論文の内容の要旨

論文題目

Label-free detection of drug responses of cancer cells by machine-learning-assisted high-throughput bright-field imaging

（機械学習を用いたハイスループット明視野顕微法による
がん細胞薬剤応答性の無標識検出）

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1. Introduction

Cellular drug responses are the most fundamental reactions in biology and medicine, which provides valuable information for understanding the fundamental mechanisms of biology and the development of new drugs. To investigate the drug responses efficiently and massively, high-throughput screening has played a major role over the past decades. This technique is powerful in exploring a large number of samples, but is often limited by the fact that it only acquires a single variate from each sample, for example, live or dead. Owing to the technological advances of imaging and robotics, image-based high-throughput screening, namely high-content screening, has been developed to acquire a significantly larger number of variables in each sample to capture more information in the cellular drug responses. Such a multivariate image-based technique has been proven effective in a broad spectrum of quantitative analysis not simply in drug responses but also associated with genome and proteome analysis.

Despite the utility of image-based screening, it falls short in addressing the full needs of biological applications as it inherently requires fluorescent labeling which has several drawbacks. First, fluorescent probes are not available for all target molecules and may interfere with natural cellular functions. Although a wide range of immunofluorescent probes are commercially available for cellular imaging, they are costly and require time-consuming labeling processes including cell fixation which kills the cells, and thus hinders live-cell and large-scale assays. In addition, while fluorescently tagged cell lines can offer live-cell assays without such a labeling process, the development of such cell lines requires even more efforts than immunofluorescent labeling. Therefore, a method for image-based high-content screening without using fluorophores is desired for easy manipulation and economical assays.

In my doctoral work, I studied cellular drug responses in a label-free manner by high-throughput bright-field imaging with the aid of machine-learning techniques in various settings. The first chapter introduce the background of cellular drug responses, high throughput screening, thigh content screening and high-speed imaging modalities. Chapter 2 discusses the principles of the major components that were employed in my study including optofluidic time-stretch (OTS) microscopy, microfluidic design, image processing and machine learning. Chapter 3 describes the experimental demonstration of distinguishing cellular drug responses through morphological changes as a proof-of-concept. Specifically, I treated MCF-7, a human breast cancer cell with different concentrations of paclitaxel, a conventional anticancer drug to induce morphological changes on cells. While those morphological changes are too subtle to be observed by human eyes, they were consistently detected on images through the method I developed in my thesis. Chapter 4 describes a challenging application of label-free detection of drug responses with whole blood. In this chapter, K562, a chronic myeloid leukemia cell line, and its drug resistant strain K562/ADM were treated with adriamycin, another conventional anticancer drug. which drug responses of cancer cells were evaluated as they were spiked in undiluted blood. The drug treated cells were further spiked into whole blood to mimic a practical situation in blood diagnosis, and their morphological changes were successfully captured by our system. Chapter 5 describes another challenging application where the drug responses of cancer cells harvested from mouse xenografts were evaluated. In this chapter, A549/EGFP and PC-9/mRuby (two lung cancer cell lines into which fluorescence proteins, mEGFP and mRuby2 were genetically inserted respectively) were generated to discriminate them from somatic murine cells. These cells were transplanted into athymic nude mice, and harvested after grown. The collected cells were further treated with gefitinib, an anticancer drug as targeted medicine for the evaluation of drug responses. Finally, chapter 6 discusses the advantages and limitations in label-free detection of drug responses as well as possible future work.

2. Label-free detection of drug responses of cancer cells: experimental demonstration (Chapter 3)

The workflow for label-free detection of morphological changes is schematically illustrated in Fig. 1. The whole process can be divided into four parts: (1) drug treatment, (2) optofluidic time-stretch imaging, (3) feature extraction, and (4) classification. In this study, I used MCF-7, a human breast adenocarcinoma cell line, as a model cell, and paclitaxel, a classic anti-cancer drug, as a model drug to induce morphological changes to the cells. In order to acquire high-quality bright-field images of the cells in a high-throughput manner, OTS microscopy was employed. OTS microscopy is a high-speed imaging flow cytometry technique in which cells are scanned by spatially mapped broadband optical pulses while they are flowing in a

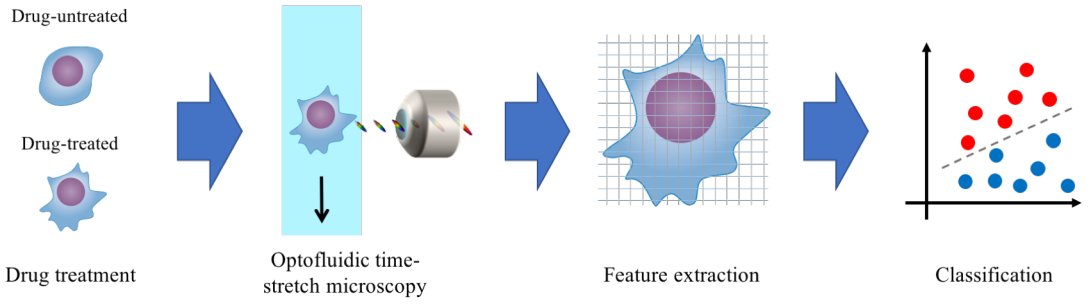

Figure 1. Workflow. Drug-treated and -untreated cells were imaged by the optofluidic time-stretch microscope. Then, the morphological features were extracted from the acquired images, based on which the two types of cells were classified into two groups.

microfluidic channel. My group has previously developed an OTS microscope with a spatial resolution of 780 nm and a flow speed of approximately 1 m/s. However, the 1-m/s flow speed of the microfluidic channel had been limited the throughput of the system, hindering its application in the measurement of a large number of cells. By optimizing the structure and design of the microfluidic channel, I successfully increased the flow speed by a factor of 10, achieving 10 m/s to enable higher throughput without sacrificing spatial resolution. Each blur-free image acquired with the OTS microscope shown in Fig. 2 was obtained in as short as 4 µs, which corresponded to a frame rate of 250,000 frames per second. Those images acquired by the OTS microscope maintain the same pixel resolution as the static images acquired by a CMOS camera on a conventional microscope.

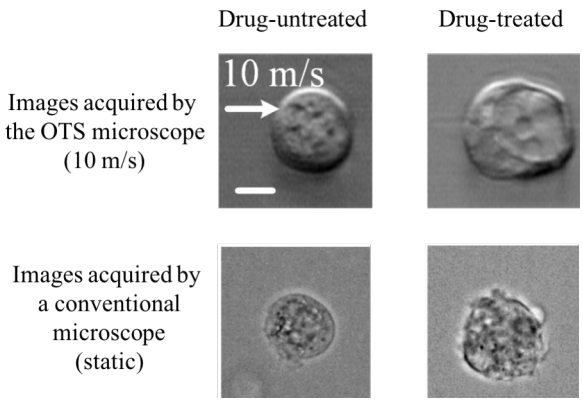

Figure 2. Images of drug-treated and -untreated MCF-7 cells. The top row shows the images acquired by the OTS microscope in which cells were flowing at a speed of 10 m/s. The right column shows the images of MCF7 cells treated with paclitaxel for 24 hours. Scale bar: 10 µm.

To evaluate whether the drug induced morphological changes on cells can be detected with the proposed approach, MCF-7 cells treated with various concentrations of paclitaxel were examined following the workflow shown in Fig. 1. A total number of 548 features were extracted from each image by using CellProfiler, an open source image analysis software, and then support vector machine (SVM) classificatio was implemented on MATLAB. Figure 3 shows the distributions of drug-treated and -untreated (negative control) populations after classification in various concentrations. The separation between these two populations increases along with drug concentrations up to 1 µM. This trend was constantly observed in two replicates of experiments and four trials of SVM classification. This result indicates that the morphological changes due to drug treatment can be perceived through label-free bright-field imaging and machine learning based classification. The result also suggests that the morphological changes are dependent on drug concentration, which is investigated in the following paragraph.

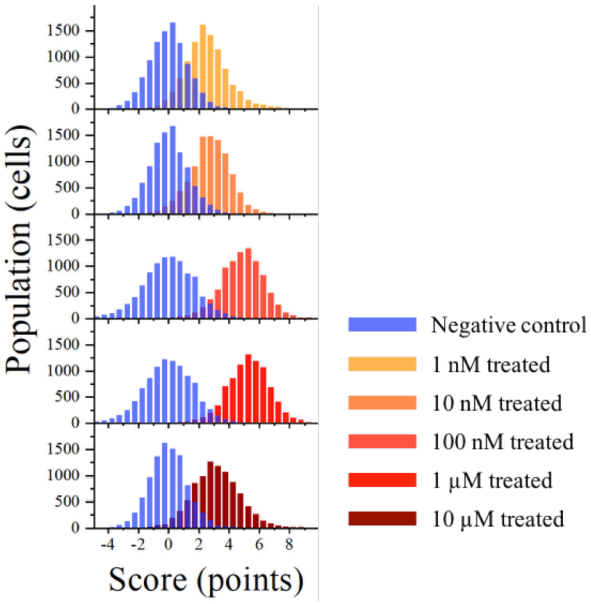

Figure 3. Classification of drug-treated and -untreated cells. Histogram of SVM classification scores for MCF-7 cells treated with various concentrations of paclitaxel for 24 hours. Each population consists of up to 10,000 cells.

3. Label-free detection of drug responses of cancer cells in human whole blood (Chapter 4)

With the successful demonstration of cellular drug responses expressed in morphological changes, I further investigated the feasibility of evaluating cellular drug responses in a more

practical situation such as using whole blood. In this chapter, I demonstrate an improved microfluidic channel which enables cells flow at 15 m/s, and increasing the throughput more than one million cells per second. I also demonstrate the classification of drug treated and untreated cells with K562 and K562/ADM, a chronic myeloid leukemia cell line and its drug resistant strain, and adriamycin, an anticancer drug often used for leukemia. Furthermore, I spiked the drug treated K562 and K562/ADM in whole blood and clearly captured the images of these cells along with other blood cells at the throughput of 10^6 cells/s (Figure 4). Their dose dependent drug responses were identified from these images suggesting that the method I developed in this thesis can be applied to evaluate the drug responses as a blood diagnosis.

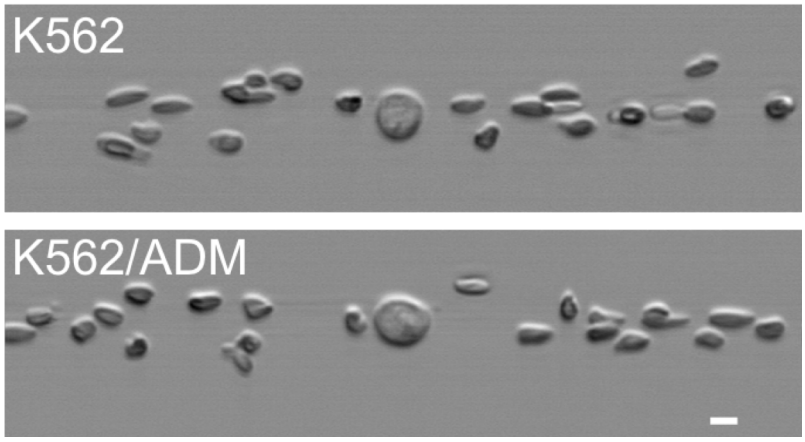

Figure 4. K562 and K562/ADM cells spiked in whole blood were clearly captured in the flow. More than 20 cells can be seen in each frame which was acquired in 20 μs resulting in a throughput of over 10^6 cells/s. Flow speed: 15 m/s, scale bar: 10 μm.

4. Label-free detection of drug responses of ex vivo cancer cells in mice (Chapter 5)

In this chapter, I examined the feasibility of using dissociated xenograft as target cells to investigate their drug responses through my method. For this purpose, A549/EGFP and PC-9/mRuby (two lung cancer cell lines into which fluorescence proteins, mEGFP and mRuby2 were genetically inserted respectively) were generated to discriminate them from somatic murine cells. These cells were transplanted into athymic nude mice, and harvested after grown. The collected cells were further treated with gefitinib, an anticancer drug as targeted medicine for the evaluation of drug responses. The drug responses of A549/EGFP and PC-9/mRuby were also tested without transplanted in mice, which means they were tested with identical processes as I discussed in previous chapters. Both *in vitro* and *ex vivo* results showed differences in drug response, suggesting my method can also applicable to evaluate the drug responses of *ex vivo* samples.