LOCAL TEMPERATURE MEASUREMENT AND CONTROL USING FUNCTIONAL GEL-TOOL CONTAINING A QUANTUM DOT BY COLOR ANALYSIS OF FLUORESCENCE SPECTRUM

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ABSTRACT

We developed a novel temperature measurement and control method that uses color analysis of fluorescence spectrum. Functional gel-tools encapsulating quantum dots (Q-dots) was used. These tools can be manipulated in a solution by optical tweezers. We used color analysis of the fluorescence for temperature measurement. YCrCb information (Y: brightness, Cr: red color difference, Cb: blue color difference) is used for temperature measurement. From calibrations, we confirmed Cr information is suitable for temperature measurement with high sensitivity and stability. Moreover, we also demonstrated heat control of local environment using focused laser by simultaneous heating and temperature monitoring using the gel-tool.

KEYWORDS: Gel-tool, Quantum dot, Temperature, Color

INTRODUCTION

Several methods have been developed for temperature measurement with high spatial resolutions and sensitivities. Fluorescence measurement is major for on-chip measurement [1]. Intensity distribution of fluorescent indicator reveals environmental information. However, the fluorescent intensity methods suffer from problems associated with absorption, quenching, and photo degradation even if use of Q-dots. Microspheres whose surfaces have been modified by color indicators have been developed [2]. However, color indicator method is difficult to miniaturize such sensors due to the difficulty in detecting low levels of indicators. We propose gel-tool that employs a biocompatible hydrogel containing Q-dots for temperature measurement and control. Q-dots are considered to be suitable for temperature sensing because of lower photo degradation rather than fluorescence dye. [3] However, Q-dots also suffer from photodegradation and blinking. We overcame this problem by performing temperature measurements based on fluorescence spectrum analysis. Spectral information is detected by obtaining the color of the gel-tool using the color CCD. The color information of the gel-tool was calibrated with the YCrCb color spaces to avoid affection of the intensity fluctuation. Optical temperature control is also possible because Q-dots absorb the near infrared (IR) laser. We demonstrated temperature measurement and control using color analysis of the fluorescence of the gel-tool.

THEORY

We have used Q-dots as temperature indicators for optical thermometry applications. These Q-dots are semiconductors and have cadmium selenide (CdSe) cores encased in zinc sulfide (ZnS). These Q-dots have size-tunable absorption and emission wavelengths, exceptional photo stabilities, and high photoluminescence quantum yields. Q-dots that are strongly dependent on temperature and are insensitive to quenching by oxygen have been developed. The fluorescence from Q-dots decreases and the peak emission wavelength shifts to longer wavelengths with increasing temperature. However, the fluorescence intensity method suffers from photo degradation and blinking even if use of Q-dots.

To solve these problems, we proposed a fluorescence spectral method that uses color information. The color information is obtained by using a color CCD (WAT-250D2, Watec co. Ltd.). The color information in each pixel can be analyzed from the data obtained by a CCD camera (in RGB color space). The color information of the system must be converted from RGB color space to another color space YCrCb for color analysis. This is because RGB information is unsuitable for image analysis since all parameters are affected by brightness fluctuations, whereas Cr and Cb are robust against the fluctuations.

In addition to robustness against brightness fluctuations, it is important that the parameter varies monotonically with temperature and has a strong correlation with temperature for an automatic measurement system. RGB information was converted to YCrCb color spaces using the following equations 1. A filter of the WAT-250 filter was a complementary filter. Therefore, equation 1 was converted to equation 2 using equation 3.

\[
Y = 0.299R + 0.587G + 0.114B
\]
\[
Cr = 0.5000R - 0.419G - 0.081B \tag{1}
\]
\[
Cb = -0.169R - 0.331G + 0.500B
\]
\[
Mg = R + B, Cy = G + B
\]
\[
Ye = R + G, G = G \tag{2}
\]
\[
Ye = R + G, G = G \tag{3}
\]

Equations 1 and 3 were used for temperature calibration. Cr and Cb are used in the spectral method, while Y is used in the intensity method.
EXPERIMENTAL

Lumidot 560 Q-dots (Sigma-Aldrich, Co.) were used as temperature indicators. They have an excitation wavelength of 545 nm, a peak emission wavelength of 560 nm. We used a hydrophilic photo-crosslinkable resin (ENT-2000, Kansai Paint, Co. Ltd.) as the body of the gel-tool. This resin can incorporate functional materials inside itself. It is suitable for encapsulating Q-dots because they aggregate in hydrophobic materials. We prepared the gel-tool containing Q-dots by the simple process. 1 g ENT-2000 and 0.1 g Q-dot solution (0.865 g/cm³) were mixed. A mixture of this resin and Q-dots were aggregated by introducing them into an 1M K₂HPO₄ solution. The aggregated resin forms a spherical structure due to the surface tension of the solution. This resin mixed with the Q-dots was polymerized by UV illumination (around 366 nm). The polymerized gel-tool can maintain its structure in pure water. The systems can be sorted according to size by using a filtering process. Figure 1 (a) shows a fluorescence image of the cell investigation system. The system could be manipulated by optical tweezers in the solution because the refractive index of PEG (1.4) is higher than that of water (1.3), as shown in Figures 1(b),(c).

All experiment was performed an inverted microscope (IX71, Olympus) equipped with a fluorescent light source. A near-infrared laser (maximum power: 5.5 W; wavelength: 1064 nm) was used for the optical tweezers. We used a mirror unit (U-MWIG3) for exciting the system. A cell incubation chamber (Zilcos, Tokai Hit Co. Ltd) was used to control the temperature (accuracy of 0.3 K). Fluorescence information of the system is acquired using a CCD camera (WAT-250D2, Watec).

RESULTS AND DISCUSSION

Figure 2 shows the fluorescent images of the system at 30, 35, 40, 45, and 48°C. Figure 3 shows the temperature calibration results for Cr, Cb, and Y. Cb did not vary monotonically with the temperature. In contrast, Cr and Y were strongly correlated with the temperature. The sensitivity and accuracy of the intensity method were −1.1%/K and ±0.5 K, respectively. Those of the Cr method were −1.3%/K and ±0.3 K, respectively. These results can be explained by the spectral sensitivity characteristics of our system. The peak wavelength of the emission spectrum of Lumidot 560 was 560 nm. The mirror unit blocked fluorescence under 570 nm so that the CCD detects fluorescence longer 570 nm. The emission spectrum intensity decreases monotonically at uniform ratio according to the temperature increase. In this wavelength range, decreasing ratio of Mg is higher than that of Cy. Therefore, Cr value represented monotone decrease according to the temperature increase.

We also compared the measurement stabilities of the intensity and spectral methods. Figure 4 compares the measurement stabilities of the intensity and Cr methods. This comparison was performed using the same samples. The fluorescence intensity decreases with increasing measurement time. After 57 s, the fluorescence intensity decreased to about 20% its initial value. In contrast, Cr was almost constant throughout the measurement. These experiments confirm that the spectral method using Cr is suitable for high sensitivity, stable measurements.

We demonstrated temperature control by laser heating and temperature monitoring with the gel-tool. The laser absorption rate of the gel-tool was 1.8×10⁻³%/μm. Finally, we measured the temperature shift between before and after laser heating as shown in Fig. 8. Laser power was adjusted to 30.8 mW. Diameter of the laser spot was about 2 μm. We succeeded in measurement of the temperature distribution inside the tool, and confirmation of the temperature increase at both edges of the tool (about 2.6 K). From these results, the possibility of the wireless feedback control of the local temperature was indicated.

Figure 1: Photograph of gel-tool containing Q-dots. (a) Fluorescent image of gel-tools. (b) Gel-tool trapped by optical tweezers (in bright field observation). (c) Transport of gel-tool by optical tweezers (in fluorescent observation).

Figure 2: Fluorescence images of the gel-tools at each temperature. (a) 303 K. (b) 308 K. (c) 313 K. (d) 318 K.
CONCLUSION

We developed temperature measurement method using the gel-tool containing Q-dots by color analysis. Temperature was calibrated by fluorescent color using YCrCb color spaces. The calibration results indicated Cr is suitable for temperature measurement. Cr method can perform stable long-lifetime measurements because Cr is robust against brightness fluctuations. It is thus superior to the conventional method that uses fluorescence intensity. This tool is also applicable to local heat input by laser irradiation to the tool. This technique has the potential to contribute to advances in cell biology.

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REFERENCES


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