

FRAP Microscopy

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Fluorescence recovery after photobleaching (FRAP) microscopy is a method for imaging intracellular molecular dynamics. Introduced in the 1970s to study the diffusion of biomolecules in living cells,^{1,2} the technique has become an increasingly popular method for examining variables such as nuclear protein complex dynamics,³ diffusional mobility of membrane proteins,⁴ and cytoskeletal dynamics.⁵

FRAP is a powerful mode of fluorescence-light microscopy in which a specialized illumination strategy is implemented in order to permit perturbation of the steady-state fluorescence distribution by bleaching fluorescence in selected regions. After the bleaching step, researchers can observe and analyze how the fluorescence distribution returns to the steady state (see **Figure 1**). Because the photobleaching of fluorophores is permanent, changes in the fluorescence intensity in both the bleached and unbleached regions are attributable to the exchange of bleached and unbleached fluorescent molecules between those regions.

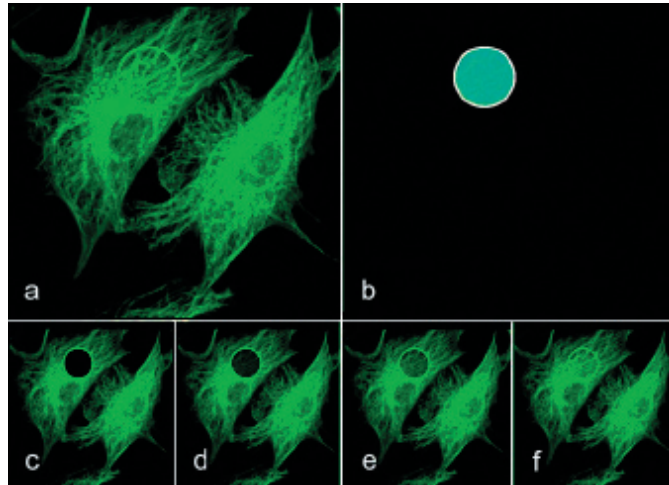
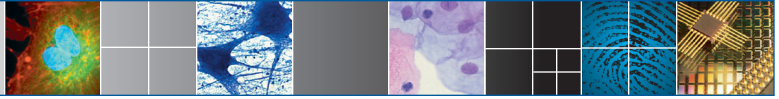


Figure 1. Generalized FRAP concept. **(a)** A region of interest is selected in the sample. **(b)** The region is irradiated with high-intensity illumination until the fluorochromes within the region are photobleached. **(c-f)** The recovery of the fluorescence to the bleached region of interest is monitored as a function of time.

FRAP microscopy is typically geared toward dynamic, low-light endeavors. To make the most of the limited signal available in these investigations, it is important to use a detector that offers high quantum efficiency. Furthermore, exposure times should be kept short to allow the capture of rapidly changing phenomena. Given this requirement of superb low-light-level sensitivity at fast frame rates, electron-multiplying CCD (EMCCD) technology represents an ideal solution for FRAP microscopy.

The Rolera-MGi camera from QImaging® utilizes an EMCCD that enables the detection of extremely weak signals above the readout noise floor during high-speed applications. Not only does this advanced Rolera™ camera feature a back-thinned EMCCD with up to 95% quantum efficiency in the visible spectrum, its quantitative 14-bit digitization allows a wide range of brightness values to be recorded. The Rolera-MGi also provides a state-of-the-art IEEE 1394 interface, thus ensuring convenient, standardized connectivity to the researcher's FRAP workstation as well as rapid data transit for fast, low-light imaging.



Citations

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