Single Molecule Spectroscopy using the Luca-EMCCD platform

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Application Note

Introduction
Single molecule spectroscopy usually requires a well elaborated setup which is necessary to obtain a sufficient S/N. This is crucial for single molecule detection because photons from a single emitter have to be discriminated from the background signal. Traditionally this is done by suppressing the background with confocal microscopy or total internal reflection in combination with sensitive detectors, e.g. photomultipliers, avalanche photodiodes or EMCCD cameras, the latter preferred for single molecule imaging. In this application note we present a simple single molecule experiment using the cost effective Luca-EMCCD platform.

Experiment
For the experiment an inverse wide field fluorescence microscope setup (Zeiss, Axio Observer Z1) equipped with a Zeiss Plan-Fluar ojective (100x, NA 1.45) was used. Excitation of the samples was performed with an X-Cite 120 PC lamp unit. Excitation and fluorescence light were separated with a dichroic filter set for 565 nm (excitation) and 630 nm (emission). For detection a Luca-S EMCCD camera with gain setting 255 and 50 ms exposure time was used.
Samples were red emitting quantum dots (CdSe/ZnS ellipsoids 6 x 12 nm, Qdot®655, Invitrogen) functionalized with streptavidin. The stock solution was diluted to a concentration of 100 pM. For measurement, one drop was placed on a coverslide and incubated for 10 min to allow the protein coated quantum dots to stick on the glass surface

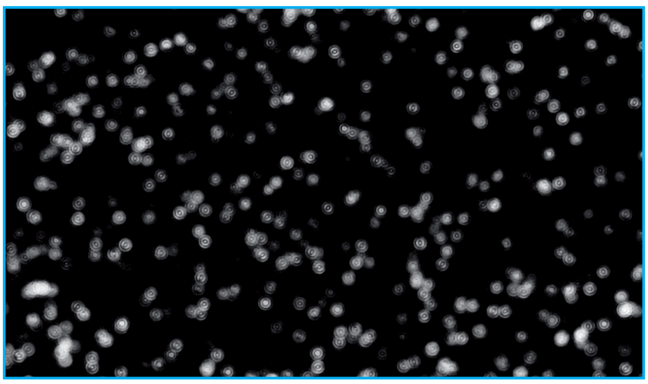

Fig 1: Immobilized quantum dots on glass surface

Results
Due to the protein coating the quantum dots rapidly attached to the glass surface and could be observed in immobilized state. For recording, the microscope was slightly defocused in order to avoid projection of quantum dot emission on a single pixel what would result in saturation of the pixel. Isolated quantum dots were characterized by a well defined concentric diffraction pattern (Fig 1). These isolated quantum dots showed a blinking effect, which is typical for single emitters with dark states (Fig. 2; video sequence 1 in supplementary data). This is no diffusion effect as the blinking is still present after complete drying of the sample. However, given the time resolution provided by the Luca-S (30 fps), the observation of this effect was only possible because the quantum dots used are characterized by very long off-times. With other quantum dot samples no blinking was observed although in all samples single particles were detected.

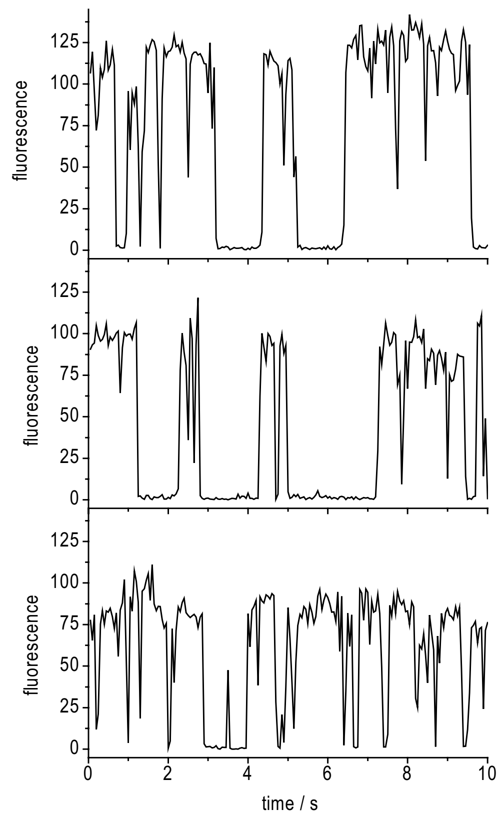

Fig. 2: Representative intensity-time profiles of blinking quantum dots

It was also possible to observe freely diffusing quantum dots in the solution, but S/N ratio and average resting time in the focus were too low to observe blinking in the solution. (Shown in video sequence 2 in supplementary data.)

Summary

In this application note we demonstrated the possibility to perform single molecule experiments with low cost equipment using a Luca-S EMCCD camera. The sensitivity of the Luca-S is sufficient not only for the detection of immobilized single emitters but also for single particle tracking in solution.

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