

## **COMBINATION OF MULTI-COLOUR 4D VIDEO MICROSCOPY WITH LASER ASSISTED TECHNIQUES**

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Understanding the complexity of cellular processes requires a better knowledge of their dynamical properties and geometric structure. Recent progresses in cellular and molecular biology and in microscopy make it possible to visualize cells and acquire multidimensional data of fast cellular activities. Beside microscopic observations, laser-assisted techniques (FRAP, FLIP, Photoactivation, FRET, ...) allow a variety of manipulation and measurements of cellular activities, such as intracellular transport, and in vivo interactions between molecules. However, laser-assisted technique and fast imaging have remained separated.

We have combined, on the same microscope, a position-controlled laser input together with "rapid" multidimensional acquisition followed by image restoration. Practically, a fast 4-5D deconvolution microscope and an argon laser and 405 nm blue diode have been combined in a way that permits to simultaneously acquire image stacks and perform FRAP or photoactivation. By using galvanometric mirrors and a specific laser-injection device, the laser beam can be precisely steered to locations dynamically selected by the user inside a moving sample. This work was done in collaboration with Universal Imaging, Roper Scientific and Leica companies.

These developments are illustrated with preliminary results of fast 4-5D acquisition + FRAP on living cells expressing Rab6A-GFP and Rab6A'-GFP proteins. These small GTPases are involved in membranes transport and display multiple behaviours within the cell. They exchange continuously between a cytosolic and a membrane pools. They are concentrated on the inner face of one specific cellular organelle, the Golgi. They also exist as associated to transport intermediates moving rapidly along microtubules and defining trajectories that are related to the specific function of these members of the regulatory machineries of intracellular traffic.