FRONT VERSUS BACK-ILLUMINATION IN SINGLE-MOLECULE DETECTION: A QUANTITATIVE COMPARISON

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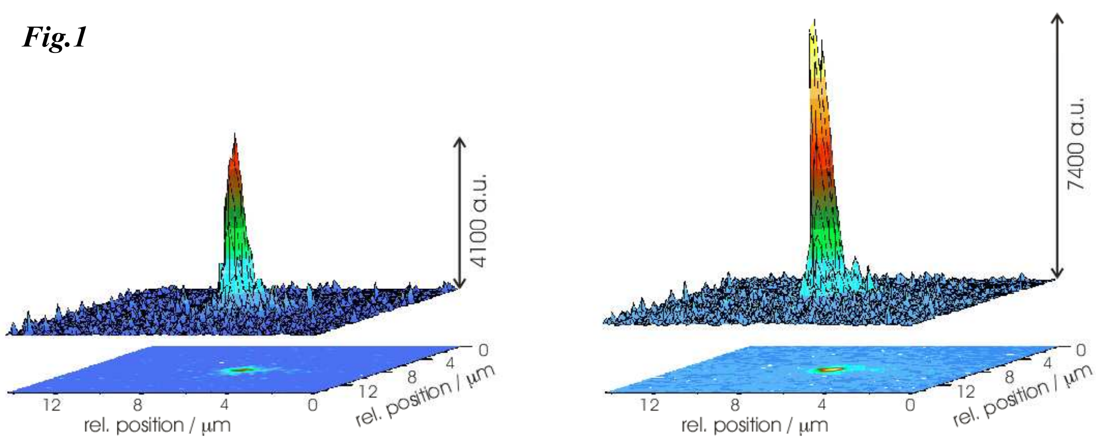
Optical single molecule detection schemes rely on the registration of the laser-induced fluorescence of the molecule under study and demand an efficient detection of the emitted photons. The temporal resolution in single-molecule experiments is limited by the requirement to detect a sufficiently large amount of photons in order to accumulate a reasonable signal-to-noise ratio to keep track of the probe molecule. Consequently, sensitive photo-detectors are of crucial interest for researchers in the field. A more sensitive detector allows to obtain the same signal-to-noise ratio at a reduced illumination time thereby increasing the temporal resolution of the experiment.

Here we compare the detection of exactly the same single molecule for two different detectors under identical experimental conditions. The two detectors were a front-illuminated IXON DV 887 FI (quantum yield ≈ 47 % @ 700 nm) and a back-illuminated IXON BV 887 (quantum yield ≈ 90 % @ 700 nm). Both detectors were operated at a chip temperature of -65°, maximum amplification and an illumination time of 30 ms. The intensity of the excitation laser was 0.48 W/cm^2 for both cases. In Fig.1 a diffraction-limited fluorescence-

Fig.1



microscopy image of a perylene-bisimide molecule embedded in a hexadecane polycrystalline matrix is shown. The molecule is excited at 601.59 nm and the emission is registered after suitable longpass filtering (cut-off < 610 nm) to suppress residual stray light from the excitation. The size of the displayed sample region is $14 \times 14\ \mu m^2$. The image on the left hand side of Fig.1 has been recorded with the DV 887 FI. The maximum signal amounts to 4100 units and more importantly the signal-to-noise ratio of the image is 75. On the right hand side of Fig.1 we depict the image recorded with the back-illuminated BV 887 which features a maximum signal of 7400 units and a signal-to-noise ratio of 120. The ratio of the peak signals, $\frac{7400}{4100} \cong 1.8$ is in agreement with the ratio of $\frac{90}{47} \cong 1.9$ for the quantum yields of the cameras. Correspondingly, the BV 887 allows to accumulate the same amount of photons as the DV 887 within half of the experimental time.