FAST 4D-FRAP IMAGING FOR ANALYSING DIFFUSION AND MEMBRANE EXCHANGES IN LIVING CELLS

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Recent progresses in biology and microscopy have made it possible to acquire and analyse multidimensional data of fast cellular activities within living cells. Photo-perturbation techniques, such as FRAP, also make complementary analyses possible at the molecular level. However, these techniques have remained separated from fast imaging and remain associated with confocal imaging, strongly restricting quantification to simple dynamic studies.

We have combined on the same microscope position-controlled laser illumination in order to photobleach the sample and “rapid” full field multidimensional acquisition. We have developed user-friendly customized tools in order to easily drive the system. We first describe our fast 4D-FRAP imaging method and validate its performance, using quantitative analyses of diffusion measurements for two-dimensional (2D) lipid layers. We then apply this technique to living cells expressing Rab6-GFP, a protein cycling between a cytosolic pool and a membrane form partly concentrated on the inner face of the Golgi apparatus.

We demonstrate the power of this combination of fast 4D microscopy and FRAP for studying complex dynamics within cells.