Quantum dot thermal spectroscopy for biological optical tweezer applications

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Abstract:

Bridging the realms of physics and materials science with the life sciences, nanoparticles have spent many years at the forefront of biological and medical research. Quantum dots can be fabricated to hold particular emission properties and can be labelled to target specific binding sites in biological samples to act as biomarkers [1]. Many quantum dots have been observed to shift in emission linearly in relation to temperature changes[2], these have the potential to become useful thermal probes for biological materials. This particular application seeks to illustrate optimisations which can be applied to dispel the heating

We investigate the laser-induced thermal effects presented when using optical tweezers in aqueous samples, with the use of quantum dot nanothermometers. A conventional optical tweezer system was modified to incorporate a number of different laser sources to controllably heat our sample, an excitation source and spectrometer. Using samples which contained quantum dots in solution, we optically trapped particles using laser sources of different wavelengths and trapping powers whilst recording the emission spectrum of the quantum dots. Shift in emission spectra indicated temperature shift within and nearby the optical trap. Commercially available CdTe QDs, λ_{em} = 540 nm at 25 °C (which exhibit an emission shift of 0.6n m/°C) and CdSe QDs, λ_{em} = 655 nm at 25 °C (which exhibit an emission shift of 0.1 nm/°C) were used to quantify localised heating by focused laser beams in a variety of samples. We discuss the limitations of the technique including quenching by surrounding liquid and cytotoxicity.

We have demonstrated the use of quantum dots as nanothermometers to measure induced heating in samples caused by the optical trap with sensitivities of 0.6˚C. These particles hold promise for performing temperature measurements with high spatial resolution in biological environments.

Experimental design:

To test temperature generation from optical tweezers, we initially speculated what common wavelengths of laser we could use for tweezing and which should yield notable differences in sample heating. With biological samples typically suspended in an aqueous environment, we considered the absorption spectrum of water, shown below in Figure 1, as a starting point.

We then designed and built a system which combines multiple optical tweezer source and fluorescence spectroscopy, as shown below in Figure 2.

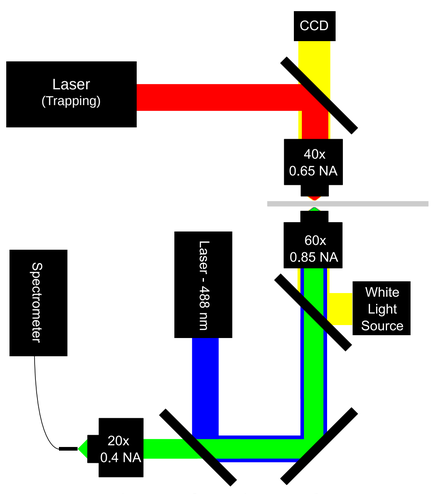

Figure 2 – Schematic of optical tweezer & fluorescence spectroscopy experiment

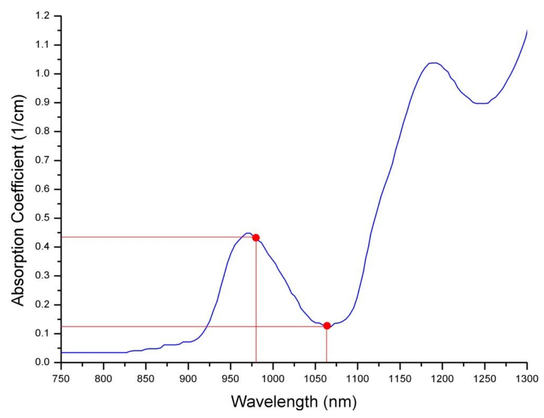

Figure 1 – Absorption spectrum of water with a peak at 980 nm and a low point of 1064 nm marked

- Multiple laser sources for optical trapping.
- Two alternative objectives for trapping40x 0.65NA & 100x 1.25NA.
- A 488 nm laser as the excitation source, focused onto the sample using a 60x 0.85NA objective.
- Emitted fluorescence was retrieved from a shared beam path with excitation and coupled via a 20x 0.4NA into an optical fibre, connected to an Ocean Optics HR2000 spectrometer.
- A CCD was used to image the sample, which was held on a micrometer stage between the tweezing and fluorescence microscope objectives.

Spectroscopy results:

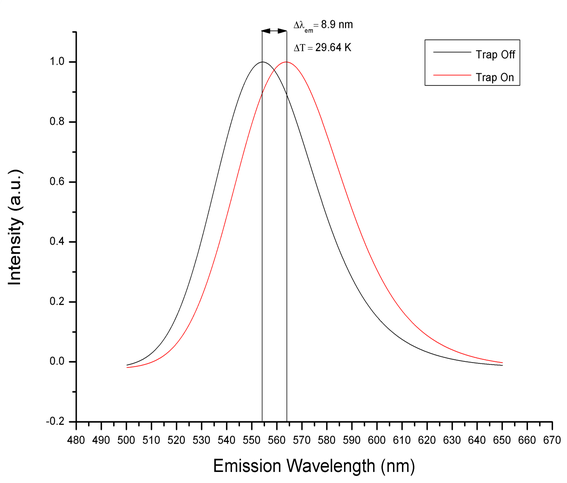

Figure 3 – Shift in emission peak between no tweezing and 2000 mW of 1064 nm (40x, 0.65 NA)

By comparing the heating rates of many laser sources, at different trapping powers, we are able to illustrate the thermal impact of optical tweezers on the sample.

The resulting collaborative results, from an upcoming article [3], are illustrated below in Figure 5.

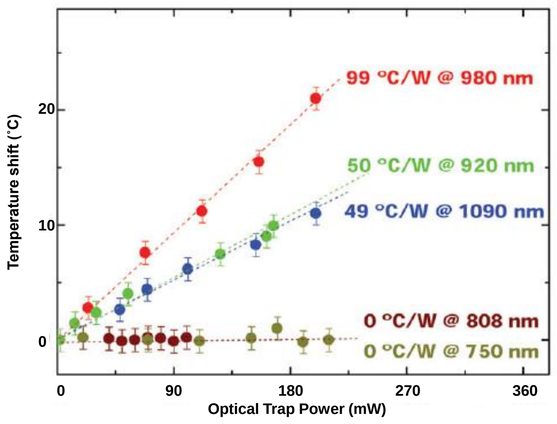

Figure 5 – Temperature shift observed from multiple optical tweezer sources [4]

Initial results using alternative environments indicated a shift in the emission peak of the quantum dots, as shown in Figure 6. This is an important factor to consider when extending the range of applications for these techniques.

A microspheres added to the quantum dot samples were trapped using the optical tweezer. The sample was then excited using the 488 nm laser (5 mW). The emissions spectrum was recorded using a 5 s integration time. A curve is fitted to the raw data and the shift in peaks is then recorded and compared to the emission where there was no optical trapping, an example of this is shown in Figure 3.

Peak shifts are recorded for multiple measurements, we then convert the emission peak shift to represent a temperature shift. This data is plotted and a line is fitted. As shown in Figure 4.

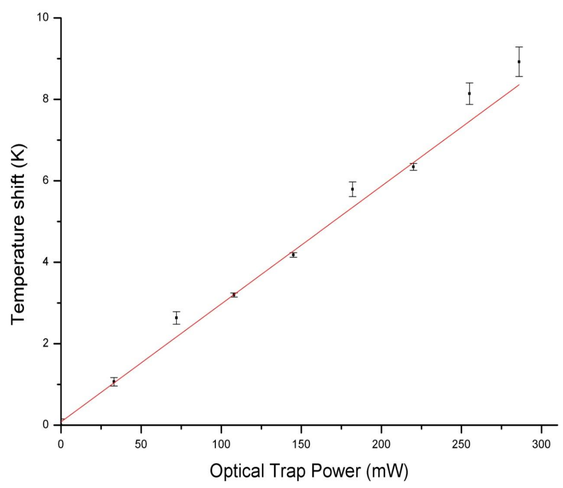

Figure 4 – Shift in sample temperature as optical trap power increases

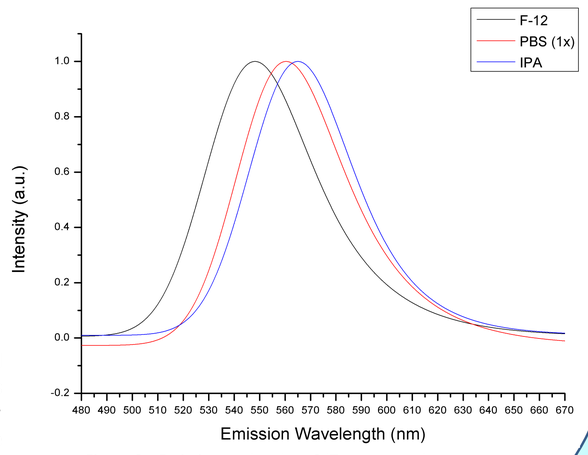

Figure 6 – Emission peaks when CdTe quantum dots are suspended in different liquid mediums.

Toxicity:

Quantum dots are regularly praised as biomarkers and probes (illustrated in Figure 7), however as a combination of heavy metals, this results in toxicity concerns. The cadmium component in particular is noted for it's damaging effect on cell membranes, mitochondrial activity and DNA integrity. The specific toxic effects of quantum dots has been shown to vary depending on quantum dot size [4]. To test the toxicity of the ⌀2.3 nm CdTe quantum dots we ran two assays. The alamarBlue assay which illustrates metabolic activity and the lactate dehydrogenase (LDH) assay which shows catastrophic cell death.

Mitochondria are the 'power plants' of a cell, generating the cell's supply of adenosine triphosphate (ADP), this activity can be used as an indicator of cell health. The alamarBlue (resazurin) assay allows us to test the mitochondrial metabolic activity. Healthy cells reduce the resazurin in the media to resorufin, a fluorescent red dye, as shown in Figure 8.

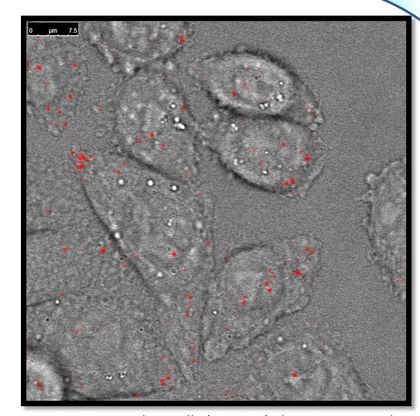

Figure 7 – Mammalian cells (CHO-K1) showing an uptake of CdSe quantum dots.

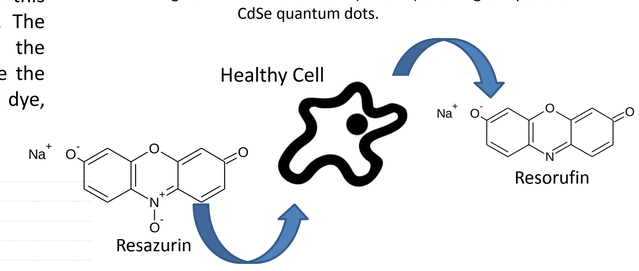

Figure 8 – Testing mitochondrial metabolic activity by the reduction of resazurin to resorufin

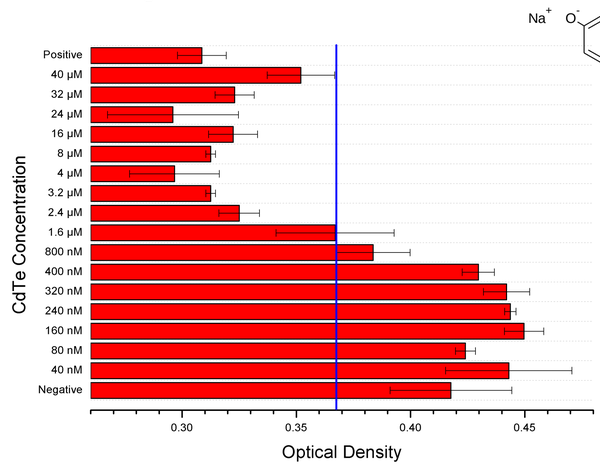

Figure 9 – Reduction of resazurin to resorufin. OD below the blue line indicates zero to low mitochondrial metabolic activity.

- J774 (macrophage) cells, at a concentration of $5x10^6$ per ml are supplemented with CdTe quantum dots at concentrations ranging from 40 µM to 40 nM
- Incubated at 37 °C, 5% CO_2 for 24 hours. Positive control contained 10% Triton, negative containing media only.
- The non-fluorescent blue dye, resazurin, is added to the wells in a 1:10 dilution with fresh media, fluorescence is measured over time. The results of the assay after 24 hours are shown in Figure 9.

Damage to the cell membrane, apoptosis, or necrosis, will cause the contents of the cell will be released into the surrounding media. LDH is an enzyme which is released when the cell membrane is damaged. LDH catalyses the interconversion of pyruvate to lactate, a reaction which also controls an interconversion of NADH to NAD+, as shown in Figure 10. This assay allows us to test the integrity of the cell membrane, after exposure to toxins.

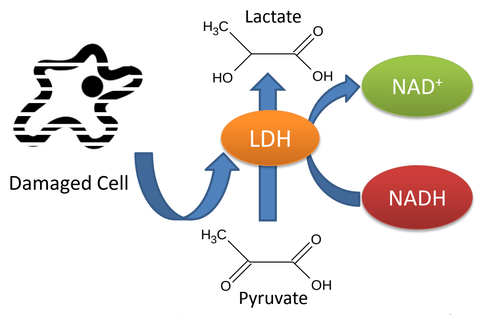

Figure 10 – Testing cell membrane integrity. LDH is released from cells with compromised membranes. LDH converts pyruvate to lactate in the presence of NADH.

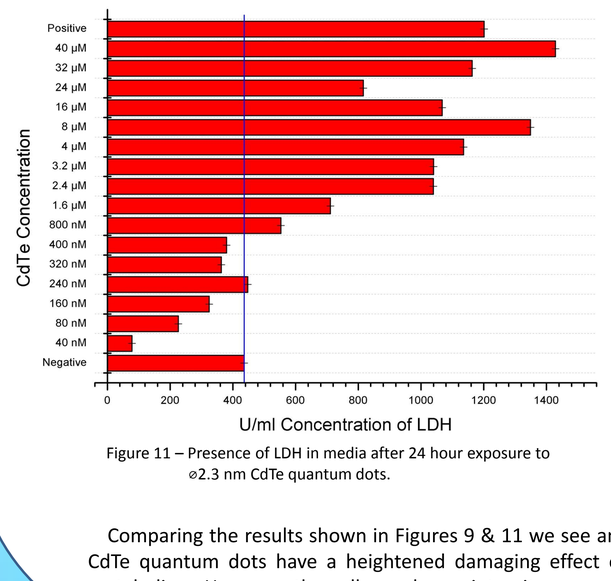

Figure 11 – Presence of LDH in media after 24 hour exposure to ⌀2.3 nm CdTe quantum dots.

- To quantify the presence of LDH we take a sample of the media, which formed the external environment for the cells, after the cells have experienced 24 hours of exposure to the CdTe quantum dots.
- Samples are supplemented with sodium pyruvate and NADH, the mixture is incubated at 37 °C, 5% CO_2 for 30 minutes.
- 2,4-dinitrophenylhydrazine is added as a colorimetric marker for pyruvate, 4M NaOH is added to halt the reactions.
- We use a set of standard pyruvate dilutions to produce a curve and compare the colorimetric response of the samples to convert optical density to a value of units of LDH per ml, such as the results shown in Figure 11.

Comparing the results shown in Figures 9 & 11 we see an expected trend, where the higher concentrations of CdTe quantum dots have a heightened damaging effect on both cell membrane integrity and mitochondrial metabolism. However, the cell membrane integrity appears to be more susceptible to lower concentrations of CdTe quantum dots than the mitochondrial activity.

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References:

1. Gao, X., Cui, Y. *et al* (2004) "*In vivo* cancer targeting and imaging with semiconductor quantum dots" *Nat. Biotechnol.* **22**, 969–76
2. Maestro, L. M., Jacinto, C. *et al* (2011) "CdTe Quantum Dots as Nanothermometers: Towards Highly Sensitive Thermal Imaging" *Small* **7**, 1774–1778
3. Haro-González, P., Ramsay, W. T. *et al* (2013) "Quantum Dot-Based Thermal Spectroscopy and Imaging of Optically Trapped Microspheres and Single Cells" *Small* [In Press]
4. Lovrić, J., Bazzi, H. S. *et al* (2005) "Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots" *J Mol Med* **83**: 377–385

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