FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING

Fluorescence Recovery After Photobleaching (FRAP) is used to measure the dynamics of 2D or 3D molecular mobility e.g. diffusion, transport or any other kind of movement of fluorescently labeled molecules in membranes or in living cells. In this technique, the recovery of fluorescence in a defined region of interest (ROI) of a sample after a bleaching event is monitored by taking a time series of images, see Fig.1.

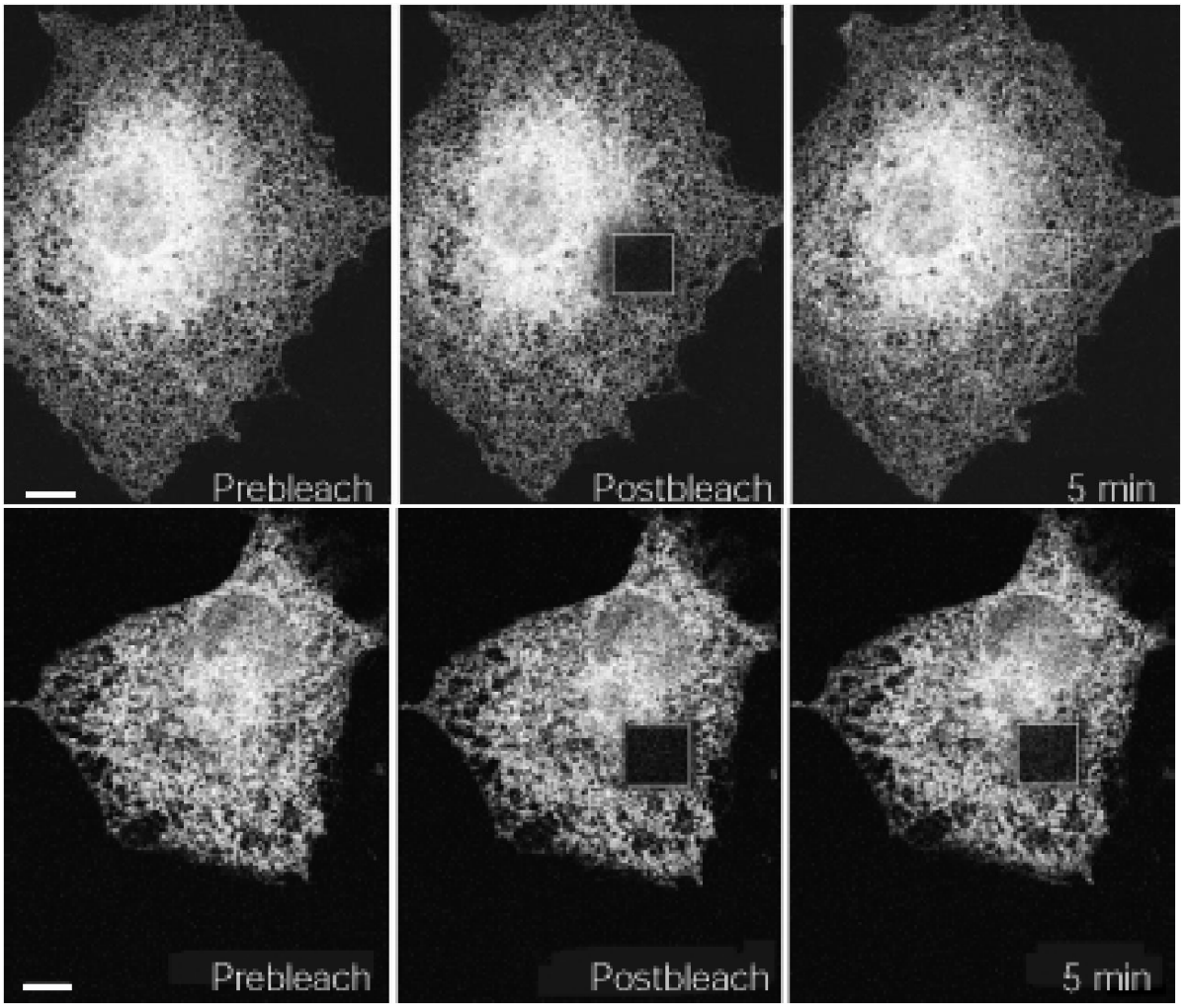

Fig.1 Upper panel: Images of a live cell prebleach, postbleach and after five minutes. Lower panel: Corresponding images of a fixed cell (J. Lippincott-Schwartz, Nature Rev Mol Cell Biol 2 (2001) 444).

The recovery of fluorescence results from the movement of unbleached fluorophores from the surroundings into the bleached area.

The mean intensity in the ROI is plotted versus time, where the recovery time (half-time) indicates the speed of this mobility, e.g. diffusion time, and the level of fully recovered intensity gives information on mobile/immobile species of the fluorescent molecule, see Fig. 2.

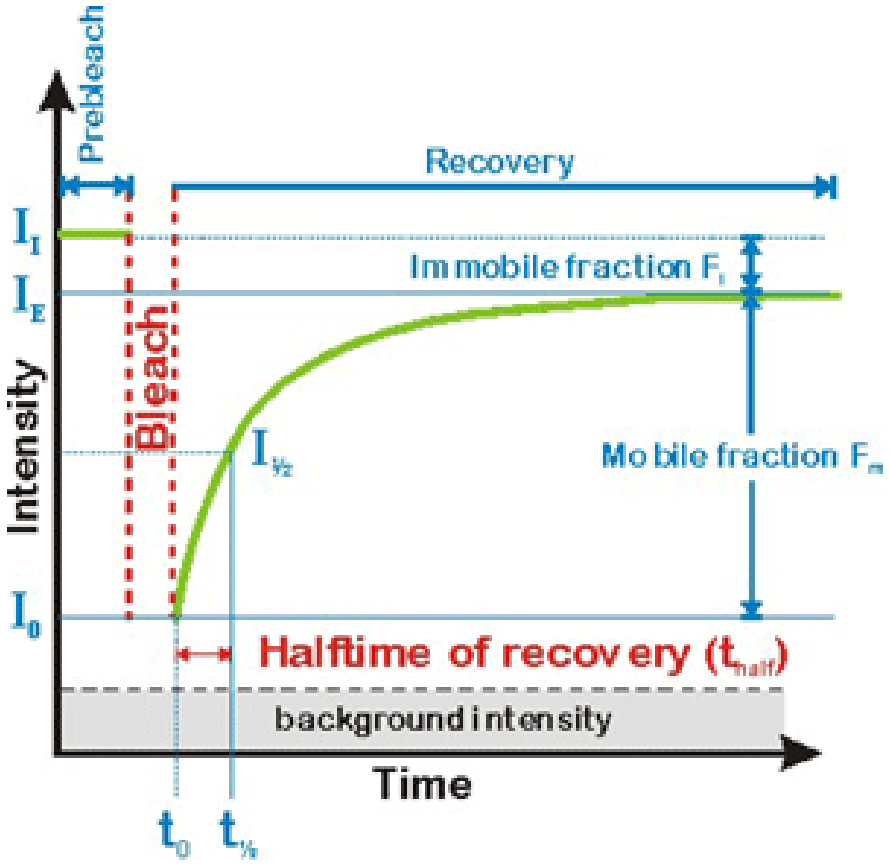

Fig. 2 The FRAP recovery curve gives information about the mobility of a molecule and the fraction of immobile species. (From EAMNET FRAP on-line teaching module, EMBL)

A related method is FLIP (Fluorescence Loss in Photobleaching), which is the decrease/disappearance of fluorescence in a defined region adjacent to a repetitively bleached region. Like FRAP, FLIP is used to measure the dynamics of molecular mobility in membranes or in living cells, especially the ability of a molecule to move between different organelles in the cell.