VISITRON SYSTEMS GmbH
Microscopy and Imaging

2D-VisiFRAP Realtime Scanner

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

Photo-Bleaching and Photo-Activation are well established fluorescence imaging techniques for photo manipulation. A laser beam is used to perform photo bleaching or activation in user defined free selectable regions, lines or dots. The 2D-galvanometer scan head can easily be used on the standard epi illumination port of the microscope.

VisiFRAP-DC
Direct Coupling

FRAP
PA
Ablation

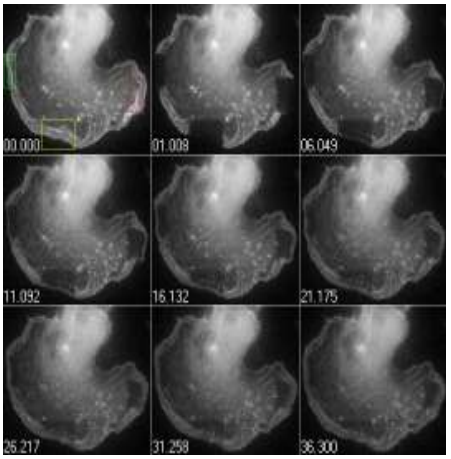
Olympus IX-83 microscope with VisiFRAP-DC-355

FRAP on the fly

The optimised system components allow simultaneous FRAP and imaging at single mouse click on any position in the sample FOV. This 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 “FRAP on the fly” meets perfectly the major demand in 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 the laser spot in several regions on the display and the accuracy of the calibration. This tool makes it easy to use different objectives and filters. It saves time and improves your work.



Actin polymerization of Melanoma cells.
Image courtesy of Prof. Rottner,
University of Bonn

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VisiFRAP-DC
Direct Coupling

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2D-VisiFRAP-DC Realtime Scanner

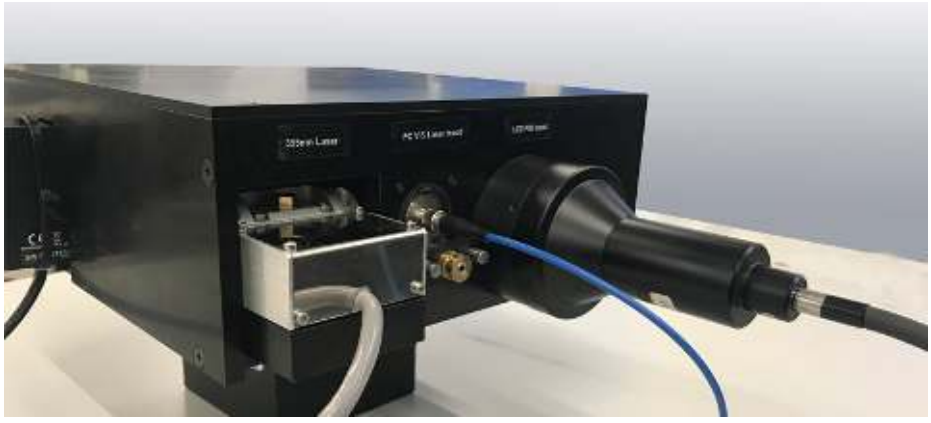
The latest addition to the Visitron Systems 2D-FRAP scanner family convinces by its compactness and flexibility. The design allows the direct coupling of one laser in the Galvo-scan-head without laser fibers. An additional laser fiber input offers the combination of the system with other VIS-laser lines if required. As usual with our VisiFRAP solutions, the unit is designed to allow FRAP or Ablation while imaging in widefield, confocal or TIRF mode without the need to switch filter cubes or other hardware.

VisiFRAP-DC355 with control unit

FRAP Microscopy Detection

Fluorescence recovery after photobleaching (FRAP) microscopy has been widely used to study the diffusion, binding and transport of biomolecules in living cells. With the advance of photoswitchable fluorochromes, the same instrumentation can now be used to photoactivate molecules of interest.
To allow the capture of rapidly changing phenomena, it is important to use a detector that offers high quantum efficiency, such as an EMCCD or sCMOS-BI camera. If required, these detectors can yield millisecond time resolution at single-photon sensitivity, clearly outperforming conventional CCD detectors.

Leica MDI microscope ith VisiFRAP-DC355 and CSU-W1 confocal with Ham Flash camera



VisiFRAP-DC355 side panel with FC-VIS laser input and LED Epi illumination fiber coupler

VISITRON SYSTEMS GmbH
Microscopy and Imaging

VisiFRAP-DC for Ablation

Combining state-of-the-art cutting pulsed laser technology with our successful VisiFRAP Scanner, the VisiFRAP-DC Ablation System offers maximum flexibility and ease of use. The system is a perfect tool for Subcellular Nano-Surgery, Irradiation, DNA repair, Microstrokes or other Organelle destruction.

VisiFRAP-DC

Ablation
355nm or
532nm
pulsed laser

Pre Ablation | Post Ablation-I | Post Ablation-II

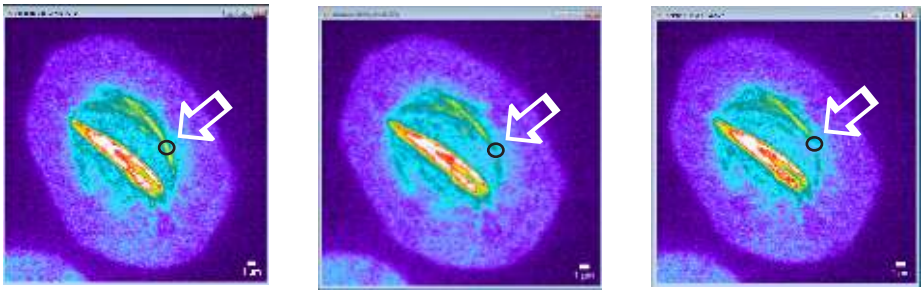
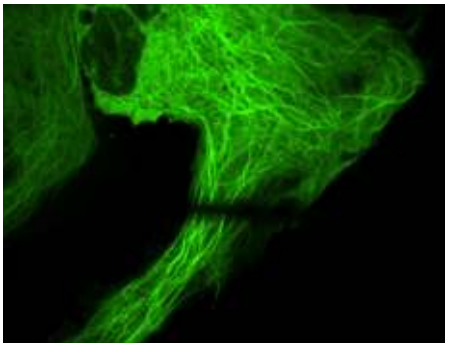


Figure: Ablation of microtubule structures using the VisiView® FRAP/Ablation on the fly. The scale bar shows 1 µm.



Ablation of microtubules inside U2OS cells

Features:

- Interactively cuts submicron-sized objects
- Combines Ablation with FRAP
- Operates at kHz-rates
- Compatible with TIRF/Confocal/Widefield

Typical Applications:

- Cutting subcellular structures
- Nano Surgery
- DNA damage or irradiation
- Microstrokes e.g. thrombosis
- Microengraving into glass
- Nucleocytoplasmic transport
- Protein diffusion studies
- Single Cell optical transfection

The following models are available:

- Model VisiFRAP-405nm
- Model VisiFRAP-405nm-VIS
- Model VisiFRAP-UV355nm
- Model VisiFRAP-UV355nm-VIS
- Model VisiFRAP-UV532nm
- Model VisiFRAP-UV532nm-VIS

Nikon-Ti microscope withVisiFRAP-DC 355nm

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VisiFRAP-DC

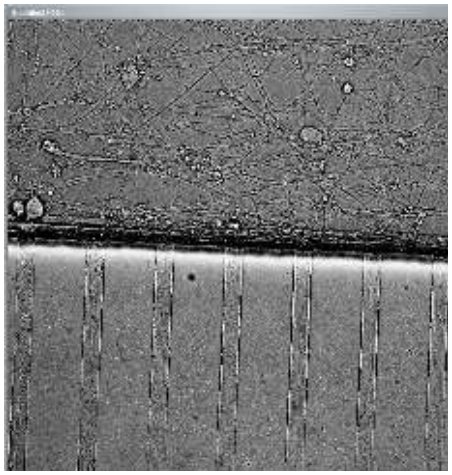
Ablation Application

Ablation Exeriment Application and Image Analysis

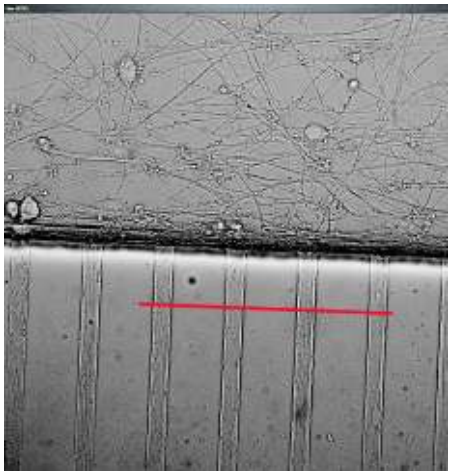
The 2D VisiFRAP-DC module offers great versatility and ease-of-use when it comes to performing experiments where precise localization of a laser spot is required:
FRAP and uncaging experiments come to mind. By implementing a directly coupled pulsed laser (355 nm and 532 nm wavelength are available), the 2D VisiFRAP module's function can be extended to perform ablation experiments: the energy of the pulsed laser is high enough to cut axons, DNA strands and even glass.

FRAP 2D-VisiFRAP-DC355 Ablation

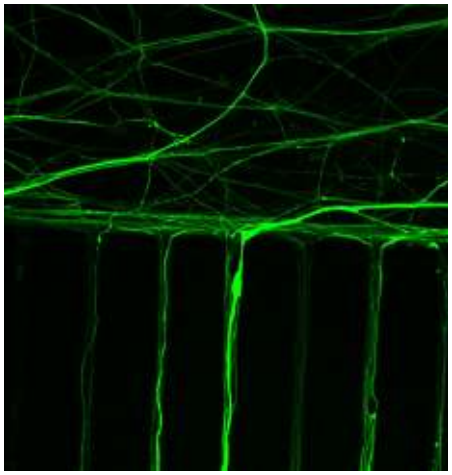
This example shows an ablation experiment were afferent axons from dorsal root ganglion neurons passing through microgrooves of microfluidic devices were cut with a 355 nm UV laser. The fluorescent signal was acquired by live cell image acquisition and the neurons were infected with GFP-expressing lentiviral vectors. Images courtesy of: Gianluigi Nocera, Laboratory of Prof. Dr. Claire Jacob, Institute of Developmental Biology and Neurobiology, Johannes Gutenberg University Mainz.



Pre-Ablation_Brightfield



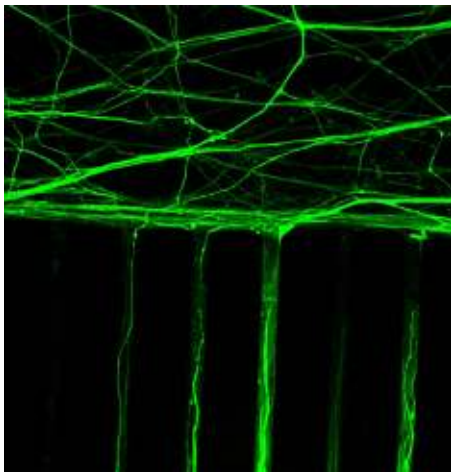
ROI for FRAP



Pre-Ablation_sdc488 green

Figure shows axons from dorsal root ganglion neurons: Images are pre- and post Ablation

Post-Ablation_Brightfield.



Post-Ablation_sdc488 green

VisiFRAP-DC-TIRF

Combination

VisiFRAP-DC-TIRF combination with Orbital Ring-TIRF Technology

The VisiFRAP-DC scan head can extended with our Orbital RingTIRF condenser. In that case UV lenses will be used because of 355nm pulsed laser for Ablation and TIRF combination. The Total Internal Reflection Fluorescence (TIRF) technique is the ideal method for observations of cells close to the coverslip surface. By total reflection of the excitation light at the coverslip / medium interface, the fluorescence emission is limited to a very thin space in the vicinity of the glass surface.

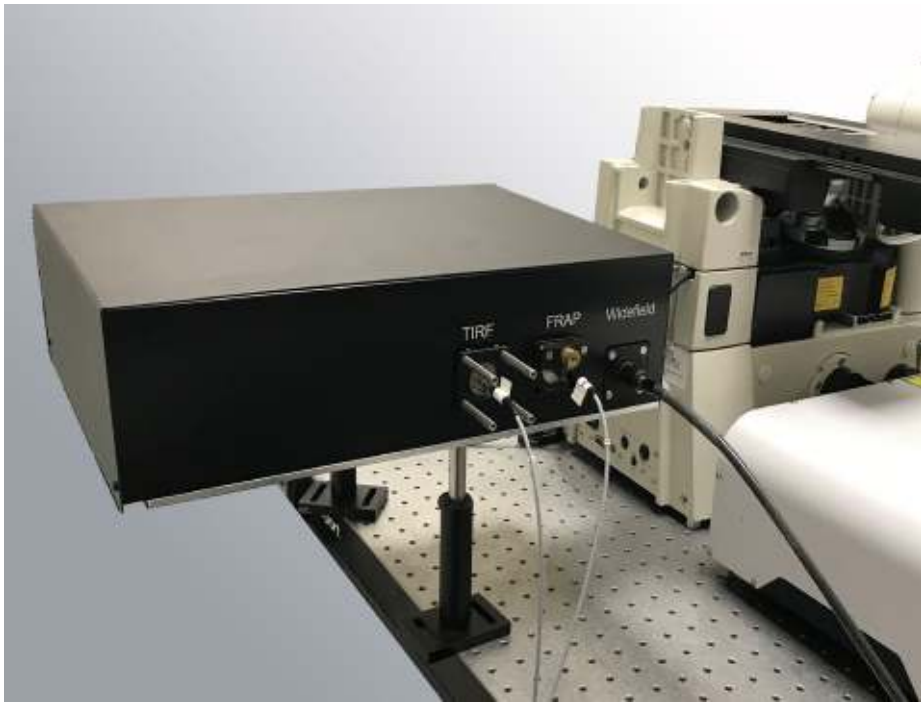


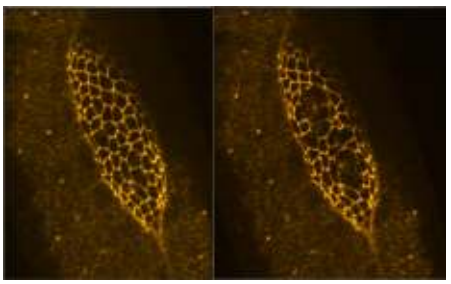
Figure shows VisiFRAP-DC355-TIRF with FC laser VIS input TIRF, FRAP and LED Widefield LLG illumination

Simultaneous Spinning TIRF illumination with Ablation

To go even further, the VisiFRAP-DC-TIRF adds photo-ablation, TIRF illumination with widefield or confocal microscopy in living cells.The TIRF 360 degree spinning of the laser excitation light at the back focal plane of the objective, allows for an uniform imaging of samples without shadowing or artifacts. With the traditional single point illumination an interference pattern is often disturbing the quality of the image.

Widefield - FRAP - TIRF Illumination

Each optical input for FRAP and TIRF is coupled by a single-mode optical fiber with FC-input to the Visitron laser merge system with multiple outputs. The widefield illumination input is coupled via an additional Liquid Light Guide, typically to a LED light source.



VisiFRAP-DC-TIRF with TIRF condenser for microscope coupling

VisiFRAP-DC

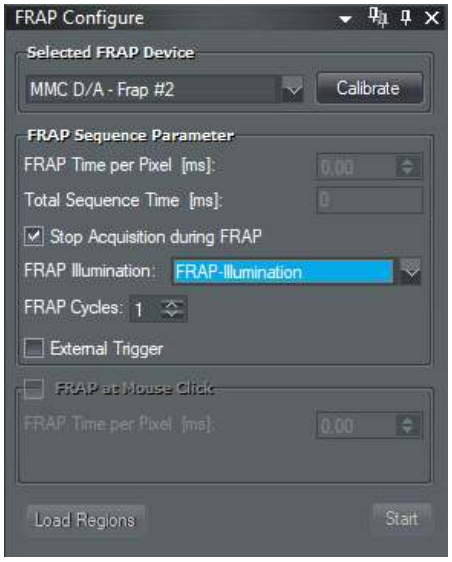
FRAP, PA, Ablation

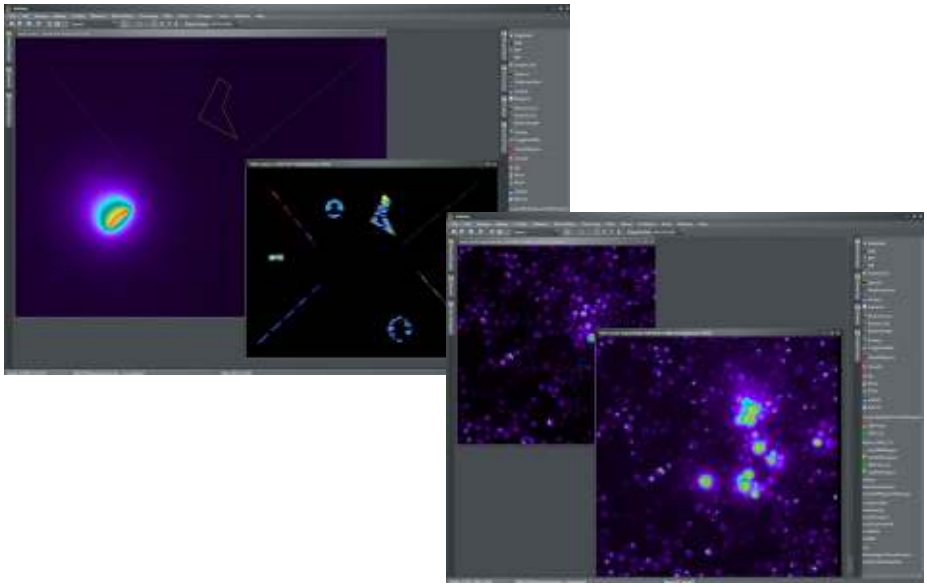
VisiView® Software

VisiView® FRAP - Ablation Module

Easy to use FRAP Scanner

The VisiView® FRAP option in conjunction with the 2D-VisiFRAP gives you control over high power lasers, which are focused down to the µm-scale. The co-evolution of Software and 2D-VisiFRAP results in a perfect interplay and high time resolution when switching lasers. Moreover, flexible ROI selection and fast laser deflection provide the freedom to specifically excite multiple parts of your sample almost at once.





FRAP Acquisition Dialog

The FRAP configuration dialog is directly accessible from the time lapse tab of the clear-cut VisiView acquire dialog. It gives you control over FRAP parameters as well as access to the simple auto-calibration procedure. Further, you can easily test the FRAP parameters using a live preview before you start the real experiment.

Automatic Calibration Algorithm

A calibration which matches the camera coordinates with the laser scanner galvo coordinates needs to be done once for each microscope objective used for FRAP. This is accomplished automatically by moving the beam to some pre-defined positions in the live image and marking their coordinates. Subsequently, the appropriate calibration is selected automatically when the objective is changed.

FRAP on the Fly Function

For fast kinetics, FRAP on the Fly mode can be used. During the acquisition sequence e.g. cells can be laser activated by clicking with the mouse pointer within the image and can be recorded in Real-Time.