Lab Automation for Biology: a practice to implement an “eye” for a robot to see cell condition

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Background: Challenges of the Biotechnology Industry

Regenerative Medicine  
Cancer Treatment  
Diagnostics  
New Drug Development

the industry is supported by many experts

But we rely too much on the experts…

Lack on manpower  
Reproducibility  
Working environment  
Data quality

Reproducibility Crisis


Lab Automation is inevitable
Cell Culture Market

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Global Market Growth Forecast

- 2017: $13.82 Billion
- 2023: $26.28 Billion

Fast Growing Regenerative Medicine Market

- 2018: JPY 50 Million
- 2022: JPY 100 Million
- 2025: JPY 200 Million
- 2030: JPY 500 Million

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https://www.marketsandmarkets.com/Market-Reports/cell-culture-market-media-sera-reagents-559.html
https://www.marketsandmarkets.com/PressReleases/cell-culture.asp
https://www.fuji-keizai.co.jp/market/18093.html
Cell Culture Automation

- Cell culturing requires:
  - Professional training
  - 3 to 4 weeks of incubation
  - Daily careful and repetitive operations
- Automation
  - Pharmaceutical companies need high volume cell cultures
  - Definitely needed to boost rapidly growing demand for large volume cell cultures

Nearly 60% of my research activity is occupied by cell culturing.
Eye is the key

- Cell culture involves routine observation of cells with a microscope.
- Need technology to identify a cell culture condition with a microscope.
- A limited number of developments has been done in this field.
- The AI-Robot-Microscope combination enables automated rapid AI process.

A generic process of AI.

Learn → Test → Observe
LabDroid Maholo

Multi-purpose laboratory automation system:
a general purpose industrial robot equipped with a set of lab tools which you can find in an ordinary laboratory.

- pipet
- 50ml tube
- centrifuge
- vortex mixer
- microscope

YASKAWA MOTOMAN

7-axis dual arm = very flexible
Varieties of experiments can be performed
How Maholo Works


Control PC compiles a protocol to a robot program by referring 540 pre-defined motions. Each motion is connected with an inter-mediating motion which is automatically generated using a random search algorithm.
Automated Cell Culturing System

- Incubate cells
- MAHOLO draw out a 12-well plate containing cells
- Performs media exchange every 24 hours
- Puts a plate on a fully-automated microscope

Key Technology
- Automated Identification and Analysis of Cell Differentiation Stages

Non-healthy culture will be abandoned or applied recovery protocols

Bright field images for each well are acquired
Differentiation stage identification

C2C12 is known to show apparent morphological changes after 24 hours since differentiation initiation. For human eyes, it is difficult to recognize morphological changes before 24 hours.

If we can identify the differentiation stage earlier, we can save our precious time from culturing unnecessary cells. **We develop a machine learning technology to identify cell differentiation stages using b.f. cell images.**
We kept a C2C12 plate in a CO2 incubator for microscope which enabled us to capture 44x12 images every 4 hours.
Feature extraction

Input:
Cell differentiation images

Extract features

Build a cell differentiation stage predictor

Apply Multiple Machine Learning Algorithms for Cell differentiation stage classification

Wavelet (haar, level=8)

HLAC higher-order local auto-correlation

BCF blurred circle function

0 h

64h

Fujita, Hasegawa "High-order local autocorrelation of high-order local autocorrelation" Image Science and Engineering Journal 34(1) (2005)
Higher-order Local Auto-Correlation

\[ C^h = (c_{xy}^h) = \text{convolution}(I_{xy}, h^*) \]

\[ F_{HLAC} = \left( \text{hist}(C^{h_1}), \ldots, \text{hist}(C^{h_{50}}) \right) \]

bin=25 builds 1250 dim. feature vector
Regression Test

To test if our feature extraction method effectively captures C2C12 images’ essential features associated to the differentiation and predict an actual differentiation stage from an image.
Regression Result: RMSD

- RMSD = root mean square distance between actual and predicted time.
- W: wavelet, H: HLAC, B: BCF, WHB = W + H + B
- At least 20 images for a label are necessary.
- HLAC shows slightly better results than others but WHB performs generally better and more robust.
- This result tells that you can predict cell culture condition every 3 hours or longer which enables much finer control for cell culturing.
We applied our method to more practical cell line HepaRG which is a human hepatic cell line commonly used for drug toxicity tests. We acquired 0, 96, 192, 288, 480h HepaRG bright field images during differentiation.

HepaRG shows different morphological changes during the differentiation.

Regression Test Result

RMSD=3.4h
Culture Condition Optimization

- Images of cells cultured with different conditions give skewed prediction results.
- We utilize this feature to automated optimization of culture conditions.

The figure shows distributions of differences between actual and predicted time for several culture conditions (regular (2:2) and others (3:1, 4:0, 0:4)).

3:1 Faster than the regular
4:0 some even faster and slower
0:4 slower

85% accuracy to distinguish faster/normal/slower
Future Plan

Automated Cell Culturing System + Super Resolution Microscope

Yokogawa’s super resolution microscope (spinning disk laser confocal) is attached to LabDroid Maholo via robot arm, which realizes high-throughput automated cultured cell quality control system.
We developed an automated cell culturing system using LabDroid MAHOLO.

We developed a machine learning method for cell differentiation condition identification with microscope image data.

Our method shows promising results for two morphologically different types of cells.
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