

SINGLE MOLECULE STUDIES OF LIVING COLOURS.

M.F. Garcia-Parajo, M. Koopman, B. I. de Bakker, N.F. van Hulst: Applied Optics group, MESA Research Institute & Faculty of Applied Physics. University of Twente, P.O. Box 217, 7500AE Enschede, The Netherlands.

The green fluorescent protein (GFP) has become an invaluable marker for monitoring protein localisation and gene expression *in vivo*. Mutagenesis of the original wild type GFP has led to variants fluorescing in colours ranging from blue to yellow. The use of these different mutants at the individual molecular level requires however a thorough study of their complex photodynamical behaviour. Furthermore, in single pair fluorescence energy transfer (spFRET) experiments it is crucial to dispose of pairs with emission spectra clearly separable and exact knowledge of the emission dipole moments of both chromophores. We have studied a number of GFP mutants at the individual molecular level using a polarisation sensitive scanning near-field optical microscope and have characterised their complex behaviour. We present recent data concerning the photodynamics of the S65T mutant, the RsGFP and the new red fluorescent protein drFP583. A comparative study is presented in terms of the absorption cross section, quantum efficiency, fluorescence lifetime and blinking behaviour of the different mutants obtained from single molecule data.