MULTIMODAL-FRAP IMAGING FOR ANALYSING LIVING CELL ACTIVITY: WHICH TECHNIQUE FOR WHICH APPLICATION?

Charles Gueudry[1], Cyril Favard[2], Vincent Fraisier[1], Victor Racine[3], Jean Salamero[1], Jean Baptiste Sibarita[1]
1 UMR144 CNRS, Tissue and Cell Imaging, Institut Curie - Research Center, 12 rue Lhomond F-75248 Paris, France.
2 UMR 6133 CNRS, Institut Fresnel, 13397 Marseille Cedex 20, France.
3 Institute of Molecular and Cell Biology, 61 Biopolis Drive (Proteos), Singapore 138673
E-mail: JBS@curie.fr

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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. We previously combined a position-controlled laser illumination module with “fast” multidimensional CCD-based acquisition systems. This module that can be adapted to any kind of CCD-based acquisition setup is now interfaced under the same instrument with wide-field, confocal spinning-disk and TIRF modalities. This unique instrument allowed us to precisely ask the question: “which technique or combination of techniques is the best for my application?”

We first describe our multimodal-FRAP imaging system and validate its performances using quantitative analyses of diffusion measurements for two-dimensional lipid layers. We then apply this technique to living cells expressing different xFP tagged proteins with their own behaviors and dynamics such as 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, through this biological question, the power of combining fast multidimensional microscopy and FRAP for studying complex dynamics within cells, and we discuss the interest and limitation of each technique depending on the biological question.