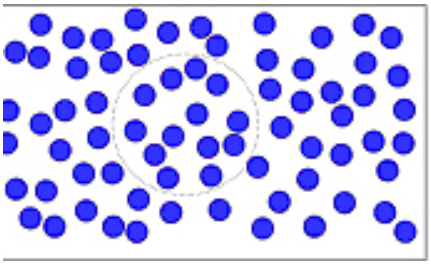
# Intelligent BioMed-Solutions from Visitron Systems

## Application Note: VisiFRAP

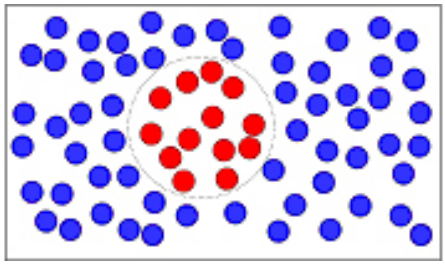
07-2010

Fluorescence recovery after photobleaching (FRAP) microscopy[1] has been widely used to study the diffusion, binding and transport[2] of biomolecules in living cells. With the advance of photoswitchable fluorochromes, the same instrumentation can now be used to photoactivate molecules of interest.

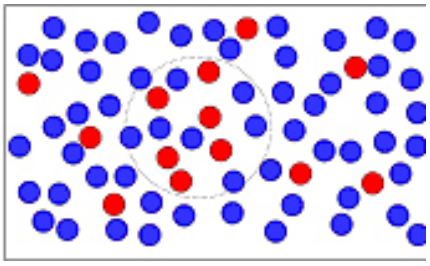
The VisiFRAP system enables its user to photomanipulate and observe labelled molecules with micrometer precision. It allows biologists, chemists and physicists to study diffusion, binding and transport processes within cells, membranes[3], hydrogels and other microsystems.



Fluorescently labelled molecules form part of a cellular structure, such as the cell membrane or the cell cytoskeleton. Depending on their function, these molecules are either bound to other cell components or diffuse freely.

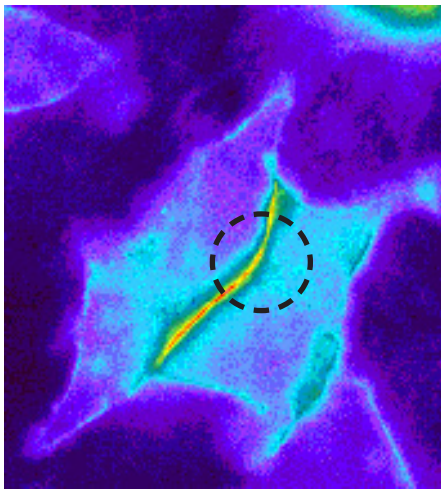


After selecting a region of interest, a high-intensity laser spot is scanned over the molecules. The laser wavelength and intensity can be controlled, allowing either photoactivation or photobleaching of fluorophores.

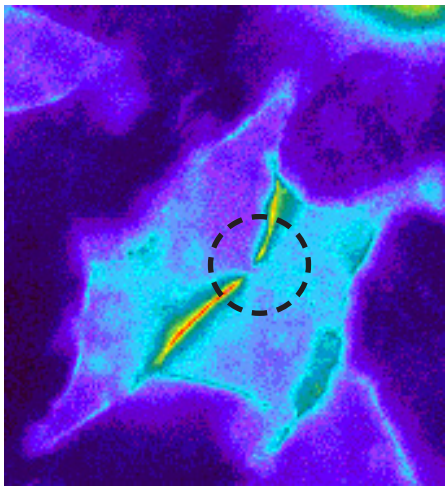


On the time scale of seconds to minutes, the photomanipulated molecules diffuse or are transported out of the region of interest. Since they have been “marked” by the laser, their movement can be studied.

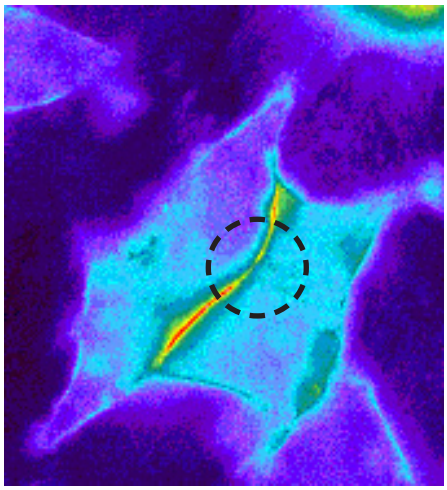
To allow the capture of rapidly changing phenomena, it is important to use a detector that offers high quantum efficiency, such as an electron-multiplying CCD (EMCCD) camera. If required, these detectors can yield millisecond time resolution at single-photon sensitivity, clearly outperforming conventional CCD detectors.



Two endothelial cells link their cell membranes into an adherens junction. A protein which forms part of this adherens junction is labelled with a fluorescent dye. In this example, the protein of interest is p120 catenin and the fluorophore is Green Fluorescent Protein (GFP).



A region of interest is selected and bleached within less than 100 milliseconds using a high intensity laser spot. The fluorescence intensity is shown in pseudocolor.



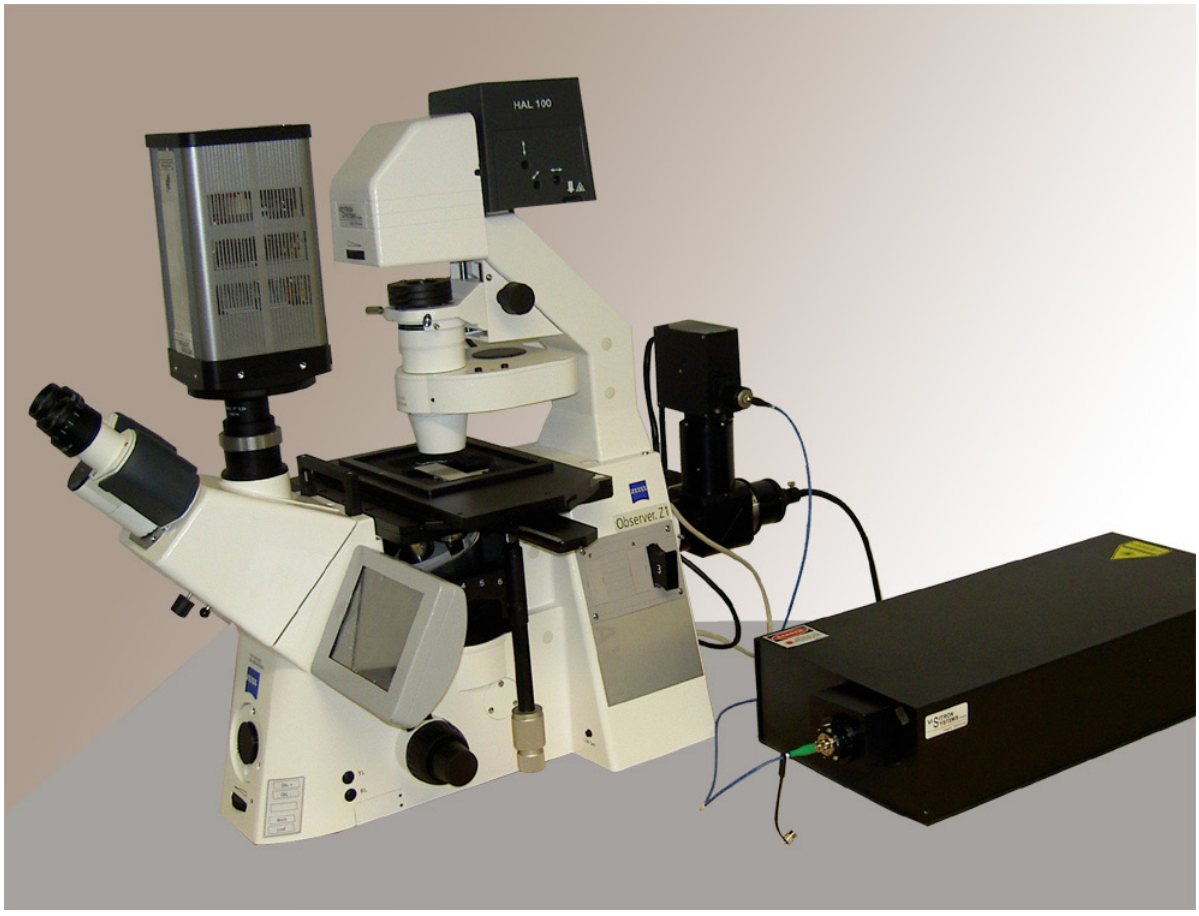
The molecules that were bleached in this region unbind and diffuse out of the adherens junction. New molecules bind, slowly recovering the fluorescence signal at the region of interest.

The 2D-VisiFRAP system from Visitron Systems GmbH is a microscope based imaging solution specially designed for fluorescence recovery or photoactivated studies. The system is based on a 2D galvanometer scanner, flexible laser system with high speed VS-AOTF, high sensitive scientific grade CCD camera, a research microscope and powerful imaging software.

The 2D galvanometer scanner head is typically mounted on the epi fluorescence condenser of the microscope. The laser light can be simpl adapted via standard FC-connector. Customer`s requirements can be easily integrated due to the flexibility of the system. Realtime multi-point confocal technology can be combined to enhance resolution- and image quality.

## Key Features

- Bleaching of multiple regions, shapes and choice of single or multiple wavelengths
- Instantaneous switching between FRAP and imaging modes
- Confocal spinning disk FRAP option
- Optimised optics and camera provide very high photon collection efficiency
- Flexible VisiView software support
- Highly automated MDA “multi dimensional data acquisition”
- Option: experimental control over vital systems (temperature, environment, perfusion)




**Citations**

1. Axelrod, D., Koppel, D.E., Schlessinger, J., Elson, E., and Webb, W.W. (1976). Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. Biophys. J. 16:1055-1069.

2. Wehrle-Haller B. (2007). Analysis of integrin dynamics by fluorescence recovery after photobleaching. Methods Mol. Biol. 370:173-202.

3. Edidin, M., Zagyansky, Y., and Lardner, T.J. (1976). Measurement of membrane protein lateral diffusion in single cells. Science 191:466-468.