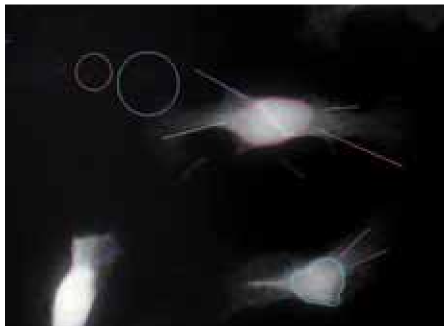
2D-VisiFRAP

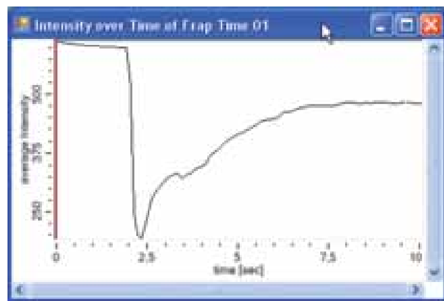
Fluorescence Recovery After Photobleaching

2D-VisiFRAP Realtime Scanner

New: With unlimited number and size of regions and with auto-calibration

Photo-Bleaching and Photo-Activation are established fluorescence imaging techniques. A laser beam is used to perform photo bleaching or activation in a user defined free selectable regions, lines or dots. The 2D-galvanometer scan head can either be used on the epi- or the emission port of the microscope. It can also be combined with a CSU spinning disc confocal.





Zeiss Axio-Observer Z1 with Coolsnap HQ2 and VS-2D FRAP Scanner.

VisiFRAP input for laser and epi light source.

FRAP on the fly

The optimised system components allow simultaneous FRAP and imaging at single mouse click on any position in the live image. This new feature in the VisiView FRAP software is minimising any loss of temporal information and shows the flexibility and high speed positioning of the VS-FRAP scanner. The unique "FRAP on the fly" solves perfectly the major demand for FRAP experiments.

Auto-Calibration

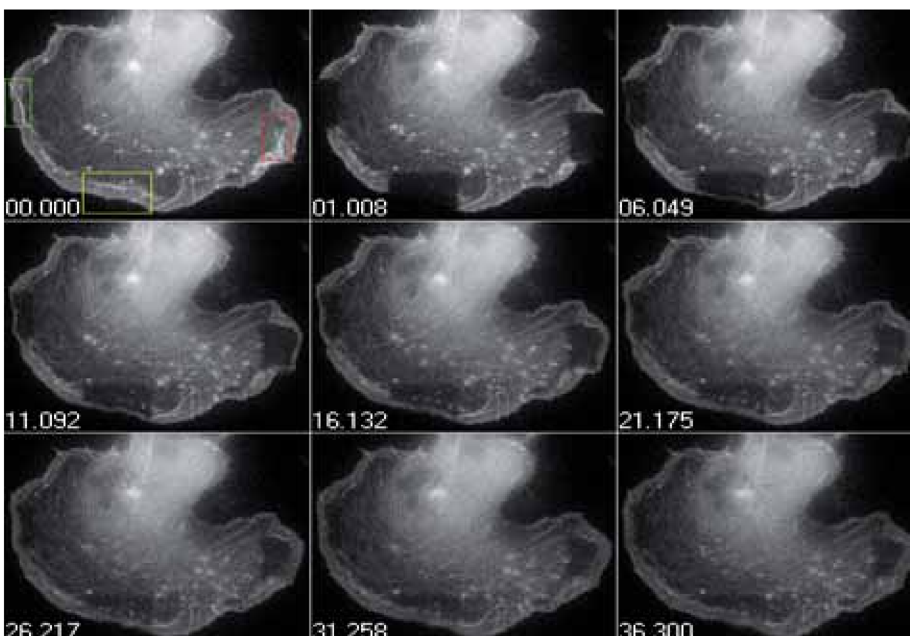
With the automatic signal and spot detection of our VisiView imaging software, the auto-calibration algorithm calibrates the FRAP scanner. It shows in several region on the display the laser spot and the accuracy of the calibration. This tool makes it easy to use different objectives and filters. It saves time and improves your work.

2D-VisiFRAP Realtime Scanner

Fluorescent dyes show an emission of a specific wavelength after they have absorbed light of a shorter wavelength. Dyes, exposed to high intensity light, e.g. in the near UV, respond with permanent photobleaching. The intense light renders the dyes unable to emit fluorescence anymore. Fluorescence recovery is based on this phenomenon and is typically used to measure the dynamics of molecular motility of fluorescence labeled molecules. It is also possible to measure the exchange of molecules between separate compartments within cells.

2D-VisiFRAP

Fluorescence Recovery After Photobleaching



Actin polymerization at the tip of lamellopodium of Melanoma cells.
Image courtesy of Prof. Rottner, University of Bonn

Features:

- Galvanometer controlled 2D module for illumination of multiple ROIs
- Optimized optics for high photon efficiency
- Choice of single or multiple wavelengths and laser lines
- Simultaneous observation of stained cells and laser while the FRAP process is running
- Point size of laser spot about 1 µm for 100x objective with high aperture of > 1.33 NA
- FC fiber connector for laser system
- VisiView® FRAP software module

Applications:

- FRAP, FLIM, Photo-Activation
- Uncaging
- Photo-ablation
- Photo manipulation of intracellular dynamics
- Photoconversion studies

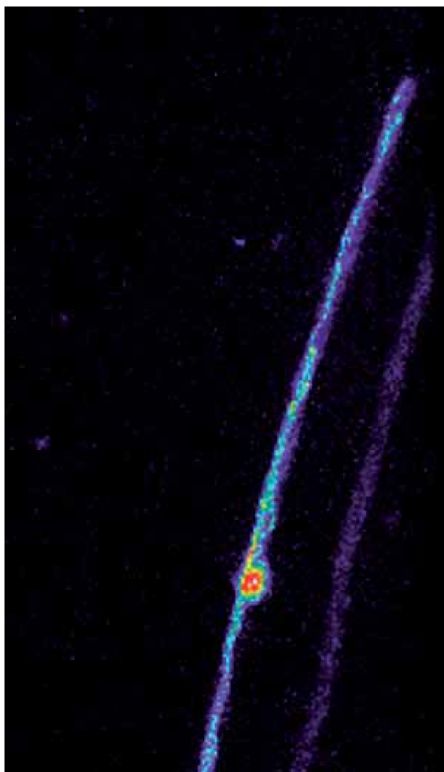
VisiFRAP support for Zeiss, Leica, Olympus and Nikon microscopes.

2D-VisiFRAP-EM

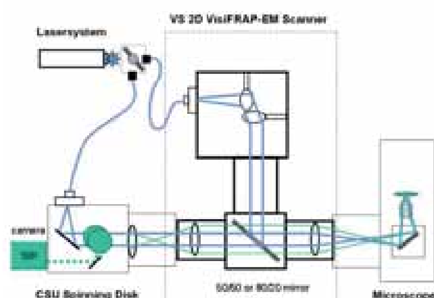
Emission
FRAP
Scanner

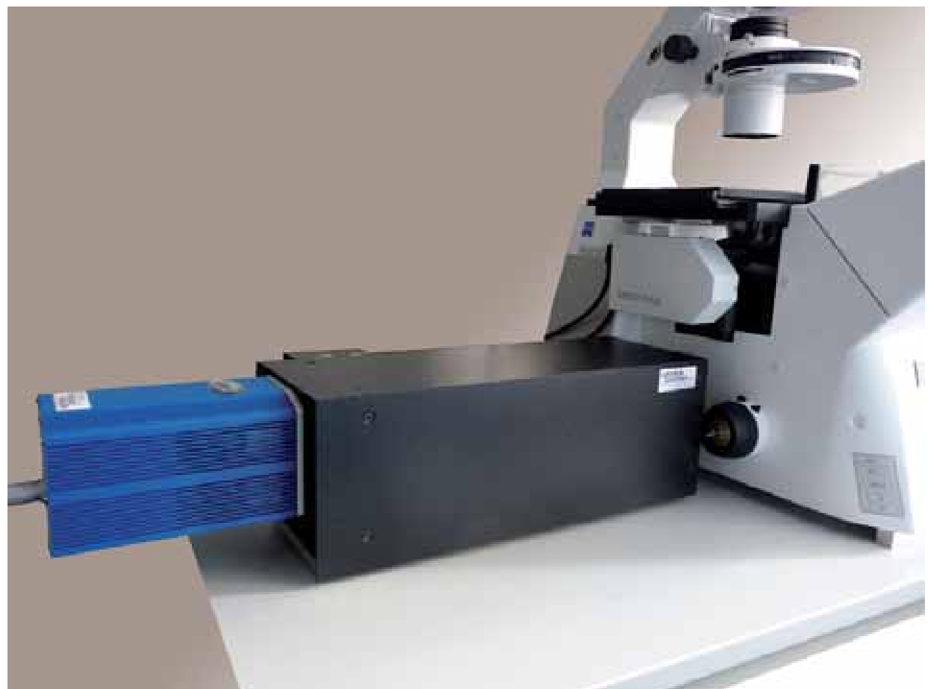
2D-VisiFRAP-EM Emission Realtime Scanner

The Emission FRAP scanner is positioned at the camera port of the microscope. It can be combined with CSU confocal or directly connected to a scientific grade CCD camera. No moving parts or optics are needed because of a special polydichroic design. In order to use the same laser lines for multiple image applications the VS-DOL100 Dual Output Laser option with high speed galvanometer scanner is used.



Photoactivation of GTPase Rab5 with N-terminally tagged paGFP.
Image courtesy of Prof. Steinberg, University of Exeter





Zeiss Axio-Observer with 2D Emission FRAP including emission filter wheel and Coolsnap-HQ camera.

Versatile and Flexible

The Visitron emission FRAP can also be used without CSU confocal head. In that case, a 6-position emission filter wheel is included in the FRAP head. The reason is, that the standard emission filter from the microscope filter cube must be removed to get the FRAP laser active simultaneously to the epi fluorescence.

Features:

» "VisiFRAP-EM" performs flexible microscope laser scanning
» Allows simultaneous imaging and FRAP
» Diffraction spot size < 5 µm FWHM
» Integrated control with VisiView® imaging software
» Arbitrary multi-region scanning of points, rectangles and polygons

Application Note

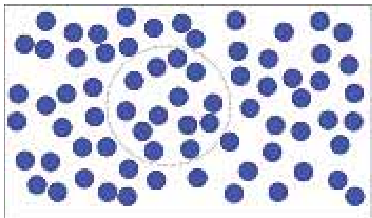
Fluorescence recovery after photobleaching (FRAP) microscopy[1] has been widely used to study the diffusion, binding and transport[2] of biomolecules in living cells. With the advance of photoswitchable fluorochromes, the same instrumentation can now be used to photoactivate molecules of interest.

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.

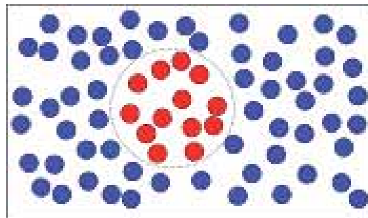
VisiFRAP

Application Note

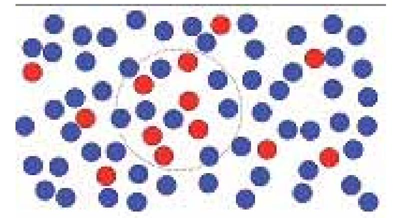
The VisiFRAP system enables its user to photomanipulate and observe labelled molecules with micrometer precision. It allows biologists, chemists and physicists to study diffusion, binding and transport processes within cells, membranes[3], hydrogels and other microsystems.



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.

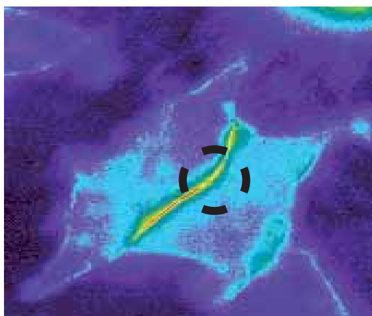


After selecting a region of interest, a high-intensity laser spot is scanned over the molecules. The laser wavelength and intensity can be controlled, allowing either photoactivation or photobleaching of fluorophores.

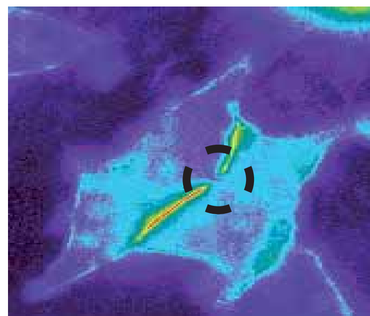


On the time scale of seconds to minutes, the photomanipulated molecules diffuse or are transported out of the region of interest. Since they have been "marked" by the laser, their movement can be studied.

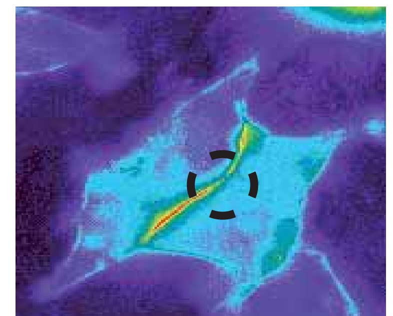
To allow the capture of rapidly changing phenomena, it is important to use a detector that offers high quantum efficiency, such as an electron-multiplying CCD (EMCCD) camera. If required, these detectors can yield millisecond time resolution at single-photon sensitivity, clearly outperforming conventional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



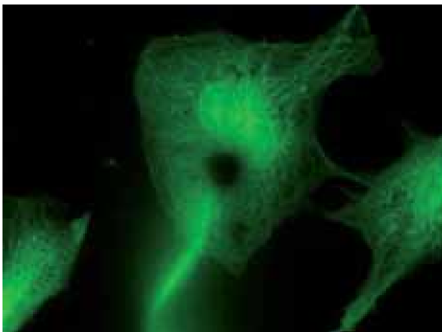
The molecules that were bleached in this region unbind and diffuse out of the adherens junction. New molecules bind, slowly recovering the fluorescence signal at the region of interest.

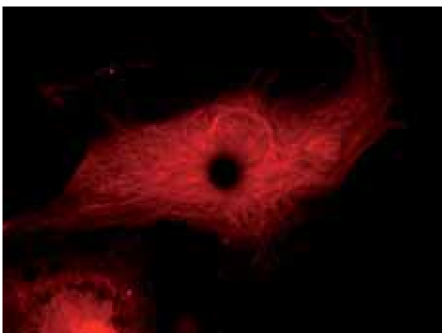
1D-VisiFRAP Point Illumination

Fluorescence Recovery After Photobleaching

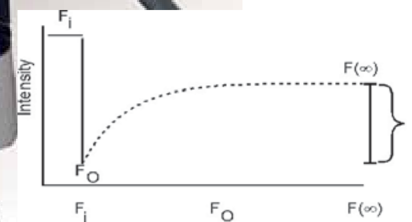
1D VisiFRAP Point Illumination

The 1D-VisiFRAP optics from Visitron Systems GmbH are specially designed for fluorescence recovery or photoactivation at a single point. The system is based on a high sensitive CCD camera system, typically a scientific microscope, motorized microscope xy stage and special designed 1D point laser condenser. All components are automatically controlled by the VisiView® imaging software.

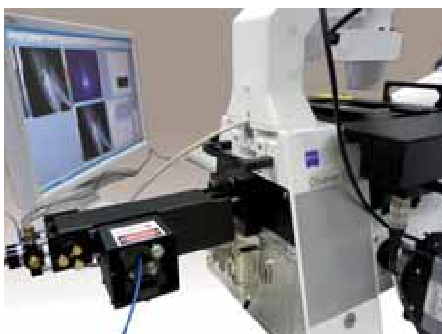




Endothelial cells used with VS-1D single point FRAP.



Zeiss Axio-Imager with 1D FRAP optics and motorized emission filter wheel.



Combination of VisiTIRF condenser with 1D FRAP optics.

Smooth Illumination

The positioning of the selected point or area for bleaching or photoactivation is either done manually or by the motorized xy stage. Single points as well as ROI's can be scanned by moving the XY-stage. An area of interest can be simply selected with single mouse click. The area moves automatically to the middle of the image field. With „FRAP on the fly" every mouse click activates the laser for bleaching.
Several laser combinations from 405 nm up to 640 nm allow flexible photomanipulation of different fluorochromes or switchable proteins.