

**CYCLIC DYNAMICS OF THE EPITHELIAL CYTOSKELETON :
MOLECULAR ANALYSIS AND MODELING OF TWO-PHOTON FRAP EXPERIMENTS.**

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The intrinsic localization of two-photon (TP) fluorescence excitation combined with laser-scanning microscopy and fluorescence recovery after photobleaching (FRAP) makes it possible to analyze the transport dynamics of intracellular components with a high spatial and temporal accuracy. A TP-FRAP instrument was developed to study the apical cytoskeleton of epithelial cells in relation with cell morphogenesis and morphological maintenance. Following the assumption that the maintenance of a stationary cytoskeleton might be highly dynamic, we mapped the transport dynamics of various GFP-fused cytoskeleton components at different intracellular location, and assessed the molecular basis of transport with the help of molecular mutants. Our experiments led to a large set of FRAP data, for which a dedicated modelling/simulation approach was developed to extract as much information as possible. Care was taken to treat the full diffusion / convection / bleaching equation, and especially to take into account a fluorescence depletion effect. This led to a quantitative description of a molecular assembly-disassembly cycle of ezrin, and the analysis actin renewal and myosin Myo1a transport. We will present the quantitative aspects of our instrumental developments, theoretical background for modeling FRAP data, as well as key biological results and our current efforts. The present work should be more generally useful to study and locally assess the dynamics of protein turnover in submicroscopic structures.

Coscoy et al. "Molecular analysis of microscopic ezrin dynamics by two-photon FRAP". PNAS, 2002, 99, 12813-8.

Waharte et al. "A two-photon FRAP analysis of the cytoskeleton dynamics in microvilli of intestinal cells". Biophys. J. online, 13-12-04.