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Luminescent Gold Nanoparticles for Temperature Sensing

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Abstract **-** Temperature is a critical parameter that influences activities of living organisms at various levels and also governs biochemical reactions in molecules as it defines their state and dynamics. Several methods have been applied for the sensing of temperature, but this work concentrates on using fluorescence-based nano-sensors detection methods due to their fast response, high spatial resolution, high-temperature resolution, and safety of remote handling.
In this review, the emphasis is made on gold nanoparticles utilized as nanothermometers for both local and intracellular thermometry due to its good biocompatibility, high stability, and low cytotoxicity. Furthermore, these fluorescence temperature-dependent AuNPs were used as nanoprobes to measure temperatures at a different range. Moreover, this process is reversible, which illustrates that the fluorescence intensity of AuNPs can be regained to the initial intensity.

Keywords **-** Temperature, Fluorescence, Gold nanoparticles, Gold nanoclusters, Nanothermometer.

I. INTRODUCTION

One of the most important physical parameters in our daily lives is temperature, as we always experience a change in season by feeling a subtle variation in ambient temperature and also recognize illness due to elevation of body temperature [1]. In science, the temperature is one of the most frequently measured parameters and also a fundamental thermodynamic variable that strongly affects biochemical and physiological actions and processes [2], [3]. Temperature is a central physical quantity that governs biochemical reactions in molecules as it defines their state and dynamics [4]. Accurate determination of temperature is of increasing importance due to its widespread applications in micro/nanoelectronics, integrated photonics, and nanobiotechnology [3], [5].

At the cellular level, several events such as cell division, gene expression, enzyme reaction, and metabolism result in a change in temperature in living cells [6], [7]. In a disease state, for example; cancer, malignant cells inside tissues can be at a higher temperature likened to vigorous cells because of variances in cellular metabolism. Therefore, accurate and sensitive thermal measurement of living cells has drawn much attention to obtain techniques and devices that are proficient in precisely observing temperature within biological systems. [7].

Unluckily, traditional methods based on thermocouples, although sensitive and accurate cannot measure temperatures remotely within small confined spaces or at many locations concurrently, for example, within individual cells of a complex cellular network [8]. This has resulted in many promising approaches to local temperature sensing explored at the moment which includes scanning probe microscopy, Raman spectroscopy, and fluorescence-based measurements using nanoparticles and organic dyes [9]. Among them, fluorescence-based nano-sensors are promising analytical tools because of their fast response, high spatial resolution, high-temperature resolution and safety of remote handling [3], [10]. However, they possess the merits of being non-invasive and inherently parallel making them suitable for microfluidic and biological imaging experiments.

According to Fangmao et al, there are several qualities a fluorescent temperature sensor used for in-vivo measurements should possess concerning the complex cellular environment. These include, firstly, the fluorescent signal from the sensor must be bright enough to overcome autofluorescence and background signals from cellular materials. Secondly, the fluorescent temperature sensor should be non-toxic to the cell, a characteristic that is specifically significant for long-term cellular studies. Also, given the complex biochemical medium in a physiological solution, the fluorescent readout from the temperature sensor should preferably be ratiometric [8]. A few examples of fluorescent-based nanosensors include; metallic nanoparticles, quantum dots, polymers, organic dyes and biomaterials [11]. For example; AuNPs, CdSe-CdS quantum dot [12], PFBT-RhB and NIPAM based materials [8].

In this work, the review is concentrated on metallic nanoparticles mainly gold nanoparticles as nanothermometers that have been used for both local and in-vitro thermometry.

Gold nanoparticles (AuNPs) are the most stable metal NPs, possess non-cytotoxic properties, have good biocompatibility and have a strong light absorption and scattering effect [13]. AuNPs have been extensively used as fluorescent probes adept of high-contrast cellular imaging by multi photon-excited fluorescence microscopy in a great diversity of patterns [11]. AuNPs linger to obtain a reasonable amount of consideration because of their exclusive optical, electrical and photothermal properties [14]. These optical properties are dependent on nanoparticle size, shape, and dielectric environment, which enables their application as novel imaging and sensing probes [15]. The collective oscillation of conduction electrons, also recognized as surface Plasmon, demonstrates a resonance in the observable to near-infrared wavelengths. This resonance can be tuned by altering the shape of the AuNPs and is accountable for a large absorption and scattering cross-section at the resonance wavelength [14].

AuNPs are also regarded as one of the most promising photothermal agents with extreme properties, as they transform optical energy into heat via nonradiative electron relaxation dynamics [11], [15].

II. GOLD NANOPARTICLES

On the nano-scale, gold is a well-studied metal because of its tunable electronic structures and broad material properties [16]. Gold nanoparticles (AuNPs) have been broadly used in bio-nanotechnology which relies on their exclusive properties and numerous surface functionalities. The ability of AuNP functionalization offers a multipurpose platform for nano-biological assemblies with antibodies, proteins, and oligonucleotides [17].

According to Carattino et al, several metallic nano-objects are being used as agents for photothermal therapy or drug delivery [18], [19]. One of the benefits of AuNPs is the likelihood of fine-tuning their resonance to the near-infrared range, where the penetration of light into tissues can be of numerous centimeters [14]. Furthermore, the particles can be used for treatment and also for imaging [18], [20]. For photothermal therapy, AuNPs are used as heat sources to locally increase the temperature to prompt the death of specific cells in a tissue [19]. Moreover, the temperatures gotten can only be projected from models or an ad-hoc calibration [20]. Hence, a method to concurrently increase and monitor the local temperature will be of countless interest in a comprehensive range of fields [14].

Since the first observation of emission of light from bulk gold, the luminescence of metallic nanoparticles has been the subject of broad study in recent years [14]. The luminescence signal of AuNPs is constant over time; hence do not blink nor bleach making it a valuable labelling agent for processes that involve lengthy periods of observation [21]. However, most luminescent NPs are cytotoxic to living cells, possessing highly toxic elements such as Cd, Se, and Te, which would induce damage to samples [22]. This makes AuNPs advantageous to other metallic NPs (such as copper, silver and platinum) because its non-cytotoxic, chemically stable and also produces good biological response [23].

Within several spherical AuNPs, gold nanorods have gained considerable attention due to their different plasmonic properties. Mohamed et al. demonstrated that luminescence from gold metal is a million times lower as compared to gold nanorods [24]. This is due to the improvement effect of the electric fields through coupling to the surface plasmon resonance in the rods. These characteristic properties of nanorods which are the several plasmon resonances and the luminescence are relevant for surface-enhanced Raman spectroscopy, biosensor development, and fluorescence enhanced spectroscopy [25]. It is widely known that the main unique property of metal nanoparticles is plasmon absorption, which initiates from coherent oscillations of the electrons in the conduction band induced by the electromagnetic field. Concerning gold nanorods, the plasmon absorption spectrum shows two unique bands which are transversal plasmon resonance and longitudinal plasmon absorption [25], [26]. Many theoretical studies have been suggested to clarify the luminescence properties of gold nanorods which include, excitation of gold nanorods at 480 nm resulting in the excitation of the surface plasmon coherent motion and also the excitation of the d-electrons in the metal. Releasing these electronic motions followed by the recombination of the sp-electrons with holes in the d-band was accepted to lead to emission [24], [25].

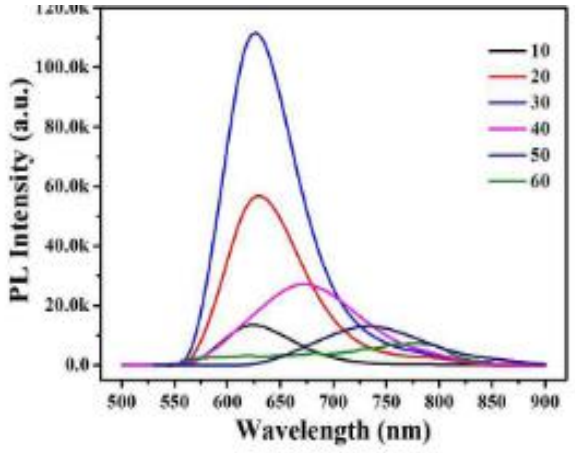

Fig. 1; Fluorescence spectra of Gold Nanoparticles under various times.

There are several shapes in which AuNPs can be produced, this includes sphere, shells, cages, stars, clusters, rods, etc. According to their sizes and shapes, AuNPs can absorb and scatter light in the visible and near-infrared wavelength regions. In recent developments, the widely used AuNPs for cancer therapy include gold nanorods, nanospheres, nanoclusters, nanoshells and nanocages [27]. However, in this review, nanoclusters (NCs) will gain much attention in the following sections.

GOLD NANOCLUSTERS

Gold Nanoclusters (AuNCs) is conceivably the kind of NCs that are gaining more attention in analytical chemistry because of their remarkable optical properties. Like other nanomaterials (such as quantum dots), their Stokes' shift and emission peaks (λem) depend on the number of gold atoms forming the AuNCs [16]. Over the last decade, lots of different types of luminescent metal NCs have been established and this includes gold (Au), silver (Ag), copper (Cu), and platinum (Pt). However, AuNCs are less toxic, more stable chemically and very easy to synthesize making it more encouraging for biological and biomedical applications [23].

According to Alba et al., methods for AuNCs fluorescence for sensing and bio-imaging depends on three mechanisms: that is; fluorescence quenching, which is the foundation of the determination of ion metals such as Hg (II) and Cu (II), or small molecules such as dopamine or histamine; secondly, AuNCs accumulation for protein determination; and lastly energy transfer (FRET) for bio-imaging. Unlike other nanomaterials, AuNCs have been barely used to sense gases [28].

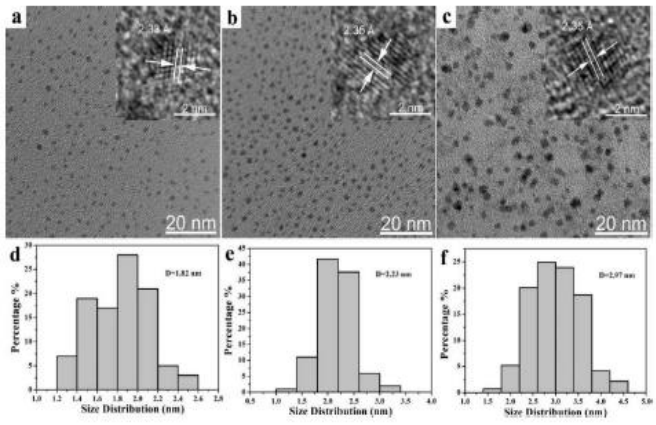

Fig. 2; TEM image and size distribution of AuNCs at a different wavelength.

III. MECHANISM OF GOLD NANOPARTICLES FOR TEMPERATURE SENSING

Several factors contribute to the dependency of fluorescent nanothermometers on temperature. These factors give a vivid reason why the fluorescence intensity of gold nanothermometers increases with a decrease in temperature and vice versa. Few mechanisms from previous works are outlined as follows;

Wu et al. produced a temperature-dependent sensor which consists of bovine serum albumin-stabilized gold nanoclusters (BSA-AuNCs) and fluorescein-5-isothiocyanate (FITC). They precluded that, the reduction of fluorescence intensity of FITC/BSA-AuNCs concerning increment in temperature was due to an increase in the diffusion rate of oxygen gas (O_2). Because the extent of protein aggregation relies on temperature, high temperatures can bring about the aggregation of FITC/BSA-AuNCs and this will lead to their fluorescence quenching. After experimenting to support this hypothesis by using Tween 20 (non-ionic surfactant) to prevent aggregation of FITC/BSA-AuNCs at high temperatures, it was noticed that the fluorescence intensity still reduced with increasing temperature.

According to series of experiments and studies, they then proposed that at high temperatures gold- thiol (Au-S) bond between the cysteine residues and Au core gets weakened and also the hydrophobic interaction (i.e., a decrease in the net charge of BSA) between AuNCs and BSA becomes stronger. This resulted in the decrease in charge transfer from BSA ligands to AuNCs via the Au-S bond which triggers the fluorescence quenching of AuNCs [29].

According to Sun et al., they reported AuNCs as nanothermometer with its fluorescent intensity decreasing with increasing temperature and vice versa. They demonstrated that this phenomenon was because as temperature increases, the molecule collision frequency, and the non-radiative transition rate increase; whereas the radiative transition rate remains constant, reducing the fluorescence intensity from the excited state [30].

Thermosensitive luminescent gold nanodots were synthesized which had an increment in the photoluminescence quantum yield and the lifetime when the temperature was decreased and when temperature increased, the photoluminescence quantum yield and lifetime depreciated. According to Boom et.al, this phenomenon was because, at high temperatures, there are more non-radiative recombinations of electrons and holes than at room temperature. And after cooling down the temperature, photoluminescence quantum yield reverses back in few minutes to the initial photoluminescence value obtained at room temperature [31].

Quenching of the fluorescence intensity of a magnetofluorescent nanothermometer which comprises Fe3O4@SiO2@(pNIPAM-co-RhBITC)/ AuNPs was understood to be due to the distance between the RhBITC

and the AuNPs. This is because, RhBITC acted as the fluorophore whiles the AuNPs acted as the quencher, hence under different temperatures, the distance between the RhBITC and the AuNPs will be different which will result in different fluorescence quenching efficiency and intensity. When the temperature is lower than the lower critical solution temperature (LCST) of pNIPAM, the pNIPAM layer gets swollen leading to the AuNPs and RhBITC far apart from each other. Concerning this, AuNPs can barely affect the fluorescence emitting from the fluorophore (RhBITC). On the other hand, high temperatures lead to shrinking of the pNIPAM layer making the distance between the AuNPs and the RhBITC shorter hence AuNPs efficiently quench the fluorescent intensity of the fluorophore [32].

GOLD THERMOMETER FOR LOCAL TEMPERATURE

The capability to devise and measure the local temperature of a medium at the nanometer scale is of considerable value in various nanotechnology applications, including nanoelectronics [33], spectroscopy [34], nanofluidics, nanoscale catalysis and photothermal therapeutic medicine [35].

Lately, numerous approaches have been designed to either execute high-resolution thermal mapping (for example, fluorescent molecular/polymeric thermometers [36], scanning thermal microscopy [37] and fluorescence polarization anisotropy [38]) or remotely control the local temperature using plasmonic or magnetic nanoparticles. Nevertheless, none of these practices can attain both local temperature sensing and heating [35].

Chen et al. developed a fluorescent probe consisting of AuNCs protected by bovine serum albumin (AuNCs@BSA) which displayed dual emission under ultraviolet (UV) excitation. Thus, a blue peak is attributed to protein surface oxidation species and an important red emission obtained from the AuNCs unit. The emission from the AuNCs@BSA which is red showed greater sensitivity to temperature which exhibited a roughly 40% drop in intensity upon lifting the temperature from 10 to 45^{o}C. Even though the red emission from AuNCs@BSA shows a repetitious decrease upon increasing the temperature from 10 to 45^{o}C, upon re-cooling to 10^{o}C, pronounced hysteresis is shown, leading to a signal 12% higher than the initial value [39].

Barreiro et al. also produced fluorescent AuNCs capped with lipoic acid (LA) to be used as probes for oxygen determination. But before the usage for oxygen determination, the effect of temperature was analyzed on the AuNCs@LA and fluorescence was studied. Increasing the temperature from 30 to 210^{o}C (10^{o}C stepwise), as expected, the fluorescence intensity decreased with temperature and vice versa [28].

To explore more potential applications of AuNCs as a nanothermometer, Sun et al. made findings of their thermo-responsive property in aqueous solution. They used series of GSH-AuNCs samples at temperatures increasing from 0 to 80 °C to measure the fluorescence in the space of 10 °C which led to the decrease in fluorescence intensity of the GSH−AuNCs by 75%. This shows that the sensitivity is approximately 0.94 %°C^{-1}, which is competitive compared with previous studies. Furthermore, decreasing the temperature from 80 to 0 °C led to the emission intensity restoring accordingly. Conversely, to increase the fluorescence intensity of AuNCs, low temperature is useful. The repeatability of the fluorescent intensity measured at different temperatures shows that GSH−AuNCs have an excellent thermo-sensitivity against temperature variations [30].

Shang et al. developed a fluorescent-based AuNC thermometer by taking advantage of the temperature sensitivity of their fluorescence lifetime and emission intensity, which change considerably over the normal temperature range (15–45^{o}C). Thus, the absolute temperature was estimated from the fluorescence lifetime [40], [41]. AuNCs protected by lipoic acid were synthesized which emit bright fluorescence in the near-infrared region (NIR). Prominently, they showed excellent colloidal stability in biological media which is essential for the performance of fluorescence probes in their biological applications. The fluorescence emission spectra of the AuNCs in phosphate-buffered saline were reported where the intensity decreased by 67% upon increasing the temperature from 10 to 45^{o}C [40].

According to Zhang et al., peptide nanofiber gold nanoclusters (PNF-AuNCs) was used as an optical thermometer. The emission spectra of PNF-AuNCs in phosphate-buffered saline (PBS) solution was determined. Increment of temperature from 10 to 45°C, led to their luminescence intensity decreased by 52%. Further quantitative analysis showed a good linear relationship between the intensity and the solution temperature in the investigated range. Concurrent with the intensity, the luminescence lifetime of PNF-AuNCs decreases with increasing temperature. A good linear relationship was observed between the average lifetime of PNF-AuNCs and the surrounding temperature which yielded a correlation coefficient of 0.97. However, not only the emission intensity, the lifetime of the PNF-AuNCs but also the lifetime of the PNFAuNCs changes sensitively with temperature [23]. The luminescence lifetime is more attractive than the intensity as is observed when it comes to thermometry applications because it is independent of the probe excitation conditions and concentration [23], [42].

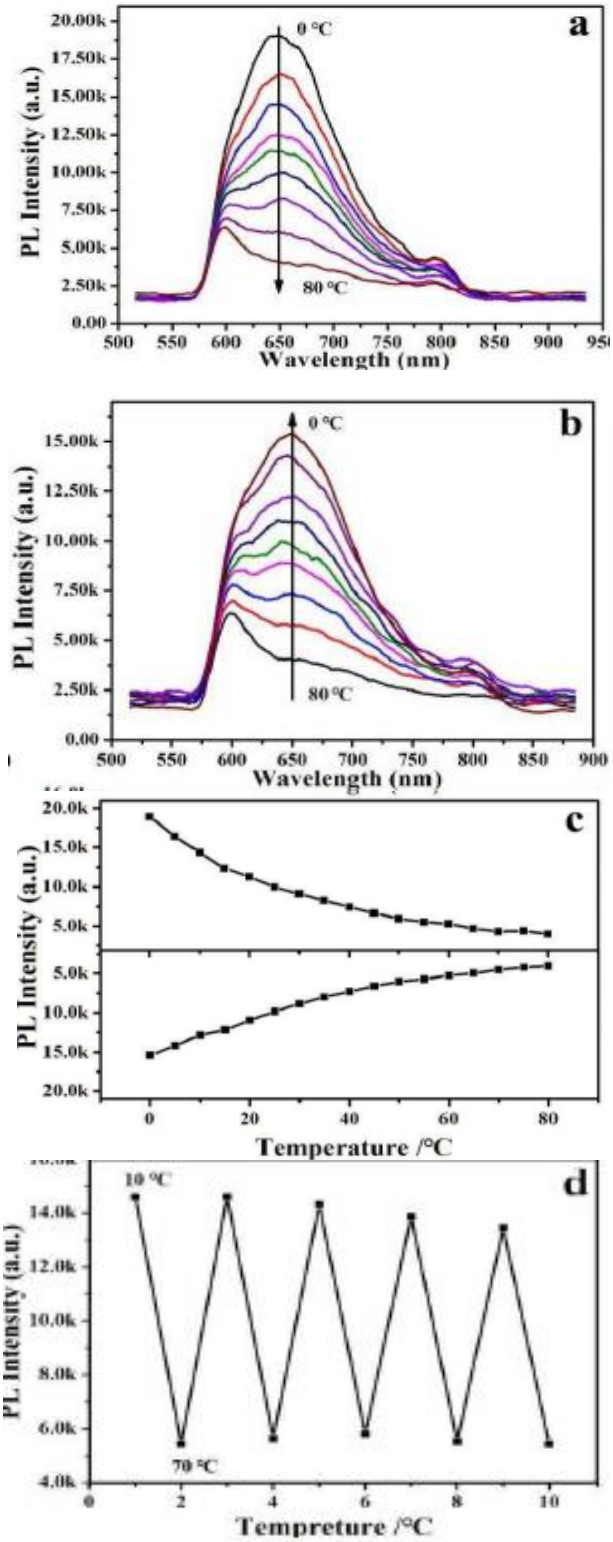

Fig. 3; a) Increase in temperature from 0-80oC led to the decrease in fluorescent intensity, b) Decrease in temperature from 80-0oC resulted in restoring the fluorescent intensity as it decreased in (a). c) Summarizes the temperature effect on the fluorescent intensity of the AuNCs as obtained in (a) and (b), d) emission peak intensity of GSH-AuNPs during five heat up and cool down cycle.

GOLD THERMOMETER IN LIVING CELLS

Gold nanoparticles (AuNPs) are essential for intracellular thermometry because of its stability, good biocompatibility and also non-cytotoxic to living cells [13]. Remarkably, the fluorescence lifetime of AuNCs is much larger in cells than in buffer solution over the whole temperature range studied. This effect most likely arises from the creation of a biomolecular corona around the adopted nanoparticles that transforms their photophysical properties [43].

Shang et al. used time-correlated single-photon counting (TCSPC)-based fluorescence lifetime imaging microscopy (FLIM) to illustrate the great potential of AuNCs for spatially resolved temperature measurements in living cells. They examined the ability of AuNCs for temperature sensing in HeLa human cancer cells using FLIM. Lipoic acid-capped AuNCs were prepared which can be efficiently internalized by HeLa cells by energy-dependent, clathrin-mediated endocytosis. AuNCs were incubated in serum-free cell culture medium for 2 hours, significant amounts of AuNCs were seen inside the HeLa cells and also assured by intensity-based confocal microscopy. The temperature of the environment of the HeLa cells was then varied using a temperature-controlled sample stage. Increasing the temperature within the cells led to a noticeable decrease in the fluorescence lifetime [40].

According to Wang et al., AuNPs with a copolymer shell (Fe3O4@SiO2@(pNIPAM-co-RhBITC)/ AuNPs) were used as nanothermometer for measuring intracellular temperature. Here, the AuNP thermometer was incubated in live Hela cells through endocytosis without any additional reagent. Fluorescence images were taken as quickly as possible after placing the cells at 4^oC and 25^oC for 20min. Cells placed at 4^oC showed stronger fluorescence than those placed at 25^oC. These results illustrated that the nano-thermometer could be used to sense the intracellular temperature changes in living cells, therefore fluorescence images of HeLa cells included with the nano-thermometer were gathered at different temperatures in the range of 4- 41^oC. This showed a decrease in fluorescent intensity as the temperature kept rising [32].

Wu et al. constructed a fluorescent probe comprised of bovine serum albumin-stabilized gold nanoclusters (BSA–AuNCs) and fluorescein-5-isothiocyanate (FITC) which displayed temperature-responsive fluorescence signals. The peaks of the fluorescence of FITC and BSA-AuNCs were seen at wavelengths of 525 nm and 670 nm, respectively. Moreover, the fluorescence intensity of FITC displayed a lower temperature sensitivity than that of BSA-AuNCs. Concerning these properties, they executed fluorescence intensity ratiometric-based temperature measurements in HeLa cells which decreased upon increasing the temperature [29].

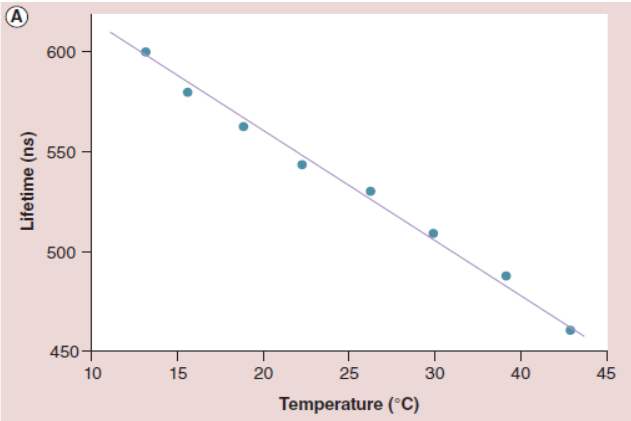

Fig. 4; Temperature-dependent fluorescence lifetime of AuNC and its line of best fit.

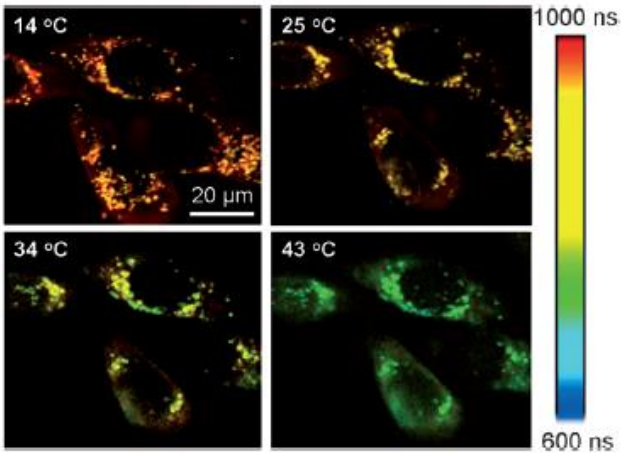

Fig.5; Fluorescence images of Hela cells incubated with AuNCs under different temperatures.

IV. CONCLUSION

In summary, these nanothermometers form a high-temperature sensitivity of both their fluorescence intensity and excellent stability over the temperature ranges. They provide precise temperature readings in biological systems in a spatially resolved manner by using fluorescence imaging. This fluorescence is reversibly responsive to the external environmental temperature with good reproducibility. Moreover, this novel fluorescence nanothermomter shows a reversible linear temperature response to the temperature in a wide range. The gold nanothermometer exhibited excellent biocompatibility and low cytotoxicity hence further used for fluorescence imaging studies on Hela cells where the AuNPs were incubated into the cells by simple endocytosis.

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Author Profile

Mr. Joseph Afrifa Owusu obtained a Bachelor of Science degree in Chemistry from the Kwame Nkrumah University of Science and Technology in 2014. He was an active member of the Ghana Students Chemical Society where he won many awards in research and experimental work. He further moved on to work as a Quality Control Chemist at Kinapharma Pharmaceutical Company Limited in Ghana. From there he pursued my education in Master of Science degree program in Chemical Engineering and Technology from the Jiangsu University of Science and Technology.

