On the Use of Core-Shell Type Semiconductor Nanocrystals as Sensors

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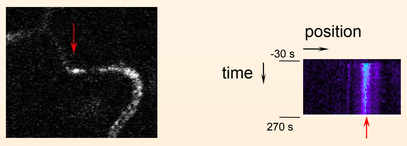
Abstract

Here we describe progress towards our objective of non contact transition metal ion sensing. Semiconductor nanocrystals show complex photophysical properties and require a very careful setup of the measurement parameters. Under these conditions they allow for very high resolution sensing of ions.

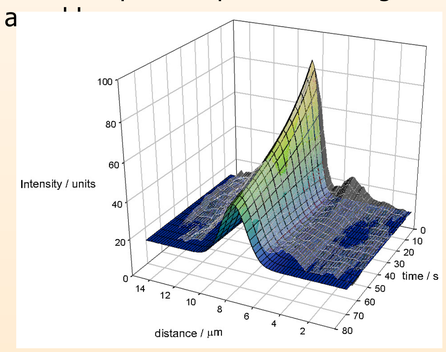
1. Why look for new fluorescent sensors ?

Fluorescent imaging has over the last two decades undergone rapid development with the discovery and exploitation of the fluorescent proteins and further development of organic fluorophors. While fluorescent proteins provide exciting opportunities, fluorescence stability remains a

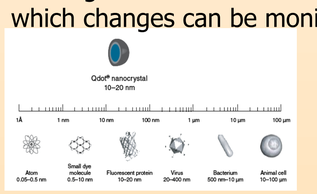


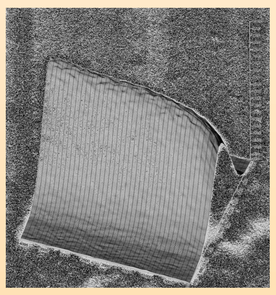
Photodamage to fluorescent proteins

Fluorescence from green fluorescent protein at the periphery of a plant epidermis cell fades over time [1]. Within 12 imaging frames the fluorescence intensity is fallen to half its initial value.



More recently nanocrystals have added promising opportunities to fluorescence imaging providing strong emission and good photostability. Yet some features of nanocrystals render them challenging tools.
Sensing much more than tracking requires a stable baseline fluorescence from which changes can be monitored.





Properties of semiconductor nanocrystals

Scale of quantum dots according to manufacturers (INVITROGEN QDot® range). Growing the crystals to different size allows for fine tuning of the emission wavelength. Repetitive scanning of the emission spectrum of quantum dots QDot800 shows photo-brightening.

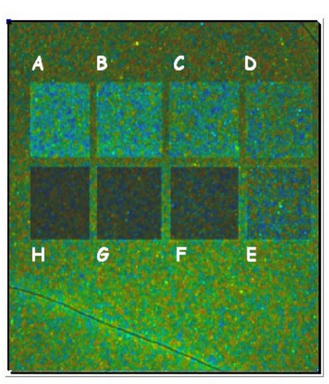
Repetitive scanning of the emission spectrum of quantum dots shows a change in fluorescence intensity over time. **Can semiconductor nanocrystals be used as sensors?**

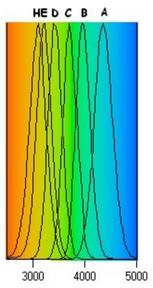
2. Brightening and Quenching

Further investigation of the fluorescence characteristics of commercially available quantum dots showed that the flu
lifetime is a function of the excitat

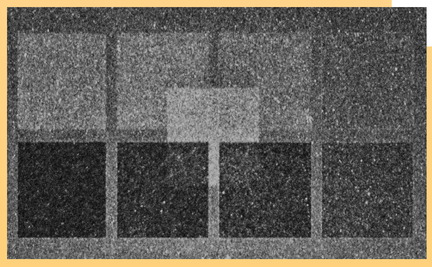
Quantum dots QDot525® (INVITROGEN) are immobilized in
gel matrix and excited at different
energy levels. The fluorescence life
as well as the fluorescence intensit
change with the previous excitatio

This effect is seen in other types o
core shell type semiconductor
nanocrystals as well. Quenching as
as brightening is fully reversible.





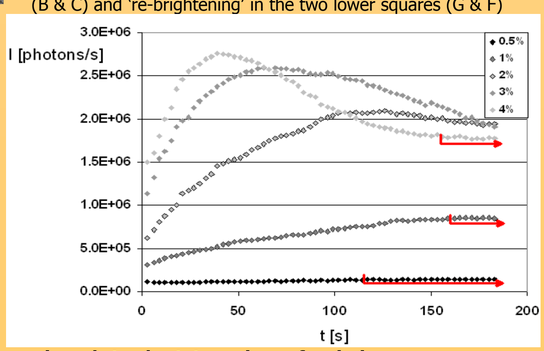
Lifetimes in ps
Square 36 x 36 µm



Fluorescence lifetime of quantum dots in a sol gel

Above: The squares have been excited at energies corresponding attenuation levels of:
0.5 % 1 % 2 % 3 %
10 % 8 % 6 % 4 %,
with the total photon number kept constant by adjusting the time of excitation.
Left: The effects observed are fully reversible. Further excitation of the central square at low (brightening) energy levels yields further brightening in the two top central squares (B & C) and 're-brightening' in the two lower squares (G & F)

Investigation of the photon count rate during excitation at different energy levels shows complex kinetics. We analyzed these kinetics to identify conditions where the fluorescence characteristics of the quantum dots remain stable. **Once in steady state conditions the quantum dots can be successfully used as sensors.**



Dynamic and steady state regions of emission

Repetitive excitation at different energies leads to photo-brightening and –quenching. Depending on time of excitation and energy the emission can reach a steady state (red bar).

3. Non-Contact Ion Sensing

We aim to achieve non-contact interaction between sensor and target ion by means of Förster Resonance Energy Transfer (FRET) [2]. In that case we should be able to describe the fluorescence according to a Förster formalism with the fluorescence decay given by:

$$I(t) = I(0)\exp\left(-\frac{t}{\tau_0} - 2\gamma\left(\frac{t}{\tau_0}\right)^{\frac{1}{2}}\right)$$

With γ being the transfer coefficient between donor and acceptor, defined as:

$$\gamma = \frac{[A]}{C_A}$$

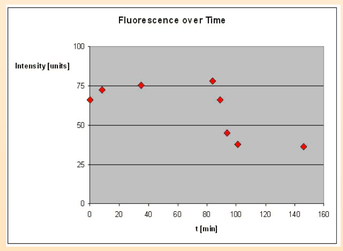
$[A]$ is the concentration of the acceptor and C_A is the critical acceptor concentration as calculated from the overlap integral between donor emission and acceptor absorbance.

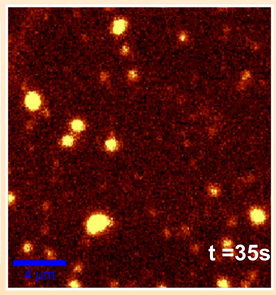
Fitting our data to FRET kinetics showed good agreement between measurement and model and suggested the interaction to be without contact between sensor and target.

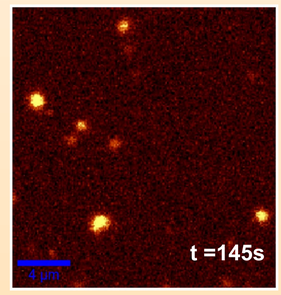
4. Transition Metal Ion Sensing

Transition metal ions like copper and nickel play an important role in biology as nutritional microelements as well as important ligands in proteins e.g. manganese in the photosystem II of plant cells or copper in the regulation of intracellular transport [3]. Sensing transition metal ions in biological systems by chemical methods proves to be difficult for concentrations are small and chemical sensing interferes with the very process one desires to monitor.

Using a sol gel to immobilize the quantum dots, we obtain a matrix which we can perfuse with ion solution to measure the effect of quantum dot – ion interaction. QDot800 provides a suitable sensor for copper ions.





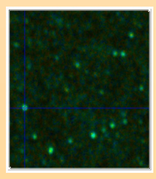


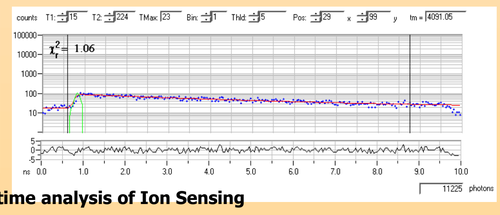
Copper ion sensing in a sol gel

Quantum dots QDot800 ® are embedded in a sol gel matrix and infused with a copper sulphate solution. The fluorescence intensity is monitored before and during perfusion. The very slight rise of the baseline before addition of ions at t=80s shows that the quantum dots were at t+0s not yet at steady state.

Are we looking at non-contact interaction?

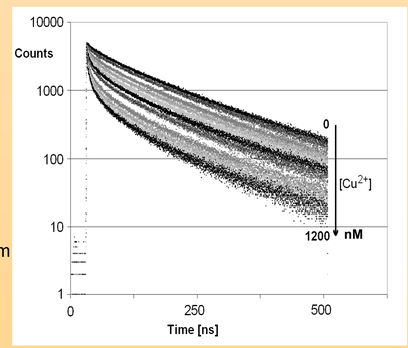
We analyse the fluorescence lifetime of the quantum dots. The fluorescence decay of the quantum dot can be described by a single exponential decay. Upon addition of copper we observe a Förster Type quenching; suggesting indeed non-contact interaction via resonance energy transfer.





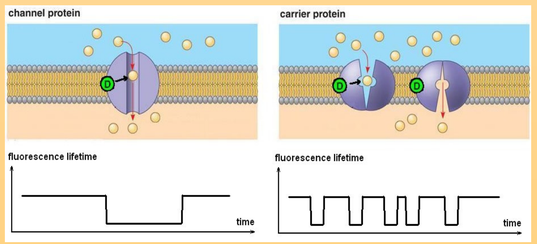
Fluorescence lifetime analysis of Ion Sensing

Precise fluorescence lifetime data can be observed from single quantum dots immobilized in a sol gel matrix. Upon addition of copper ion solution (0 to 1200 nM) to an ensemble of quantum dots we observe quenching indicative of Förster Resonance Energy Transfer. Fitting the curves allows precise measurement of the ion concentration.



5. Quo Vadis ?

Biosensing: Non Contact Ion Sensing
We have shown that semiconductor nanocrystals demonstrate kinetics consistent with non contact sensing of single transition metal ions. This technique will open exciting possibilities for ion sensing in biological samples with minimal interference with the target.



Placing a sensor within the Förster distance of the ion passage will allow non interfeering sensing of cellular ion transport.

Biotracking: Photo-activated quantum dots for protein and molecule tracking
It has been shown that fluorescent proteins which can be activated by excitation light greatly enhance the possibilities of protein tracking in cells [1]. The time of observation though is short due to photodamage to the fluorophore. We propose using the unique characteristics of semiconductor nanocrystals to develop a photo-activated quantum dot for greatly enhanced observation times.

Information Technology: Optical switches
Understanding and utilizing the changes in fluorescent characteristics in quantum dots might pave the way towards an optical storage device in information technology.

References:
[1] Sutter JU, Campanoni P, Tyrrell M & Blatt MR. "Selective Mobility and Sensitivity to SNAREs exhibited by the KAT1 K+ Channel at the Plasma Membrane." Plant Cell. 2006
[2] Birch DJS, Holmes AS & Darbyshire M. "Intelligent Sensor for Metal Ions based on Fluorescence Resonance Energy Transfer" Meas. Sci. Technol. 1995
[3] Liu J, Sitaram A, Burd CG. "Regulation of Copper-Dependent Endocytosis and Vacuolar Degradation of the Yeast Copper Transporter, Ctr1p, by the Rsp5 Ubiquitin Ligase." Traffic. 2007

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