

# LOOKING THROUGH THE MICROSCOPE : WHAT CAN WE LEARN FROM FLUORESCENCE LIFETIME MEASUREMENTS ABOUT REACTIVE OXYGEN SPECIES VARIATIONS IN LIVING CELLS?

*A.C. Ribou<sup>(1)</sup>, T. Rharass<sup>(1)</sup>, J. Vigo<sup>(1)</sup>, J.M. Salmon<sup>(1)</sup>*

<sup>(1)</sup> Institut de Modélisation et d'Analyse en Géo-Environnement et Santé, Université de Perpignan Via Domitia, 52 avenue Paul Alduy, 66860 Perpignan. ribou@univ-perp.fr Fax: 0468662144.

Reactive oxygen species (ROS) are involved in numerous cellular processes. ROS can act as signal transducers, and their presence is essential for proper cell function. But ROS can also cause oxidative stress, and they can induce cell death. Numerous diseases are caused by aberrant ROS production. Despite a growing interest in bio-medical research, direct quantification of ROS remains difficult. Since their detections on cellular level is of great interest new methods are continually developed such as new fluorescence probes or spin trapping. We have recently established a new method based on the fluorescence lifetime of well-known oxygen probes. The detection were successfully performed on the cellular level with the PBA (1-pyrenebutyric acid). We recorded the fluorescence lifetime decays using time-resolved microfluorimetry in human lymphoblastic cells CCRF-CEM loaded with less than micromolar concentrations of PBA. Emission was recorded through an adapted band-pass filter (404nm for PBA) after excitation of single living cells with a pulsed nitrogen laser (337nm, 3ns). Both PBA lifetimes and NAD(P)H intensities were simultaneously recorded. ROS concentration variations were calculated from the Stern-Volmer equation. We demonstrated that ROS concentrations change upon treatment with inductor an inhibitor of intracellular ROS. Recently our studies focus on chemotherapy with the anti-cancer drug adriamycin. The results will be discussed.