Purpose of the visit

The purpose of the visit was to carry out first measurements to elucidate changes to the behaviour of fluorophores in the vicinity of nanoantennas. This work is part of a project to develop multiplexed biosensors based on plasmon-assisted energy transfer between functionalised quantum dots and labelled analytes. Contrary to FRET in absence of plasmonic structures, several effects must be accounted for in the plasmon-assisted FRET. First of all, field enhancement can increase the amount of incident energy that is absorbed by the donor and acceptor, or both. Also, radiative behaviour changes of both components can occur and that could lead to changes of the QE of the donor, which could in turn result in changes of the Förster radius. Finally, energy losses are increased in the presence of plasmonic structures, with direct excitation of plasmons leading to strongest dissipation of energy. The combination of all these effects makes it difficult to predict the efficiency of the plasmon-assisted FRET, and the latter is likely to be different for each donor-acceptor pair. Consequently, the aim of the visit was to show that energy transfer occurs between the chosen donor and acceptor moieties (CdTe QDs and AlexaFluor594 dye) and to monitor their radiative behaviour in the vicinity of two-coupled-disks nanoantennas (prepared prior to visit).

Work carried out during visit

- Energy transfer measurements were made using both steady-state and time-resolved spectroscopies.
- FLIM technique was used to study the behaviour of fluorophores in the vicinity of plasmonic nanoantennas.
- Several meetings were held to establish collaborations and to determine future directions for the project.

Main results obtained

First of all, steady-state photoluminescence and absorbance measurements were used to check for the occurrence of energy transfer between the AlexaFluor594 dye (acceptor) and two QD samples, emitting at 550 and 570 nm. The spectral properties of these samples and the dye are shown in Figure 1.

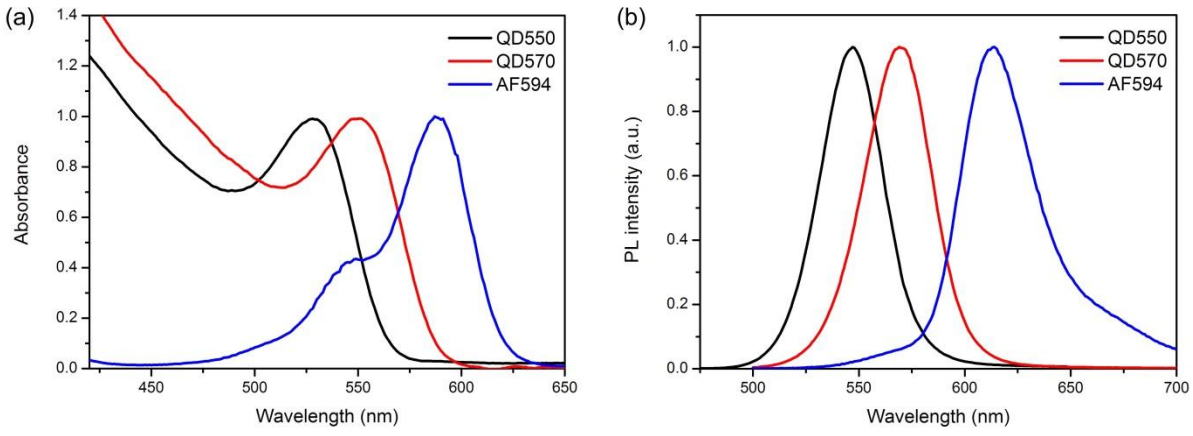

Figure 1| Spectral characteristics of CdTe QDs and AlexaFluor 594 dye. (a) Absorbance and (b) photoluminescence spectra of QD550 (black line), QD570 (red line) and AlexaFluor594 dye (blue line).

The QD samples were mixed with the AF594 dye in different proportions to give two sets of samples, one for each QDs, with varying qd-to-dye molar ratios. Absorption and photoluminescence spectra of all of these samples were taken and then separated into contributions from QDs and dye. Such separation was achieved using the linear regression analysis function of Origin. The contributions to the absorption spectra allowed accurate determination of the QD and dye molecules concentrations in each sample. These values, combined with the evaluation of contributions of QDs and dye to the PL spectra, were then used to determine the amount by which the photoluminescence intensity of the two constituents changed. The results of these measurements are shown in Figure 2.

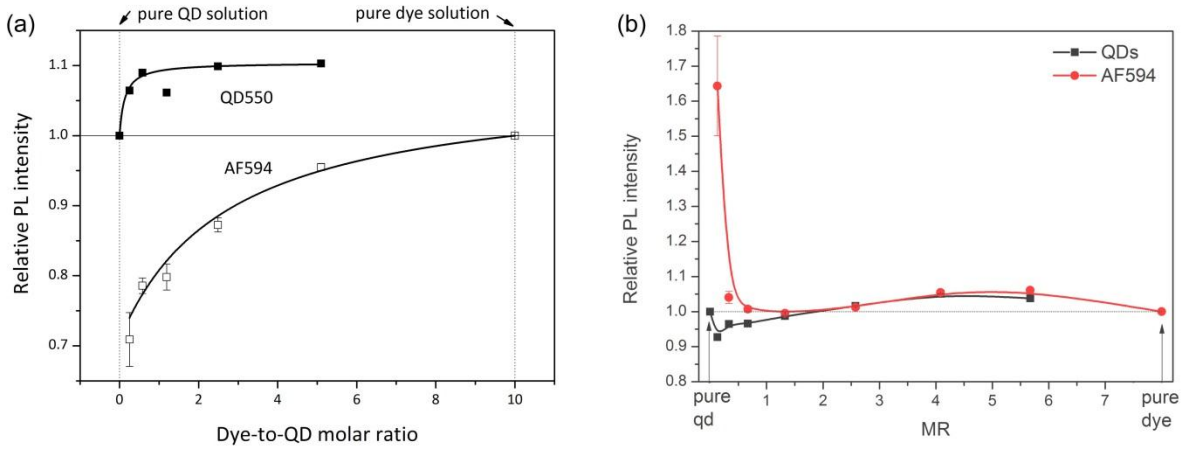

Figure 2|Summary of PL titrations of AF594 and (a) QD550 and (b) QD570 samples. The graphs show the intensities of emission of QDs and AF594 dye in the mixed samples relative to pure QD and dye solutions (respectively) of the same concentration. Note that the lines are shown to guide the eye only.

As it can be seen in the figure, the changes in the photoluminescence of QDs in most samples did not exceed 10%. The emission of the dye changed more, but not in proportion to changes of QDs' fluorescence. Considering that inner filter and re-absorption effects have not yet been taken into account, it is likely that changes to QDs fluorescence intensity are due to experimental error and errors incurred during the linear regression analysis and not due to the existence of FRET between the constituents. Furthermore, for QD550 sample, it is the QDs' fluorescence that increased and dye's fluorescence increased. If energy transfer was the cause of photoluminescence intensity changes, the opposite effect should have been observed. To confirm this conclusion, lifetime measurements for all these samples were performed.

The photoluminescence decay curves obtained from these measurements are shown in Figure 3. Panels (a) and (c) show the decay behaviour of samples in the green channel (524 ± 12 nm). At these wavelengths the major contribution to fluorescence is that from QDs. In accordance with previous conclusions, no change in the lifetime of QDs is observed for any of the mixed samples. The behaviour in the red channel (614 ± 20 nm) was complicated by the fact that QDs contributed to the fluorescence in this channel, QD570 especially had a significant contribution. As the amount of the dye increased, the lifetime of the mixed samples decreased proportionally.

As a consequence of the above, it was not possible to monitor the FRET process in the vicinity of plasmonic nanoantennas. Some attempts were made, however to optimise the conditions under

which such measurements can be performed in the future. It was determined that fluorophore concentration of ~0.01 μM was optimum, allowing best resolution between interacting and non-interacting species. The inset of Figure 4 shows a FLIM image obtained using 0.01 μM concentration of QDs on top of a quartz slide with 9 gold double-disk nanoantennas placed with 2 μm between them.

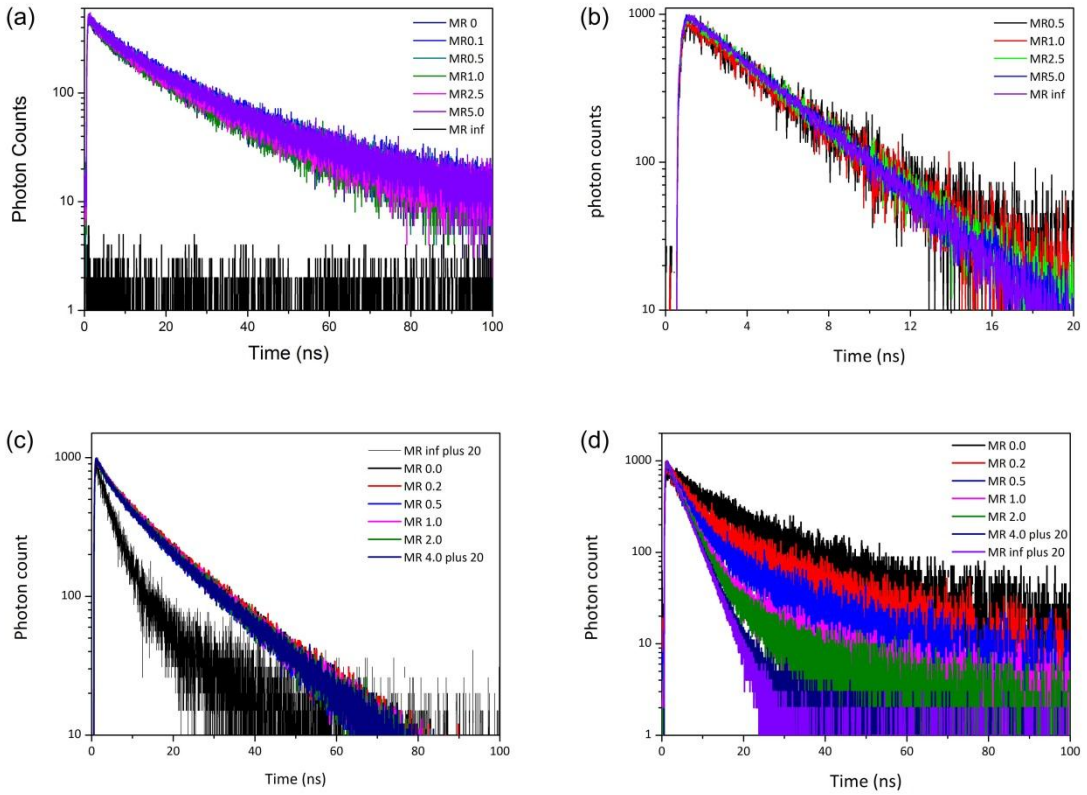

Figure 3| Photoluminescence decay of mixed CdTe QD and AlexaFluor dyes. (a) and (c) show the PL decay of QD550/AF594 and QD570/AF594 samples (resp.) in the green channel, and (b) and (d) show the corresponding behaviour in the red channel.

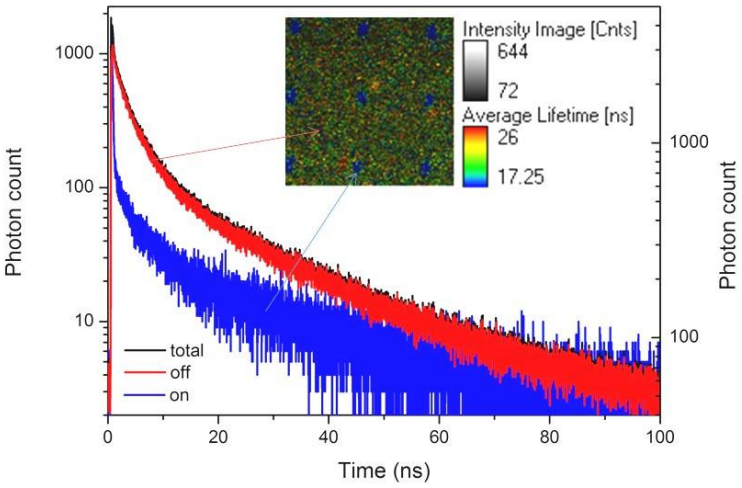

Figure 4| Photoluminescence behaviour of QD610 on top of a quartz substrate with gold 2-disk nanoantennas. The lifetime image is shown in the inset, while the main figure show the lifetime curves obtain from regions with laser excitation on and off the nanoantennas. The concentration of QDs was 0.01 μM.

Future collaborations with host institution

It is planned that this work will develop into a continuing collaboration.

Projected publications resulting from grant

The work performed was preliminary, and as such will probably not be published. However, the settings for imaging and monitoring the interactions between fluorophores and plasmonic structures were optimised, and this will be extremely important for future progress on this project.

Other comments

None