

VIDEO FLIM USING A VERSATILE SHORT PULSE LASER SOURCE

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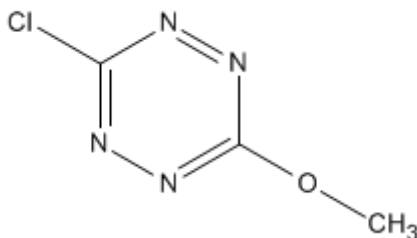
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Nanosensors have a strong potential in biology sensing. Indeed nanocrystals contains much more dyes than latex of similar volume. In a crystal, the excited state can be trapped by a local reorganization of the lattice or diffuse among the molecules. The first type of crystal will give labels whereas the second type will give sensors.

Tetrazines are the smallest fluorophores absorbing (510nm) and emitting (590nm) in the red.



Chloromethoxytetrazine :

MeOTzCl has a long fluorescence lifetime in solution of 150ns and a yield of 0.38. It makes disk like crystal at room temperature. Nanocrystals were produced by precipitation of an ethanol solution in water. Crystals were produced by a slow evaporation from dichloromethane. Through this process, a wide variety of microcrystals are produced, which are studied using full field FLIM microscopy. This setup allows video recording of the photo aging kinetics as well as of the phase transition kinetics. Moreover, purification or phase selective preparation are not needed.

The experimental setup [1] consists in a versatile laser source coupled with an inverted microscope Nikon 2000U and video FLIM detector. The laser source consists in a low repetition-rate (10MHz) femtosecond oscillator (t-Pulse 200) delivering 200nJ pulse energy at 1030nm. Part of the energy is used to produce a continuum subsequently frequency-doubled delivering a pulsed radiation from 420 to 650nm. The other part is converted through SHG and THG, providing short-pulsed radiation at 515 and 343 nm.

The low repetition rate of the source allows long lifetime fluorescence, while the large choice of wavelength allows both full field excitation and full field photochemistry.

We have studied the energy transfer process in MeOTzCl, and the influence of impurities produced by burning the crystals with intense green irradiation. The fluorescence decay becomes faster in presence of photoproducts. The fluorescence decays of the irradiated crystals are monoexponential all along the burning process. This shows that excitation is moving faster from dye to dye in the crystal than from dye to photoproduct. But the concentration of quencher and thus the diffusion length of the excitation in the crystals cannot be measured. Indeed during the burning process neither the absorbance nor the number of fluorescent molecules does change. Less than 1% of the molecules are transformed.

[1] J.-A. Spitz, R. Yasukuni, N. Sandeau, M. Takano, J.-J. Vachon, R. Méallet-Renault, R. B. Pansu, *Journal of Microscopy* **2008**, *11*.