Low-cost Parallelized Microfluidic Based Single-Cell Morphological Image Cytometry

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1. Introduction

Cell morphology imaging techniques are commonly used to assess the quality and attributes of cells in culture. However, these methods are often batch processed, requiring manual cell handling steps, microscope capture and downstream image processing and identification. Current imaging methods for cell cytometry and morphological characterization uses expensive tools with high computational and/or manpower requirements which limits the wide-spread use of such techniques in clinical and biological applications. We develop a machine learning model based on a low-cost microfluidic device integrated with a portable microscope system for deployable and real-time morphological profiling of immune cells within a biological sample [1,2].

2. Substantial section

Our approach uses a 6-class support vector machine (SVM) learning model based on 20x20 monochrome cell image pixels. The model performs up to 1000 cell classifications per second using a standard i7 laptop without graphics processing unit (GPU) acceleration. The 6-class SVM model has an overall validation accuracy of approximately 85%.

2.1 Materials and Methods

We labelled and classified approximately 22,000 cell images with 6 distinct morphological classes based on their size and shape. (Figure 1). Image data was collected using a low-cost imaging system with a camera (BFS-U3-16S2M, FLIR Blackfly2) with a fixed objective lens, 2 x 0.15 NA (Edmund Optics). Each captured image has a resolution of 20x20 pixels with a scale of 0.738 micron per pixel.

The dataset was enhanced using image augmentation techniques due to imbalances in the data distribution (Figure 2). The applied augmentations were flip, rotate, and circular shift [3]. The enhanced dataset was then split into training, test and validations datasets.

2.2 Results and Discussion

With the single cell imaging and model development and creation of the dataset as discussed above, a Support Vector Machine (SVM) was implemented using Python 3.8 with scikit-learn 1.3.2 on a 64-bit Windows system, powered by an 11th Gen Intel® Core™ i7-1165G7 processor, without Graphics Processing Units (GPU) acceleration. Cross-validation was applied to the model during the train-test phase to measure the generalization ability of the model. The results of the cross-validation resulted in an 84.4% average accuracy for the model. To further validate the model, unseen data was introduced to the model for prediction and manual adjudication was performed post-prediction to determine the generalization ability of the model. We observe that the model was indeed able to generalize on most of the classes except for class 4 and class 6. The inability to generalize those classes is hypothesized to be related to the high variability in the morphologies presented within those classes. The model, however, is shown to be able to generalize and detect single cell images with high accuracy despite the lower performance for class 4 and class 6.

2.3 Concluding remarks

In closing, our technology offers significant advancement in the field of cell profiling by providing a label-free, cost-effective method for single cell profiling. Leveraging on machine learning algorithms, we can obtain detailed morphological information of individual cells, which was previously unattainable.

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2.4 Figures and tables

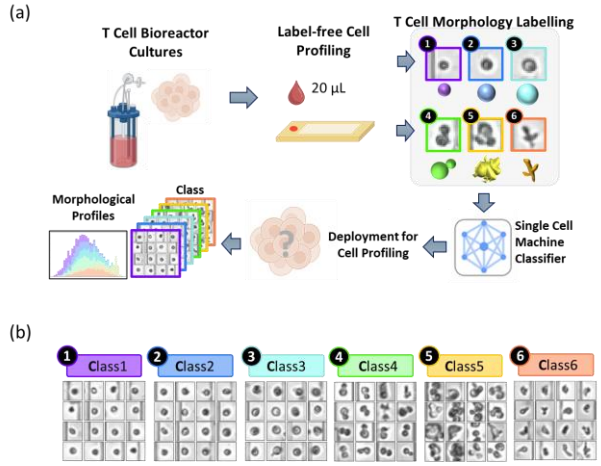

Fig. 1: Experimental workflow on the observation and categorization of distinct cell morphologies. (a) Schematic overview of the experimental protocol. 20µL of cultured cells were profiled using a custom microscope setup and microfluidic device. The cells were categorized into 6 distinct class labels based on observed features and used to train a machine learning model. (b) Snapshot of observed cell images, each image is 20 x 20-pixels with a resolution of 1.35 µm per pixel

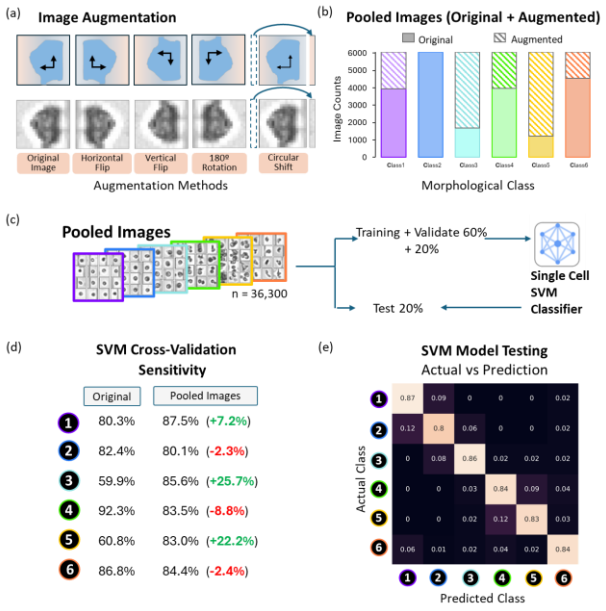

Fig. 2: Image classifier model development and validation (a) Image augmentation was performed on the dataset to balance class distributions. (b) A comparison of pooled images illustrates the dataset growth resulting from augmentation in contrast with the original dataset size. (c) Train-validate-test split was applied to the pooled images before training a support vector machine (SVM). (d) Cross-validation performance of the SVM on the original and pooled datasets, emphasizing differences in individual class sensitivities after pooling. (e) Normalized confusion matrix of the SVM model.

Acknowledgments

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References

[1] K. K. Zeming et al., Small, vol. 17, no. 12, p. 2006123, 2021, doi:https://doi.org/10.1002/smll.202006123.
[2] K. K. Zeming, T. Salafi, C.-H. Chen, and Y. Zhang, Scientific Reports, vol. 6, no. 1, p. 22934, 2016/03/10 2016, doi: 10.1038/srep22934.
[3] K. Zhang, Z. Cao, and J. Wu, in 2020 IEEE International Conference on Image Processing (ICIP), 25-28 Oct. 2020 2020, pp. 1676-1680, doi: 10.1109/ICIP40778.2020.9191303.
[4] S. Ota et al. Science, vol. 360, no. 6394, pp. 1246-1251, 2018, doi: doi:10.1126/science.aan0096.
[5] C. Helbig, G. Ammann, T. Menzen, W. Friess, K. Wuchner, and A. Hawe, J Pharm Sci, vol. 109, no. 1, pp. 264-276, Jan 2020, doi: 10.1016/j.xphs.2019.03.024.