscopy

1. INTRODUCTION
Light microscopy has played a critical role in medical science for more than a century. It combines a unique
capacity to extract detailed pictures of the interrelated structure and dynamics of biologic systems, which can
be used to explore fundamental processes such as cell metabolism, cell movement, cell cycle progression, cell
differentiation and cell death. Light microscopy can identify changes in cell behavior and morphology that reflect
the combined activity of entire networks of genes and proteins and serve both as a marker of cell health and as
an early indicator of incipient pathology. Because of the complexity of the cell shape and intensity distribution
in brightfield images, the classic methods that work fluorescence are no longer sufficient. Furthermore, because
of the high throughput nature of the systems that generate the images, the quality of data generated requires us
to have an algorithm that minimizes user intervention.

Images from phase-contrast microscopy are recorded in a time series, for instance, taken every fifteen minutes
over several days. Typical data sets contain hundreds of images that render manual analysis infeasible. Robust
automated tracking of cellular behaviors becomes central in analyzing the cell images. Typically, tracking is
carried out by frame-by-frame segmentation. First, a segmentation model is used to locate the position and
determine the shape of a cell in the current image. In this process, a cell boundary is identified by a closed curve.

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Then this closed curve is evolved so that it will become the segmentation of the same cell in the next image. Thus, the quality of the tracking system depends heavily on the quality of the segmentation model.

Several segmentation techniques have been used for analyzing cell images. Active contour segmentation methods such as snakes\textsuperscript{1,2} are often used in cell images. Cells change positions and shapes over time and active contour techniques are very suitable for capturing the dynamic shape changes. However, in the case of cell division where the closed curve is topologically changed into two closed curves, the parametric active contour approach may not be easily applicable. Modifications\textsuperscript{3} will be needed to handle such cases. The level set active contour methods,\textsuperscript{4,5} on the other hand, represent closed curves by the zero level set of a function. This implicit representation\textsuperscript{6} allows topological changes to occur without special manual changes. However, the implicit representation also allows two closed curves to merge. This may happen when two cells are close to each other. One solution is to introduce extra energy terms\textsuperscript{5} in the model. Another is to use multiple level set functions\textsuperscript{3,7} for multiple cells. In Ref. 8, a topology control is applied to prevent cell fusion. In general, level set segmentation is usually more computational expensive since nonlinear partial differential equations need to be solved. Statistical classification techniques and morphological operations are used for cell segmentation in Ref. 9. In Ref 10, instead of incorporating large number of adaptations in order to apply active contour or other segmentation techniques for cell boundaries, only the cell locations are tracked.

Most of the above previous work are developed for fluorescent images, in which only part of a cell (e.g. nucleus) is visible; see Figure 1 (top left). The cells are typically appear round shape and the intensities are more uniform within the cell. However, for biological reasons, fluorescent cells may not be preferable. In that case, brightfield images are used, which are usually poorer quality due to low contrast. In brightfield images, both the nucleus and cytoplasm of the cell are visible. Thus, the shape of a cell usually appears highly irregular. Also, the intensities within the cell interior show more variation. In addition, the cell is usually surrounded by “halo”, an artifact commonly seen in phase-contrast microscopy; see Figure 1 (top right). All these problems have presented difficulties to standard segmentation methods. Only a few techniques have been developed for brightfield images. In Ref 11, a semi-automatic segmentation technique is developed for brightfield images. It combines the techniques of texture-analysis approach and the gradient-detection approach. An active contour method based on snakes is developed in Ref 12. In order to eliminate the intensity variation within the cell, a level set segmentation is applied to a defocused cell image in Ref 13. As the defocusing decreases, the segmentation obtained approaches to the cell boundary. Brightfield images with many cells are tracked and segmented in Ref 8. Although there are many cells to deal with, each cell is relatively small. Thus they appear like dark patches which resemble the fluorescent images. Also, many of these methods do not address the issue of cell division.

In this paper, we present a robust segmentation method for brightfield images. The images we are interested in typically consists of tens of cells and so the details of each cell are visible. However, the cell interior also shows high intensity variation but low contrast compared with the background. The proposed segmentation method combines the advantages of the level set and watershed segmentation algorithms. By assigning initial markers at the appropriate locations, it is able to resolve the low contrast and cell division problems.

In Section 2, we discuss the issues of segmenting brightfield images. Then we present our segmentation method in Section 3. The algorithm combines watershed and the level set approaches. The former is designed to handle the issues of low contrast and cell division, and the latter provides the initial markers for the region growing process of watershed and further enhancement. Numerical results are presented in Section 4. Finally, conclusion is made in Section 5.

2. ISSUES OF BRIGHTFIELD IMAGE SEGMENTATION

Segmentation for brightfield images in general is more challenging than segmentation for fluorescent images. The latter usually has better contrast and segmentation techniques such as active contour usually work well. The former, however, have low contrast. Some part of the cell have similar intensities as the background. Standard segmentation techniques either does not work or require substantial manual effort to fine tune model parameters.

As an example, an active contour level set segmentation\textsuperscript{4} is applied to segment a fluorescent and brightfield image of the same cell. see Figure 1 (bottom left & right). This segmentation technique has been used in many image processing applications. In this example, it works quite well to capture the boundary of the fluorescent
cell image. Only a few iterations are needed to converge to the correct boundary. However, it does not work for the brightfield image. The problem is caused by the “halo”, bright white patches surrounding the cells, typically present in most brightfield images. The halo is usually very distinctive, and so the active contour segmentation regards it as the object of interest, instead of the cell interior which has similar intensities as the background. In fact, one might argue that the active contour segmentation is doing the right thing by capturing the most distinctive feature in the image. However, in the context of cell image analyses, it is not what we desire.

3. METHODOLOGY

Interestingly, because of the presence of the bright halo surrounding the cell interior, we find that watershed\textsuperscript{14,15} is especially suitable for segmenting brightfield images. The reason is that once initial markers are placed appropriately within the cell, the region growing process of watershed will capture the entire cell interior until it hits the halo which serves as a “wall” that stops the expansion. However, due to low image contrast and broken halo, the region growing of the cell may “leak” into the background; or vice versa, the region growing of the background may also “leak” into the cell interior. As it turns out, good initial markers are crucial. We first describe the issues of marker extraction, and then present a level set approach. We will also explain how the combined method can detect cell division.

3.1 Watershed segmentation

Watershed has been used for segmentation of cell or other images.\textsuperscript{16–18} For cell images, it is often used as a technique for dividing many cells in one image.\textsuperscript{17} In this paper, we are interested in brightfield cell images that have relatively fewer cells. However, each cell shows more details and the intensities within the cell have relatively larger variation; i.e. some parts of the cell are darker or lighter than the background but other parts may be similar to the background intensities.

The watershed segmentation algorithm is a region growing method that simulates a flooding process. A 2D image can be viewed as a topographical surface by visualizing the pixel intensity as height. Intuitively, by putting a number of water sources at various pixel locations and allow the water to flow out and flood freely, water coming from different sources will eventually meet along watershed lines. Viewing these watershed lines as
boundaries of different regions, a segmentation result is obtained. Mathematically, in the first step, a number of markers (pixel positions of water sources; extracted either manually or automatically) are given different labels. For each non-marker pixel, topographical distances between it and each marker is calculated. The pixel is then assigned the same label as the marker having the shortest topographical distance to it among all markers. In rare situations where there are more than one marker attaining the same minimum distance, a tie breaker is used. In our implementation, we use a FIFO queue data structure to handle tie breakers automatically. After assigning labels to each non-marker pixel, the different labels associated to different pixels give the segmentation result.

3.2 Extraction of markers

For the cell images we are interested in, the contrast is low. Moreover, the halo is usually broken; i.e. it does not completely surround the cell interior. If there exists a path connecting a background point to a cell interior point such that the pixel intensity variation along the path is small, then watershed tends to group this path under a single region. To prevent this from happening, our idea is to place more cell interior markers inside the cell, and place more background markers outside the cell.

Figure 2. (Top right) Segmentation result using only 2 cell interior markers (2 white dots) and 4 lines of background markers (top left). (Bottom right) Segmentation result using more cell interior markers (white patches) and 4 lines of background markers (bottom right).

Figure 2 shows an example that motivates this idea. As shown in Figure 2 (top), only two pixels are used for the cell interior markers and four lines of pixels are used for background markers. Note that the pixel intensities within the cell typically vary from dark (below background) to light (above background). If the pixel markers happen to have intensities that are close to the darker side (or lighter side), then the region it grows into will tend to have a similar darker (or lighter) intensities. Now, if the halo more or less surrounds the cell, then the cell interior markers will eventually capture all intensities within the cell. However, if the halo is broken as in this case, the cell boundary is “weaker” and those cell interior pixels with intensities similar to the background will tend to connect to the background region. As a result, the watershed segmentation misses part of a cell.

The problem is that the two intensities of the two cell markers unlikely represent most intensities within the cell. A natural remedy is to include more markers so that more intensities are included; see Figure 2 (bottom). With more cell markers, the segmented contour captures better the “weaker” cell boundary.
To heuristically find a point or two as a marker within a cell is relatively easy. However, it is not as obvious to find a marker patch within a cell. Note that we do not want the cell marker patch to overlap the background at all; otherwise, we would obtain a cell region which covers a lot of the background since the background has low intensity variation. An intuitive approach is to make use of the segmentation result of the previous frame. Since cells do not move much between two frames, the interior of the previous cell has a large overlap with that of the current cell. We may obtain a marker patch by shrinking the previous cell region. It works in some cases but it is not robust.

3.3 Level set marker extraction

Our approach is to use level set method to determine the cell marker. We show in Figure 1 that standard level set segmentation does not work well for brightfield cell images. However, for marker extraction, we just need it to identify the main body of the cell approximately.

The active contour level set segmentation used in Figure 1 is a two-phase level set approach. That is, the level set function divides the image into the region inside the cell and the region outside the cell (background) such that the intensities in each region are more or less the same. In other words, it tries to identify the two intensities that best approximate the entire image. It works well for fluorescent images where the intensities of the cells and the background are different. It does not work well for brightfield images where the cells have much more intensity variation and there is very little contrast between the cells and the background. Although we only need to find an approximate cell interior, we found that the two-phase level set segmentation is just not enough.

Instead, our marker extraction method is based on a multiphase level set framework. We use $m$ level set functions to identify $2^m$ intensity levels. The bigger the $m$, the more refined partition of the image is obtained. In our experience, $m = 3$ is sufficient for cell images. Let $\phi_i, i = 1, \ldots, m$ be the level set functions. For each of the $2^m$ disjoint regions, let $c_I$ be the mean intensity of the cell image $u(x, y)$, $I = 1, \ldots, 2^m$. We minimize the energy functional:

$$
\min_{\{\phi_i\}} \sum_{i=1}^{2^m} \int (u - c_I)^2 \chi_I \, dx \, dy + \sum_{i=1}^{m} \nu \int |\nabla H(\phi_i)|,
$$

where $\chi_I$ is the characteristic function of region $I$ and $H(x)$ is the Heaviside function. To find the solution of the minimization problem, the Euler-Lagrange equations are solved for $\{\phi_i\}$. For the case $m = 2$, we have 2 level set functions which divide the image into 4 disjoint regions. (Note that each region may not necessarily be a connected region.) The corresponding Euler-Lagrange equations are:

$$
\frac{\partial \phi_1}{\partial t} = \delta_{e}(\phi_1) \left\{ \nu \nabla \cdot \left( \frac{\nabla \phi_1}{|\nabla \phi_1|} \right) - \left[ ((u - c_1)^2 - (u - c_{01})^2)H(\phi_2) + ((u - c_{10})^2 - (u - c_{00})^2)(1 - H(\phi_2)) \right] \right\},
$$

$$
\frac{\partial \phi_2}{\partial t} = \delta_{e}(\phi_2) \left\{ \nu \nabla \cdot \left( \frac{\nabla \phi_2}{|\nabla \phi_2|} \right) - \left[ ((u - c_{11})^2 - (u - c_{01})^2)H(\phi_1) + ((u - c_{10})^2 - (u - c_{00})^2)(1 - H(\phi_1)) \right] \right\},
$$

where $\delta_{e}$ is an approximate Delta function, $c_{11}$ is the mean value of $u$ in the region where $\phi_1 > 0$ and $\phi_2 > 0$, and the other $c_{ij}$ are defined similarly. The parameter $\nu$ is to control the smoothness of the level set curves. The equations can be easily extended to general $m$.

After the equations are solved, we obtain $m$ level set functions and $2^m$ regions. We pick up the region where all $\phi_i < 0$. Since the background and cell interior usually have similar intensities compared to the halo and nearby pixels, the region we chosen often contains the cell interior as well as the background. We postprocess this region by removing the background and small isolated subregions. The resulting region is used as the initial marker for watershed. We remark that the marker region may not necessarily be connected and in fact, typically it consists of several patches. Since we only use it as an initial marker for watershed, it does not matter how it looks precisely at this point.
3.4 Cell division detection

Once the cell and background markers are determined, we apply the watershed algorithm to provide an initial segmentation of the cell. As shown in Figure 2, by choosing the cell interior markers appropriately, the watershed segmentation can capture the cell interior quite well, even the contrast between the cell and the background is low. However, note that these cells are living and they move and change shape. More importantly, they may undergo cell division; i.e. a cell is dividing into two separate cells. Mathematically speaking, the cell boundary undergoes a topological change from one closed curve into two separate closed curves.

To handle the topological change resulting from cell division, active contour segmentation using level set has been used for example in Refs.3–5. Level set segmentation methods represent a closed curve by the zero level set of a function.6 This implicit representation allows the zero level set to be a single or multiple closed curves. Thus, topological changes can be handled naturally. The standard watershed algorithm, however, is not designed to handle topological changes. It is because the number of watershed segmented regions is predetermined before segmentation (region growing process) and it is equal to the number of initial marker by default. For instance, suppose there is only one cell in the previous image. Then, naturally, one cell interior marker will be defined for the current image and hence only one cell region will be found. Heuristics may be used to guess the number of cells in the current image, but it will then lead to the robustness issue which may result in false cell division.

Here, we present a novel technique for watershed to detect cell division. At the end of the level set marker extraction, there are usually multiple (disconnected) patches obtained. In standard watershed, disconnected patches would be regarded as two separate regions and hence two different labels. But here, we put one label to all (disconnected) patches within the cell. Then we apply the standard region growing watershed algorithm. Suppose there is no cell division. In this case, the disconnected markers will grow into one single connected region. Note that although the markers are physically separated initially, as they all have the same label, they belong to the same region and hence merging of disconnected patches is allowed to take place. This is in contrast with the usual watershed in which the boundary where two separate regions meet forms a segmentation. In the case where there is a cell division, then the disconnected markers will grow into two separate regions and remain disconnected.

After the region growing process, we just need to examine the connectedness of the watershed segmentation result to determine if a cell has divided. Specifically, we examine the pixels with the same region label. If they are all connected, no cell division. Otherwise, the cell is divided.

We emphasize that our approach does not try to detect cell division before running watershed. Thus it differs from the standard watershed where a cell division is first detected and if so, markers with different labels are assigned to the two cell interiors. In our approach, all the markers placed in the cell interior are of the same label. Interestingly, by using only one label, cell division or no cell division can be handled uniformly without any special modification.

3.5 Final level set segmentation

The watershed segmentation only provides an initial segmentation of the cell. There are two issues of watershed segmentation. One is that the segmentation results given by watershed often have a saw-tooth boundary. In practice, a smooth boundary is usually preferred. Another issue is that the cell detection using watershed may not be very robust. For instance, watershed would consider no cell division if two essentially separated regions are touched only at a pixel. It becomes difficult to judge if there should be a cell division.

We apply the level set active contour segmentation4 to the binary image obtained from watershed. We note above that level set segmentation usually would not work well for the original brightfield image due to low contrast and bright halos. However, to apply the level set segmentation in the post-processing phase is much more efficient and accurate. Usually a few iteration would be sufficient to obtain a nice cell boundary.

The watershed and level set segmentation algorithms complement each other. The watershed segmentation alone would not be robust enough to accurately detect topological changes in cell division. The level set algorithm alone, on the hand, would have trouble dealing with the low contrast. In combining the two, a robust and effective segmentation method is obtained.
4. NUMERICAL RESULTS

We apply the combined watershed and level set segmentation algorithm for brightfield cell images showing live C2C12 cells obtained from experiments performed at the Genomic Laboratory, McGill University. We illustrate the effectiveness of our segmentation approach by the following examples. All computation are performed on a PC using MATLAB. The original image size of the cell images is $512 \times 512$, but for illustration purpose, only the portion of a cell is shown whose size is around $100 \times 100$.

![Figure 3. (Top) A cell is shrinking to a round shape which typically happens before a cell division, (bottom) watershed segmentation results.](image)

Figure 3 shows a sequence of images where a cell is shrinking to a round shape. This usually happens before a cell division. In this example, we apply our watershed segmentation using the heuristic marker extraction obtained from previous frame. It works well in some cases, but in general, it is better to use the level set marker. At the end, level set segmentation is still needed to obtain smooth segmentation contours.

![Figure 4. The 8 disjoint regions obtained from the multiphase level set marker extraction using 3 level sets.](image)
Figure 4 shows the eight different disjoint regions obtained from the multiphase level set marker extraction. The two images on the top left capture the halo. The one on the bottom right captures the cell interior and the background. The latter is used as the initial marker for the watershed segmentation after the removal of the background and small isolated regions.

![Figure 4](image)

Figure 5. (Top left) The brightfield image of a cell undergoing cell division, (top right) the initial marker obtained from the level set marker extraction, (bottom left) watershed segmentation, (bottom right) final segmentation result.

We illustrate the combined watershed and level set segmentation process in Figure 5. The initial marker (top right) obtained from the multiphase level set marker extraction (Figure 4) covers a significant but incomplete portion of the cell. The watershed segmentation (bottom left) captures the remaining parts; however, notice the staircase effect appear on the segmented boundary. Note also that the top and bottom parts of the cell are barely connected by a thin pixel line. The final level set segmentation (bottom right) gives a nice and smooth boundary. Moreover, it splits the region into two to signal a cell division has taken place.

Figure 6 shows a sequence of images where a cell is dividing from one cell into two. Our cell division detection algorithm (Section 3.4) nicely captures the splitting of the cell boundary.

Finally, Figure 7 shows the segmentation results obtained from the combined watershed and level set method for cell images that are very difficult to segment by standard methods. The first one (top left) has very low contrast. The intensities for the background, cell boundary and cell interior are close. As such, the right cell boundary appears very fuzzy. The next two images (top middle & right) have “broken halos” but our segmentation method does well where the boundary is weak (absent of halo). The last two images (bottom) have very bright and very dark intensities within the cell, which makes it difficult to detect whether the bright parts are the halos and the dark parts the background. These results demonstrate that our segmentation method is quite robust and works for many different types of cell images.

5. CONCLUSION

We have presented a robust segmentation method for brightfield images. It combines the techniques of watershed and level set algorithms. By applying the multiphase level set extraction, an initial marker with sufficient number and appropriately located pixel points inside the cell can be obtained. The growing region process of watershed captures the rest of the cell even the halo may be broken. Also, we have explained how the method can detect cell
Figure 6. Segmentation results of a dividing cell.

Figure 7. Segmentation results for cell images that have very low contrast, broken halo, or high intensity variation within the cell.
division and no cell division in a uniform way with no special attention. We have demonstrated the effectiveness of the method by a number of examples from live cell images.

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