2D Light scattering images analyzed by deep learning algorithm for label-free differentiation of dead and live colonic adenocarcinoma cells

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Abstract. The detection of cell viability or the detection of the percentage of live and dead cells in a sample of cells is an important parameter. At present, the common methods for cell viability determination mainly rely on the responses to cell dyes. However, the additional need for cell staining will consequently cause time-consuming and laborious efforts. Furthermore, the determination of cell viability by cell staining is invasive and may damage the internal structure of cells. In this work, we proposed a label-free method to classify live and dead colonic adenocarcinoma cells by 2D light scattering combined with deep learning algorithm. The deep convolutional network of YOLO-v3 was used to identify and classify light scattering images of live and dead HT29 cells. This method achieved an excellent sensitivity (92.16%), specificity (94.23%), and accuracy (93.2%). The results show that the combination of 2D light scattering images and deep neural network may provide a new label-free method for cellular analysis.

Key words: Label-free; Colon cancer cells; 2D Light scattering; Deep learning

1. Introduction

Accurate and efficient assessment of cell viability is fundamental for cellular analysis in the biomedical field [1]. It is often used to monitor cell proliferation and health status for cell culture; to in vitro evaluate drug or environmental effects in cell-mediated cytotoxicity assays; to confirm the cell status after a series of operations, such as cell separation [2] and capture [3, 4], etc. Commonly used assay depends on two-color fluorescence with calcein AM and ethidium homodimer-I staining to distinguish metabolically active cells from dead cells[5]. However, this method is tedious and susceptible to subjective factors of the operators. In addition, the staining with cell dye is invasive and affect reuse in subsequent experiments [6]. The 2D light scattering technology is developed from the 1D forward and side light scattering. Su et al. have successfully discriminated against the normal granulocytes and leukemic cells [7]. However, the 2D light scattering pattern is complex and it is difficult to obtain the precise correspondence between image spots and cell organelles [8]. Previously, machine learning algorithms especially support vector machine (SVM) were used to label-free differentiate cell types. For example, Domenico et al. had successfully classified the subclasses of T lymphocytes. However, the accuracy rate (79%) is not satisfactory solely by machine learning image analysis.[9] However, the SVM algorithms are limited by poor pattern recognition ability. It
performed well in simple tasks such as the shapes of the scattered spots were obvious distinct, but failed for challenging tasks of distinguishing complex scattering images for most cases. Deep learning frameworks exhibited the ability to deal with complicated target detection/location tasks. In this work, we proposed a label-free approach for live and dead HT29 cells classification based on deep learning assisted 2D light scattering images analysis. An excellent sensitivity (92.16%), specificity (94.23%), and accuracy (93.2%) were achieved by our method. Although, the experiments were only carried on live and dead HT29 cells, it is highly promising for accurate label-free analysis of other cells.

2. Materials and methods

2.1. Experimental Setup and Working Principle
For light scattering imaging experiments, the incident light angle is critical since different angles corresponding to cell characteristics. As shown in figure 1, Side scattering based 2D light scattering imaging system was built to interrogate cell viability in this study. The image generation module and image collection modules are incorporated to obtain scattering images and the latter one responses for cell-type determination.

![Image 1](image.png)

Figure 1. The experimental setup of 2D light scattering system.

2.2. Cell Culture and Cell Viability Confirmation
The HT29 cells were cultured in McCoy’s 5A medium complement with 10% FBS at 37°C in a humidified atmosphere with 5% (v/v) CO2. To obtain pure live HT29 cells, the cultured live cells were collected and washed with PBS solution. To get dead HT29 cells, the live HT29 cells were heated at 65°C for 30 minutes. The treatment effects of HT29 cells were confirmed by the stain process. Two dyes Calcein AM and Ethidium homodimer-1 were used to stain live and dead cells, respectively. Live cells and dead cells showed green and red fluorescence, respectively.

2.3. Neural Network Architectures and training process
The YOLO deep neural network which has been proposed in CVPR was utilized in this article. The core of the YOLO algorithms is progressively approaching the detection target. By comparing the characteristics of the network with our task, the network is expected to perform outstanding to discriminate live and dead HT29 cells with high accuracy.
The training process was carried out in Ubuntu system environment with the programming language of Python 2.7.12, and Tensorflow 1.6.0. The model was trained 30k times on the training set, and the batch_size value was set as 16. The initial learning rate is 0.001, and decay is 0.0005 during the training process. The results is shown in figure 2(a) and figure 2b.

3. Results and discussion

3.1. The analysis of light scattering patterns
Figure 3 shows the images of the HT29 cells under a bright field microscope and the proposed 2D light scattering system, respectively. It is difficult to discriminate these two types of cells only by bright field microscope.

3.2. The results of the classification of live and dead HT29 cells
In order to evaluate the performance of YOLO-v3 model for label-free cell viability identification, other two classical target detection frameworks which are Fast-FCNN and SSD were compared. In the test phase, the image size of the input was the same as the training phase (416×416 pixels). Three key parameters, the overall accuracy, the sensitivity and the specificity were introduced to evaluate the difference between judge results and ground truth. They were defined by the formula (1) ~ (3),

![Figure 2. The (a) loss function and (b) IoU value in training process.](image)
respectively.

\[
\text{Accuracy} = \frac{\sum_{i=0}^{k} P_{ii} + \sum_{j=0}^{k} P_{jj}}{\sum_{i=0}^{k} \sum_{j=0}^{k} P_{ij}} \tag{1}
\]

\[
\text{Sensitivity} = \frac{\sum P_{jj}}{\sum P_{jj} + \sum P_{ji}} \tag{2}
\]

\[
\text{Specificity} = \frac{\sum P_{ii}}{\sum P_{ii} + \sum P_{ij}} \tag{3}
\]

Where \( k \) is the number of category, and it can be divided as \( k+1 \) classes (including the background). \( P_{ij} \) was denoted as the number of cells that in class \( i \) but were decided as class \( j \). Here \( P_{ii}, P_{jj}, P_{ij} \) and \( P_{ji} \) presented the true negative (TN), the true positive (TP), false positive (FP), and false negative (FN), respectively.

|            | TP  | TN  | FP  | FN  | Sensitivity | Specificity | Accuracy |
|------------|-----|-----|-----|-----|-------------|-------------|----------|
| Faster-RCNN| 46  | 47  | 5   | 5   | 90.2%       | 90.38%      | 90.29%   |
| SSD        | 45  | 47  | 5   | 6   | 88.24%      | 90.38%      | 89.32%   |
| YOLO-v3    | 47  | 49  | 3   | 4   | 92.16%      | 94.23%      | 93.2%    |

Table 1. Classification results of live and dead HT29 cells.

Table 1 evaluated the three deep neural networks of accuracy, sensitivity, and specificity. Compared with Fast-RCNN and SSD, YOLO-v3 model has a remarkably high value of 93.2%, compared to 90.29% for Fast-RCNN and 89.32% for SSD. The YOLO-v3 model also performed best at sensitivity (92.16%) and specificity (94.23%). In addition, the YOLO-v3 took only 0.05s to judge one image, faster than the Fast-RCNN and SSD. The performance of the YOLO-v3 model outperforms other two methods in our dataset both in accuracy, sensitivity, specificity, and speed.

4. Conclusion

In this work, we proposed a label-free method for the classification of dead and live HT29. The method for cell viability testing is usually to stain dead and live cells with different dyes. However, this way needs a professional operation, laborious and aggressive to cells. We used the YOLO-v3 network to train the light scattering images of HT29 cells and result with a sensitivity of 92.16%, specificity of 94.23%, and the accuracy of 93.2% was achieved. We demonstrate a high capability of the combination of light scattering patterns and deep learning for label-free cellular analysis.

5. References

[1] Nkupiou-Kenfack E, Engel H, Fakih S, and Nocker A 2013 J. Microbiol. Meth 93 20
[2] Kamande J W, Hupert M L, Witek M A, Wang H, Torphy R J and Dharmasiri U 2013 Anal. Chem. 85 9092
[3] Zhou H, Wang Q, Yuan D, Wang J, Yang H and Wu H 2016 Analyst. 141 4293
[4] Hejazian M, Li W and Nguyen N T 2015 Lab. Chip. 15 959
[5] APAHiles R A 1984 J. Pharm. Sci. 79 371
[6] Boyd V, Cholewa O M and Papas K K 2008 Current Trends in Biotechnology & Pharmacy 2 66
[7] Xie L, Liu Q, Shao C D 2016 Opt. Express. 24 21700
[8] Maltsev V P 2000 Rev. Sci. Instrum. 71 243
[9] Rossi D, Dannhauser D, Telesco M, Netti P A and Causa F 2019 Lab. Chip. 15 3888

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