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High-Throughput Fluorometric Assay of Enzymatic Reactions on a Microreactor Array Chip

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1. Introduction

Microarray chip technology such as DNA chip, peptide chip and protein chip is one of the promising approaches for achieving high-throughput screening (HTS) of biomolecule function [1-3]. It has great advantages in feasibility of automated information processing due to one-to-one indexing between array position and molecular function as well as massively parallel analysis of molecular properties as a benefit of down-sizing and large-scale integration. Mostly, however, the function that can be evaluated by such microarray chips is limited to the affinity against certain target molecules. HTS of enzymatic activity is considered to be an important technology that leads to the discovery or artificial creation of new useful enzymes, and its realization is expected to have great influences on various fields in bio-applications such as drug discovery, bio-energy and food industries. To evaluate enzymatic function, one needs to measure the change in substrates or products through enzymatic reactions. Hence, the enzyme microarray chip as similarly fabricated as protein microarray chip does not work and another ingenious device, namlely, microreactor array (μRA) chip is required [4-6]. By applying microfabrication technology, one can integrate vast numbers of microreactors with the nanoliter or picoliter-level volume a μRA chip. Some researchers reported the potential usefulness of μRA chips in various biochemical applications other than affinity assary, e.g., polymerase chain reactios or cell-based assary for drug testing. However, the necessary measurement platform for μRA chips is rather complicated than DNA or protein microarray chips. Thus there still remains lots of issues to be overcome before realizing its practical applications. In this paper, we propose a new HTS system of enzymatic activity based on μRA chip technology. A prototype of the automated and massively parallel measurement system for fluorometric assay of enzymatic reactions was developed by the combination of μRA chips and a highly-sensitive fluorescence microscope. Design strategy of μRA chips and an optical measurement platform for the high-throughput enzyme assay are discussed.

2. Experimental

A prototype of the automated and massively parallel measurement system for fluorometric assay of enzymatic reactions was developed by the combination of microreactor array chips and a highly-sensitive fluorescence microscope (Figs 1 and 2).

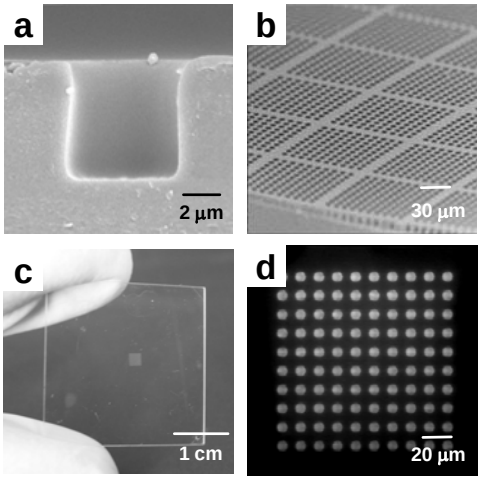

Fig. 1. (a) Cross-sectional view of a microreactor. (b)A bird's-eye view SEM photo of the microreactor array chip. (c) A photograph of a microreactor array chip (10×10 units/chip, 10×10 reactors/unit). (d) Fluorescent image of 1 mM fluorescein in the microreactors (ex. 488 nm, em. 510 nm).

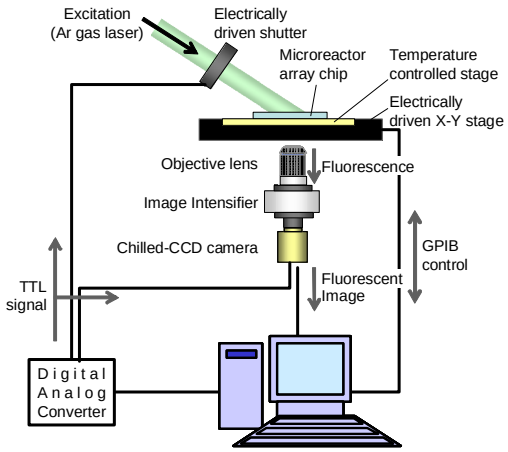

Fig. 2. Schematic diagram of high-throughput screening system for enzymatic activity using μRA chips.

2. Results and discussion

Down-sizing of microreactor into 1-10 μm level brings great advantages, for example, in reducing the sample volume of enzyme or substrate, high-density packing of reactors on a chip, shortening the time for completing chemical reaction. On the other hand, one should pay special attentions to the geometric optics when developing an optical assay system. According to experimental and simulation results, some guiding principles in system design are shown. It is emphasized that adopting an objective lens with good compatibility to the reactor size is important for improving detection sensitivity, and also the optical property of the chip material is not negligible.

Requisites in screening of enzyme activity are precise and highly-sensitive detection in short sampling time and massively parallel analysis. With due consideration of these factors, the automatically controlled measurement platform was developed for fluorometric assay of reaction products on a μRA chip. The system achieved the highly sensitive detection of 10 nM fluorescein and the block analysis of 100 reactors in a few second by real-time image processing (Fig. 3), and was demonstrated to be useful for detecting enzyme reaction of a tiny amount of β-galactosidase as a model enzyme (Fig. 4). Here fluorescein-di-beta-D-galactopyranoside (FDG) was used as a substrate.

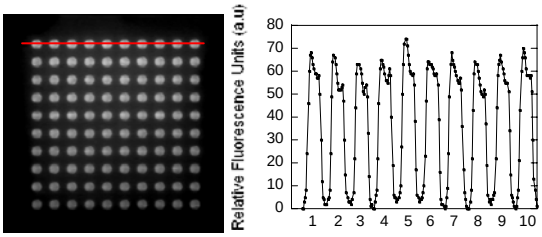

Fig. 3. Fluorescence intensity distribution of microreactor array filled with 1 mM fluorescein.

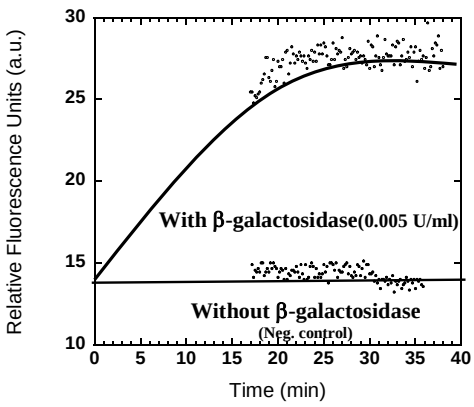

Fig. 4. Time-lapse measurement of β-galactosidase enzyme reaction in a microreactor. Approximately 10 enzyme molecules exist in a single reactor.

Moreover, the system is considered to be extendable to the massively parallel assay by adopting step and repeat motion of the chip stage according to the synchronized control using a personal computer (Figs. 5 and 6).

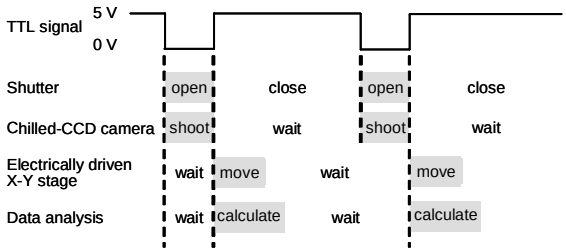

Fig. 5. A timing diagram for controlling the present measurement system.

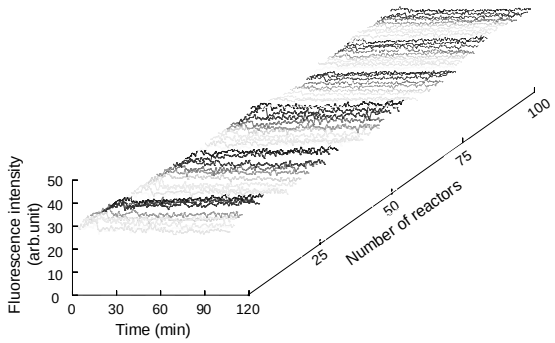

Fig. 6. Parallel time-lapse measurement of β-galactosidase enzyme reaction proceeding inside 100 microreactors.

4. Conclusions

In conclusion, we have proposed and demonstrated a microreactor array-based HTS system for enzymatic function. Such a HTS tool would be useful for various kinds of enzyme reaction measurement and be applied to the creation of useful biomolecules in the near future.

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