THERMOGENESIS DETECTION OF SINGLE LIVING CELLS VIA QUANTUM DOTS

Jui-Ming Yang¹, Haw Yang², and Liwei Lin¹

¹ Berkeley Sensor and Actuator Center, Department of Mechanical Engineering
University of California at Berkeley, Berkeley, California, USA
² Department of Chemistry, Princeton University, Princeton, New Jersey, USA

ABSTRACT
Cellular endocytic quantum dots (QDs) have been successfully used as thermometers to sense temperature variations inside single living cells. Temperature changes have been detected remotely through optical readout. We showed the increased extracellular calcium concentration can trigger the thermogenesis of NIH/3T3 cells and therefore elevated the cellular temperature about 1.5 °C. This technique has the potential as nanoscale thermometers for intracellular temperature measurements using non-contact, far field optical instruments with combination of both diffraction-limited spatial resolution at about 700 nm and sampling-rate-limited temporal resolution at about 30-100 ms.

INTRODUCTION
Analyses of cell temperature dependence can be useful to advance the fundamental understanding of cellular activities. Notable research in these areas include heat shock proteins and adaptive thermogenesis [1]. Heat generation inside cells has been used as a measure for metabolic actions, immune-modulators, intracellular enzyme activities as well as the effectiveness of drugs. However, accurate temperature assessments inside living cells are very challenging and the current state-of-the-art tools do not provide good spatial resolution and reading accuracy. For example, several groups have explored various techniques for thermal imaging in single living cells, such as micro-thermocouples and fluorescent dyes [2, 3], but with limited spatial resolution and accuracy. Here, semiconductor nanocrystalline particles, quantum dots (QDs) are proposed to be temperature indicators in single living cells. Previously, photoluminescence peaks of the quantum dots have been demonstrated to exhibit a red-shift with increasing temperature [4-6]. This phenomenon provides an opportunity for recording temperature variations based on non-contact, far-field optical readouts, which is advantageous for biological studies. Furthermore, several groups have reported that the placement of QDs (with bio-compatible coating and/or micelle encapsulation) inside living cells can be realized by passive endocytosis into cytoplasm [7]. Moreover, QDs with antibodies and specific sub-cellular compartments can be delivered to specific organelles inside living cells [8]. Therefore, it could be possible to use QDs as tiny thermometers in single living cells for various applications. In this work, we demonstrate the basic feasibility studies and a demonstration of detection of thermogenesis using QDs.

EXPERIMENTAL SETUP
Figure 1 shows a schematic diagram of the experimental setup by using CdSe/ZnS core/shell QDs coated with streptavidin for the temperature detection scheme. A mercury lamp was used as excitation light source. The attenuated and spectrally filtered light was directed through the microscope objective (20× or 40×) and focused onto the sample. The emission from endocytotic QDs was collected by the same objective and directed through a dichroic mirror (560lp; Chroma), channeled through an emission spectral filter and a spectrograph (SP2150i, Princeton Instruments/Acton-Research, grating 600 g/mm blazed at 500 nm) for resolving the quantum-dot emission spectra, and finally imaged by an intensified camera (Cascade 512B; Roper Scientific) with single-molecule sensitivity.

Figure 1. Schematic diagram of non-contact temperature sensing of single living cells using emission spectral shifts of quantum dots.

The entrance slit of the spectrograph served to minimize the influence of other QDs outside the centerline region and had an opening at 100-µm wide in the spectral imaging mode. To locate intracellular
QDs, the grating of the spectrograph was set at the zero-th order for imaging (Figure 2). Once a suitable cell and a proper QD spot were located, the grating was switch to the first order for time-dependent spectroscopic investigation. The spectral images were collected at integration times from 30 to 100 ms, which depended on loading concentration of quantum dots. The central wavelength of the spectrograph was set to 655 nm. The dispersion of the grating at 655 nm was 8.857 nm/mm, which corresponds to 0.14 nm/pixel at the imaging plan of the camera. With a 20× objective, the special resolution of each pixel was 700 nm at the sample.

To distinguish QDs’ signal from background noise and cell auto fluorescence, a threshold value was manually chosen in each experiment to cut off the unwanted intensity profiles. The threshold values varied from experiment to experiment, but were kept at the same value for a given experiment. When the intensity profile was below the threshold value, temperature reading was not available at that point, resulting in a blank data point. The emission peak of each row, which corresponded to the location along the observation slit, was calculated by the Gaussian fit of the intensity-wavelength profile. The fitted emission peaks were then compared with the initial wavelength data to obtain the relative spectral shift, which served as temperature indicator.

In a typical experiment, the quantum dots were introduced inside NIH/3T3 cells using the Qtracker Cell Labeling Kits (Q25021MP, Invitrogen). The kit used a custom targeting peptide to deliver QDs into the cytoplasm of live cells. In order to obtain reference temperature for the cell surroundings, a protocol was developed to leave portion of QDs remain at the outside of living cells. A homogeneous layer of collagen I (C3867, Sigma-Aldrich) with QDs was coated on top of the glass in a Petri dish. Cells were then seeded onto the QDs layer. The cells engulfed the QDs as they migrated across the substrate. A portion of the surface was not in the cell migration path such that some QDs were left outside of cells, as illustrated in Figure 3, serving as the reference temperature marker.

![Figure 2](image1.png)

**Figure 2.** (top-left) Fluorescent image of cells overlaid with quantum dots shown in red. (top-right) The spectra of QDs at corresponding vertical axis of the observation slit. (bottom) A representative spectrum (blue dots) and its fitted curve (red solid line).

![Figure 3](image2.png)

**Figure 3** (top) A schematic drawing showing the collagen-QDs layer serve as the reference local temperature markers, while the engulfed QDs providing the cellular temperature information. (bottom) The fluorescent image shows the both cell and collagen-QDs layer. The yellow lines indicate the outlines of the cells.

**QUANTUM DOTS CALIBRATION**

Cells were cultured in a Petri dish at normal conditions (37 °C and 5% CO₂), and incubated with QDs mixture (10 nM) for 1 hour. After washing out excess QDs, cells were fixed by formaldehyde (3.7%) and incubated in phosphate buffered saline (PBS) at pH 7.4. The Petri dish was then placed on the temperature-controlled stage under microscope with an external thermocouple (5SC-TT-K-36-36, Omega, Inc.) in the solution for observations. The thermocouple was used for temperature readouts and served as a feedback sensor for temperature regulations. The temperature for the culture dish was controlled from 17.3 to 47.2 °C, with about 5°C increments. The spectral images were captured after temperature reaches steady states and analyzed by MATLAB. Figure 4 shows the results of peak wavelength as a function of temperature. The spectra of QDs exhibited red-shift toward longer wavelength with increasing temperature. The fitted temperature sensitivity is about 0.057 nm/°C. This linear spectral
shift relationship is similar to previous single QDs results [5].

In general, the spectroscopic properties of organic dyes tend to be a strong function of pH. A control experiment has been performed to explore the potential spectral shift of QDs due to intracellular pH changes. Dead cells with quantum dots were incubated at different pH levels in PBS for examining the spectral shift at room temperature. Before changing the pH levels, cells were washed with PBS three times and incubated in fresh buffer solution with different pH. The results are displayed in Figure 5. It is observed that with pH value changes from 4 to 10, the emission peak wavelength of QDs changes about 0.0025 nm per 0.1 pH. This implies that the influences of pH changes (as huge swing of pH values is not expected inside living cells) to the spectra shift of QDs is negligible as compared with the temperature changes.

**TEMPERATURE MEASUREMENT WITH CALCIUM INFLUX**

The verifications of temperature measurements inside living cells inspire us for further studies on possible applications. One commonly known effect is the thermogenesis of living cells with the infusion of calcium. This effect has been reported qualitatively but has not been quantitatively characterized in single living cells due to the challenges in temperature measurements. Here, we used ionomycin to induce calcium ions into the extracellular space, which in turn triggers cellular thermogenesis and generates heat. NIH/3T3 cells have been chosen in this experiment and fluorescent dye, fluo-4 (F10471, Molecular Probes) in a separate control experiment is used to quantify cellular calcium concentrations. First, fluo-4 was loaded according to the manufacture recipe and intracellular [Ca\(^{2+}\)] was measured. The extracellular calcium concentration was increased by ionomycin to a final concentration of 1 µM, ~40 seconds into the experiment. The fluorescent images in the left side of Figure 6 show the intensity changes before and after the infusion of ionomycin. The measured average intensity of fluo-4 is displayed in the right panel of Figure 6. These results imply that the calcium concentration has been elevated by about two orders magnitude higher than the original condition after the infusion of ionomycin into the extracellular space.

![Figure 6](image)

For the cellular temperature experiments, NIH/3T3 cells were cultured in a Petri dish at standard condition (37 °C and 5% CO\(_2\)) with up-taken quantum dots as temperature markers. Prior to the experiments, the culture dish containing 500 µl of the culture medium was lowered to room temperature to be the same temperature as other chemicals. The culture dish was placed on the imaging platform to record the emission spectra of QDs. After 2 minutes into the experiment, 10 µl of 0.5 mM ionomycin solution was added to the culture dish, to reach a final concentration 1 µM of intracellular [Ca\(^{2+}\)]. We recorded and calculated the temperature responses for 15 minutes, with 6 seconds interval and 150 frames.

Figure 7 shows typical measurement results of temperature responses from two live samples and one dead sample. The average spectra shift was calculated by Gaussian fitted spectra. Results show approximately 0.1 nm shift, which corresponds to 1.5
°C temperature rise, three minutes after the calcium infusion for live cells. In contrast, dead cells show no measureable temperature changes. Using z-test statistics, the dead cell experiments showed a 95% confidence interval of -0.005 to +0.009 nm in average spectra-shift, which implied calcium ions concentration didn’t affect the emission spectra of QDs.

Figure 8 shows the temperature map of a single living cell as function of position and time when adding calcium ions 2 minutes into the experiment. The y-axis represents the location of the cell along the entrance slit of the spectrograph, and the color of pixels indicates the relative temperature changes. The thermal image displays several hot spots in red and yellow within a single living cell a few minutes into the experiment and the temperature profile across a single cell became non-uniform. This suggests that the intracellular temperature difference can exist within a single living cells and the temperature distribution can be measured using the proposed methodology. The result demonstrated that QDs are capable of providing intracellular temperature difference and this thermal image methodology is capable of providing spatial resolution of 700 nm per pixel with 20× objective.

CONCLUSION

This work successfully characterized the spectrum shift of QDs in cellular environment under different temperatures as 0.057 nm/°C as the foundation for intracellular temperature measurements. We have achieved and reconstructed the thermal imaging of the living cells which has the potential to advance insightful knowledge for subcellular activities with respect to temperature variations. Experimentally, cellular temperature increases about 1.5 °C after the infusion of high concentration calcium. This observation implies that heat generation in living NIH/3T3 cells can be significant and measureable with calcium stimulants.

REFERENCES