INDUCING SPARSITY AT LN_2 TEMPERATURES: TOWARDS POLARIZATION-SELECTIVE SINGLE-MOLECULE EXCITATION AND EMISSION DEPLETION

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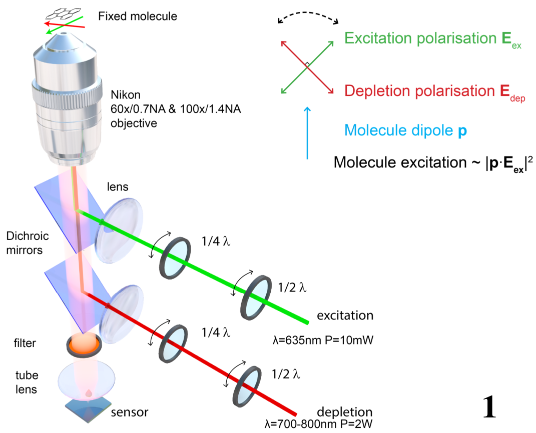

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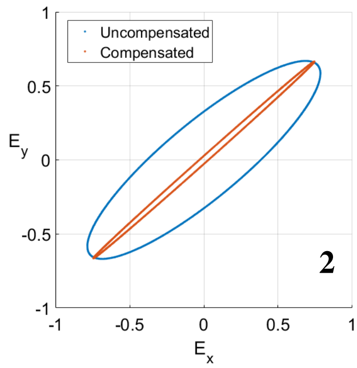
The accuracy with which a molecule can be localized is ultimately limited by the amount of emitted photons that can be collected before photobleaching. At cryogenic temperatures, photobleaching is strongly suppressed, and a localization precision of better than 1 nm has been reached [1]. However, room-temperature techniques that induce sparsity in a fluorescently labeled sample, such as photoswitching, may not work or only very inefficiently. In order to maintain typical labeling densities, alternative routes are required to introduce a sparse activation of fluophores at low temperatures.



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Here, we show our latest results on promoting sparsity with polarization-selective single-molecule excitation and emission depletion. We use the fact that, below saturation, the excitation of a dye molecule with a fixed transition dipole moment is proportional to $|\mathbf{p}\cdot\mathbf{E}|^2$, where **p** is the dipole moment of the molecule and **E** is the electric field vector. Hence, rotating the polarization results in a $\cos^2 \alpha$ dependency of the emission, leading to a slight increase in sparsity in the sample. Moreover, we will show our progress towards implementing a non-linear polarization-dependent emission depletion scheme [2, 3], to obtain a $\cos^n \alpha$ dependency with n substantially larger than 2 (Fig. 1). We aim at a linear polarization state with a better than 1:100 polarization extinction ratio, regardless of the chosen angle or the additional optics required between excitation and the sample. Therefore, we fully calibrate our optical system, and compensate for spurious polarization changes due to, e.g., dichroic mirrors and non-perfect waveplates. Careful calibration of both excitation and emission depletion light paths allows us to drastically improve on a naive implementation of polarization selectivity. For the excitation path alone, we have improved on the linearity of the polarization by up to a factor of 12, which in intensities means a suppression of the unfavorable polarization of over two orders of magnitude.

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