Accurate nanophotonic control over the local density of optical states

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Controlled modification of the local density of optical states is an increasingly important tool for investigating photophysical properties of complex fluorescent emitters. Several recent publications have exploited radiative rate modification to determine the quantum efficiency of fluorescent probes. The most often used technique employs a metal mirror positioned at nanometer-scale distances to the fluorophores. The main challenge here lies in controlling the mirror-fluorophore distance accurately. We present a method where the deflection of an atomic force microscope is used as feedback to control the fluorophore-mirror distance, enabling accurate modification of the radiative rate over a larger range.

Introduction

Fluorescence spectroscopy and microscopy are important tools in material sciences, analytical chemistry, biochemistry, biophysics, environmental monitoring and medicine, and are primary research tools in the life sciences. The success of fluorescence-based methods relies on the availability of well-characterized fluorescent marker molecules. The commonly used methods to determine key parameters like the fluorescence quantum yield (review see [1]) were developed decades ago, and are based on the assumption of an ensemble of identical fluorophores. For commonly used photophysically non-complex emitters, this assumption is valid.

However, for mixtures of dyes or for heterogeneous fluorophores exhibiting interchangeable and coexisting states, the current methods are bound to fail. The fluorescence quantum efficiency determined for these complex fluorophores is an average from all absorbing and emitting fluorophores in the sample. The results obtained are hence strongly dependent on the sample composition. An example of the failure of the current methods are the variations in reported quantum efficiencies from some fluorescent proteins, a group of fluorophores known for their photophysical complexity, that have been studied extensively on the single molecule level[2-3]. The quantum efficiency of the fluorescent protein DsRed given in the literature ranges from 29% [4] to 79% [5], which is an exceptionally wide span.

The unavailability of reliable quantum efficiencies for different states or subensembles might still be tolerable for fluorescent proteins used for standard applications, but is becoming a severe problem for fluorescence superresolution microscopy leading to fluorescence *nanoscopy* [6-8].

One particular class of superresolution microscopy is based on complex heterogeneous fluorophores that exhibit dynamically interchangeable coexisting emitting and non-emitting states. In these techniques fluorophores are used that switch “on” and “off” so that only a small fraction of the total fluorophores present is imaged in each subsequent

frame. The fact that each image has a dilute fraction of fluorophores allows accurate localization of the fluorophores and by the subsequent imaging of many frames with different fluorophores a complete image of the biological structure can be obtained. One important factor in obtaining the highest possible spatial resolution with these techniques is that the spatial resolution roughly scales with the square root of the number of photons collected from a molecule. Clearly, for these applications the parameter of interest is the quantum efficiency of the bright, emitting state, without any bias from dark, absorbing but not emitting states.

Determining the quantum efficiency of the bright fraction

A method that can determine the quantum efficiency (QE) of the bright fraction alone does exist. It relies on the expression

$$QE = \frac{\gamma_{rad}}{\gamma_{tot}} = \frac{\gamma_{rad}}{\gamma_{rad} + \gamma_{nonrad}}$$

where the quantum efficiency is expressed as the ratio of radiative and total decay rates. The essence lies in the fact that any measurement of the total decay rate γ_{tot} will only measure molecules that are "on". To use this relationship to obtain the QE of the bright fraction one more step has to be made. It is well known that by modifying the optical environment of the emitter the radiative decay rate is modified according to the local density of optical states (LDOS). Thus by tuning accurately the LDOS the radiative rate γ_{rad} is changed while the non-radiative rate γ_{nonrad} is intrinsically constant. Upon inspection of the equivalence above it thus becomes clear that it is possible to retrieve the QE by careful modification of the photonic environment.

The most common way to accurately modify the LDOS is by positioning the fluorophores at a well-defined distance from a flat mirror as first demonstrated by Drexhage [9]. The LDOS depends on the exact distance between the emitter and the mirror, the emission wavelength and on the orientation of the transition dipole moment with respect to the mirror. The distance-dependent modification of the local density of states results in a characteristic oscillation in the fluorescence decay rate that can be accurately modeled [10-13]. It is essential to control the LDOS precisely by controlling the distance between the fluorophores and the mirror between a few nanometers to some hundreds of nanometers with a precision of better than 10 nm. Lately this method has been used to study a number of different complex emitters.[14-18] Yet, the precise control of the distance between the emitters and the mirror remains a challenge.

Accurate mirror positioning with an AFM

To achieve accurate positioning of the mirror-fluorophore distance we adapted an atomic force microscope (AFM) to be able to perform real time feedback on the position. To do this the mirror is glued to the cantilever chip and the deflection of the in-contact cantilever is used to make a closed loop feedback, see figure 1. The essential advantage that this technique has over many alternatives is that the mechanical construction between the mirror - in this case a 100 µm large polystyrene sphere coated with 100 nm thick layer of gold – is only a few 100 micrometers long. This drastically reduces temperature related drifts. Also it is advantageous that the same point of the mirror is used for all measurements; in this way the same set of fluorophores is tested on the same reflective coating and effects due to spatial inhomogeneity are ignored.

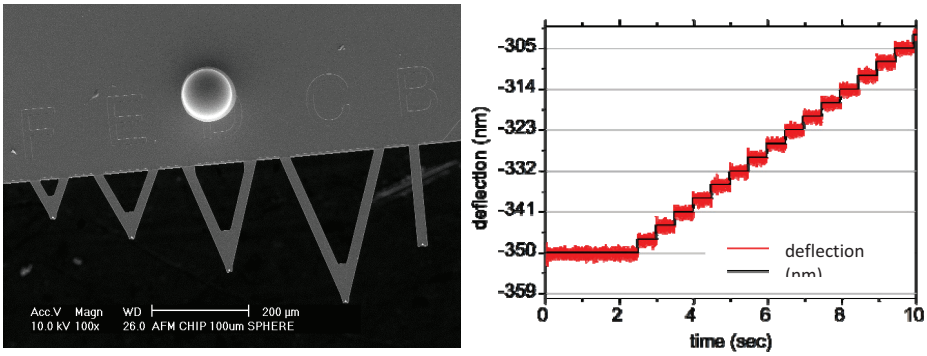

Figure 1a) SEM image of AFM cantilever with ball mirror glued to it. b) feedback controlled steps in height of 3 nm.

Results

Using our feedback system we are able to control in real-time the mirror-fluorophore distances as shown in Figure 1b. The pre-programmed set-points are reached within 100 ms and the remaining surface noise is about 3 nm peak-to-peak. Several conditions have to be met to achieve this high accuracy. First of all the setup must be appropriately isolated from sources of vibrations. Secondly for optimized performance the choice of the cantilever is of great importance. Also the feedback parameters of the PID loop are of significant importance; the most optimal settings for these parameters can vary from tip to tip and depend on the substrate on which the fluorophores are deposited.

Conclusions

We demonstrate excellent accuracy of positioning in this nanophotonic application. Since the AFM is fully integrated into a confocal microscope, we believe our device is easily converted into a quantum efficiency microscope, allowing for the detailed examinations of samples with spatial inhomogeneous quenching.

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