Cell Classification in 3D Phase-Contrast Microscopy Images via Self-Organizing Maps

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Abstract. Cancer cell morphology can be used as an indicator of metastasizing behaviors. To analyze cancer cell morphology, we used 3D phase-contrast microscopy. This is one of the most common imaging modalities for the observation of long-term multi-cellular processes of living cells without phototoxicity and photobleaching, which is common in other fluorescent labeling techniques. However, it also has certain drawbacks at the image level, such as non-uniform illumination and phase-contrast interference rings. Our first step compensates for row-contrast artifacts via single cell detection using intensity-based global segmentation. We extracted cross-sections using principle component analysis; this was due to the interference's non-symmetric diffusion pattern, which appeared around each individual cell. Then, we analyzed cell morphology by an intensity gradient, considering local peaks as bright ring regions. Finally, we applied a self-organizing map method that has potential viability for cancer cell classification into active and inactive categories.

1 Introduction

Metastatic cancer cells are spread easily through blood vessels to other parts of the body, making treatment more difficult. It is important to characterize putative drug targets for discovery and development of anti-cancer drugs and to quantify the drug's anti-migratory effects [1].

Cell invasion and migration are closely related to cell morphology [2]. Active cancer cells have an irregular-shape, whereas inactive cells have a spherical topology. Therefore, analysis of cell morphology is an important aspect for the efficient and realistic screening of newly developed drugs.

To analyze cell morphology, phase-contrast microscopy is commonly used because it enables long-term multi-cellular process observation of living cells without the use of chemical staining techniques. Additionally, it does not require fluorescent labeling techniques [3].

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Phase-contrast microscopy generates large volumes of images during observation of live cells. The manual analysis of large volumes of data is extremely time-consuming and error-prone. Therefore, it is necessary to develop a computer-aided method to automatically and accurately detect individual cells and measure cell morphological characteristics [4]. When a computer-aided method is developed, the characteristics of phase-contrast microscopy images such as halo phase-contrast artifacts, non-uniform illumination and low contrast between cells and background should be considered. This automatic approach can also be efficiently used in large data experiments that screening cell cultures [5].

In previous studies of cell behavior using phase-contrast microscopy, images have been obtained using several methods specific for two-dimensional environments [6-8]. Local and global thresholding methods do not effectively separate cells due to non-uniform illumination and low contrast. Active contour and level-set-based approaches also yield poor results due to halo artifacts. Moreover, commonly used 3D segmentation methods are extremely time consuming and produce inaccurate results because phase-contrast microscopy images have issues such as light diffusion, low contrast etc… Therefore, approaches for cell analysis based on image intensity must be capable of compensating for interference [4].

Figure 1 shows the detailed flowchart of the proposed classification method. In this paper, we propose a three-dimensional method that is independent of phase-contrast microscopy image artifacts. In Section 2, we introduce methods that correct for non-uniform illumination and detect the cell area by a global segmentation method. In Section 3, we investigate an unsupervised classification method to cluster cancer cells into active and inactive categories. The experimental results are presented in Section 4. Finally, the conclusions are presented in Section 5.

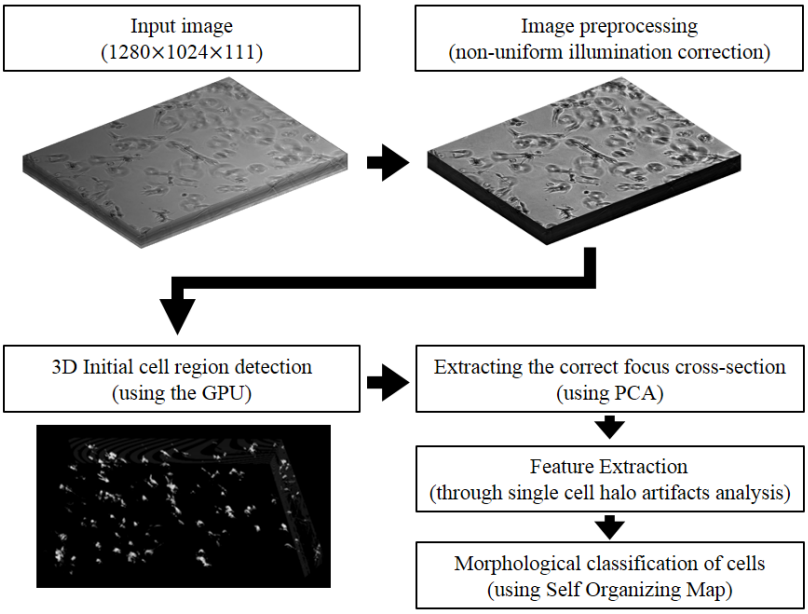

Fig. 1. The flowchart of the proposed procedure

2 Single-Cell Detection

Living U87 glioblastoma cells were dispersed in a 3D cell matrix in a Matrigel environment. Using a phase-contrast microscope, cells were imaged at 10× magnification with a z-step distance of 5 μm.

2.1 Non-uniform Illumination Correction

Phase-contrast microscopy can be used for monitoring live cell migration and activation without fluorescent labeling techniques. However, due to differences in light absorption and the material properties of a biological gel, the image brightness and contrast are not uniform [9, 10].

Non-uniform illumination correction methods have been developed using entropy minimization, homomorphic filtering-based methods, and an estimation parameter of non-uniform illumination [11]. However, it is difficult to find optimal parameters that consider characteristics of phase-contrast microscopy.

In this study, we used the modification framework histogram-smoothing (MF-HS) algorithm, which is resistant to the over-enhancing problem and allows for preservation of edge details [12].

Figure 2 shows a sample result of non-uniform illumination correction. This algorithm resolves non-uniform illumination issues and enhances low-contrast problems for analysis of phase-contrast microscopy image.

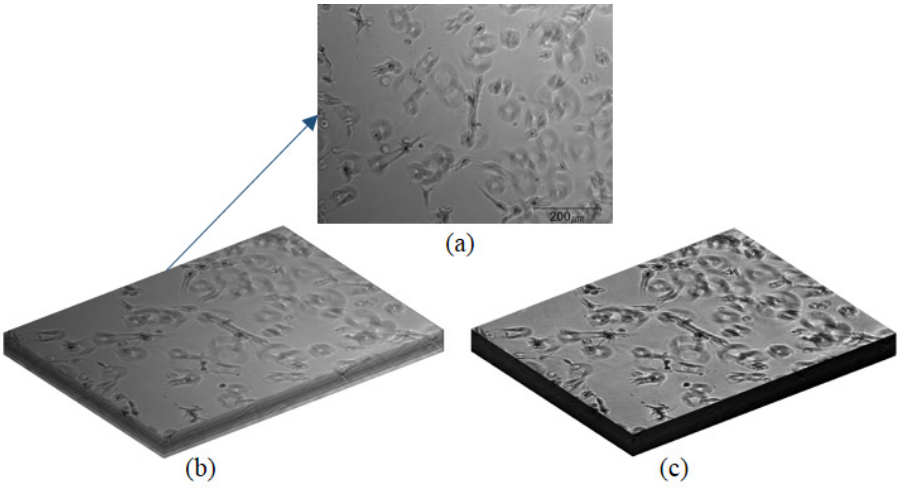

Fig. 2. Non-uniform illumination correction results. (a) First section of the input image (1280 × 1024), (b) Original images (1280 × 1024 × 111), (c) Histogram equalization results (1280×1024×111).

2.2 Global Approach to Image Segmentation

The performance of various threshold algorithms to identify the approximate cell boundary was based on local maxima values. The minimum threshold was selected, similarly to the Intermodes method, which takes a bimodal histogram. The histogram was iteratively smoothed using a running average of size of three until only two local maxima remained [13].

To visualize the detection of individual cells, a rendering method is required. General rendering methods of 3D microscopy images are time consuming for a CPU. In order to ameliorate this problem, a region of interest (ROI) in the original image was cropped to reduce computational burden. However, the results are strongly dependent on the user's selection, and images must be analyzed with a loss of original information [14].

Therefore, we used a method for rendering high-capacity 3D microscopy images using the GPU. We implemented the proposed method using texture-based volume rendering and ray casting with accelerating CUDA. These techniques made it easier to process and visualize the 3D microscopic images quickly and efficiently. Table 1 shows that the proposed method significantly reduced processing time for large data sizes [14, 15]. Figure 3 shows an example GPU-based visualization result of the detected cells.

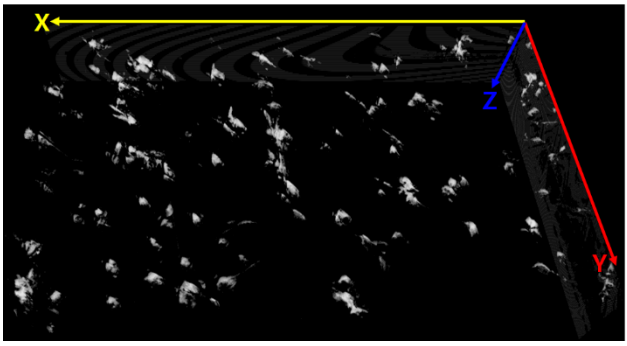

Fig. 3. 3D visualization of detected cells

Table 1. Comparison of GPU-based and CPU-based image processing performance

Number of Image slices	30	65	85	111
CPU (sec)	6.96	16.63	28.03	39.19
GPU (sec)	6.53	8.23	9.62	11.73

3 Cell Classification by Self-Organized Mapping

In this section, we used a self-organizing map (SOM) algorithm [19] to classify each cell in the phase-contrast microscopy image, considering the inherent characteristics of the generated image.

3.1 Halo Pattern Analysis

To analyze individual cells, the detected 3D objects were labeled within all phase-contrast microscopy images and pattern information on each labeled object was obtained. In 3D phase-contrast microscopy images, halo patterns appear around cells as bright disks in the XY plane, and they provide information about off-focus phase-contrast interference rings as they move away in the z direction [1] (Fig. 4).

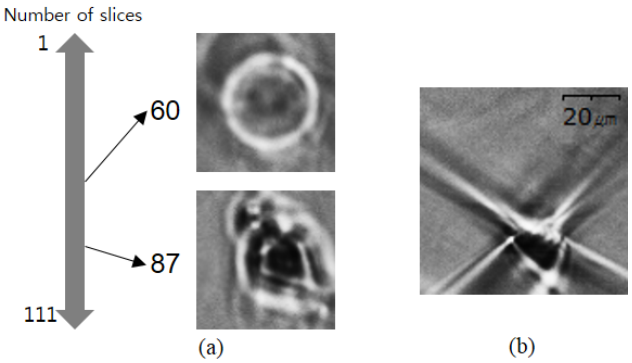

Fig. 4. Cell appearance correlation in phase-contrast microscopy. (a) XY plane, (b) XZ plane.

The cells in phase-contrast microscopy image were oriented obliquely in an array of directions; as a result, the halo pattern was limited due to the 2D analysis. In order to estimate the orientation of each cell and extract the lighter diffusion plane, the first principal axis vector of each cell's initial region orientation was calculated and the cross-section that intersects the center of each cell, orthogonal to the first principal axis, and passes through the region's center of mass was identified. To achieve this, principal component analysis (PCA) [17] was applied to be able to clearly determine the halo pattern around each cell (Fig. 5) [10, 18]. PCA is a commonly used statistical data analysis technique; it involves a simple yet powerful calculation that helps to reduce the dimensionality of complex data [19].

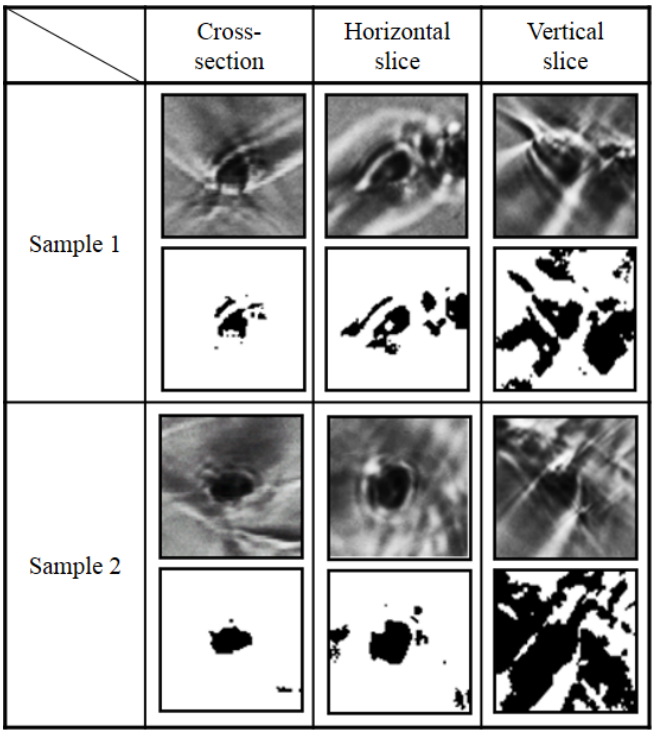

Fig. 5. Comparison of threshold segmentation results. Gray-level images after MF-HS-based histogram equalization (top) and binary images after thresholding (bottom).

3.2 Morphological Classification of Cancer Cells

Unsupervised learning is a challenging problem in many areas of data analysis. Since ground truth data in remote sensing is expensive and hard to obtain, the use of unsupervised procedures has become more relevant in this field [16, 20].

Neural network models are able to find clusters of closely related cell morphology patterns, and thus can be used to achieve the desired classification; it can also be used to categorize without supervision. In this paper, we used SOMs [21], which are a type of artificial neural network. A neuron is a processing unit. Neurons are connected by synapses or connecting links, each of them characterized by weights that modifiable [20].

The SOM is based on competitive learning. Its approach has been successfully applied in many different fields and is powerful enough to perform extremely demanding computational tasks, including image analysis, image classification, and computer vision. SOM is different from other artificial neural network in that it preserves topological properties of the input data. This forms a representation of both the relative density of input vectors in that space, and a two-dimensional delineation of the space topology [22]. Then, they can be simplified to allow for coding and manipulation of data, thus conferring more flexibility in analysis.

4 Experimental Results

The performance of the proposed method of feature extraction is shown in Figure 6. We identified cross-sections that passed through the detected individual cell area's center of mass and were orthogonal to the first principal axis. Moreover, two gradient peak values from the center of the cross-section image were obtained. These peaks represent the halo pattern around the cell boundary [9]. Once the feature vectors were extracted from this information, they could be used to analyze the differences in gradient value between active and inactive cells.

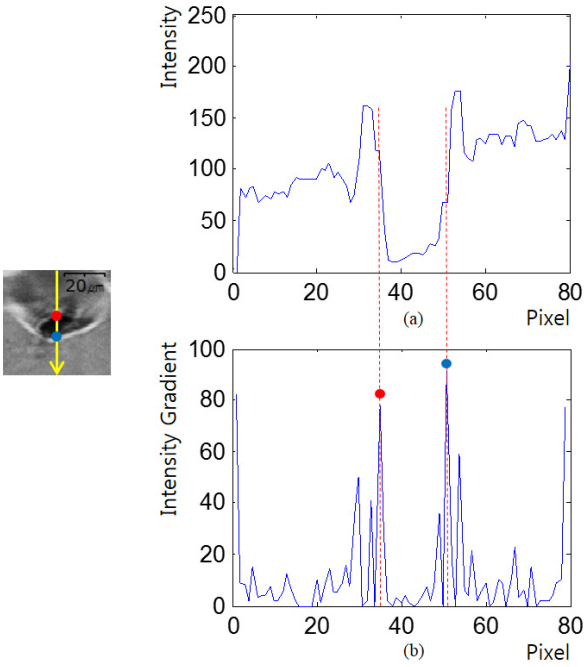

Fig. 6. Correlation of the intensity profile and intensity gradient of a cell cross-section sample image. (a) Intensity profile graph, (b) Intensity gradient graph.

Classifying each cell in the phase-contrast microscopy images was performed using SOMs. For experiments, parameters of a SOM-based classifier were adjusted to classify cancer cells as active or inactive. A minimal output layer size (2x2) was chosen. Figure 7 shows the results of cell classification. Circles represent spherical cells (3 clusters: green, blue, red) and stars represent irregular-shaped cells (1 cluster). The green, blue and red clusters were commonly characterized as having spherical morphology, suggesting they are inactive cells, although the halo pattern of the respective groups were slightly different.

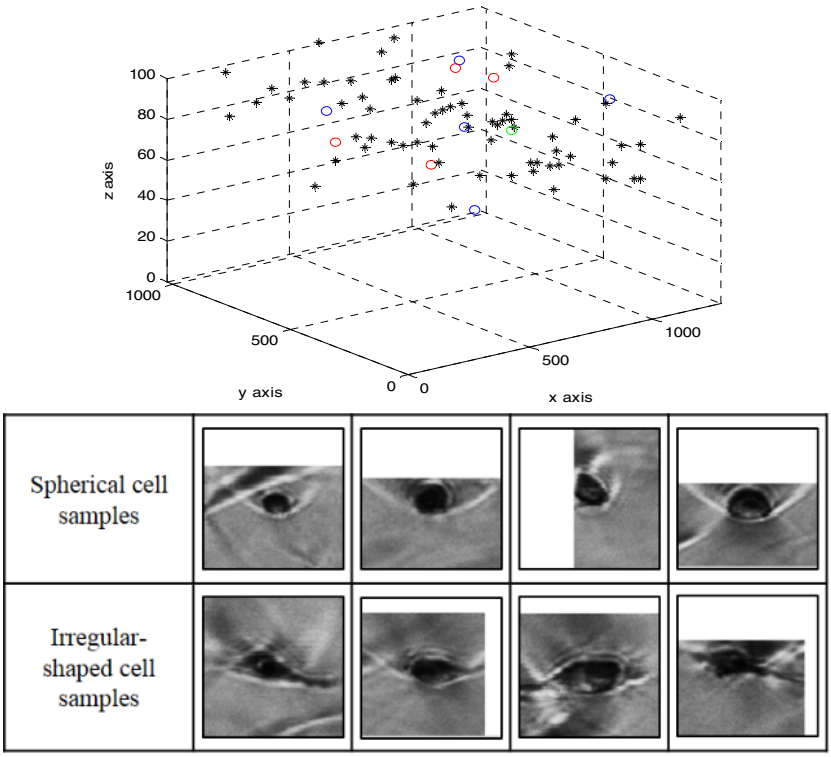

Fig. 7. SOM-based cell classification results
(Circles: spherical cells, Stars: irregular-shaped cells)

The accuracy of the proposed method was compared with other feature sets using SVM. The value of accuracy was measured by comparing the output with manually obtained results conducted by an expert. Our method provided 78.95% accuracy, while that of other methods did not exceed 73.68% (Table 2).

Table 2. Feature and classifier selection

Classifier	SVM (Support Vector Machine)		SOM
Feature	Cell size and roundness	Fourier descriptor	Intensity gradient
Accuracy	59.45%	73.68%	78.95%

5 Conclusion

For cancer cell screening, the use of phase-contrast microscopy is usually required. It provides an efficient modality for monitoring the morphological behavior of label-free cells in a 3D environment. We proposed a method to classify single cells in dense cell populations by compensating for the characteristics and artifacts produced by phase-contrast microscopic imaging. The PCA algorithm was used to account for the halo patterns on 3D cell images and then the SOM algorithm was used for classification of cell shape. As the SOM was based on competitive learning, it was not necessary to extract training data, so the characteristics of classified cell types had higher categorical resolution. In addition, the classification results did not depend on the segmentation methods because the features of the intensity profile were used. All of this information was extracted from the system despite the lack of cell boundaries in the phase-microscopic images.

As demonstrated, this paper proposes a method of extracting information about morphological cell features by accounting for image artifacts generated by the 3D nature of the system [9]. These techniques could potentially be used in the screening of novel cancer therapies. In the near future, 3D segmentation could be investigated more exhaustively; in particular, taking into account the heterogeneity of U-87 cells.

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