Jurnal Teknologi

Full paper

Intracellular Thermal Sensor for Single Cell Analysis -Short review

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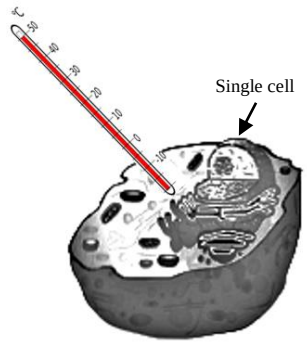
Article history

Received : 15 August 2014
Received in revised form :
5 January 2015
Accepted : 10 February 2015

Graphical abstract



Abstract

Temperature is a key environmental variable that affects almost all natural and engineered systems from the system level down to the molecular level. The first attempt to measure temperature goes back to 1592 when Galileo Galilei tried to develop a thermometer. Since then having accurate temperature measurements has been a challenging research topic. Recently, in single cell analysis, internal temperature and heat generation inside a living cell has proven to have important roles in the survival of cells, controls many cellular activities for instance; cell division and gene expression. Moreover, cancerous cells are identified with excessive heat production. Studies have been done by researchers from different fields in the attempt to develop sensors that can accurately report the temperature inside living cells. This short review presents the most recent developments in nanoscale thermometry for biological applications, highlighting the recent advances in the near field and the far field methods. The far field thermometry cover sensors that depend on the luminescence's of the material, for example: quantum dots, nanoparticles, and fluorescents based compounds. While, near field thermometry is based on different principles depending on the sensing mechanism used. Some of the examples mentioned are thermocouple thermometry, RNA thermometry, resonant thermometry, photoacoustic thermometry and carbon nanotubes thermometry.

Keywords: Luminescent thermometry; non-luminescent thermometry; sensitivity; single cell

1.0 INTRODUCTION

Temperature is an important physical property of a matter that can determine the internal energy contained within a system, and can describe a change or a situation a system is undergoing. It can be defined as the measurement of the average kinetic energy of molecules in an object or system [1]. The understanding of temperature goes back several decades i.e. around 100 BC when Hero Alexandria observed that the volume of air varies notably with the temperature. However, his observation became significant in the 1590's when Galileo Galilei performed that idea experimentally [2] and developed "the thermoscope"; a device that is very important in the history of science and is responsible for the growth of the thermometer and temperature sensing [3].

Temperature plays an important role in understanding changes that can happen in a system, for instance; when a person gets sick, the body temperature would be higher compared to other healthy individuals. Furthermore, temperature to influence the efficiency of electrical devices; higher temperatures usually mean greater resistance and noise which leads to a decrease in the reliability and an increase in the occurrence of failure. The knowledge obtained from temperature detections can help overcome and solve many drawbacks [4].

In single cell analysis, high demands by researchers from different fields have been increasing towards measuring the internal temperature of a biological cell. Temperature has proven to have important roles in the survival of cells; affects cell metabolism, cell division, gene expression and many other cellular activities [5-7]. Besides that, cells that are characterized with an abnormality i.e. cancer tends to have extraordinary heat production [8], [9]. Therefore, by knowing the internal temperature of a cell, valuable information about the cell's mechanisms and activities and also cell survival can be understood.

This short article presents an overview of the current developments and techniques used to measure the internal temperature of living cells. The methods are categorized based on the sensing mechanism into luminescent and non-luminescent thermometry; which cover a discussion on quantum dots, nanoparticles, fluorescents based compounds,

thermocouple, thermal resonant, photoacoustic, and carbon nanotubes.

2.0 LUMINESCENCE THERMOMETERS

Luminescence thermometers refer to thermometers that use the fluorescence emitted from materials to measure the temperature. They use far field detection method, considerably fast, and allow the measurement of temperature on static and moving parts [10]. The detection of temperature happens when the temperature of the luminescent material changes, and as a result the intensity of the fluorescence, the excitation spectra, the decay lifetime, or the wavelength of the fluorescence changes [11, 12]. The use of fluorescence spectroscopy in combination with high spatial resolution fluorescence microscopes enable intracellular monitoring of many different species for medical and biological purposes [13]. Below is a discussion of different examples that use luminescence to investigate the temperature profile in living cells.

2.1 Nanoparticles based Thermometry

Nanoparticles (NPs) are particles that are very small in size, they can be smaller than cells or channels in nano-fluidic devices [14]. The particle sizes generally range from 2 nm to several µm, but in most cases they are in the nm scale. They can be made of different materials and can be altered to have different shapes [15]. Their small size gives them the advantage of having high signal to noise response ratio and signal amplification that improves the analytical sensitivity and response time.

Fluorescent nanoparticles, depending on their structure and properties, exhibit specific excitation and emission spectra. The temperature sensing mechanism differs for the different type of NPs used, depending on the material they are made from. Some depend on the quenching mechanism to enhance the florescent, others are doped with fluorescent dyes to form a particle matrix and some are intrinsically fluorescent for example, quantum dots (QDs).

Nanoparticles are good candidates for single cell intracellular temperature measurement. Due to their small size the physical damage that can be caused to the living cells is minimal. They have a high spatial resolution, for instance, according to Raleigh's approximation, the strength of light coming out of an isotropic particle is approximately equal to the sixth power of the particle diameter meaning that for a particle having a diameter of 10 nm, the strength of light emitted will be 1000000 [11]. Furthermore, they are less likely to be affected by photobleaching, and can be easily controlled to target a specific location for detection by performing surface modification with biomolecules, such as antibodies.

Scientists have tried to study the effect of different nanoparticles towards living cells and have assessed their feasibility to become an actual thermal sensor for biological cells. J. Davda *et al*. [7] demonstrated the biocompatibility of nanoparticles having different sizes with biological cells. They used endothelial cells for the study and nanoparticles sizes ranging from 277 to 372 nm. They showed that cells react optimistically towards the NPs and did not affect the cell viability in a 48h mitogenic assay. However, the uptake rate of the cells depended on the concentration of the nanoparticles used i.e. the higher the concentration the more was the uptake of NPs.

Aznar *et al*. [16] developed a temperature dependent nanoscopic solids that can be internalized in a cell. The sensor consisted of mesoporous silica NPs loaded with Safranin O and functioned with paraffin (hydrophobic layer that block the pores), as a capping molecule. The principle was that when the temperature reached the melting point of the paraffin, the paraffin would melt and result in the uncapping of the pores (Figure 1(A)). The NPs were taken by HeLa cells and incubated in temperatures above the melting point of Safranine O. The release of Safranine O was observed by confocal microscopy (Figure 1(B)). This sensor has not been applied in particular for cell temperature measurement; however, it did indicate the temperature value in the cell, when the cell reached the melting point of Safranine O.

F. Ye *et al*. [17] developed an ultra-bright single nanoparticle radiometric temperature sensors based on semiconducting polymer dots (Pdots). A temperature sensitive dye Rhodamine B (RhB) whose emission intensity decreases with increasing temperature was attached to the matrix of Pdots. The Pdot-RhB nanoparticles showed excellent temperature sensitivity and high brightness because of light harvesting and amplified energy transfer capabilities of the Pdots. The Pdot-RhB nanoparticle obtained temperature sensing under a single wavelength excitation and had a linear temperature sensing range that matched well with the physiologically relevant temperatures. Pdot-RhB was tested on Hela cells for intracellular temperature measurements. The results showed a decrease in brightness as the temperature increased from 13.5°C to 36.5°C (Figure 2). The exceptional brightness of Pdot-RhB permits this nanoscale temperature sensor to also be used as a fluorescent probe for cellular imaging.

K. Oyama *et al*. [18] designed fluorescent NPs in which Eu-TTA was the temperature sensor inserted into a polymer network of polymethylmethacrylate (PMMA). The polymer network protected the dye from changes in pH and ionic strength since fluorescence intensity of Eu-TTA was affected by those parameters. The particles were further coated with the cationic polymer polyallylaminehydrochloride (PAH) that facilitated binding to the anionic cell membrane (Figure 3(A)). The core of the nanothermometer had a spherical shape with a diameter of approximately 113 nm while the whole structure had a diameter of approximately 211 nm. The nanothermometer was tested on HeLa cells. Coating the NPs with PAH facilitated spontaneous cell penetration through endocytosis. The pH-sensitive fluorescent dye (pHrodo) with PAH facilitated spontaneous cell penetration through endocytosis. The pH-sensitive fluorescent dye (pHrodo) conjugated to dextran was used as the marker of endocytosis (Figure 3(B)). The results showed that through pulse heating of the outer part of the cells for 1s, the average temperatures before and during heating were 36 ± 0.5°C and 47.2 ± 3.9°C respectively.

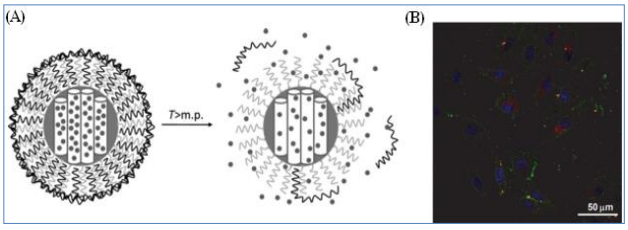


Figure 1 (A) schematic diagram of nanoscopic mesoporous silica NPs loaded with Safranin O that releases when the temperature increases above the melting point. (B) Fluorescent image of the Hela cell showing Safranin O probe (in red) spread in the cell cytoplasm in cells incubated at 42°C [16]

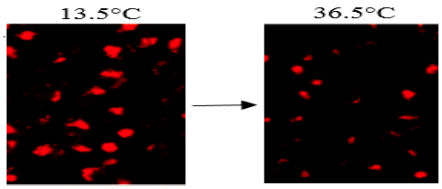


Figure 2 Fluorescent image of Hela cells using Pdot-RhB temperature sensor showing a decrease in brightness with an increase in temperature (property of RhB) [17]

Nanoparticle properties proved to have an enhanced emission spectra when exposed to multiple photon excitation. D. Jaque *et al*. [19] studied the temperature sensitivity of a multi-photon excitation Er/Yb co-doped NaYF4 NPs and showed that a maximum temperature sensitivity of 1ºC could be achieved using those particles. They also examined their capabilities in the measurement of the temperature in HeLa cells, and concluded that for the typical excitation intensities used in bioimaging experiments, pump induced thermal loadings can be neglected in the necrosis and the local temperature increments could be large enough to cause cell deaths.

From the advantageous structure manipulation property of NPs, scientists studied NPs not only for temperature mapping but also for the treatment of tumor cells [20]. I. El-Sayed *et al*. [21–23] demonstrated that gold NPs are a novel class of photothermal agents causing cell injury and death through conversion of the strongly absorbed light to thermal energy. They have shown that Au NPs absorb light and convert it into heat in picoseconds. They also showed that by altering the shape of the solid pure gold NPs i.e. the shell thickness and the core size, will enable the NP to absorb light strongly in near infrared regions.

Nanoparticles provide great advantages over traditional fluorescent dyes as they have unique fluorescent properties that could be used and altered for various applications. Furthermore they provide greater biocompatibility and reduced toxicity compared to conventional chemical agents. However, more research and expanded studies are needed to improve the current steps forward to develop NPs that could be used commercially.

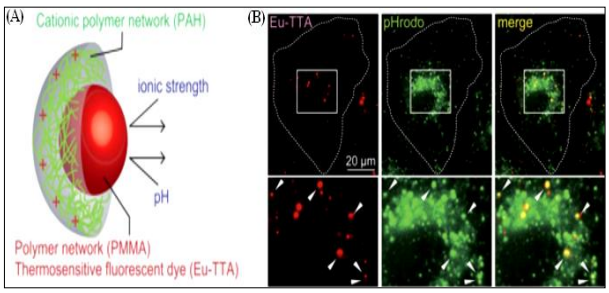


Figure 3(A) Schematic diagram of the fluorescent nanothermometer. The red sphere represents the core which is made of a polymer network (PMMA) and thermosensitive fluorescent dye (Eu-TTA), and the green part refers to the cationic polymer network (PAH). (B) Fluorescence images of Eu-TTA of nano- thermometers (red), pHrodo–dextran (green) colocalized (yellow) which determines endocytosis [18]

2.1.1 Quantum dots based thermometry

Quantum dots (QDs) are one of the pioneer methods proposed that show promising results to measure the internal temperature of a single cell. They are nanoparticles made of semiconducting materials with a very small size range, reaching up to 1 or 2 nm's [5]. They have autofluorescence properties which mean that they emit light naturally due to their material properties when exposed to energy [24]. Temperature measurements are done by exciting the QDs with a laser for the electrons to shift from ground state to the excitation state forming a hole and an excited electron. Autofluorescence happens when these excited electrons transition to the ground state and combine with the holes in a radiative transition. However, at higher temperatures the thermal energy reduces the gap between the ground and excited in a non-radiative transitions to the ground state because the states overlap at a higher energy [11]. The emission spectra of this fluorescence exhibit a red-shifted frequency which measures the variations in temperature. Such detections provide a good opportunity in biological studies to detect the temperature of single living cells [25], [26]. Quantum dots are usually made based on Cd material having a dual [27] or multiple [28] core shell structure. They have several advantages such as bright fluorescence properties [14], long fluorescence emission time [29] , good photo-stability [30] size tuneability [31]. However, some of their disadvantages are that they have poor solubility [32], agglutination [33], photo-blinking [34], unstable in changing environments [35], and being toxic to biological systems.

Scientists over the years have attempted to study the thermal detection abilities of QD, for instance, S. Li *et al*. [36] studied individual CdSe QDs (7-12 nm in diameter) and demonstrated their application in microelectromechanical (MEMS) heaters. They showed that the sensitivity of a single QD was approximately 0.1 nm/°C, which was consistent with bulk measurements that gave a sensitivity of approximately 0.093 nm/°C over a range of 24.4 to 43.6 °C. However, based on their observations they suggested that is not advisable to measure the absolute temperatures based on the results of a single QD. The group average results are more reproducible.

J Yang *et al*. [37, 38] performed several studies using QD655 on NIH/313 fibroblast cells. In their earliest analysis, they reported that CdSe/ZnS (core/shell) has a 0.057 nm/°C resolution in the cellular environment under different temperatures., They also proved that the cellular temperature increased about 1.5 °C after the infusion of high concentration of calcium ions (Ca^{+2}) [37]. Later, they were able to achieve intracellular heat generation mapping under Ca+2 stress and a cold shock (a decrease in temperature from 37 to 15°C) [38]. They have suggested that the hypothesis that individual cells use transient thermal gradients for signaling is true and can be further studied.

A series of studies on QD were done by Maestro research group [19, 29, 39, 40] over the past five years. They studied multi-photon excitation effect on QDs and how it improves temperature sensitivity. In one of their studies, they concentrated on deep tissue image. They demonstrated that CdTe QDs (8 nm in diameter) have a broadband luminescence centered at 800 nm and can be efficiently excited by 900 nm laser pulses through a multiphoton excitation, these QD are ideal optical probes for deep tissue biological imaging. From this study they were able to obtain the first deep tissue image based on infrared excited/emitting quantum dots with excitation and emission wavelengths lying within the biological window [39]. In another study, they examined the potential of CdTe QD (3.6 nm in diameter) as a two-photon excited (TPE) fluorescent nanothermometer. They have suggested that the near infrared (NIR) two-photon excitation gave a much higher sensitivity than those of a single photon excitation (SPE) because the fluorescent volume was restricted to a focus spot of the infrared excitation beam. They obtained a temperature resolution of ±

0.5 nm/°C with an optimum excitation wavelength of 880 nm [19].

Integrating the QDs into microfluidic systems, H. Maruyama *et al*. [41] developed a novel cell investigation tool impregnated with temperature sensitive cadmium selenide (CdS) QDs to measure local temperature on a microfluidic chip (Figure 4(A)). The system was made of polyethylene glycol (PEG), a material that is biocompatible and allows for cell measurements. The cells tend to encapsulate (eat) the quantum dot which then get distributed into different parts of the cell for example: the cell membrane and cell nucleus (Figure 4(B)). They calibrated the temperature by several colour spaces i.e. HSV and YCrCb (H: hue, S: saturation, V: values (brightness). Y: brightness, Cr: color difference of red, Cb: color difference of blue). From their results, they suggested that the temperature measurement using Cr is most suitable for cell analysis having a sensitivity of -1.3 %/K and an accuracy of 0.3 K (Figure 4(C)).

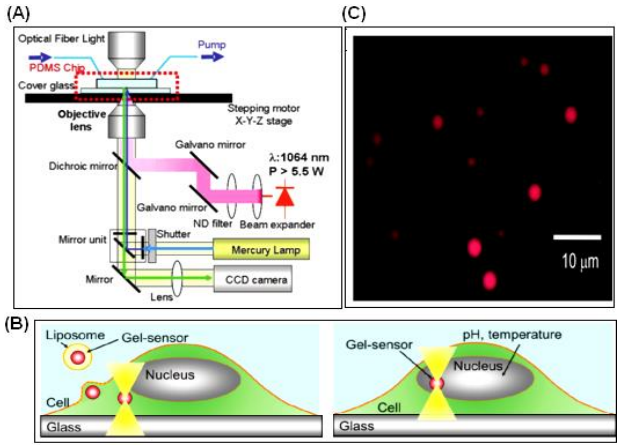


Figure 4 (A) Experimental setup of QDs excitation (B) QDs in different locations in the cell after encapsulation. (C) Fluorescent images QDs in cell [41]

Some researchers tried to study the properties of QDs to improve their behavior in biological systems for instance: Q. Ma *et al*. [27] compared the photostability of naked QDs and coated QDs with bovine serum albumin (BSA) under single photon excitation (SPE) and two-photon excitation (TPE). They tested them in human hepatocellular carcinoma (QGY) cells and human nasopharynx carcinoma (KB). They concluded that, the BSA coated CdSe/Cds/ZnS QDs improved the photostability up to 4–5 times compared to the naked QDs in QGY and KB cell lines. They also suggested that near infrared TPE decreased the QD photobleaching compared to SPE. The near-infrared (NIR) wavelengths have the best penetration depth in tissues and the TPE could produce very low background signals in biological systems. It was suggested that TPE was the best option to be used for biological applications especially cell imaging because of its ability for long term monitoring studies including the in vivo detections.

Another study on QDs properties by Nakamura *et al*. [33] aimed to overcome photo-blinking. Photo-blinking causes problems when viewing and tracing the fluorescence of QDs, photographing and recording the movement under high speed conditions. They have successfully synthesized QDs encapsulated with rhodamine B thiol-organosilica layer (thiol-OS-QDs) for biological imaging. The thiol-organosilica layers were useful for improvement and multi functioning of QDs and the emission wavelength of rhodamine B (570 nm) was similar to that of QD (605 nm), which could compensate for the decrease in photo intensity of the QDs. The thiol-OS-QDs showed a broadened absorbance wavelength range for emission, and maintenance of strong and stable fluorescent under severe conditions compared to bare QDs. In addition, the QD showed high and stable fluorescence that could be detected even at the lowest intensity for up to 30 min.

Several factors still affect the use of QDs as a temperature indicator in single living cells. For example, photo-bleaching, QDs individual responses, the movement of QDs inside cells and the possible toxicity due to the QD degradation.

2.1.2 Quantum Mechanical Spin of Diamond Based Thermometry

Researchers in physics and biology have recently discovered diamond to be an ideal material for many applications. They have found that the nitrogen-vacancy (NV) site of diamond nanoparticles have an exceptional fluorescent property with many potential applications, for instance; in biolabeling [42,43] and temperature sensing [44-46]. These nanodiamonds are highly stable with a size of only a few nanometers. Figure 5 shows a simple energy-level diagram of the NV center. The basic photophysics can be explained by three electronic levels, a ground state |g, an excited state |e, and a metastable singlet state |s. Three spin sublevels mS = 0 and mS =±1 at zero and nonzero magnetic field B. In the diagram, D refers to the zero-field splitting and 2γB refers to the Zeeman splitting, where γ is the electron gyromagnetic ratio [45]. The ground and excited transition has a resonant wavelength of 638 nm (zero phonon line) and can be efficiently excited at most wavelengths below 640 nm. NV center sensing is based on monitoring shifts in the spin resonance frequencies through the defects in the fluorescence as a function of external disturbances such as temperature.

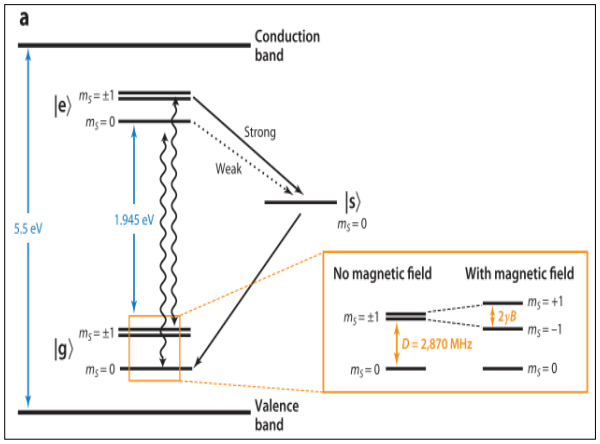


Figure 5 Characteristics of the nitrogen-vacancy (NV) center. (a) Energy level diagram of NV−. |g denotes the electronic ground state, |e the electronic excited state, and |s the metastable singlet state. Wiggly arrows indicate the radiative transition, and black arrows indicate strong and weak nonradiative decay via the singlet state [45]

The use of quantum mechanical spin based thermometers for intracellular temperature measurement has been recently introduced by G. Kucsko *et al*. [46]. They utilized the spin-1 system of nitrogen vacancy color centers in nanodiamonds, this spin state transition is thermally sensitive (Figure 6(A, B)). To optimize the detection they have used single-crystalline

nanodiamonds containing approximately 500 nitrogen–vacancy centers. The accuracy of the sensor reported was 44±10 mK having a response time of milliseconds. They tested the sensor on human embryonic fibroblast WS1 cells, and applied an internal heating environment using gold NP. This method showed promising future due to the robust structure of diamond that could tolerate the complexity of cellular environment and not be affected by enzymatic degradation or metabolic reactions. However, the injection of the nanodiamonds into the cells using nanowires is a complicated procedure and can cause difficulty in an operating environment.

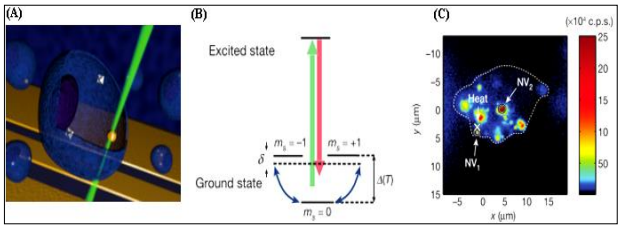


Figure 6 (A) Schematic diagram of nanodiamonds (grey diamonds) and a gold nanoparticle (yellow sphere) within a living cell (central blue object; others are similar) with coplanar waveguide (yellow stripes) in the background. (B) Simple NV energy diagram showing ground-state spin triplet and an excited state. At zero magnetic field, the |±1> sublevels are split from the|0> state by a temperature-dependent zero field splitting Δ (T) (C) Confocal image of a single cell under laser excitation. The cross marks the position of the gold nanoparticle used for heating, and circles represent the location of the nanodiamonds (NV1 and NV2) used for thermometry [46]

2.2 Fluorescent Dyes Based Thermometry

Visualization of a cell with fluorescent compounds provide a wide variety of information for the analysis of cell functions[48,49]. Intracellular temperature distribution through the cell as mentioned before has been in demand by scientists over the past several years. Fluorescent molecular thermometers show a potential as they function at the molecular level. The work of Zohar *et al*. [49] was amongst the earliest in the fluorescent molecular thermometry. They achieved a novel thermal imaging method using the temperature dependent phosphorescence intensity of europium (III) thenoyltrifluoro-acetonate (Eu-TTA), a rare earth thermosensitive dye. The method combined both the diffraction limited spatial (300 nm) and sampling rate limited time. They tested the method on a Chinese hamster ovary (CHO) cell and from the results they showed that when Eu-TTA was integrated in the liposomal membranes of CHO cells, the emission intensity of the Eu-TTA luminescence was temperature dependent between 15°C and 40°C. They also showed that when they used pressure ejected acetylcholine (ACh) onto cells, a change in phosphorescence was observed that was probably derived from the heat produced by the cells.

Using the same thermosensitive dye, Suzuki *et al*. [50] reported a technique for detecting and measuring otemperature changes in a single HeLa cancer cell using a devised microthermometer; a glass micropipette filled with the thermosensitive fluorescent Eu-TTA dye. They used two microthermometers: one was in contact with the cell by gently pressing the cell membrane and the other was a reference thermomerter that was placed 20 mm away from the cell (Figure 7). They found that heat production in a single HeLa cell is closely related to the SERCA activity. In addition, heat production occurred after the Ca2+ induction from the extracellular space but with some time delay.

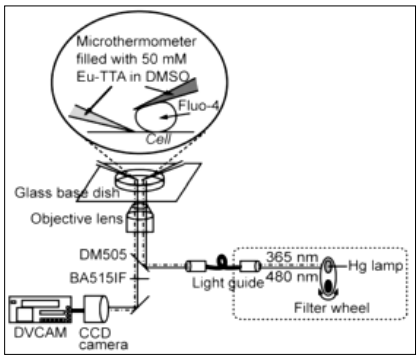


Figure 7 Schematic diagram of the experimental setup using glass micropipette system [50]

S. Uchiyama's [49, 52–54] research group conducted a series of research on cell temperature measurement. In 2010, they developed a novel fluorescent nanogel thermometer. It was designed to be biocompatible, pH insensitive and to have limited interaction with cellular components. The system was made of a thermoresponsive polyNIPAM combined with a water sensitive fluorophore, DBD-AA. At a low temperature the nanogel will absorb water into its inner part, and the water sensitive DBD-AA units will be quenched by neighboring water molecules. When the temperature increases the nanogel will shrink and release the water resulting in fluorescence from the DBD-AA units. The thermometer was tested on COS7 cells, and the temperature resolution obtained was 0.29-0.50°C in a 27-33°C range. The total fluorescence intensity was obtained from fluorescence images by summing the fluorescence intensities of all the pixels within a single cell. However, the large size and poor hydrophobicity of the gel affected its distribution within the cell [10].

They later obtained the first intracellular temperature mapping based on a fluorescent polymeric thermometer (FPT) and fluorescence lifetime imaging microscopy [51]. This method was sufficient to perform temperature mapping in living cells in which novel biological understanding, such as intracellular temperature gradients and organelle specific thermogenesis, were clearly revealed. The fluorescence properties of FPT were examined using a COS7 cell extracts and the FPT was micro injected into the cytoplasm of COS7 cells. The temperature resolution was 0.18–0.58°C within a range of 29–39°C. They also observed and proved that the temperature of the nucleus is higher compared to the temperature of the cytoplasm with an average difference of 0.96°C (Figure 8(A, B)). This difference could be due to the activities in the nucleus, such as DNA replication, transcription and RNA processing, as well as its structural separation by the nuclear membrane. They also showed that the difference depended on the cell cycle, as the cell gets older the gap becomes lesser. This decrease is explained to be the result of the temperature increase in the cytoplasm rather than its decrease in the nucleus. This method proved to be very effective by being able to provide intracellular temperature mapping with very detailed temperature distribution within the cell, however, inserting the gel into the COS7 cells require microinjection which is impractical and can be a very hectic procedure.

In one of their recent studies, they improved on their novel FPT with a cationic 3- acrylamidopropyl) trimethylammonium;

DBD-AA (APTMA) and vinylbenzyltrimethylammonium (VBTMA) units that allow for natural uptake of proteins and genes from the extracellular environment into living cells [53]. The temperature resolution obtained was 0.09−0.78°C in the range of 15−35°C. This is considered to be high in the current intracellular thermometry methods available. They tested the thermometer uptake on yeast SYT001 strain which was successful considering the yeast cell wall.

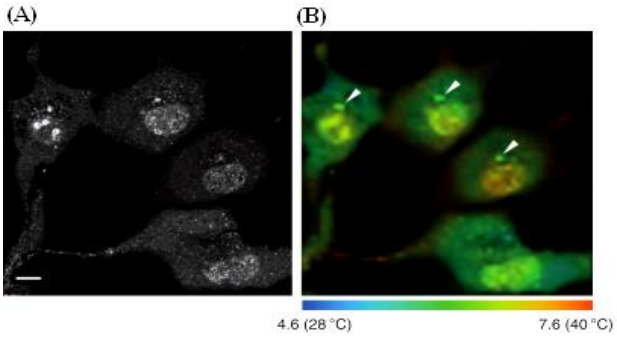


Figure 8 (A) Confocal fluorescence image. (B) Fluorescence lifetime image of FPT. The figure shows higher temperature at the nucleus [51]

So far in the above section, the materials discussed were mostly thermosensitive dyes and gels, however, attempts for developing thermosensitive materials were done on several other types including biological molecules, proteins, and DNA. J. Yang *et al*. [54] developed a novel L-DNA molecular beacons (L-MBs) based thermometer for single cell temperature measurement. The sensing mechanism happens by melting half of the hairpin structure into a single-strand DNA (ssDNA) which then reports fluorescence. The sensor should be biocompatible because the MBs consist of nucleic acids, stable because of the L-DNA (a mirror image of the natural D-DNA) that does not hybridize with any D-DNA or bind with any protein, and also as an artificial nucleic acid it resists any enzymatic degradation. The sensor accuracy was determined to be smaller than 0.7°C and the testing was on HeLa cells during Pd nanosheets treatment with 808 nm laser irradiation (suggested thermal therapy for cancer [55]).

Fluorescent proteins (FPs) have been of great interest in different research and opened the possibility for complex studies in living cells [56]. An example concerning thermal detection in single cells is the work of J. Donner *et al*. [6, 57] . They reported the use of green fluorescent proteins (GFP) as thermal nanoprobes suited for intracellular temperature mapping. The GFP proved to be biocompatible as it can be expressed in cells by genetic engineering making it a natural and non-invasive marker. The temperature probing was obtained by monitoring the fluorescence polarization anisotropy (FPA) of GFP, since FPA is a ratio of intensities it is not sensitive to changes in absolute intensities that can be caused by photo-bleaching which makes the results more reliable. This method was tested on HeLa and U-87 MG cancer cell lines by delivering local heat through photothermal approach (Figure 9(a, b, c)). A temperature accuracy of about 0.4 °C was achieved. The use of this approach provides a natural like tool that could be applied to molecular biology for therapeutic and diagnostic studies.

They then improved the nontaged GFP to GAD-GFP protein complex and tested it on Caenorhabditis elegans (C. elegans) strain. They were able to provide the first intracellular mapping of an in vivo model (whole organism rather than a single cultured cell). They obtained the results by applying an external heat source to the worm and when heat is generated inside through laser heating of a gold particle. The sensitivity was very impressive about 0.004/ °C; however the sensor did not have a notable response in the range of 24-35 °C. Also the accuracy changes when modifying the binding protein to suit the cell under investigation.

Fluorescent nano materials are being studied and developed with the aim of achieving actual intracellular mapping of a living cell to be applied in clinical and medical studies. However, these fluorescent thermometers should satisfy a couple of requirements in order to be an applicable sensor, for instance; high temperature resolution, high spatial resolution, does not get affected with the change in pH and ionic strength, cell type-independency, and should be concentration-independent output [51].

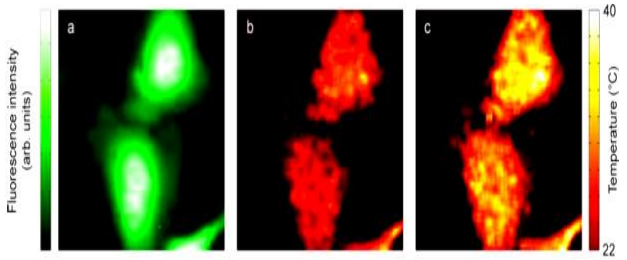


Figure 9 (a) Fluorescence intensity of GFP transfected HeLa cells. (b) Temperature map while not heating. (c) Temperature map while heating the HeLa cells with a focused infrared laser [6]

3.0 NON LUMINESCENT THERMOMETERS

Non-luminescent thermometers as the name implies are nano-sized thermometers in which their sensing properties are not directly related to luminescence. They have near field detection methods; which need direct contact with the detected object. The available literature has a limited number of examples in this category in contrast with the luminescent methods, yet, they offer a wide range of application possibilities with high thermal sensitivity.

Non-luminescent techniques differ in the sensing mechanism from one method to another. For instance; C. Wang *et al*. [8] designed a thermocouple nano-needle for detecting intracellular temperature attached to a nano-manipulator for cell penetration. The thermocouple has a sandwich structure consisting of tungsten (W) as a substrate, an insulating layer made of polyurethane (PU; except at the tip), and a platinum (Pt) film (Figure 10(A)). This method showed a precise detection of temperature with temperature resolution of 0.1 °C or less in a very short time (400 ns) (Figure 10(B)). It was verified by observing temperature fluctuation in a single U251 cell after the addition of camptothecin, a DNA topoisomerase I inhibitor that can promote tumor cell death. This novel method has a great future in clinical applications as it allows real time monitoring of intracellular temperature change, however, the use of the nano manipulator can be a drawback because it requires a lot of operating time and skilled users to undertake the experiment.

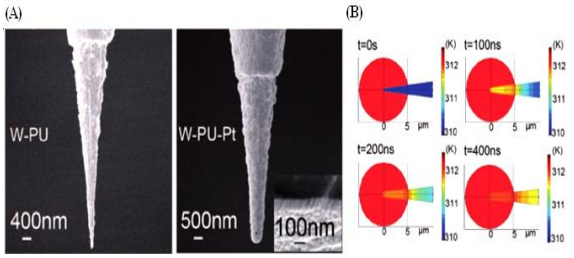


Figure 10 (A) SEM image of tungsten probe coated by polyurethane (PU; except at the tip which is uncoated) and thin platinum film as an outermost layer. (B) The simulated results of the TC probe response to a cell 2 °C higher than the environment [8]

A. Vyalikh *et al*. [58] studied a multi walled carbon nanotubes (MWCNTs) as a temperature detector for biological cells. The MWCNTs were filled with cuprous iodide, which displayed a strong temperature dependent nuclear magnetic resonance (NMR) characteristics (Figure 11) The use of the CNTs shell which acts as a barrier between the cell and the inner material, helped to increase the number of materials that could be used for temperature sensing without toxic effect on the cell. Also, the chemical modification of the outer shell of the CNTs helped to target specific locations in the cells. They have studied different properties for the sensing mechanism (spin–lattice and the spin–spin relaxation, resonance frequency, dipolar and scalar coupling, line-width and spin–lattice relaxation rate) for 63Cu and 127I. They concluded that 127I NMR spin–lattice relaxation measured on cuprous iodide filling was the most temperature-sensitive parameter and can provide 2 K detection in the range of 290 -320 K. Although not tested, this study shows a possible application in measuring internal temperature of biological cells.

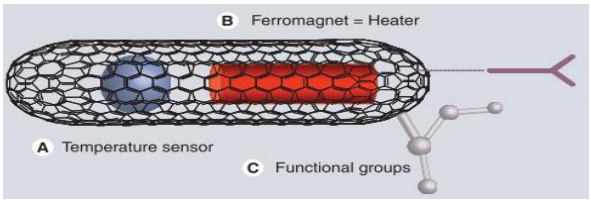


Figure 11 (A) Material with a strongly temperature-dependent nuclear magnetic resonance signal. (B) An additional filling material for further functionalities, for example a ferromagnet for heating by means of applied alternating current magnetic fields. (C) Functionalization of the outer shell for biocompatibility [58]

Another interesting idea was the work done by N. Inomata *et al*. [59]. They fabricated a microfluidic system with a resonant thermal sensor. The measurement principle relies on temperature-dependent change in the resonant frequency of Si (the resonator) as heat flows to the resonator (Figure 12(A, B)). The thermal resolution of the fabricated senor was 0.00016/°C, however, the sensitivity is dependent on the size of the resonant sensor. They tested the device on brown fat cells under three conditions i.e. non-stimulated, stimulated with norepinephrine (NE) (a stress causing hormone) and an inactive BFC. The non-stimulated cell was found to generate heat periodically over short time intervals (Figure 12(C,i)). While, heat production in the NE-stimulated cell, gave readings which were maintained over approximately 20 minutes (Figure 12(C,ii)), the inactivated BFC exhibited no obvious temperature change (Figure 12(C,iii)). This sensor is shown to have good potential in terms of biochemical reaction monitoring and miniaturization to nanoscale [59]. However, the sensor was used to measures the external temperature of BFC rather than the internal temperature which give more useful information in terms of studying the cell characteristics and metabolic reactions. Besides that, the application of this sensor is limited to the type of cell used.

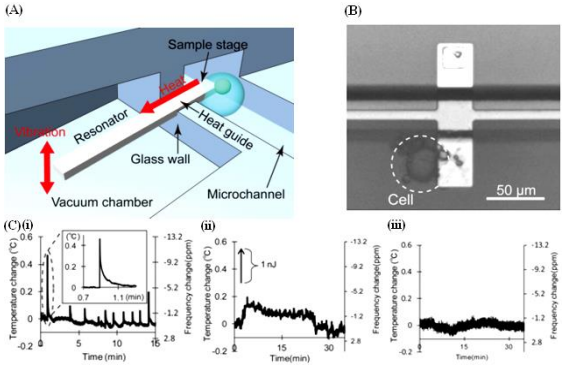


Figure 12 (A) Schematic of the resonant thermal sensor for heat detection of living cells in liquid. (B) A BFC attached to the sample stage for measurement. (C) Thermal responses of BFC over time. (i) Temperature changes in non-stimulated BFC; heat is generated from the cell in a series of pulses. (ii) Temperature changes in a NE-stimulated BFC; constant heat production over 23 min. (iii) Temperature changes in inactive cell; no temperature change is observed [59]

Photo acoustic thermometry is a novel new method proposed by L. Gao *et al*. [60] for single cell temperature measurement. Although it is a far field detection method, it is categorized in the non-luminescence thermometry because its sensing method does not require the use of fluorescent producing materials. The detection is based on measuring the ultrasound signals induced by light absorption through high-resolution photoacoustic microscopy (PAM) (Figure 13). A temperature resolution of 0.2°C with three seconds per frame imaging speed (Figure 14). They tested it on Hela cells and showed that almost 84% of the cells were viable after the measurements.

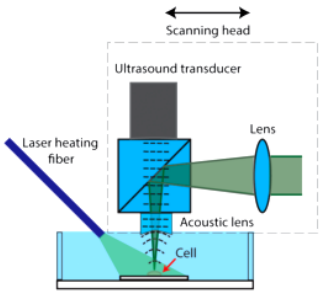


Figure 13 Photo-thermal heating. The cell was heated by a 100-mW CW laser via a multimode fiber. The intracellular temperature was monitored by a voice-coil PAM system in real time [60]

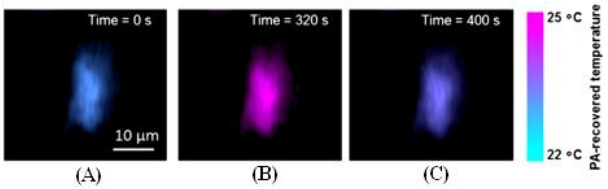


Figure 14 (A) through (C) show the cell images during photo-thermal heating at time 0 s, 320s, and 400s [60]

Although very few examples have been reported in non-fluorescent thermometry, they offer and open doors to a new field of thermometer studies that can produce sensitive temperature measurement in a very accurate way. Table 1 presents a comparison and a summary of luminescent and non-luminescent thermometry.

Table 1 Comparison and summary of intracellular thermometry

No.	Principle	Advantages	Disadvantage	Examples
Luminescent Thermometry	Far field contact	Small size	Get affected by the cell components	Nanoparticles [16-19]
	Detection through: -Wavelength -peak width -Intensity	Photostability	Data depend on the amount or the size of the sensor	Quantum dots [37-41]
		Biocompatibility	Produce qualitative data	Quantum mechanical spin of diamond [46]
		Surface alteration	Can be highly toxic	Fluorescents based materials [49=57]
Non-luminescent Thermometry	Near field contact	High sensitivity	Not user friendly and need for bulky equipment	Thermocouple [8]
	Detection through: -Electrical property	Independent of cellular components	No application on cell	Carbon Nanotubes [58]
	-Magnetic property of compounds	Considerably small	Limited to certain cells in the applications, water can affect the vibration	Thermal Resonant [59]
	-Resonance -Ultrasound signals		Sometimes toxic	Photoacoustic Thermometry [60]

■4.0 CONCLUSION AND OUTLOOKS

There has been a large amount of research on intracellular thermometry over the past four to five years. A variety of thermometers have emerged based on different physical behaviors. QDs, NPs, and the fluorescent based materials have been shown to have great potential in the biological thermometry; their properties, small size, in some cases biocompatibility and the ability to manipulate their wall structure to target specified locations in a cell, make them very attractive sensors.On the other hand, those systems need to undergo further studies to overcome their serious disadvantages. Furthermore, non-fluorescent thermometers, another category was introduced with very few examples. However, their results show precision and accuracy that encourage the development and further studies of such sensors, utilizing properties of materials other than the fluorescence such as the conductivity of semiconductor as an effect of temperature.

Regardless of the considerable amount of research, a commercial sensor that can describe the temperature distribution within living cells has not yet been reported. The major requirements that need to be fulfilled in a sensor in order to obtain reliable results are: high temperature resolution (<0.1 °C), functional independent of changes in pH and ionic strength, independent of the concentration of the sensing material, able to measure localized temperature and to be nontoxic to the cell. T Nanothermometers studies are now growing but more effort and collaboration are needed between scientists from different fields in order to develop a sensor that can monitor temperature changes for example in cancer therapy and other biomedical applications.

Acknowledgement

The research was supported by the Ministry of Education Malaysia (grant nos. 4L640 and 4F351) and Universiti Teknologi Malaysia (02G46, 03H82 and 03H80).

References

[1] T. M. Tritt. 2004. *Thermal Conductivity: Theory, Properties, and Applications*. Springer.
[2] K. M. Mccabe and M. Hernandez. 2011. Molecular Thermometry. *NIH Public Access*. 67(5): 469–475.
[3] F. Vera, R. Rivera, and C. Núñez. 2011. Burning a Candle in a Vessel, a Simple Experiment with a Long History. *Sci. Educ.* 20(9): 881–893.
[4] J. M. Adams. 1994. *Electrical Safety: A Guide to the Causes and Prevention of Electrical Hazards*. Institution of Electrical Engineers.
[5] K.-H. Lee. 2007. Quantum Dots: A Quantum Jump for Molecular Imaging? *J. Nucl. Med.* 48(9): 1408–10.
[6] J. S. Donner, S. a Thompson, M. P. Kreuzer, G. Baffou, and R. Quidant. 2012. Mapping Intracellular Temperature Using Green Fluorescent Protein. *Nano Lett.* 12(4): 2107–11.
[7] J. Davda and V. Labhasetwar. 2002. Characterization of Nanoparticle Uptake by Endothelial Cells. *Int. J. Pharm.* 233(1–2): 51–9.
[8] C. Wang, R. Xu, W. Tian, X. Jiang, Z. Cui, M. Wang, H. Sun, K. Fang, and N. Gu. 2011. Determining Intracellular Temperature at Single-Cell Level by a Novel Thermocouple Method. *Cell Res.* 21(10): 1517–9.
[9] F. Vetrone, R. Naccache, A. Zamarrón, A. Juarranz De La Fuente, F. Sanz-Rodríguez, L. Martinez Maestro, E. Martín Rodriguez, D. Jaque, J. García Solé, and J. A. Capobianco. 2010. Temperature Sensing Using Fluorescent Nanothermometers. *ACS Nano*. 4(6): 3254–3258.
[10] C. D. S. Brites, P. P. Lima, N. J. O. Silva, A. Millán, V. S. Amaral, F. Palacio, and L. D. Carlos. 2012. Thermometry at the nanoscale. *Nanoscale*. 4(16): 4799–829.
[11] S. Wang, S. Westcott, and W. Chen. 2002. Nanoparticle Luminescence Thermometry. *J. Phys. Chem. B*. 106(43): 11203–11209.
[12] J. R. Lakowicz and C. D. Geddes. 1991. *Topics in Fluorescence Spectroscopy*. Springer,
[13] M. J. Ruedas-Rama, J. D. Walters, A. Orte, and E. a H. Hall. 2012. Fluorescent Nanoparticles for Intracellular Sensing: A Review. *Anal. Chim. Acta*. 751: 1–23.
[14] J. Lee and N. A. Kotov. 2007. Thermometer Design at the Nanoscale. *Nanotoday*. 2(1): 48–51.

[15] J. Lee, A. O. Govorov, and N. a. Kotov. 2005. Nanoparticle Assemblies with Molecular Springs: A Nanoscale Thermometer. *Angew. Chemie*. 117(45): 7605–7608.
[16] E. Aznar, L. Mondragón, J. V Ros-Lis, F. Sancenón, M. D. Marcos, R. Martínez-Máñez, J. Soto, E. Pérez-Payá, and P. Amorós. 2011. Finely Tuned Temperature-controlled Cargo Release Using Paraffin-capped Mesoporous Silica Nanoparticles. *Angew. Chem. Int. Ed. Engl.* 50(47): 11172–5.
[17] F. Ye, C. Wu, Y. Jin, Y. Chan, X. Zhang, and D. T. Chiu. 2011. Ratiometric Temperature Sensing with Semiconducting Polymer Dots. 8146–8149.
[18] K. Oyama, M. Takabayashi, Y. Takei, S. Arai, S. Takeoka, S. Ishiwata, and M. Suzuki. 2012. Walking Nanothermometers: Spatiotemporal Temperature Measurement of Transported Acidic Organelles in Single Living Cells. *Lab Chip*. 12(9): 1591–3.
[19] D. Jaque, L. M. Maestro, E. Escudero, E. M. Rodríguez, J. a. Capobianco, F. Vetrone, A. Juarranz de la Fuente, F. Sanz-Rodríguez, M. C. Iglesias-de la Cruz, C. Jacinto, U. Rocha, and J. García Solé. 2012. Fluorescence Nano-particles for Multi-photon Thermal Sensing. *J. Lumin.*
[20] R. Sharma and C. J. Chen. 2009. Newer Nanoparticles in Hyperthermia Treatment and Thermometry. *J. nanoparticle Res.* 11(3): 671–689.
[21] I. H. El-Sayed, X. Huang, and M. a El-Sayed. 2006. Selective laser Photo-thermal Therapy of Epithelial Carcinoma Using Anti-EGFR Antibody Conjugated Gold Nanoparticles. *Cancer Lett.* vol. 239(1): 129–35.
[22] S. Link and M. a El-Sayed. 2003. Optical Properties and Ultrafast Dynamics of Metallic Nanocrystals. *Annu. Rev. Phys. Chem.* 54: 331–66.
[23] S. Eustis and M. a el-Sayed. 2006. Why Gold Nanoparticles are More Precious than Pretty Gold: Noble Metal Surface Plasmon Resonance and Its Enhancement of the Radiative and Nonradiative Properties of Nanocrystals of Different Shapes. *Chem. Soc. Rev.* 35(3): 209–17.
[24] M. J. Ruedas-Rama, J. D. Walters, A. Orte, and E. A. H. Hall. 2012. Fluorescent Nanoparticles for Intracellular Sensing: A Review. *Anal. Chim. Acta*. 751: 1–23.
[25] H. Chih-Hao, W. Anna, and H. Yang. 2011. An Accessible Approach to Preparing Water-SolubleMn2þ-Doped(CdSSe)ZnS (Core)Shell Nanocrystals for Ratiometric Temperature Sensing. *ACS Nano*. 5(12): 9511–9522.
[26] T. Jamieson, R. Bakhshi, D. Petrova, R. Pocock, M. Imani, and A. M. Seifalian. 2007. Biological Applications of Quantum Dots. 28: 4717–4732.
[27] Q.-F. Ma, J.-Y. Chen, X. Wu, P.-N. Wang, Y. Yue, and N. Dai. 2011. Photostability Comparison of CdTe and CdSe/CdS/ZnS Quantum Dots in Living Cells Under Single and Two-Photon Excitations. *J. Lumin.* 131(11): 2267–2272.
[28] E. J. McLaurin, V. a Vlaskin, and D. R. Gamelin. 2011. Water-soluble Dual-emitting Nanocrystals for Ratiometric Optical Thermometry. *J. Am. Chem. Soc.* 133(38): 14978–80.
[29] L. M. Maestro, E. M. Rodríguez, F. S. Rodríguez, M. C. I. la Cruz, A. Juarranz, R. Naccache, F. Vetrone, D. Jaque, J. A. Capobianco, and J. G. Solé. 2010. CdSe Quantum Dots for Two-photon Fluorescence Thermal Imaging. *Nano Lett.* 10(12): 5109–5115.
[30] S. Kim, Y. T. Lim, E. G. Soltesz, A. M. De Grand, J. Lee, A. Nakayama, J. A. Parker, T. Mihaljevic, R. G. Laurence, D. M. Dor, L. H. Cohn, M. G. Bawendi, and J. V Frangioni. 2004. Near-infrared Fluorescent Type II Quantum Dots for Sentinel Lymph Node Mapping. 22(1): 93–97.
[31] N. I. Chalmers, R. J. Palmer, L. Du-Thumm, R. Sullivan, W. Shi, and P. E. Kolenbrander. 2007. Use of Quantum Dot Luminescent Probes to Achieve Single-cell Resolution of Human Oral Bacteria In Biofilms. *Appl. Environ. Microbiol.* 73(2): 630–6.
[32] W. W. Yu, E. Chang, R. Drezek, and V. L. Colvin. 2006. Water-soluble Quantum Dots for Biomedical Applications. *Biochem. Biophys. Res. Commun.* 348: 781–786.
[33] M. Nakamura, S. Ozaki, M. Abe, T. Matsumoto, and K. Ishimura. 2011. One-pot Synthesis and Characterization of Dual Fluorescent Thiol-organosilica Nanoparticles as Non-photoblinking Quantum Dots and Their Applications for Biological Imaging. *J. Mater. Chem.* 21(12): 4689.
[34] X. Wang, L. Liu, and M. Yan. 2013. Experimental Biology and Medicine Engineering Nanomaterial Surfaces for. 1128–1139.
[35] E.-Q. Song, Z.-L. Zhang, Q.-Y. Luo, W. Lu, Y.-B. Shi, and D.-W. Pang. 2009. Tumor Cell Targeting Using Folate-conjugated Fluorescent Quantum Dots and Receptor-mediated Endocytosis. *Clin. Chem.* 55(5): 955–63.
[36] S. Li, K. Zhang, J.-M. Yang, L. Lin, and H. Yang. 2007. Single Quantum Dots as Local Temperature Markers. *Nano Lett.* 7(10): 3102–5.
[37] J.-M. Y. J.-M. Yang, H. Y. H. Yang, and L. L. L. Lin. 2010. Thermogenesis Detection of Single Living Cells Via Quantum Dots. *2010 IEEE 23rd International Conference on Micro Electro Mechanical Systems (MEMS)*, 24–28 January. 963–966.
[38] J.-M. Yang, H. Yang, and L. Lin. 2011. Quantum Dot Nano Thermometers Reveal Heterogeneous Local Thermogenesis in Living Cells. *ACS Nano*. 5(6): 5067–5071.
[39] L. M. Maestro, J. E. Ramírez-Hernández, N. Bogdan, J. a. Capobianco, F. Vetrone, J. G. Solé, and D. Jaque. 2012. Deep Tissue Bio-imaging Using Two-photon Excited Cdte Fluorescent Quantum Dots Working within the Biological Window. *Nanoscale*. 4(1): 298.
[40] L. M. Maestro, C. Jacinto, U. R. Silva, F. Vetrone, J. a Capobianco, D. Jaque, and J. G. Solé. 2011. CdTe Quantum Dots as Nanothermometers: Towards Highly Sensitive Thermal Imaging. *Small*. 7(13): 1774–8.
[41] H. Maruyama, K. Tomita, T. Masuda, and F. Arai. 2011. Temperature Measurement by Color Analysis of Fluorescent Spectrum Using Cell Investigation Tool Impregnated With Quantum Dot for Cell Measurement on a Microfluidic Chip. *2011 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS)*, 25–30 September. 13–18.
[42] C.-C. Fu, H.-Y. Lee, K. Chen, T.-S. Lim, H.-Y. Wu, P.-K. Lin, P.-K. Wei, P.-H. Tsao, H.-C. Chang, and W. Fann. 2007. Characterization and Application of Single Fluorescent Nanodiamonds as Cellular Biomarkers. *Proc. Natl. Acad. Sci.* 104(3): 727–732.
[43] N. Mohan, C.-S. Chen, H.-H. Hsieh, Y.-C. Wu, and H.-C. Chang. 2010. In Vivo Imaging and Toxicity Assessments of Fluorescent Nanodiamonds in Caenorhabditis Elegans. *Nano Lett.* 10(9): 3692–3699.
[44] D. M. Toyli, F. Charles, D. J. Christle, V. V Dobrovitski, and D. D. Awschalom. 2013. Fluorescence Thermometry Enhanced by the Quantum Coherence of Single Spins in Diamond. *Proc. Natl. Acad. Sci.* 110(21): 8417–8421.
[45] R. Schirhagl, K. Chang, M. Loretz, and C. L. Degen. 2014. Nitrogen-vacancy Centers in Diamond: Nanoscale Sensors for Physics and Biology. *Annu. Rev. Phys. Chem.* 65: 83–105.
[46] G. Kucsko, P. C. Maurer, N. Y. Yao, M. Kubo, H. J. Noh, P. K. Lo, H. Park, and M. D. Lukin. 2013. Nanometre-scale Thermometry in a Living Cell. *Nature*. 500(7460): 54–8.
[47] J. Wang, K. P. Loh, Z. Wang, Y. Yan, Y. Zhong, Q.-H. Xu, and P. C. Ho. 2009. Fluorescent Nanogel of Arsenic Sulfide Nanoclusters. *Angew. Chemie Int. Ed.* 48(34): 6282–6285.
[48] C. Gota, K. Okabe, T. Funatsu, Y. Harada, and S. Uchiyama. 2009. Hydrophilic Fluorescent Nanogel Thermometer for Intracellular Thermometry. *J. Am. Chem. Soc.* 131(8): 2766–7.
[49] O. Zohar, M. Ikeda, H. Shinagawa, H. Inoue, H. Nakamura, D. Elbaum, D. L. Alkon, and T. Yoshioka. 1998. Thermal Imaging of Receptor-activated Heat Production in Single Cells. *Biophys. J.* 4(1): 82–89.
[50] M. Suzuki, V. Tseeb, K. Oyama, and S. Ishiwata. 2007. Microscopic Detection of Thermogenesis in a Single HeLa Cell. *Biophys. J.* 92(6): L46–8.
[51] K. Okabe, N. Inada, C. Gota, Y. Harada, T. Funatsu, and S. Uchiyama. 2012. Intracellular Temperature Mapping with a Fluorescent Polymeric Thermometer and Fluorescence Lifetime Imaging Microscopy. *Nat. Commun.* 3: 705.
[52] S. Uchiyama, Y. Matsumura, A. P. De Silva, and K. Iwai. 2003. Fluorescent Molecular Thermometers Based on Polymers Showing Temperature-Induced Phase Transitions and Labeled with Polarity-Responsive Benzofurazans. 75(21): 5926–5935.
[53] T. Tsuji, S. Yoshida, A. Yoshida, and S. Uchiyama. 2013. Cationic Fluorescent Polymeric Thermometers with the Ability to Enter Yeast and Mammalian Cells for Practical Intracellular Temperature Measurements. *Anal. Chem.* 85(20): 9815–23.
[54] G. Ke, C. Wang, Y. Ge, N. Zheng, Z. Zhu, and C. J. Yang. 2012. L-DNA Molecular Beacon: A Safe, Stable, and Accurate Intracellular Nano-thermometer for Temperature Sensing in Living Cells. *J. Am. Chem. Soc.* 134(46): 18908–11.
[55] X. Huang, S. Tang, X. Mu, Y. Dai, G. Chen, Z. Zhou, F. Ruan, Z. Yang, and N. Zheng. 2011. Freestanding Palladium Nanosheets With Plasmonic and Catalytic Properties. *Nat. Nanotechnol.* 6(1): 28–32.
[56] G. a Maroniche, V. C. Mongelli, V. Alfonso, G. Llauger, O. Taboga, and M. del Vas. 2011. Development of a Novel Set of Gateway-compatible Vectors for Live Imaging in Insect Cells. *Insect Mol. Biol.* 20(5): 675–85.

[57] J. S. Donner, S. A. Thomson, C. Alonso-Ortega, J. Morales, L. G. Rico, S. I. C. O. Santos, and R. Quidant. 2013. Imaging of Plasmonic Heating in a Living Organism. *ACS Nano*. 7(10): 8666–8672.

[58] A. Vyalikh, A. U. B. Wolter, S. Hampel, D. Haase, M. Ritschel, A. Leonhardt, H.-J. Grafe, A. Taylor, K. Krämer, B. Büchner, and R. Klingeler. 2008. A Carbon-wrapped Nanoscaled Thermometer for Temperature Control in Biological Environments. *Nanomedicine (Lond)*. 3(3): 321–7.

[59] N. Inomata, M. Toda, M. Sato, A. Ishijima, and T. Ono. 2012. Pico Calorimeter for Detection of Heat Produced in an Individual Brown Fat Cell. *Appl. Phys. Lett.* 100(15): 154104.

[60] L. Gao, L. Wang, C. Li, Y. Liu, H. Ke, C. Zhang, and L. V Wang. 2013. Single-cell Photoacoustic Thermometry. *J. Biomed. Opt.* 18(2): 26003.