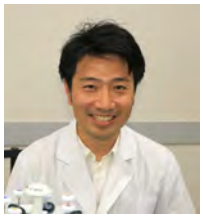
Microfluidics Hakubi Research Team（2021）
Hakubi Research Team Leader: Hirofumi Shintaku (Dr. Eng.)



(0) Research field

CPR Subcommittee**:** Engineering
Keywords: Microfluidics, single cell analysis, electrokinetics, nucleic acids, Next generation sequencing

(1) Long-term goal of laboratory and research background

We actively study problems involving fluid dynamics and transport phenomena in micro- and nanoconfined spaces. The problems are inspired by microfluidic systems for biochemical analysis and cellular engineering. Especially, we are interested in electrokinetic phenomena, e.g., electrophoresis, electroosmotic flow, and electrowetting of complex fluids, including cells and biomacromolecules. We recently developed a microfluidic system that enables the high-throughput sequencing of cytoplasmic and nuclear RNA of single cells with the physical fractionation of the subcellular RNA species via electrical lysis and isotachophoresis (ITP). We leverage our method to uncover the regulation of gene expression in single cells involving RNA localization and nuclear export.

(2) Current research activities (FY2021) and plan (until Mar. 2025)

(A) Electroporation-based lipid-bilayer assay for surface tension and transcriptomics (ELASTomics)

We have developed an approach, ELASTomics, that profiles the surface tension and gene expression in thousands of single cells by integrating nanoelectroporation and single-cell RNA-sequencing (scRNA-seq). ELASTomics quantifies the surface tension by counting the DNA tagged dextran (DTD) imported to cells through an electro-permeabilized plasma membrane via nanoelectroporation. As a proof of the concept, we experimentally demonstrated that the imported amount of DTD increases with the surface tension of cells using cells treated by cytochalasin D. We further applied ELASTomics to cancer cells with various malignancy and cells with senescence signature and discovered key genes that regulate the surface tension.
Future plan, We envision that ELASTomics is applicable to various cell types and will demonstrate the utility in various biological context.

(B) Spectrally coded hydrogel beads with barcoded DNA primers that integrates optical and genetic analysis

To integrate single-cell behavior measured by optical imaging with the gene expression analysis, we have developed spectrally coded hydrogel beads, of which surfaces are modified with single stranded DNA containing DNA barcode and poly(T) sequences for capturing mRNA. Our strategy leverages combination of codes created by cell-hydrogel bead pairs to increase the number of identifiable single cells than that of the spectral code of hydrogel beads. To this end, we stained and transfected the cells with barcode DNAs and made thousands of pairs of a single cell and a single hydrogel bead in microwells. We lyse the cells in the microwells to capture mRNA of single cells on the hydrogel beads. We decoded the combination of codes using the optical imaging data and next generation sequencing data.
Future plan, We will demonstrate the utility of our strategy by applying it to drug screening applications.

(C) Integrated microfluidic system for electrophoretic cytometry

To profile cytoplasmic component in single cells by electrophoretic analysis, we have developed an integrated microfluidic system. Unlike our previous system, the microfluidic system has 48 parallel channels for electrophoretic analysis of single cells. To monitor the electrophoretic migration of cytoplasmic molecules and organelles extracted from individual cells, we scanned and imaged the entire microfluidic system by moving the microfluidic chip on a microscope equipped with a motorized XY stage and sCMOS camera (Orca Flash 2.8, Hamamatsu Photonics). We developed an in-house program that stitches images (815 μm x 815 μm each) and outputs the entire electropherograms of 48 channels.
Future plan, We will integrate the spectrally coded hydrogel beads and the integrated microfluidic system and demonstrate multi-modal analysis integrating optical imaging, electrophoresis, and scRNA-seq.

(3) Members

(Hakubi Team Leader)
Hirofumi Shintaku
(Research Scientist)
Yusuke Oguchi (PRESTO Researcher)
(Postdoctral Researcher)
Akifumi Shiomi
Taikopaul Kaneko
Kotaro Torii
(Junior Research Associate)
Arata Tsuchida
(Technical Staff)
Kaori Nishikawa
Mayu Kawasaki
Keiko Wtanabe

(4) Representative research achievements

Article

1. Akifumi Shiomi, Kohjiro Nagao, Nobuhiro Yokota, Masaki Tsuchiya, Utako Kato, Naoto Juni, Yuji Hara, Masayuki X. Mori, Yasuo Mori, Kumiko Ui-Tei, Motohide Murate, Toshihide Kobayashi, Yuri Nishino, Atsuo Miyazawa, Akihisa Yamamoto, Ryo Suzuki, Stefan Kaufmann, Motomu Tanaka, Kazuya Tatsumi, Kazuyoshi Nakabe, Hirofumi Shintaku, Semen Yesylevsky, Mikhail Bogdanov, and Masato Umeda, Extreme Deformability of Insect Cell Membranes is Governed by Phospholipid Scrambling, Cell Reports, Vol.35, Issue 10 (2021), 109219.

Invited talk

1. Hirofumi Shintaku, Exploring transcriptional noise in subcellular compartments with on-chip electrophoretic fractionation of cytoplasmic versus nuclear RNAs, Human Cell Atlas Asia General Meeting 2021, 15th November (2021).
2. 新宅 博文, 核と細胞質に存在するトランスクリプトノイズの 1 細胞定量, 情報計算化学生物学会 2021 年大会, オンライン, 2021 年 10 月 27 日.
3. 新宅 博文, マイクロ・ナノ電気穿孔を用いた1細胞ダイナミクス分析, 日本機械学会 2021年度 年次大会, J301-01, オンライン（千葉）, 2021年9月6日.

Award

1. 塩見晃史, 金子 泰洸ポール, 西川 香里, 新宅博文, 日本機械学会 2021 年度年次大会 優秀講演論文表彰, 2022 年 1 月 18 日
2. 塩見晃史, 日本生物物理学会 若手招待講演賞, 2021 年 11 月 26 日

Supplementary

Group photo of RIKEN Microfluidics Hakubi Research Team

Laboratory Homepage

https://www.riken.jp/research/labs/hakubi/s_microfluid/
https://www.hshintaku.com/