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Temperature distribution measurement on microfabricated thermodevice for single biomolecular observation using fluorescent dye

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Abstract

Precise temperature distribution measurements with high spatial resolution are of great importance in bioassay which uses microfabricated thermodevice, such as single biomolecular observation. We propose a new method to measure the temperature distribution in water with high spatial resolution using the fluorescent dye, Rhodamine B. Since Rhodamine B solution exhibits a strong and reversible temperature-dependent variation in its fluorescent intensity, it is useful as a temperature detector. The temperature distribution on a microfabricated thermodevice was successfully calculated from the fluorescent intensity distribution of a Rhodamine B solution. Finite element method modeling was carried out to demonstrate the reliability of the proposed method. A comparison between the measured and simulated temperature distributions revealed an excellent agreement. The method allows for direct measurement of the local temperature on our microfabricated thermodevice, where the molecule of interest stands, with an accuracy of 3 °C and a spatial resolution of 5.3 μm. Precise temperature detection along with optical measurement was possible due to our new method to detect temperature distribution. This is a promising method to reveal the temperature dependent characteristics of F_1-ATPase in future study. Applying this method to other single molecular observations might also realize reliable temperature measurement and may achieve substantial results.

Keywords: Rhodamine B; Temperature distribution; Fluorescent dye; Single molecular observation; Microheater

1. Introduction

The measurement of protein molecules has commonly been performed as an average over a large number of molecules in biochemistry. Recently, the direct observation of an individual molecule has enabled the interpretation of its individual behavior and characteristics [1]. This is a cutting-edge technique in molecular biology. The development of tools suitable for single molecule handling and its characterization is one of the key issues for further progress. The size of a protein is of the order of nanometers. Minute structured tools are required to work with these proteins. Micro- and nanotechnologies of today offer an opportunity to develop such tools, making it possible to perform quantiative novel experiments, and to produce results and knowledge that would not otherwise be accessible.

In earlier research of ours, the temperature-dependent activity of a single biomolecule was studied using a MEMS-based microfabricated thermodevice [2,3]. In such kinds of works, temperature accuracy of 5 °C as well as spatial resolution of a few micrometers is required. Fig. 1 shows our system for single biomolecular observation, a rotary assay of F_1-ATPase. The device consists of microfabricated heater and thermodevice which are integrated in the flow chamber which they use for the conventional bioassay. The absolute accuracy of the thermosensor itself is 1 °C. Thus far, however, we could not measure the temperature in high spatial resolution due to the difficulty in measuring the temperature distribution in water. An infrared camera can be used to measure the temperature distri-

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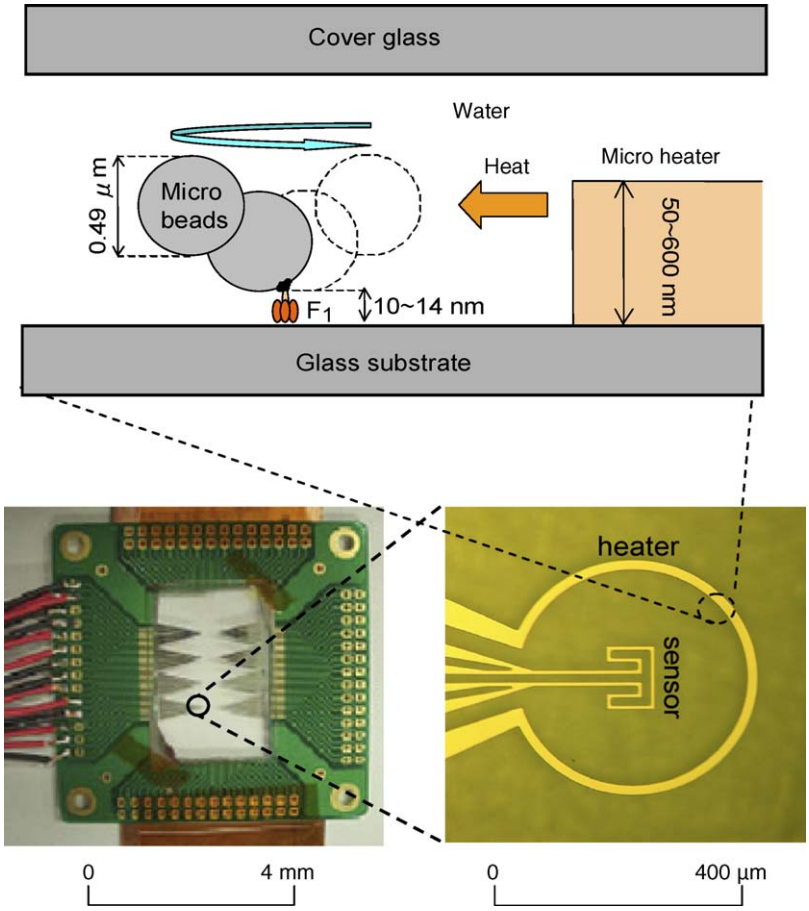

Fig. 1. Single biomolecular observation, a rotation assay of a F_1-ATPase, carried out on the microfabricated thermodevice (top). A MEMS-based microfabricated thermodevice which allows rapid temperature control under the microscope for single molecular observation (bottom). The integrated microfabricated thermosensor can detect the average temperature at the center of the circular microheater, whose diameter is 400 μm. However, the spatial resolution is poor since it cannot measure the temperature distribution.

bution with a spatial resolution of a few micrometers; however, it cannot be used for measurements on wet materials because water absorbs infrared light. A thermometer integrated in a microscale can measure the temperature in water but with poor spatial resolution. In this report, we propose a new method to measure the temperature distribution in water with high spatial resolution using a fluorescent dye. This enables us to measure the temperature distribution proceeding to the biomolecular assay.

2. Materials

Fluorescent materials such as Rhodamine B, Fluorescein, thermochromic liquid crystals (TLC) [4,5] and Quantum dots (Qdots) [6] exhibit temperature-dependent light intensities. Thanks to this, they can be used as probes to measure the temperature by calibrating the relationship between their light intensity and the temperature. However, the relation between fluorescent intensity and temperature of the plastic beads that contain the fluorescent dye on its surface is not reversible over 60 °C (Fig. 2) because the temperature stability of the plastic beads is lower than 60 °C. The measurable temperature for the TLC is limited in the range of 55–75 °C [4,5].

In the case of Qdots, the intensity is highly linear with temperature. However, the earlier study reports that the maximum temperature of Qdots is 60 °C, above which they lose their reversibility [6]. We carried out the experiment to confirm the temperature stability of Qdots using the microsystems which we used in our previous studies; microchamber and microheater [7] together with microneedle [8,9]. Qdot (#1002-1J, Quantum Dot

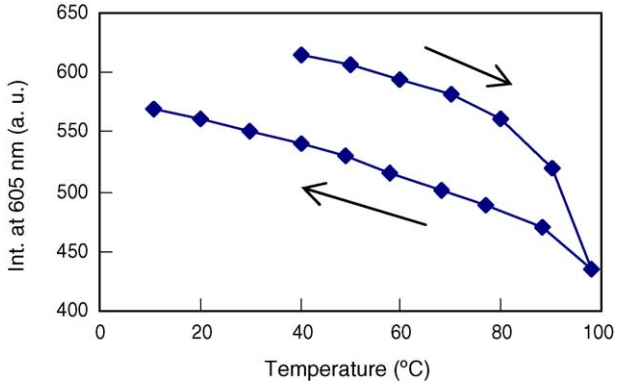

Fig. 2. Fluorescent intensity vs. temperature for fluorescent dye contained in plastic beads. The fluorescent intensity is not reversible since the plastic beads lose their physical property permanently at temperature higher than 60 °C.