IMAGE PROCESSING FOR DRUG DISCOVERY TEST WITH CULTURED CELLS

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We have been developing a Drug Discovery Test System for Genome-based Drug Discovery. This system administers chemical compounds that serve as potential candidate drugs into live cells, which are the most basic components of all living organisms, records the changes in the amount and/or localization of target molecules inside cells with our CSU confocal scanner and a highly sensitive CCD camera, and processes and quantifies the captured high-resolution image data. This screening method enables the drug efficacy and adverse drug reactions of the candidate chemicals to be verified and the candidate drugs to be confirmed in live cells. This paper describes an image processing technology we have developed for our prototype Drug Discovery Test System.

INTRODUCTION

We have been developing a prototype of a genomic drug test support system using our CSU confocal scanner. This system administers chemical compounds that serve as potential drug candidates into living cells, which are the most basic components of all living organisms, records the changes in the amount and localization of target molecules inside cells with the CSU confocal scanner and a highly sensitive CCD camera, and processes and quantifies the captured high-resolution image data. This screening method enables drug efficacy and adverse drug reactions of chemical components to be verified and drug candidates to be determined in living cells. This paper describes the image processing technology we have developed for our prototype of a genomic drug test support system.

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OUTLINE OF OBSERVED IMAGES

Even if interactions between protein and chemical compounds are observed in the test tube in the first screening process, they may not be verified because transporters that discharge chemicals and metabolizing enzymes such as cytochrome P450s exist in cells. For this reason, cultured cells which can serve as disease models are used for specimens in drug-discovery tests. Most of these cells can easily be cultured using immortalized cells. Specific areas of these cells are dyed using fluorescent reagent, images are obtained with an fluorescence microscope or a confocal microscope and processed to extract and quantify morphological changes of cells caused by chemical compounds.

For actual specimens, cultured cells are sowed on a 96-well or 384-well micro well-plate. They are fluorescently dyed after being administered various concentrations of chemical compounds to observe morphological changes, etc.

Yokogawa's image-capturing system (applying CSU) has the following features.

(1) It captures images of light cross-sections of cells (confocal images). This feature enables clear observation of microscopic structures inside cells. Therefore, intracellular

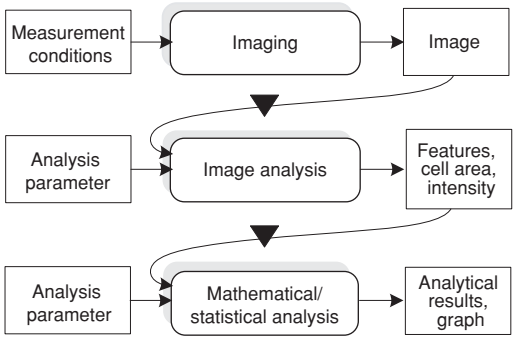

Figure 1 Analysis Flow

granules can be measured with high precision.

(2) Three-dimensional images are available by piling up the images of cross-sections of cells. The intricate structure of neurites can also be viewed accurately.

(3) Yokogawa's confocal system provides little fluorescence photobleaching, enabling continuous long-time observations. This feature makes it possible to observe dynamic changes of living cells.

The following reports on this image processing technique together with some technical cases in which the characteristics of images obtained by the sensor (CSU system) are fully used.

ANALYSIS SOFTWARE

The analysis flow of drug discovery tests consists of three steps as shown in Figure 1.

(1) Images are obtained automatically under certain measurement conditions.

(2) Obtained images are processed appropriately, and the features for various cells are converted into numerical data.

(3) Based on the numerical data, the average values of the features of cells are obtained for each concentration of the drug to draw a dose-response curve. Statistical methods are used to eliminate variation in the characteristics of cells and experimental errors to acquire required measurement data.

OUTLINE OF IMAGE ANALYSIS

This chapter describes the image processing algorithms developed for drug discovery tests.

(1) Cell tracking

Images used for cell tracking analysis are obtained by fluorescently dyeing the nucleus of cells (target specimen) and observing the same cells in the well for several days on the time lapsing basis. We apply a shape-based pattern matching method which utilizes contour data of objects (nucleus) shown in the images as models. Using the first input image (Figure 2), the model used for the matching of the second image onwards is made. To create this model, the first input image is processed binarily and labeled, and attached nucleuses are separated (Figure 3). Based on this model, the shape-based pattern matching method is applied to the second input image to recognize identical cells by checking the nucleus areas. By performing this process on several input images, the feature quantities (position, dimension, brightness, shape, etc.) of each cell (nucleus) at a certain time-point can now be calculated (Figure 4).

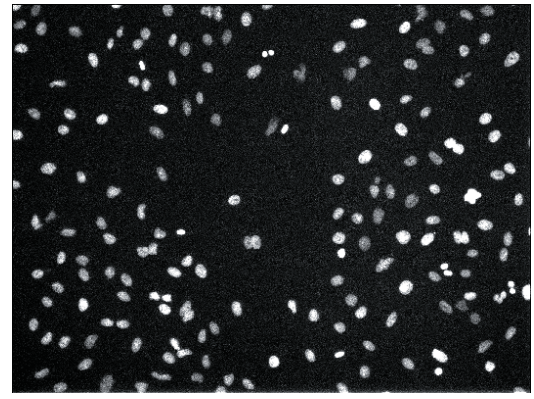

Figure 2 Input Image (First image)

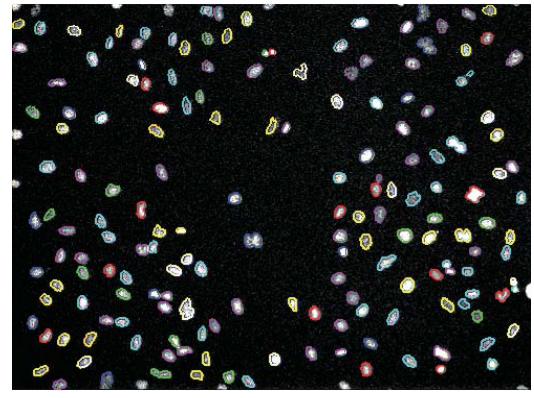

Figure 3 Modeling (Extraction of nucleus area)

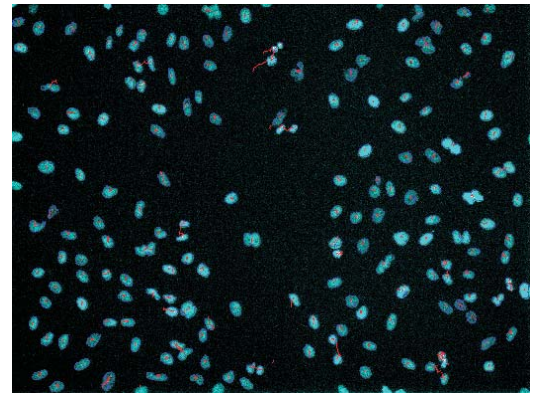

Figure 4 Tracking Results

(2) Dendrite detection

We reviewed and established algorithms for detecting

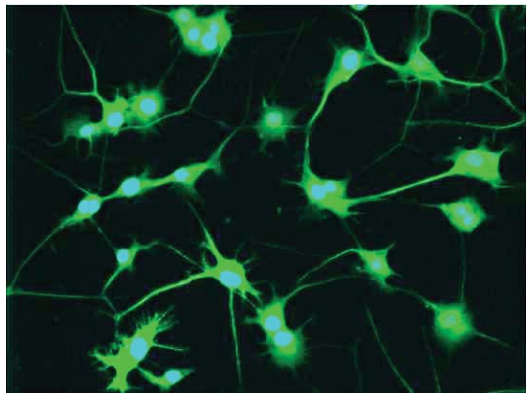

Figure 5 Original Image

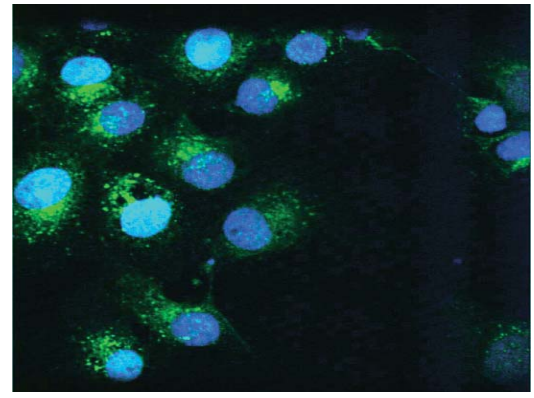

Figure 8 Original Image

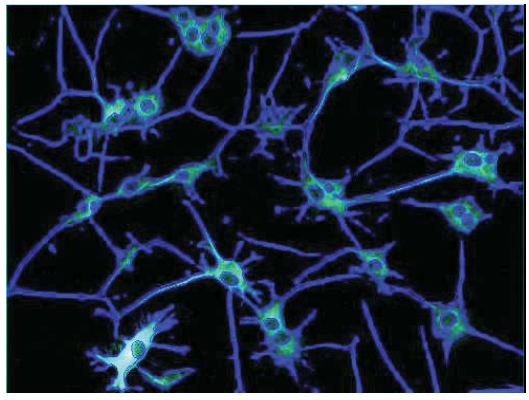

Figure 6 Cell and Dendrite Detection

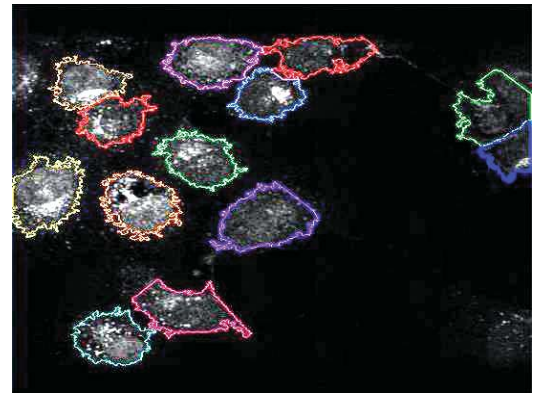

Figure 9 Cell Area Detection

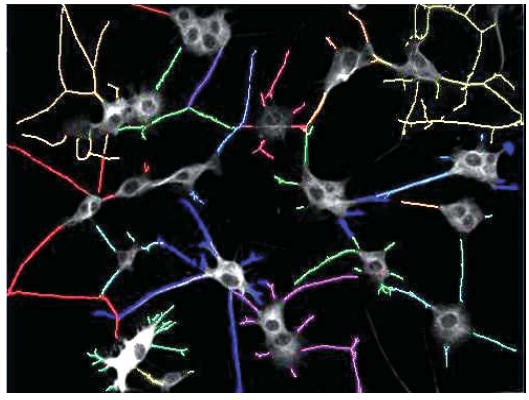

Figure 7 Cell and Dendrite Matching

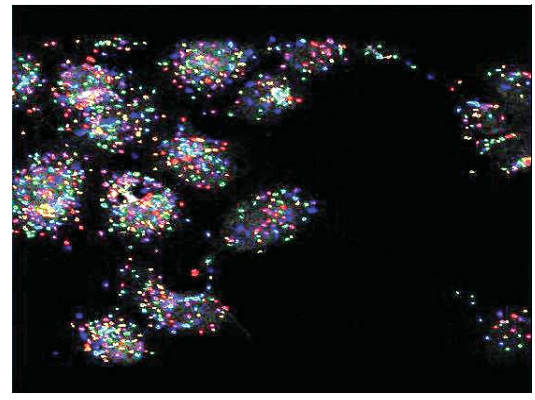

Figure 10 Intracellular Granular Area Detection

dendrites per cell. In the original image of cells (Figure 5), the areas displayed in green are the dendrites and cytoplasm, while the areas displayed in light blue are the nucleus. It can be seen that dendrites extend from the cell bodies to the areas surrounding them, and some of them are branching. First, using the original image, areas assumed to be dendrites and cytoplasm are extracted (Figure 6), and then they are separated and again extracted. Then, cell nucleus areas are estimated and matched to the cytoplasm. Finally, cells are individually matched to the dendrites (Figure 7). Figure 7 shows that cells and dendrites are matched well. This procedure enables calculation of the feature quantities (length, number of branches, thickness, and number of the extended parts) of dendrites of each cell.

(3) Intracellular granular detection

We reviewed and established algorithms for detecting

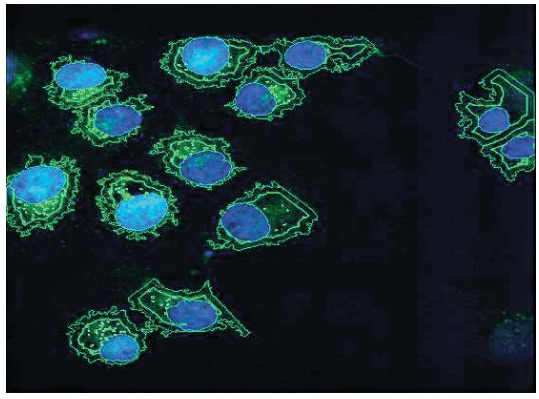

Figure 11 Matching of Cells and Granules

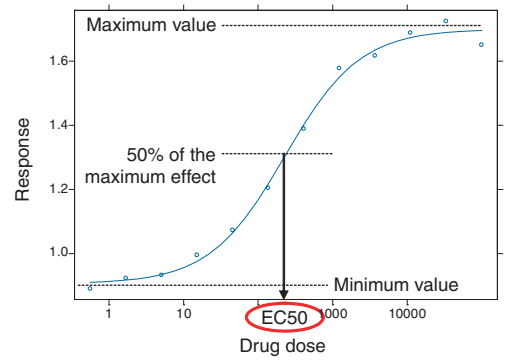

Figure 12 Dose-Response Curve and EC50

intracellular granules. There are various types of granules in cells. One of the most significant ones is G protein-coupled receptor internalization. In the original image of cells (Figure 8), the blue area is the nucleus, the green pits are the granules, and the light green area is the cytoplasm. First, the cell areas are extracted in the original image (Figure 9). Next, the granular areas are extracted (Figure 10), and granules are matched to each cell (Figure 11). Then, cell membrane areas are generated, enabling calculation of the feature quantities (number of granules, brightness, dimension, etc,) of granules on the cell membrane and in the cytoplasm of each cell.

Figure 11 shows the analysis results of intracellular granules. The red pits indicate granules on the cell membrane while the yellow pits indicate granules inside cytoplasm.

VISUALIZATION OF CELL FEATURES AND STATISTICAL PROCESSING

The cell features data obtained by image processing usually contains varies greatly. Statistical processing is thus required to extract general trends from such data. Some of them are explained as below.

The Z'-factor is an index which expresses the validity of the assay to check if the use of drug causes significant difference in cell reactions. It represents distribution differences in the features of a particular cell between negative control and positive control. When the average value and standard deviation of negative control are denoted as μ_n and σ_n, respectively,and those of positive control are denoted as μ_p and σ_p, respectively, the Z'-factor can be calculated as follows:

$$Z' = 1 - \frac{3\times(\sigma_p + \sigma_n)}{\left|\mu_p - \mu_n\right|}$$

When an inequation $0.5 < Z' < 1.0$ is formed, the quality of the assay can be regarded as excellent. If the Z'-factor is less than zero, it means that the assay needs to be reviewed.

Next, the EC50 (Effect Concentration 50) is explained. Generally speaking, when a certain drug has an effect on cells, the effect will increase as drug dose increases.

For example, when such drugs that could cause apoptosis (cell death) are used, the amount of dead cells will increase as the drug dose also increases. Then, subsequently, 100% of cells will die. The EC50 is the drug concentration level at which half of the maximum drug effect is observed. It is used as a guide to indicate the efficacy of drug concentration. If the effect of drug inhibits a certain function inside the cell, IC50 (Inhibitory Concentration 50) is used. It is the concentration of a drug which produces 50% of the maximal inhibition on the cell.

To obtain the EC50, first, it is necessary to focus on the features of cells that shows significant effects caused by drugs, and a drug's dose-response curve is drawn. Generally, the shape of the curve takes a sigmoidal form (Figure 12). Both the minimum and maximum values of the features on the curve are read, and the amount of the dosed drug for the median is obtained. This is equivalent to the EC50. The drug's dose-response curve generally includes variation in the characteristics of the specimen (cells) and experimental errors, and is therefore not an ideal response curve. So the sigmoid curve model is applied to the actually obtained drug's dose-response curve to estimate the EC50 value using a computer program performing a nonlinear least square fitting. At this point, it is recommendable to obtain the Z'-factor value to verify the validity of the evaluation process.

CONCLUSION

This paper reports on an image processing technique applied to the drug discovery tests using cultured cells. This image processing technique can be used for precisely measuring drug efficacy and toxicity of chemical compounds which can serve as potential drug candidates from the initial stage of drug development. We believe that it will contribute to reduce the development time for new genomic drugs as well as the development cost. ◆

* 'CSU' is a registered trademark of Yokogawa Electric Corporation.