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

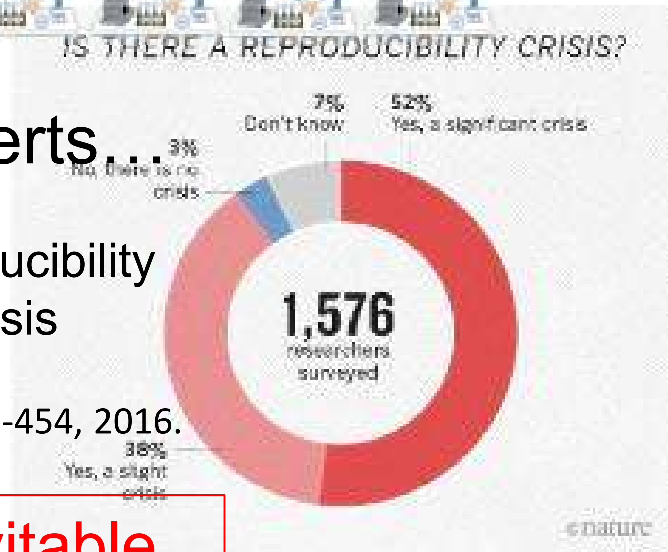
Regenerative Medicine | Cancer Treatment | Diagnostics | New Drug Development

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

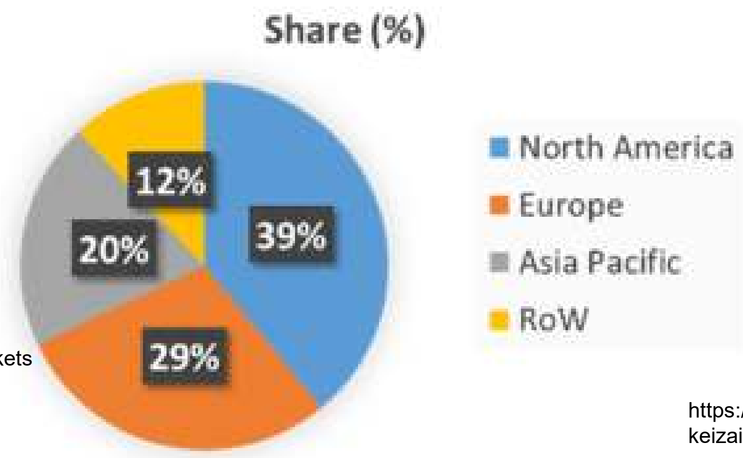


Nature 533:452-454, 2016.

Lab Automation is inevitable

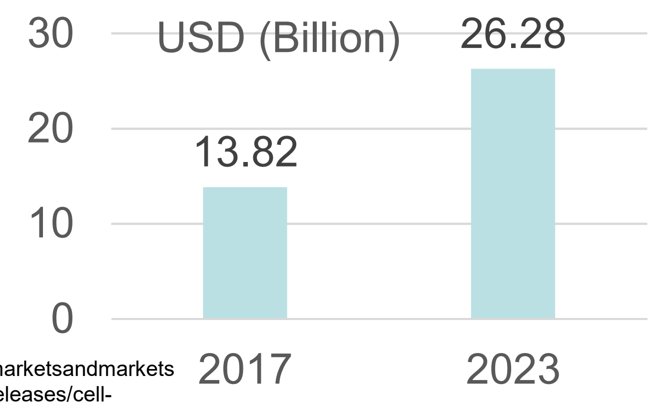
Cell Culture Market

Cell Users	%
Biopharmaceuticals	27
Tissue Culture & Engineering	21
Vaccine Production	21
Drug Development	13
Gene Therapy	7
Toxicity testing	4
Cancer Research	4
Others	3



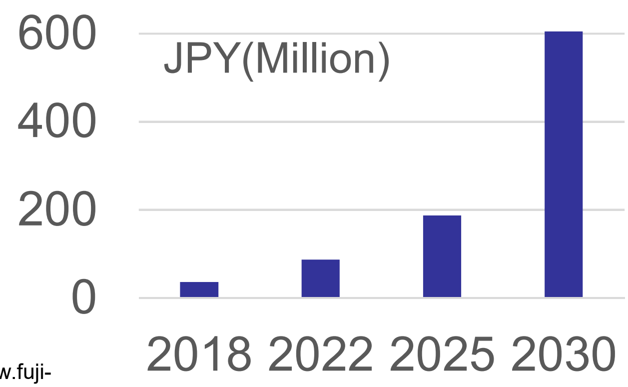
https://www.marketsandmarkets.com/Market-Reports/cell-culture-market-media-sera-reagents-559.html

Global Market Growth Forecast



https://www.marketsandmarkets.com/PressReleases/cell-culture.asp

Fast Growing Regenerative Medicine Market



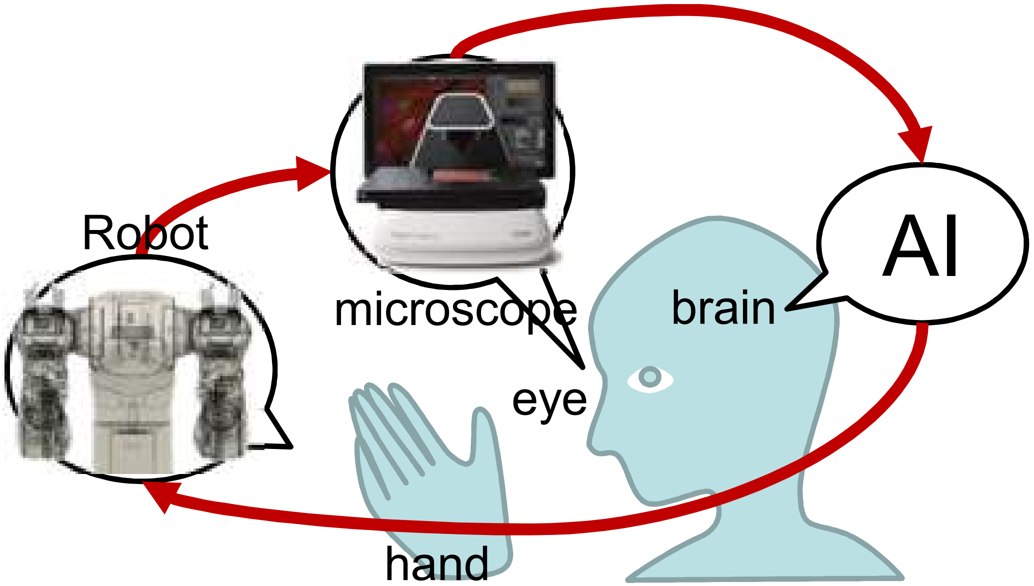
https://www.fuji-keizai.co.jp/market/18093.html

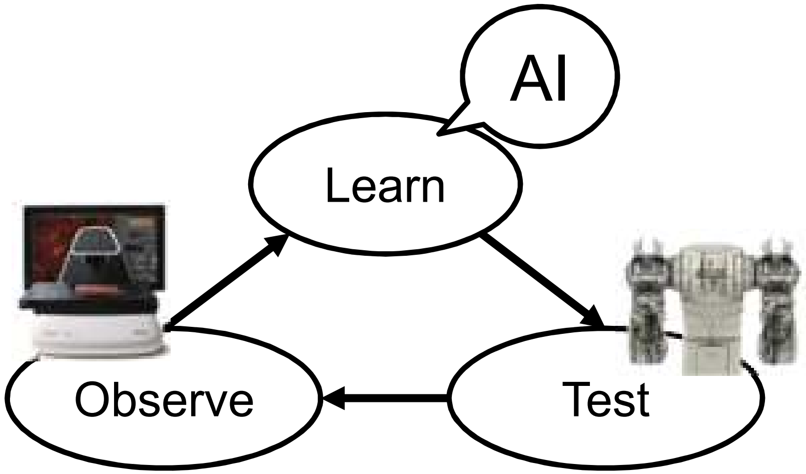
Cell Culture Automation

- Cell culturing requires:
  - Professional training
  - 3 to 4 weeks of incubation
  - Daily careful and repetitive operations
- Automation
  - Pharmaceutical companies need high volume cell cultures
  - Review paper by Kempner and Felder, “A review of cell culture automation”, JALA, 2002.
  - Definitely needed to boost rapidly growing demand for large volume cell cultures

Eye is the key

- Cell culture involves routine observation of cells with microscope
- Need technology to identify a cell culture condition with a microscope
- A limited number of development has been done in this field
- Automation Components: Hand = Robot, Brain = AI, Eye = Microscope
- the AI-Robot-Microscope combination enables automated rapid AI process

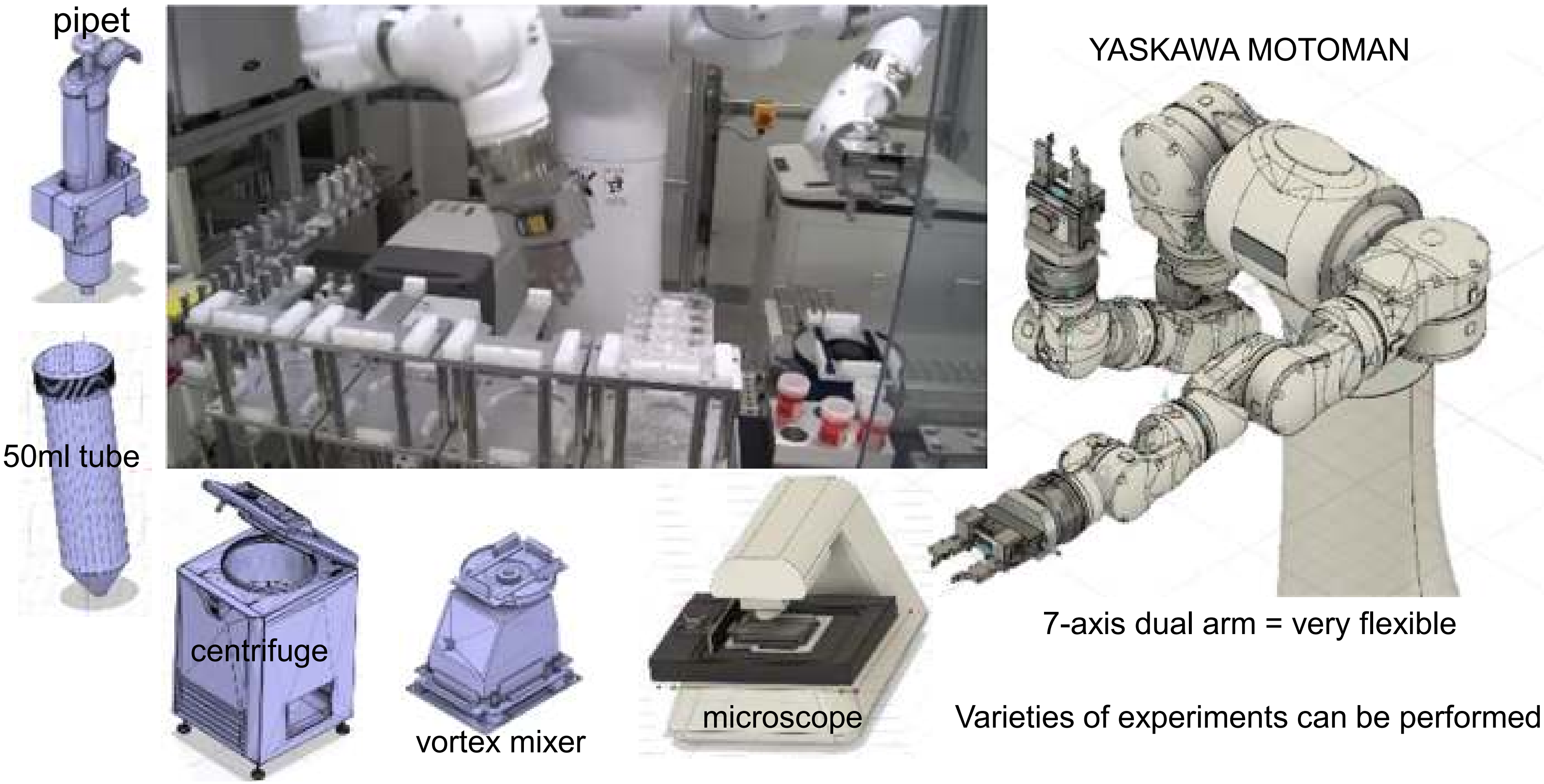




A generic process of AI

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



How Maholo Works

ProtocolMaker:
a Visual Protocol
Programming Env.

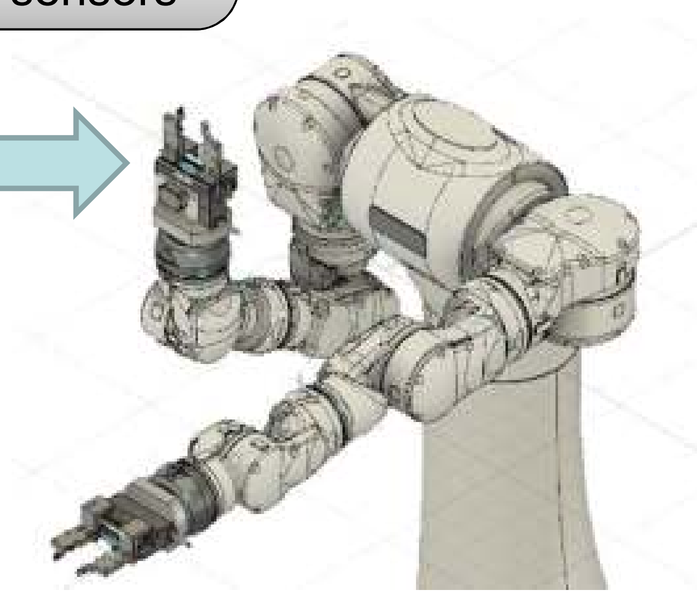
grippers

sensors

Control PC compiles a protocols 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.

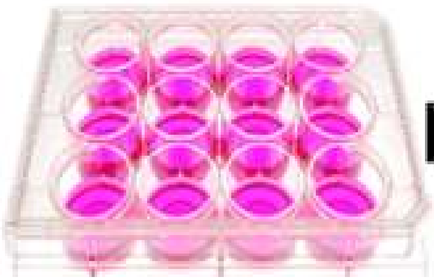
a batch program

540 pre-defined motions

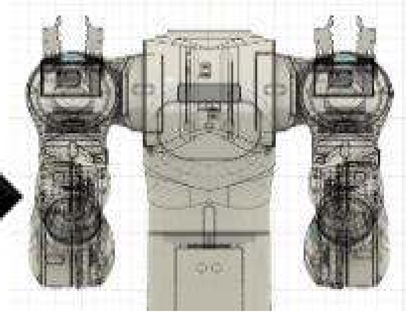


Automated Cell Culturing System

Incubate cells

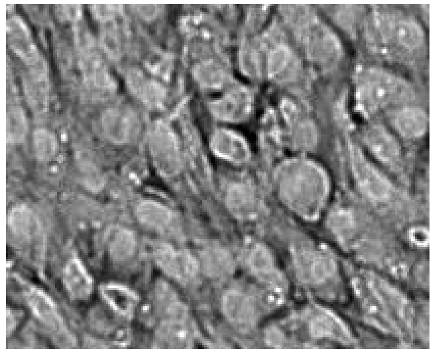


MAHOLO draw out a 12-well plate containing cells

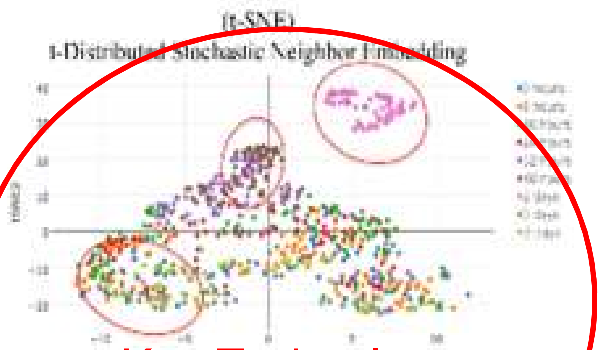


Performs media exchange every 24 hours

Puts a plate on a fully-automated microscope



Bright field images for each well are acquired



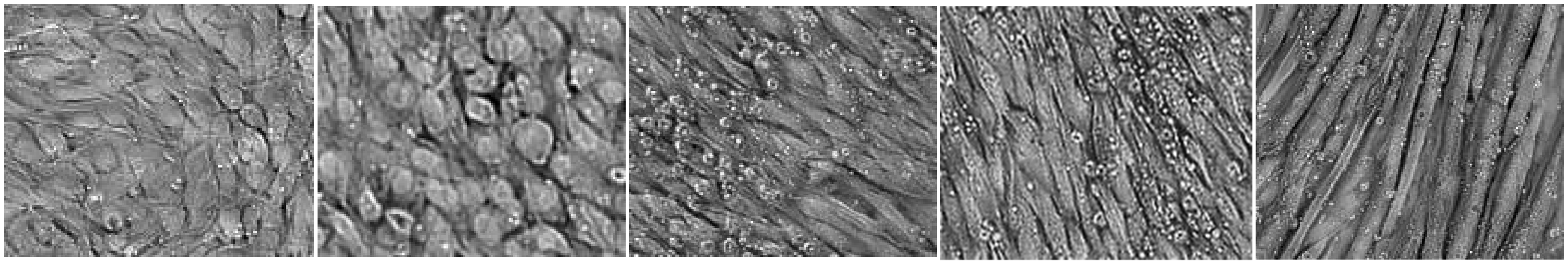
Key Technology

Automated Identification and Analysis of Cell Differentiation Stages

Non-healthy culture will be abandoned or applied recovery protocols

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.

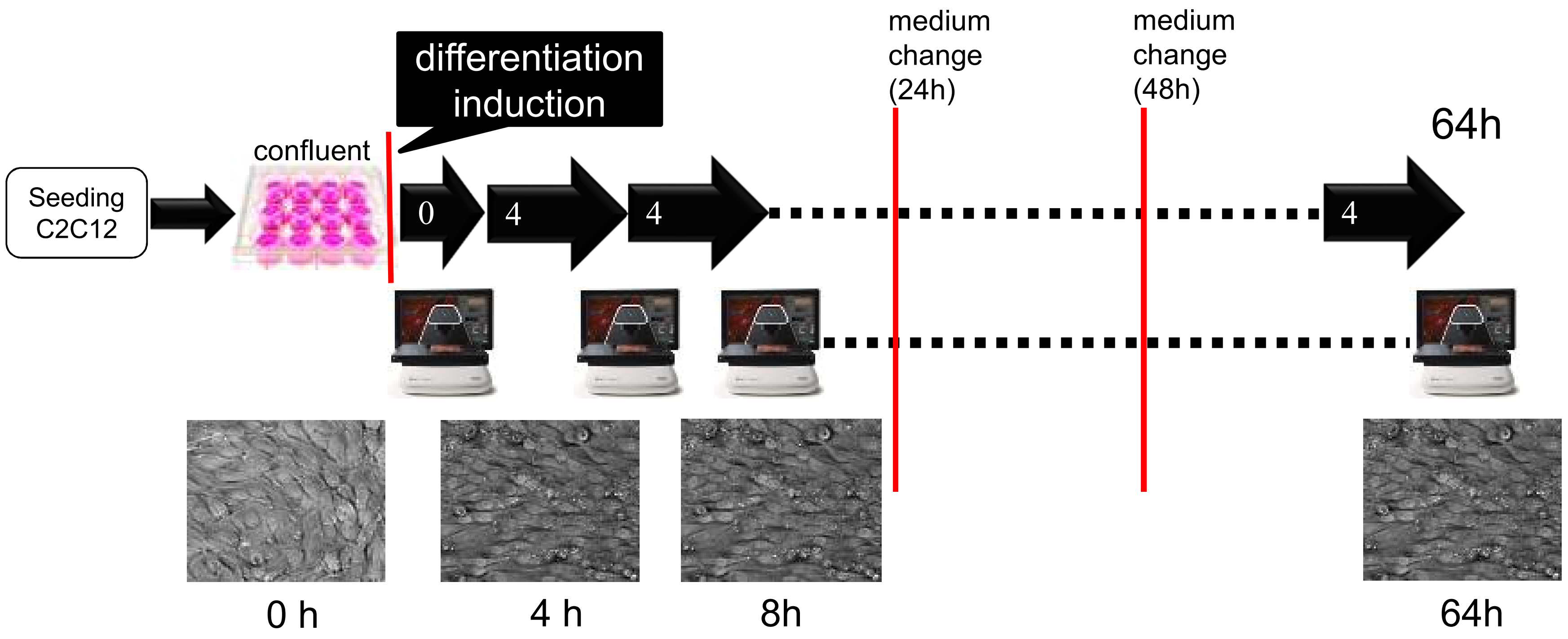


0 h 24 H 36 h 48 h 60h

If we can identify the differentiation stage earlier, we can save our precious time from culturing unnecessary cells.
<u>We develop a machine learning technology to identify cell differentiation stages using b.f. cell images.</u>

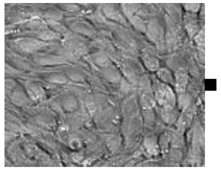
Live Cell Imaging Data

We kept a C2C12 plate in a CO2 incubator for microscope which enabled us to capture 44x12 images every 4 hours.

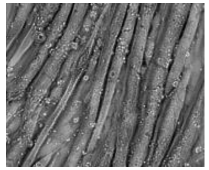


Feature extraction

Input:
Cell differentiation images



0 h

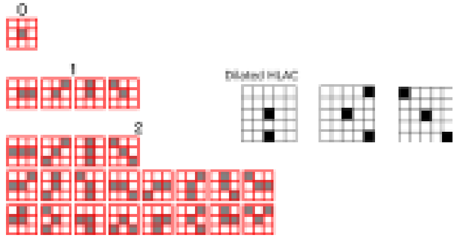


64h

Extract features

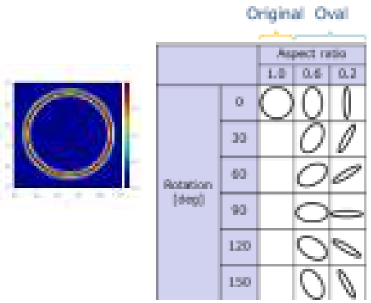
Wavelet
(haar, level=8)

HLAC
higher-order local auto-correlation



豊田, 長谷川 “高次局所自己相関特徴の拡張” 画像電子学会誌 第34巻 第1号(2005)

BCF
blurred circle function



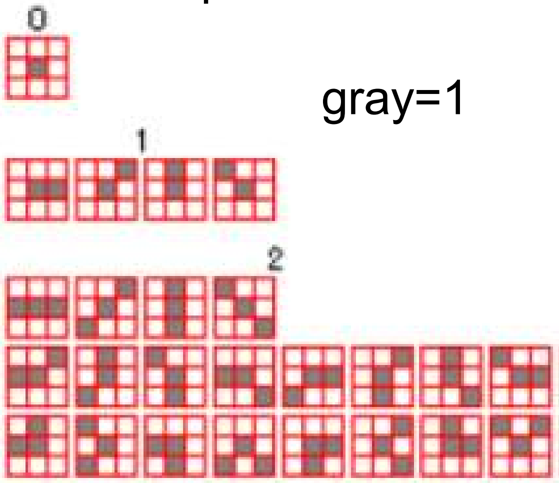
Build a cell differentiation stage predictor

Apply Multiple Machine Learning Algorithms for Cell differentiation stage classification

Higher-order Local Auto-Correlation

25 patterns

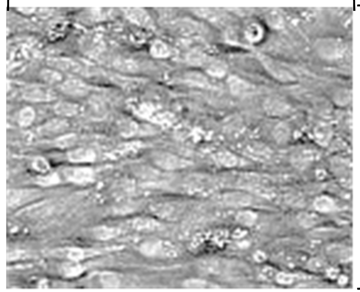
h



gray=1

I: input

x



y

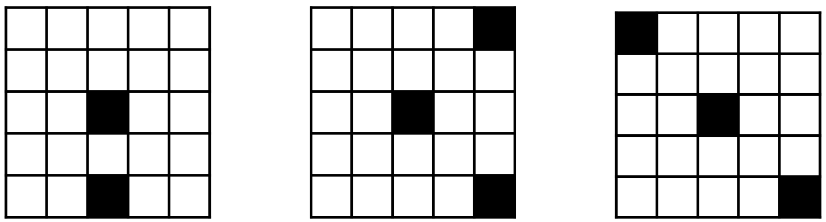
$$C^h = \left(c_{xy}^h\right) = convolution(I_{xy}, h^*)$$

$$F_{HLAC} = \left(hist\left(C^{h_1}\right), \cdots, hist\left(C^{h_{50}}\right)\right)$$

bin=25 builds 1250 dim. feature vector

+

Dilated HLAC

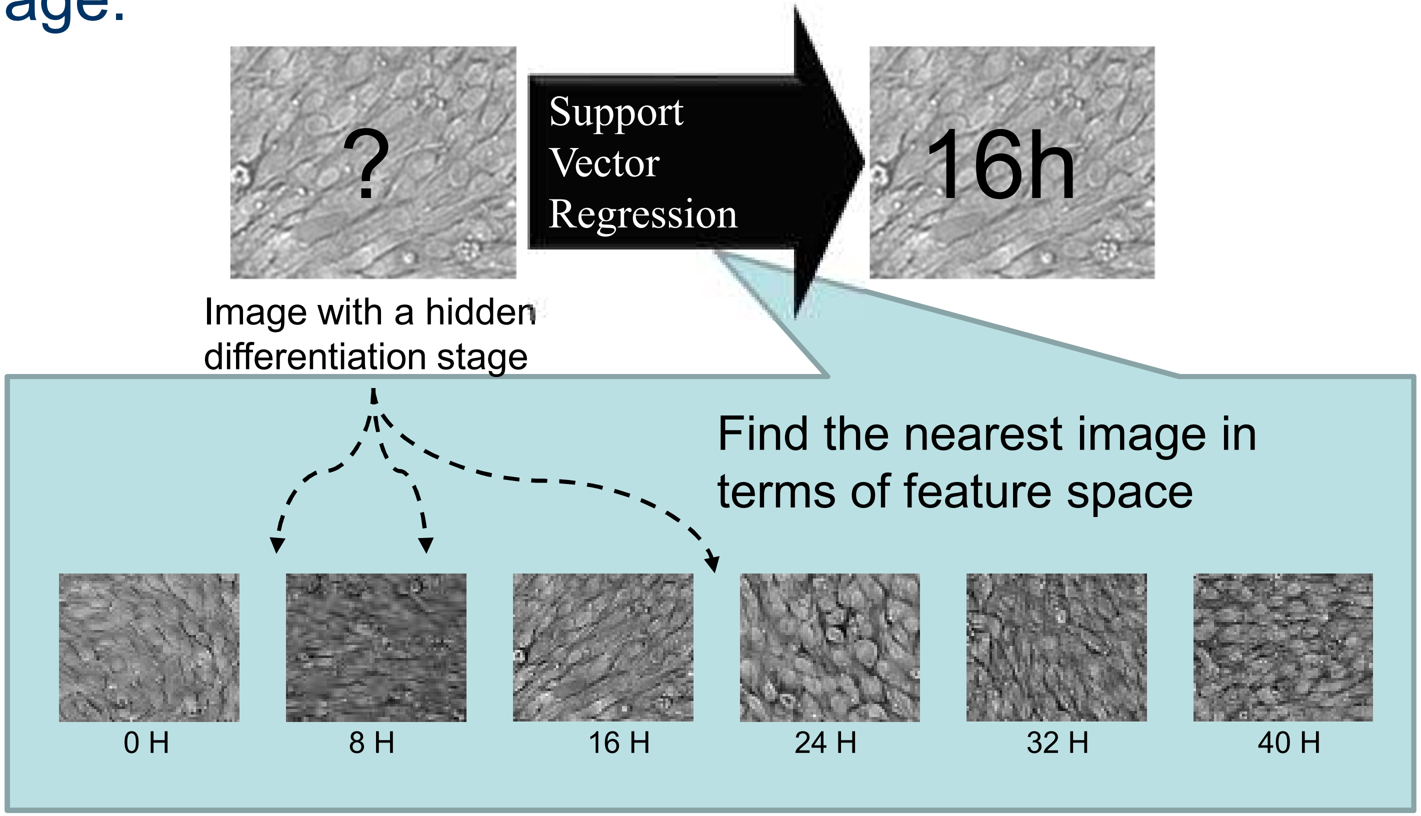


…

25 patterns

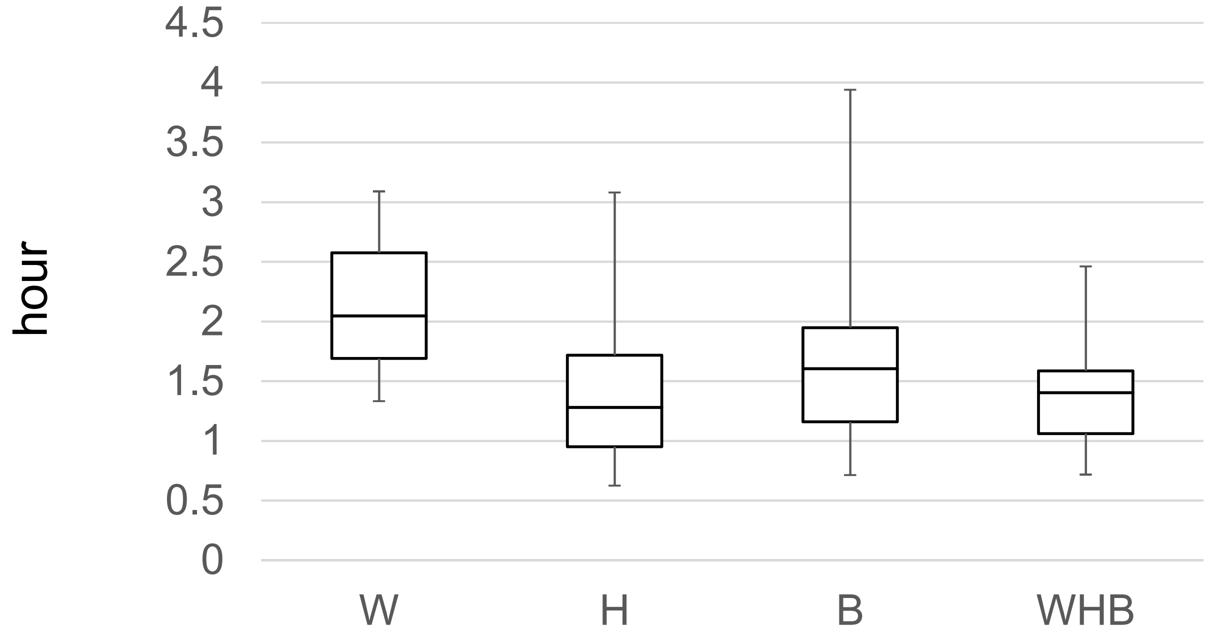
Regression Test

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

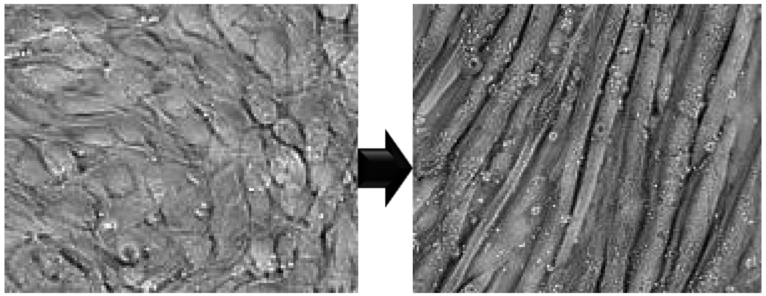
- RMSD=root mean square distance between actual and predicted time.
- W: wavelet, H: HLAC, B: BCF, WHB=W+H+B
- At least 20 images for a label are necessary.
- HLAC shows slightly better results than others but WHB performs generally better and more robust.
- This result tells that you can predict cell culture condition every 3 hours or longer which enables much finer control for cell culturing.



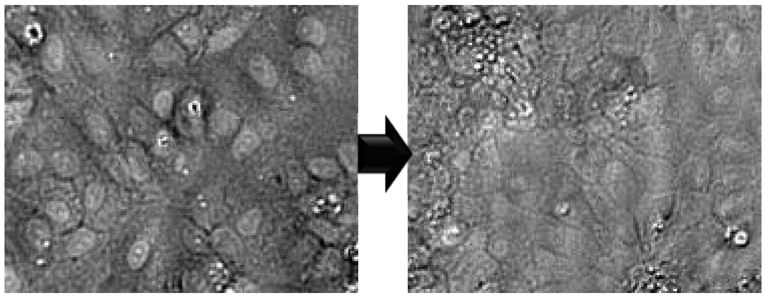
Human Hepatic Cell

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.

C2C12 0h 60h

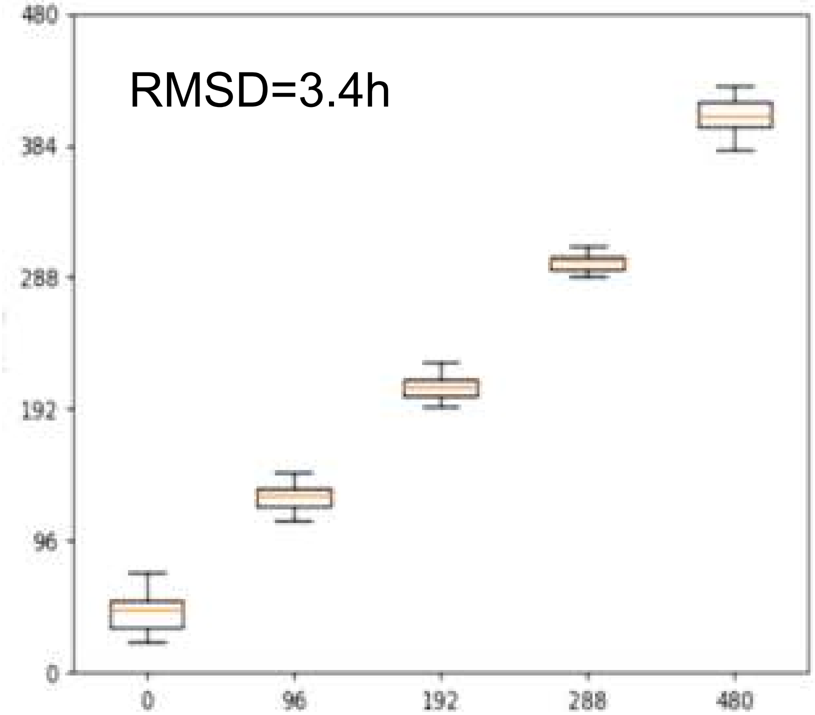


HepaRG 0h 480h



HepaRG shows different morphological changes during the differentiation.

Regression Test Result



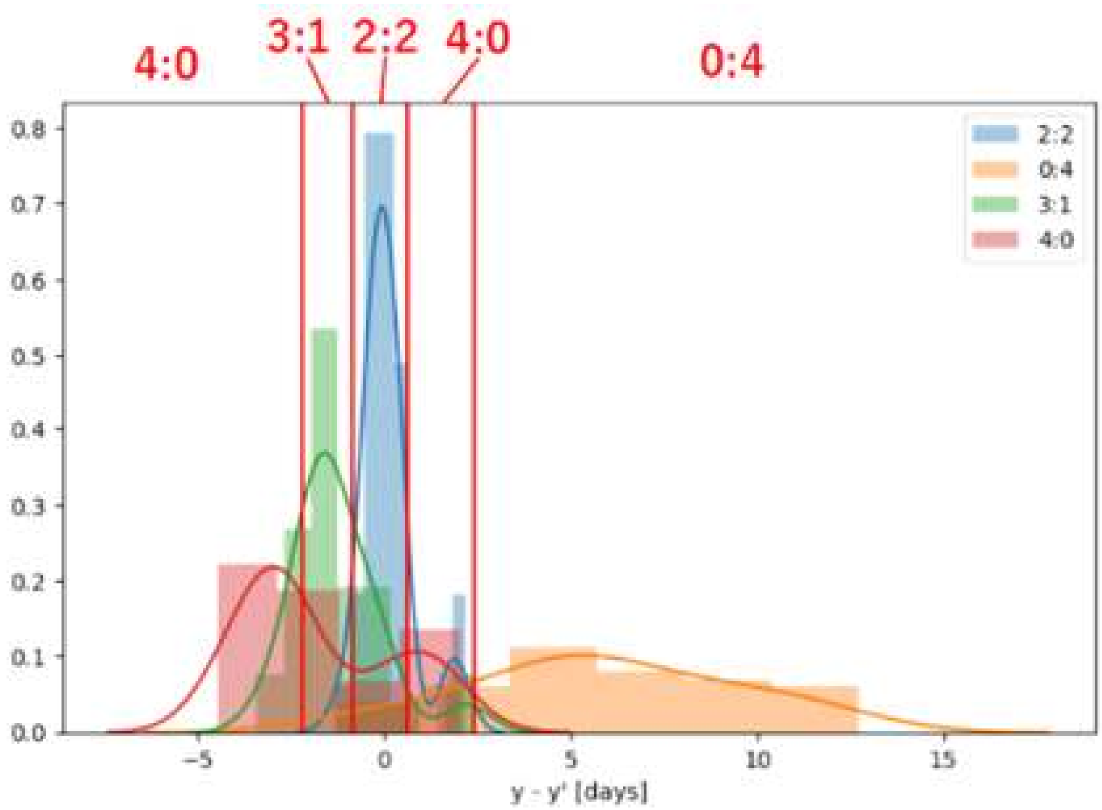
Culture Condition Optimization

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

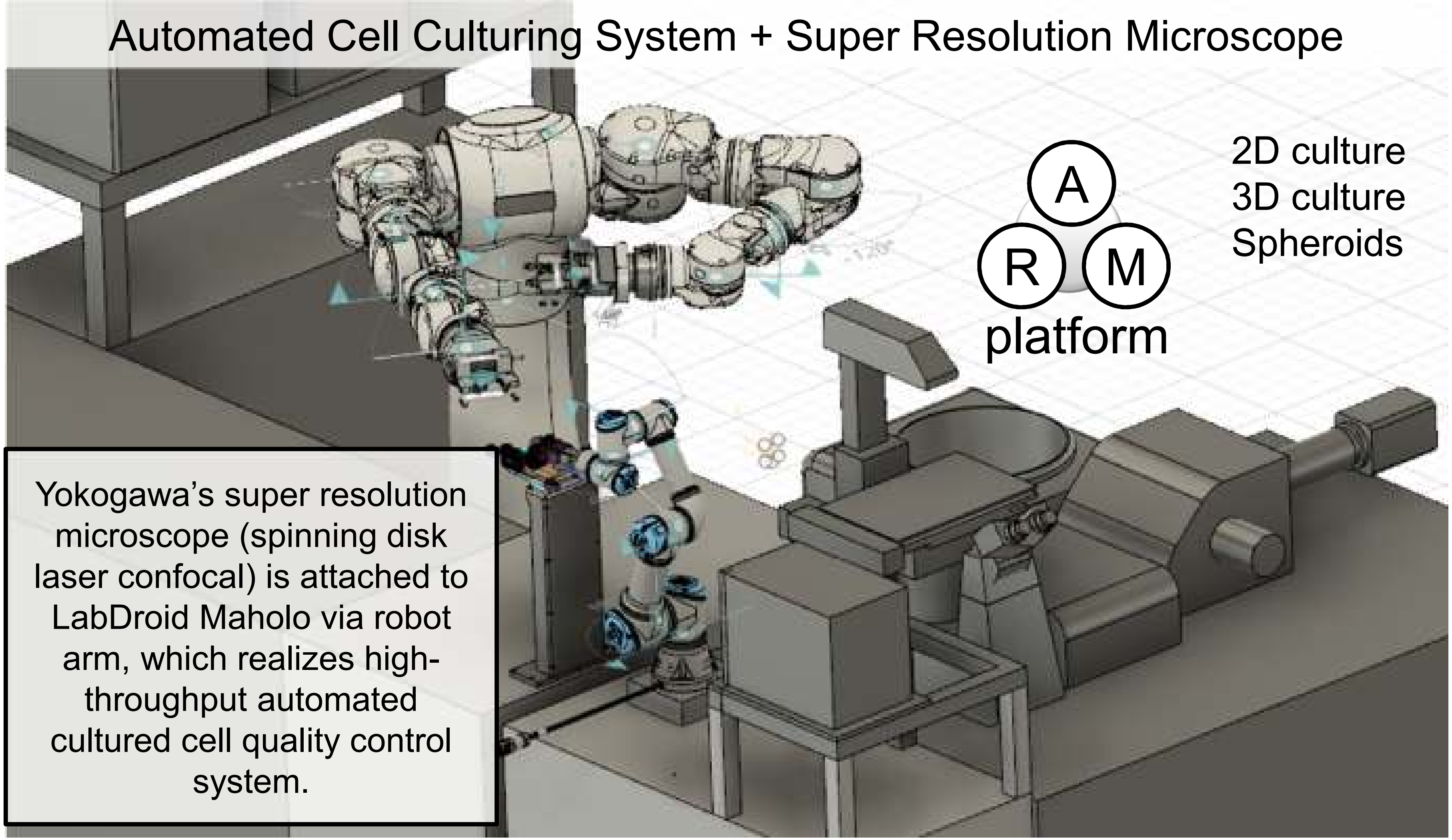
The figure shows distributions of differences between actual and predicted time for several culture conditions (regular (2:2) and others (3:1, 4:0, 0:4).

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



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.

Summary

- ✓ 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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