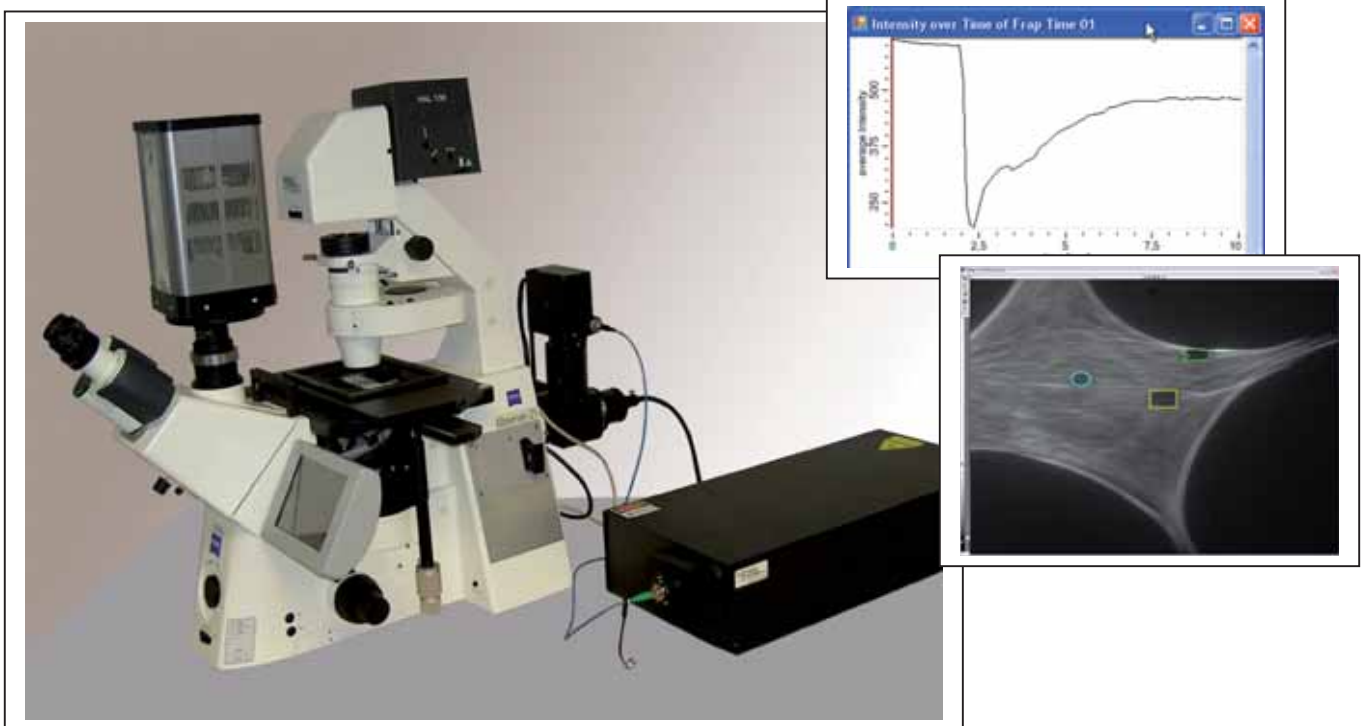


## 2D VisiFRAP Imaging System

# 2D-VisiFRAP Realtime Scanner

## Fluorescence Recovery After Photobleaching Imaging System

Fluorescent dyes show emission of a specific wavelength after they have absorbed light of a shorter wavelength. Dyes, exposed to high intensity e.g. UV light, respond by photobleaching. The high intensity light renders the dyes unable to emit fluorescence. Fluorescence recovery is based on this phenomenon and is typically used to measure the dynamics of molecular mobility or movement of fluorescently labeled molecules. It is also possible to measure the exchange of molecules between separate compartments of the cells.



The 2D-VisiFRAP system from Visitron Systems GmbH is a microscope based imaging solution specially designed for fluorescence recovery or photo activated studies. The system is based on a 2D galvanometer realtime scanner, a high sensitive CCD camera system, a research microscope like a Zeiss AxioObserver, Olympus BX/IX or Nikon E2000.

The 2D galvanometer scanner head is typically mounted on the epi fluorescence condenser of the microscope. The laser light can be simply 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 easily combined to enhance resolution- and image quality.

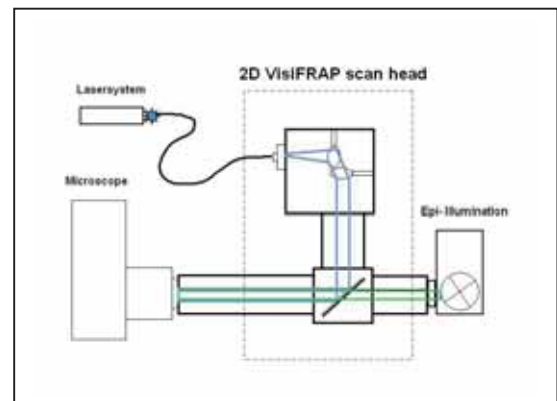
## Key Features:

- Galvanometer controlled 2D module for illumination of independent multiple points or multiple ROI's
- Optimized optics for high photon efficiency
- Choice of single or multiple wavelength and laser lines
- High speed switching between realtime confocal CSU spinning disk or Vt-Infinity multi point scanner
- Point size of laser spot about 1µm for 100x objective with high aperture of > 1.33 NA
- FC fiber connector for laser system
- Including Visitron FRAP software module



## Typical Application:

- 1D and 2D FRAP for cell biology
- FLIP
- fluorescent labeled macromolecules
- photoactivation
- acceptor photobleaching
- photoconversion studies

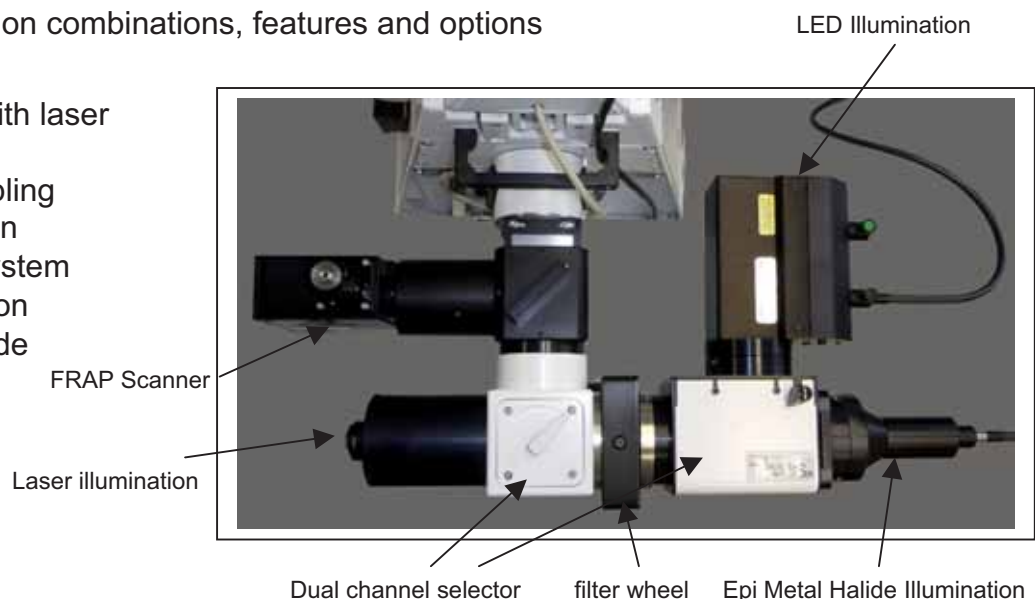


2D VisiFRAP block diagram

## Optical Alignment / Adaption to Microscope

Overview Illumination combinations, features and options

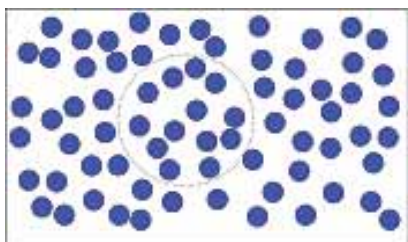
- 2D Scanner with laser input
- VS Laser coupling and illumination
- Filter wheel system
- LED Illumination
- Epi Metal Halide Illumination
- Dual light path selector



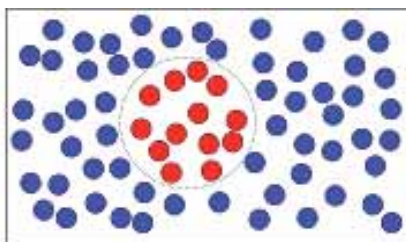
# Application Note: VisiFRAP

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

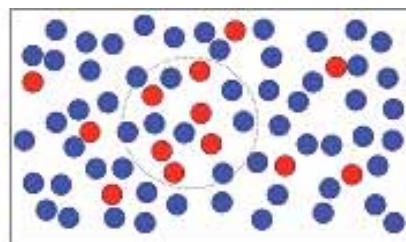
The VisiFRAP system enables its user to photomanipulate and observe labelled molecules with micrometer precision. It allows biologists, chemists and physicists to study diffusion, binding and transport processes within cells, membranes<sup>3</sup>, hydrogels and other microsystems.



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

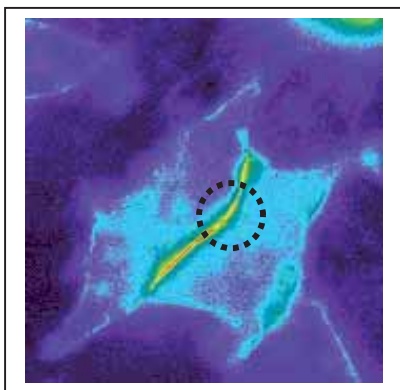


After selecting a region of interest, a high-intensity laser spot is scanned over the molecules. The laser wavelength and intensity can be controlled, allowing either photoactivation or photobleaching of fluorophores.

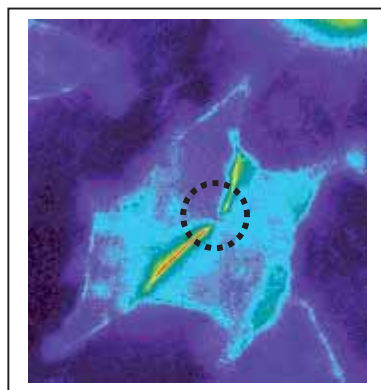


On the time scale of seconds to minutes, the photomanipulated molecules diffuse or are transported out of the region of interest. Since they have been "marked" by the laser, their movement can be studied.

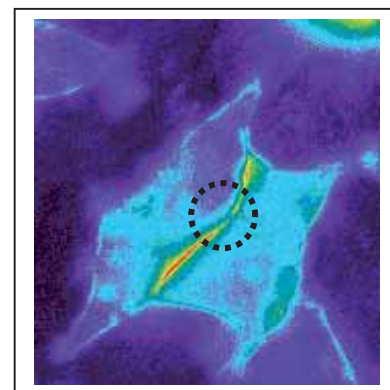
To allow the capture of rapidly changing phenomena, it is important to use a detector that offers high quantum efficiency, such as an electron-multiplying CCD (EMCCD) camera. If required, these detectors can yield millisecond time resolution at single-photon sensitivity, clearly outperforming conventional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

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