

# Nanophotonic measurement of the dark fraction in red fluorescent proteins

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All complex fluorophores such as fluorescent proteins display a fraction of the population that absorb light but do not emit fluorescence. Accurate measurement of this ‘dark’ fraction is of critical importance for quantitative microscopy in cell biology. We used a nanophotonic method to measure the dark fraction of several frequently used red fluorescent proteins.

Measuring the fraction of absorbing but not emitting fluorophores in a mixture is inherently difficult due to the simple fact that these molecules are dark. This fraction can be derived however if the quantum efficiency of the bright fraction only in the mixture ( $QE_{bright}$ ) and the averaged ensemble quantum efficiency ( $QE_{abs}$ ) are separately known.

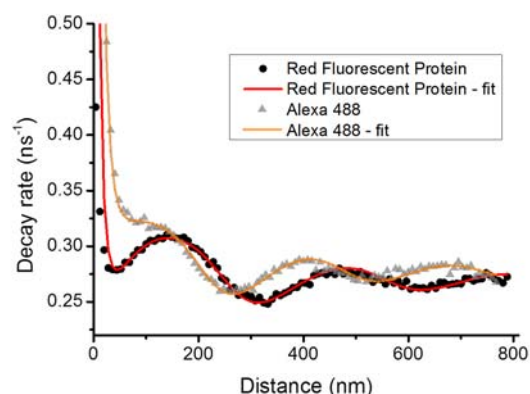
Conventional measurements of the quantum efficiency are based on the ratio of sample absorbance and brightness, which gives a value for  $QE_{abs}$ . The value for  $QE_{bright}$  can be found by a measurement that is based on decay rates.

Because such a time resolved measurement does not rely on a quantification of the absorption it will only be a measure of  $QE_{bright}$ . In our approach the total decay rate  $\gamma_{tot} = \gamma_{rad} + \gamma_{nonrad}$  is measured while the local density of optical states (LDOS) is controllably changed by modifying the nanophotonic environment of the fluorophores with a movable mirror [1-3]. Since the radiative rate ( $\gamma_{rad}$ ) is linearly dependent on the LDOS, both the radiative and non-radiative rates can be extracted, yielding an accurate determination of  $QE_{bright}$ .

The measured dependence of the total decay rate on the mirror-sample distance is shown in fig 1 for an Alexa 488 small molecule dye and one red fluorescent protein. The results we obtain show that most RFPs have a considerable 30% fraction of absorbing but not emitting fluorophores. Our findings have direct implications in quantitative microscopy such as in the interpretation of intensity-based FRET measurements, and prove an important bottleneck in the development of enhanced fluorescent proteins.

## References

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**Fig. 1** Total decay rate versus mirror position for two different fluorophores