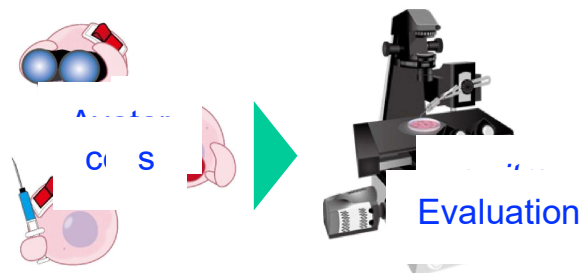


R&D Item

## 4. Intracellular CA teleoperation *in vitro*

### Progress until FY2024

#### 1. Outline of the project



In general, cells exhibit heterogeneity in their functions and characteristics. Therefore, to evaluate whether Intracellular Cybernetic Avatars (Intracellular CAs) operate as designed, it is essential to examine the time-dependent changes in the state of avatar cells, and evaluate the remote controllability of the functions designed within the intracellular CAs.

In this R&D Item, four advanced technologies will be developed for evaluating avatar cells. (4-1) High-speed, high-resolution microfluidic flow control technology to manipulate and analyze the purified avatar cells, (4-2) Live-cell imaging technology to analyze morphology, motility, activity, and



(4-1)  
High-speed and High-resolution  
Separation Technologies of Avatar Cells  
**Shinya Sakuma**  
Kyushu University



(4-2)  
Platform for Dynamic Measurement  
and Isolation of Avatar Cells  
**Yoshitaka Shirasaki**  
The University of Tokyo

cooperative behavior of avatar cells, (4-3) Detailed analytical technology utilizing deep learning-based image analysis and omics analysis to assess the remote controllability of intracellular molecular functions, (4-4) Precision detection technology for selectively detecting signals output by avatar cells through molecular recognition.

#### 2. Outcome so far

(4-1) We have developed the world's fastest on-chip cell sorting technology (Figure 1), enabling the separation of CA-loaded cells into multiple populations using fluorescence intensity as an index. Furthermore, the world's most precise picoliter pipetting technology has been established, allowing targeted cell collection under microscopic observation.

(4-2) Real-time visualization of cell-to-cell communication, mediated by cell surface molecules and secreted factors, has been successfully achieved. Dynamic measurement of interactions among three types of cells—target cells, CA-loaded inspection cells, and CA-loaded removal cells—has been initiated.



(4-3)  
Development of Data-driven Model for in-vitro  
Evaluating Teleoperation of Intracellular CAs  
**Takashi Kamatani**  
Institute of Science Tokyo



(4-4)  
Detectors for Output Signals by Intracellular  
CAs  
**Yosuke Katsuta**  
Kumamoto University

(4-3) An AI-based automatic annotation system has been constructed to extract and track cells over time from live imaging data, facilitating the quantitative evaluation of cellular functions through feature extraction. Time-series single-cell transcriptome analysis has also been conducted on target, inspection, and removal cells, leading to the identification of clusters representing the three cell types and elucidation of their characteristic gene expression profiles.

(4-4) The selection of nucleic acid aptamers capable of highly selective recognition of small compound released by avatar cells through intracellular CA activity has been initiated from a library of approximately  $10^{18}$  random sequences.



Figure 1:  
A platform for the  
dynamic measurement  
and isolation of avatar  
cells, into which the  
technologies  
developed in R&D  
Section 4 will be  
integrated.

#### 3. Future plans

*In vitro* evaluation technologies developed in R&D item 4 will be applied across the project to assess the remote-controlled functions of avatar cells, loading the Intracellular CA on cell lines as well as immune cells from human and mouse blood.