博士論文（要約）

Living Single-cell Protein Analysis by Extended-nano Fluidic Device
（拡張ナノ流体デバイスによる単一生細胞タンパク分析）

Tatsuro Nakao
中尾　達郎

Department of Bioengineering
School of Engineering
The University of Tokyo

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Summary of the dissertation

The aim of this thesis is the creation of micro/nano-integrated fluidic device and realization of living single-cell protein analysis. The summary of each chapter are described below.

System design and development of extended-nanno unit operation (Chapter 2)

In chapter 2, system of living single-cell protein analysis was designed and extended-nano unit operation of sampling was developed. Firstly, system design of living single-cell protein analysis was composed based on the integration methodology of micro unit operations. As a result, it was clarified that extended-nano unit operation of sampling, which includes volume measurement and transport, needs to be developed. Therefore, fL volumetric pipette and flask was proposed. An extended-nano fluidic device for verification was fabricated and working condition of fL volumetric pipette is investigated. Finally, working principle of fL volumetric pipette and flask was verified experimentally by demonstrating volume measurement and transport of 11 fL water as a model sample. Herewith, all micro/ extended-nano unit operations for living single-cell protein analysis were prepared.

Fabrication and investigation of micro/nano-integrated fluidic device by integration of micro/extended-nano unit operations (Chapter 3)

In chapter 3, all micro/ extended-nano unit operations were integrated into single device and the working principle of the micro/nano-integrated device was investigated. One of the two issues of realizing the integrated device is multiple partial modification

on nanochannel surfaces. Partial modification method utilizing multi-phase flow was suggested and demonstrated with the integrated device. The other issue is fluidic control on the integrated device, which has complicated nanochannel networks. Fluidic control utilizing air in the integrated device was suggested and demonstrated by investigation with standard protein solution. Herewith, micro/nano-integrated fluidic device was successfully fabricated and the working principle was verified.

Application to single B-cell analysis (Chapter 4)

In chapter 4, the micro/nano-integrated device was applied to single B-cell analysis. Firstly, analytical performance of the integrated device was investigated by drawing a calibration curve using standard solution of IL-6. LOD of the device was discussed. Secondly, living single-cell protein analysis by micro/nano-integrated device was demonstrated using immortalized cell line of B-cell. The amount of secreted IL-6 was compared with/without stimulation. Finally, the integrated device was applied to single B-cell from patient serum.

The demonstrated methodology can provide a novel research tool to fields of medicine and biology and contribute to elucidation of single-cell-level mechanism of cancer or immune diseases.