Microfluidics RIKEN Hakubi Research Team
RIKEN Hakubi Team Leader: Hirofumi Shintaku (D.Eng.)

(0) Research field

CPR Subcommittee: Engineering

Keywords: Microfluidics, Single cell, RNA sequencing, Electrokinetics, Transport phenomena

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

We actively study problems involving fluid dynamics and transport phenomena in micro/nano confined 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. We leverage our method to uncover the regulation of gene expression in single cells involving RNA localization and nuclear export.

(2) Current research activities (FY2019) and plan (until Mar. 2025)

Results:

We have developed a microfluidic approach that fractionates the cytoplasmic RNA versus nuclei of single cells leveraging selective lysis of plasma membrane and electrophoretic extraction of cytoplasmic charged molecules. In this fiscal year, we explored the physics underlying the electrophoretic extraction of cytoplasmic components. We uncovered the two distinct kinetics in the electrophoretic extraction of RNA molecules corresponding to migrations of soluble RNA molecules and mitochondrial RNAs, which were characterized by the difference in the time constants of the extraction kinetics. Fluorescence microscopy and high-throughput sequencing analyses revealed that mitochondria, in particular, required a long extraction time. Using the time constants of extraction measured with RNASelect, we estimated that the extraction efficiency after 40 s was 98.9% ± 2%, which was consistent with the average efficiency of 99% measured for mitochondrial RNA using the high-throughput sequencing. The numerical analyses revealed the complex dynamics involving electrical lysis and the electrophoretic transport of cytoplasmic RNA molecules. The numerical studies also showed that the diffusion coefficients of RNA had little impact on the time scale of the extraction, implying that our approach leverages the electrophoretic extraction to offer length-bias free fractionation of RNA molecules.

Research Plans:

We will develop a microfluidic platform that parallelly processes 48 of single cells, outputs the electropherograms of cytoplasmic molecules, and enables synthesizing the barcoded cDNA from the cytoplasmic RNAs. To this end, we will first develop an integrated microfluidic chip that fluid dynamically isolates individual cells in microfluidic channels for electrophoretic analyses of cytoplasmic molecules. We will second develop a system for controlling the fluid flow and electric field in the microfluidic chip. To integrate the on-chip measurement, e.g., microscopy images of single cells and electropherograms, with the high-throughput sequencing of cDNA products, we will also develop color-coded microbeads. The color-coded microbeads capture cytoplasmic mRNA molecules with poly(dT) sequence and incorporate barcodes to the cDNA via reverse transcription. Compiling the multi-modal data, microscopy images of single cells, electropherogram of cytoplasmic molecules, and gene-expression analyses, we will dissect the relationship between cellular phenotypes and molecular phenotypes.

(3) Members as of March, 2020

(RIKEN Hakubi Team Leader)
Hirofumi Shintaku
(Research Scientist)
Yusuke Oguchi
(Postdoctral Researcher)
Mahmoud Atta, Taikopaul Kaneko
(Technical Staff)
Kaori Nishikawa,
Sangamithirai Subramanian Parimalam
(Junior Research Associate)
Arata Tsuchida
(Assistant)
Megumi Morita

(4) Representative research achievements

1. "Distinct Kinetics in Electrophoretic Extraction of Cytoplasmic RNA from Single Cells", Mahmoud N. Abdelmoez Yusuke Oguchi, Yuka Ozaki, Ryuji Yokokawa, Hidetoshi Kotera, and Hirofumi Shintaku, **Analytical Chemistry** Vol. 92, No. 1, pp. 1485-1492 (2020).
2. "SINC-seq法による1細胞多階層解析", 新宅博文, 小口祐伴, 飯田慶, **実験医学**（羊土社) Vol.37, No.20(増刊)Vol. 6, No. 4, pp.3533-3538 (2019).

Supplementary

Laboratory Homepage
https://www.riken.jp/en/research/labs/hakubi/s_microfluid/index.html
https://www.hshintaku.com/