

# VisiScope-4Elements

The All-in-One solution

Confocal, FRAP, TIRF and Widefield

The VisiScope-4Elements System unites all basic fluorescence microscopy methods: Confocal, TIRF, FRAP (Ablation) and Widefield and makes simultaneous FRAP and Confocal or TIRF acquisition possible. Its high speed and flexibility make it an ideal tool for basic and applied research.

Solution for  
Life Science  
Research

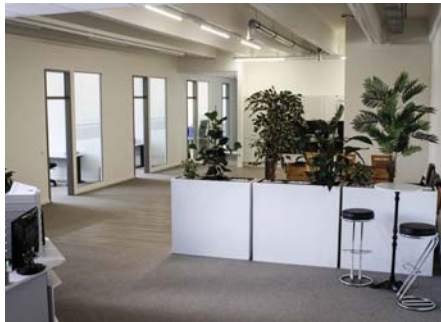


# VisiScope 4Elements

## Company Profile

### Development and manufacturing - “Made in Germany”

Visitron Systems GmbH is known as one of the leading companies supplying imaging-solutions in the field of microscopy for nearly 25 years. The company was founded 1995 by its two managing directors Dr. Gunter Köhn and Helmut Wurm. His strength is to offer a wide ranging portfolio of products for Life Science and BioMedical research. We focus on helping our customers succeed. Whether you are an existing user or prospective customer: Your success is our business.



### We have moved in a new facility end of 2017

We are happy to present in larger premises with more than 700m<sup>2</sup>. The space for manufacturing and technical support is substantially expanded. New modern offices, a large conference room and a well-equipped Demo room are now available. Everything for comprehensive presentations and high-end microscopy and imaging.



### The Visitron Sales and Application Team

A competent team of specialists from the field of molecular biology, physiology, biophysics and informatic engineers are available for extensive consulting and personal contact. The support team of Visitron Systems GmbH has a long experience in science and microscopy and is continuously trained and educated on new technology.

### The Company Architecture of Visitron Systems GmbH

Over the years Visitron has established several departments. Today the company is organized by the Software R&D, Optics R&D, Electronic R&D, Production and Service, Sales and Support and Administration department. The company is well prepared for future developments and challenges.

**VisiScope-W1 Real-Time Confocal System  
 for a wide field of view and improved image quality**

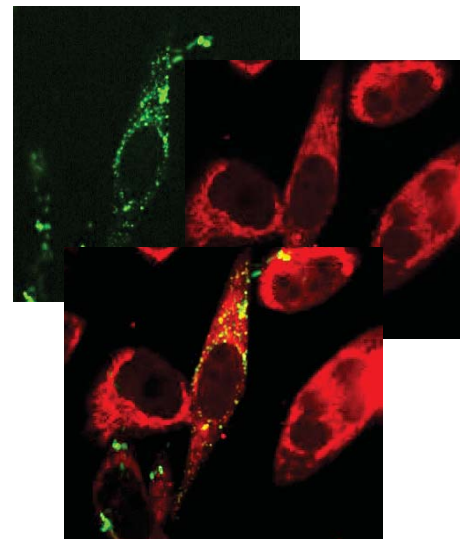
Visitron Systems GmbH has established a global distribution agreement with Yokogawa Corp. Japan. We are an established company in the field since about 25 years with 15 years experience in the Spinning Disk technique. The new CSU-W1 Confocal design for wide field of view (17 x 16 mm) and clearer images offers superior performance and functionality required in live cell research.

**VisiScope  
 4Elements**

**Confocal  
 Spinning Disk  
 CSU-W1**

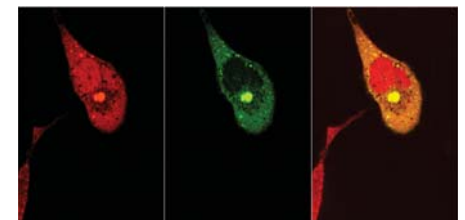


Zeiss Axio-Observer, VS-2D FRAP Scanner, CSU-W1 and two sCMOS cameras.

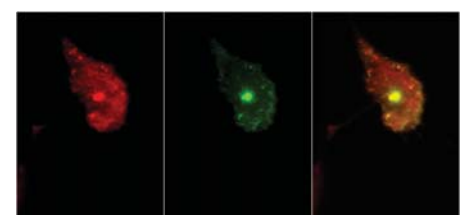


**Wide and Clear**

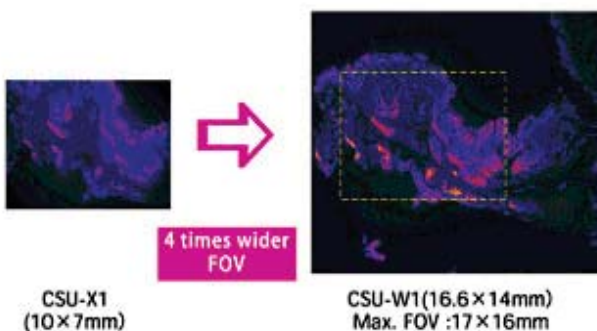
The CSU-W1 system employs a newly designed large diameter spinning disk, which gives wide and clear images with significantly reduced crosstalk. Now you can image whole mount specimen at high magnification.



RFP and GFP labeled Cells acquired with CSU-W1 confocal



RFP and GFP labeled Cells acquired TIRF illumination



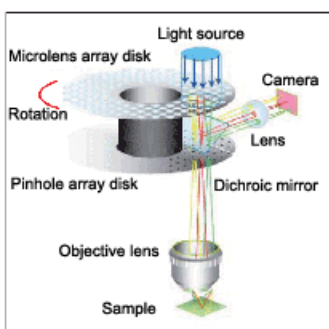
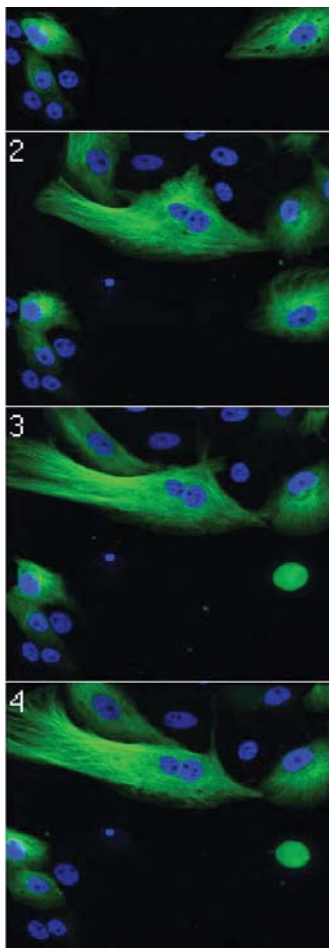


# VisiScope 4Elements

## Confocal Spinning Disk CSU-W1

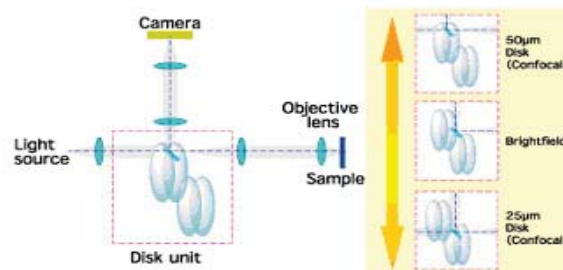
### VisiScope-W1 Real-Time Confocal System for Wide field of View and improved image quality

The CSU-W1 confocal scanner unit, a high-end model that follows the previously released CSU-X1, offers superior performance and functionality that researchers require. With its significantly larger field of view, decreased crosstalk, and extended near-infrared spectral range, it can obtain sharper images of regions deeper inside living samples.



### Selectable Pinhole Size

CSU-W1 provides an optional 25  $\mu\text{m}$  pinhole in addition to the conventional 50  $\mu\text{m}$  pinhole. Moreover, CSU-W1 provides motorized switching among the confocal paths and the brightfield path which allows direct brightfield imaging without light loss at the pinhole disk.



### Provide many models to meet versatile applications

You can select from many models and options to meet various research demands.

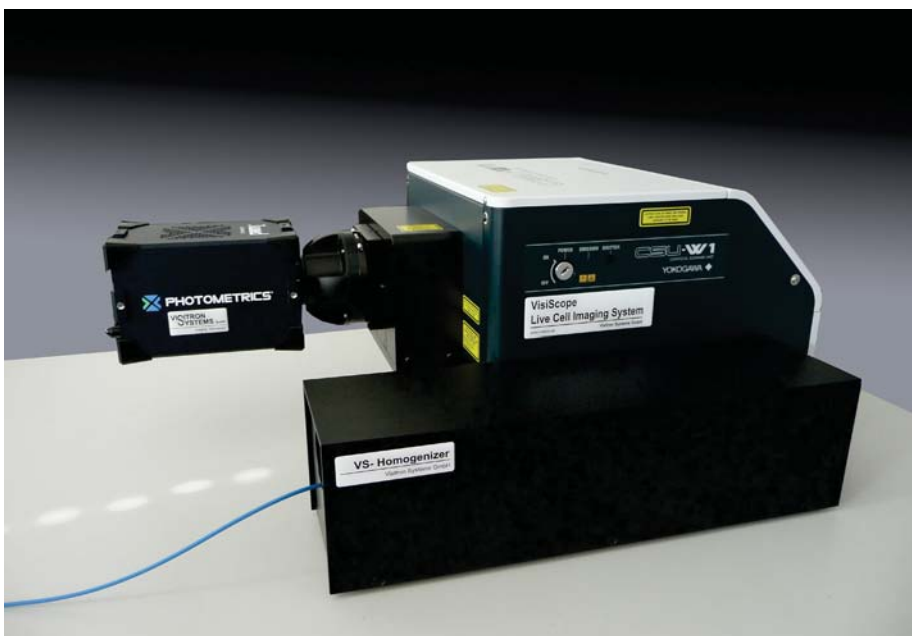
Comparison of 2 color imaging	
1 Camera model High-speed filter wheel	Sequential 
	Simultaneous dual-color imaging 
2 Camera model Simultaneous dual-color imaging with 2 cameras	Simultaneous dual-color imaging 
	Split-view model Selectable from split-view mode for simultaneous dual-color imaging, or filter wheel mode 
Split-view model Selectable from split-view mode for simultaneous dual-color imaging, or filter wheel mode	Sequential 
	Simultaneous dual-color imaging 

## VS-Homogenizer Optics

The Visitron Systems GmbH “VS-Homogenizer” optics are designed to enhance the laser illumination of spinning disk confocal CSU-W1. This optical component can be easily added to already installed CSU-W1 confocal scan heads. The existing functionality of the original CSU confocal head remains. This enhancement offers even illumination of large sample areas and allows high-sensitivity imaging of living cells without the need for mathematical shading correction.

## VisiScope 4Elements

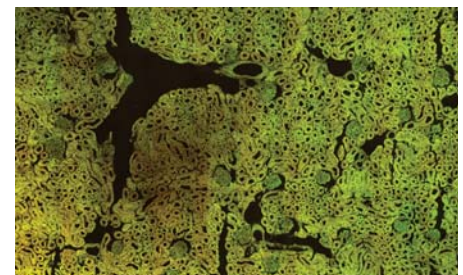
VS-Homogenizer  
 Improve  
 Uniformity



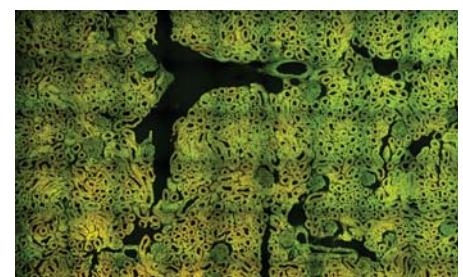
CSU-W1 and VS-Homogenizer with Prime-95B camera.

## Result of minimized stitching artifacts

13x13mm field of a sCMOS camera and 63x/1.4 oil objective



Scan slide acquisition of 8x6 images with VisiScope Homogenizer



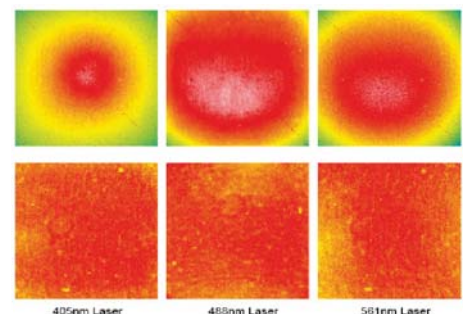
Scan slide acquisition of 8x6 images with a standard Yokogawa CSU-W1 System

## Technical Background

Laser-based microscopy generally uses gaussian beams to achieve optimal focusing of the excitation light in the sample plane. However, this requires making a trade-off between excitation uniformity and intensity. The new Visitron VS-Homogenizer tackles this challenge by providing a flat intensity profile whilst maintaining optimal focusing of the laser beam in the sample plane.

## Features and Benefits:

- » Uniformity improvement
- » Single mode fiber coupling with minimal power loss at pinholes
- » Maintains high signal to noise ratio of standard Yokogawa CSU-W1
- » No beam conditioning unit required
- » Flat intensity profile, optimal light efficiency, minimal background



Note: Patent pending

# VisiScope 4Elements

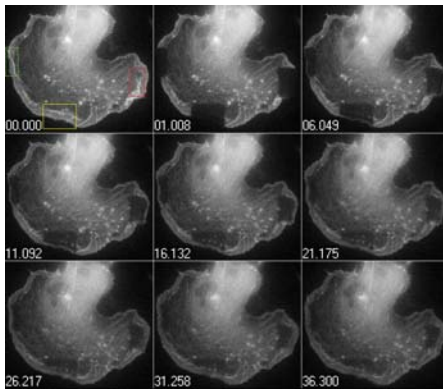
## 2D-VisiFRAP

### Fluorescence Recovery After Photobleaching

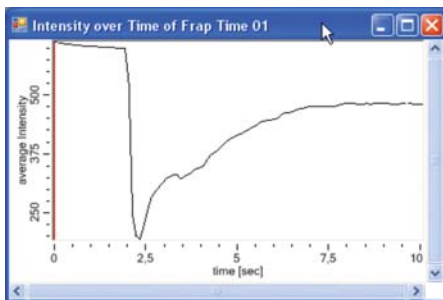
## 2D-VisiFRAP Realtime Scanner

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

Photo-Bleaching and Photo-Activation are established fluorescence imaging techniques. 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 either be used on the epi- or the emission port of the microscope. It can also be combined with a CSU spinning disc confocal.



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



Measured intensity/time recovery

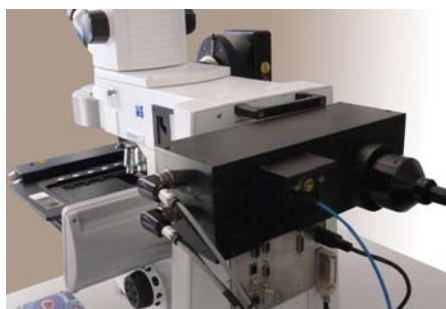


## FRAP on the fly

The optimised system components allow simultaneous FRAP and imaging at single mouse click on any position in the sample FOV. This 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” 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.



VisiFRAP mounted on upright microscope



## VisiFRAP-DC for Ablation

Combining state of the art cutting laser technology with our successful VisiFRAP Scanner, the VisiFRAP-DC Ablation System offers maximum flexibility and ease of use. Ablation is combined with standard FRAP using either directly mounted or fiber-coupled lasers.

## VisiScope 4Elements

VisiFRAP-DC

Ablation Lasers:  
355 or 532nm

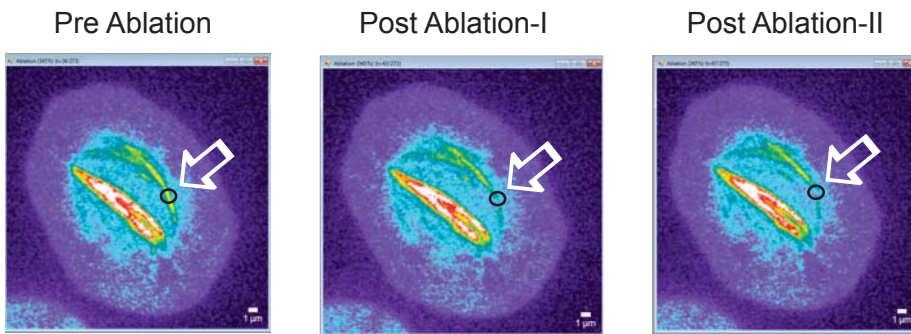


Figure: Ablation of microtubule structures using the VisiView® FRAP/Ablation on the fly. The scale bar shows 1 µm.

### Features:

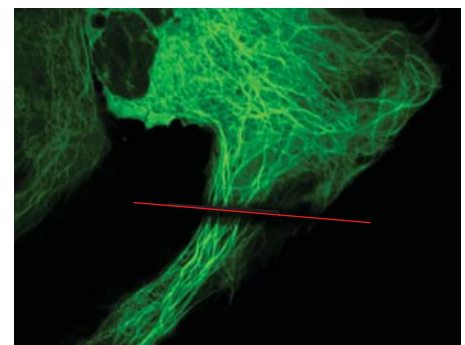
- » Interactively cuts submicron-sized objects
- » Combines Ablation with FRAP
- » Operates at kHz-rates
- » Compatible with TIRF/Confocal/Widefield

### Typical Applications:

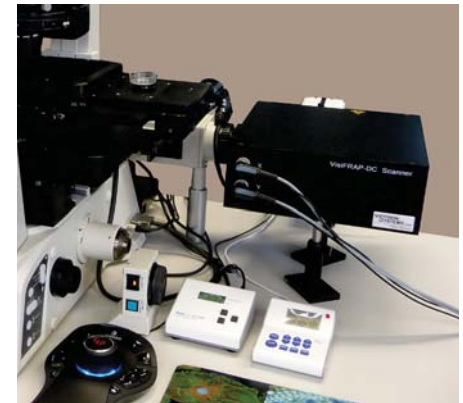
- » Cutting subcellular structures
- » DNA damage, thrombosis
- » Microengraving into glass
- » Nucleocytoplasmic transport
- » Protein diffusion studies

### The following models are available:

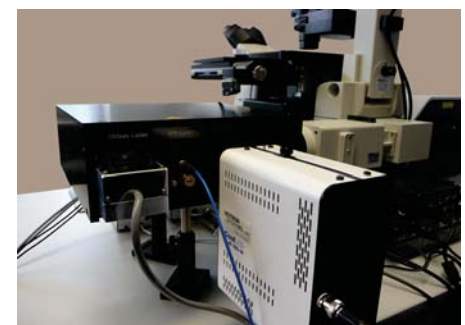
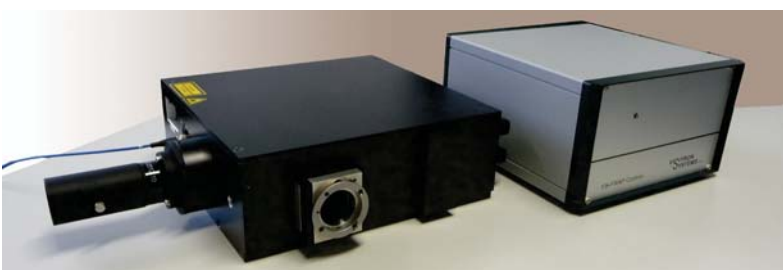
- » Model VisiFRAP-UV355      Model VisiFRAP-UV355-VIS
- » Model VisiFRAP-UV532      Model VisiFRAP-UV532-VIS



Ablation of microtubules inside of U2OS cells



Nikon-Ti microscope with VisiFRAP-DC 355nm



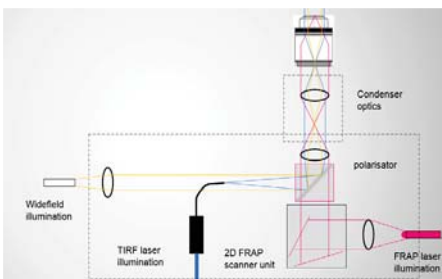
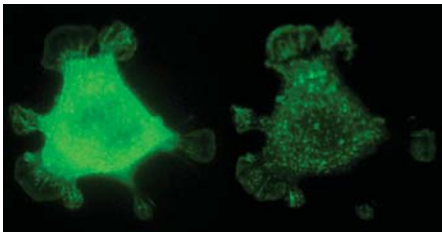
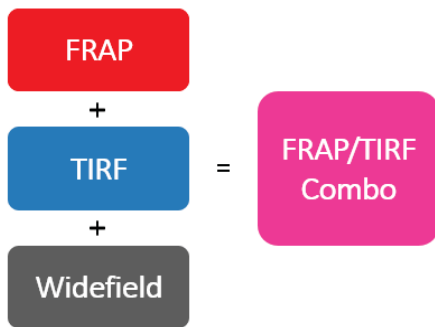
VisiFRAP-DC 355 including VIS laser option and LED illumination

# VisiScope 4Elements

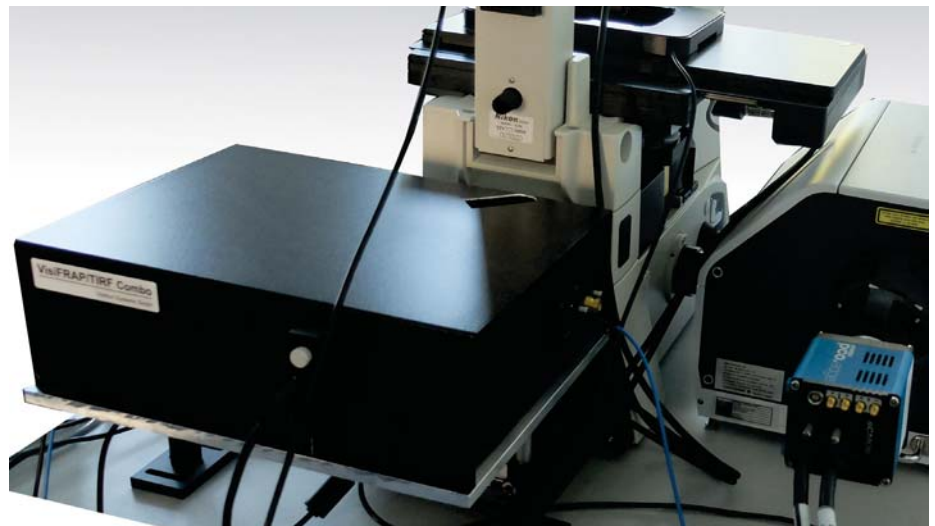
## FRAP/TIRF Combo

### VisiFRAP/TIRF Combo System in Compact Design

Why invest in two separate instruments if it is easier to integrate both functionalities in one? The VisiFRAP/TIRF Combo system is our answer to the increasing demand for combined TIRF/FRAP applications. Its compact and lightweight design avoids the use of dual-deck microscopes or multi-port switchers. Building on the proven VisiTIRF optical system, the VisiFRAP/TIRF Combo System maintains its high light efficiency and image quality while gaining the well-established 2D Galvo FRAP functionality Scanner Systems.



VisiFRAP/TIRF scan head with TIRF, FRAP and Widefield fiber cable



### Compact Design

A main factor driving the development behind this system was the lack of a compact and easy to use FRAP/TIRF combination, which often required using multi-level microscopes or complex beam combination optics. In an effort to avoid the trade-offs that come with these outdated solutions, the VisiFRAP/TIRF Combo system was developed to become the most compact and versatile solution available on the market. In addition, its modular design allows us to support a wide range of microscope platforms, even legacy systems.

### Multicolour TIRF based on High Speed Motor

VisiTIRF's high speed ultrasonic gear motor with 250mm/sec can correct wavelength dependent penetration depth correction in real time. In addition, switching between TIRF or laser widefield illumination can also be performed at the click of a button.

### Widefield - FRAP – TIRF Illumination at once

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 LLG-fiber typically to a LED light source. The special optical design requires no moving optical part.



## Widefield Microscopy

Widefield fluorescence microscopy is an imaging technique where the whole sample is illuminated with light of a specific wavelength, exciting fluorescent molecules within it. Emitted light is visualised through eye pieces or captured by a camera

Although largely replaced by confocal imaging, widefield imaging still plays an important role in optogenetic and fluorometric applications requiring low spatial but maybe higher temporal resolution as well as the wavelength flexibility of widefield illumination systems.

## VisiScope 4Elements

Widefield  
Fluorescence



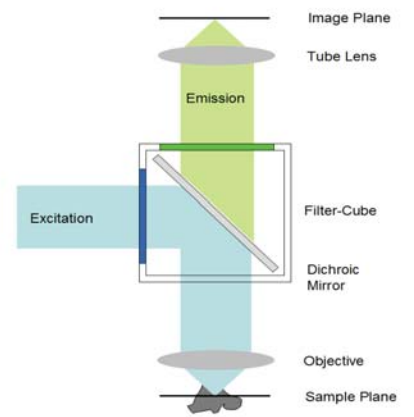
## Light Source

The most common light sources used today are light-emitting diodes LEDs. The wavelengths and intensities of light they produce can be precisely selected and controlled, they are inexpensive, do not produce excess heat, do not require alignment and are very compact. These properties make them a light source to use in comparison to arc-lamps and tungsten-halogen lamps which were commonly used in the past.

Mercury or xenon arc-lamps have very high intensities over wide spectras, however they produce a lot of excess heat, and have a much shorter lifetime than LEDs.

## Filter System

The excitation filters and dichroic mirror are usually located in a filter cube. By only allowing light of specific wavelengths to pass, the filter cube reduces the 'noise' from the sample, ensuring a clear image is produced which only shows the fluorescence of specific fluorophores.



# VisiScope 4Elements

## Incubation

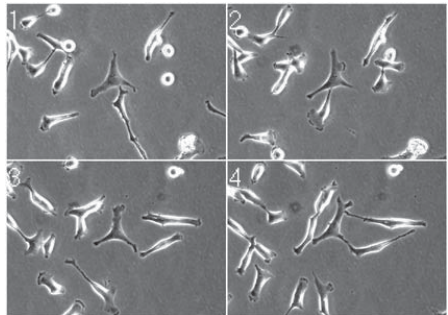
### Cells Need Perfect Climate Conditions !

#### The VisiScope Incubation System

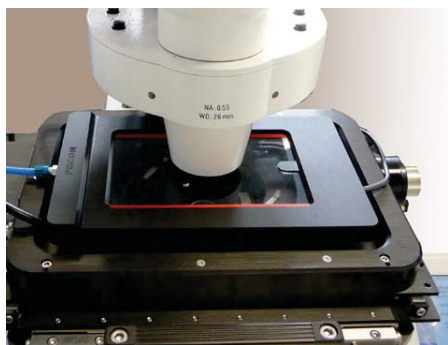
The large incubation chambers is a high performance solution for live cell applications over long time periods which are conducted at a constant temperature for the entire observation. It keeps highly stabilized conditions after a warm-up phase of the internal components e.g. slide holder, objectives. CO<sub>2</sub> and O<sub>2</sub> tightly controlled, too.



Large incubation chamber black, for laser safety



Time lapse of HeLa cells



Small incubation system with heating universal Labtek holder and heating CO<sub>2</sub> cover



#### CO<sub>2</sub> and O<sub>2</sub> modules

CO<sub>2</sub> and O<sub>2</sub> modules can be easily added with suitable CO<sub>2</sub> cover for corresponding sample holder e.g. for multiplates or universal slide holder. A O<sub>2</sub> controller controls the oxygen concentration besides the control of temperature and CO<sub>2</sub>-concentration. The O<sub>2</sub> content is reduced by displacement with nitrogen. Within the system, the O<sub>2</sub>-concentration is monitored by a zirconiumoxide sensor, an analogue PID closed loop control adds nitrogen via a piezo controlled valve into the circulating air stream. This continuous nitrogen flow gives a very homogeneous oxygen distribution with best control tolerances.

#### Objective and Mounting Frame Heater

Especially with the use of oil immersion objectives, the direct contact between the cell cultivation vessel and the colder objective leads to a significant cooling in the area of the observed cells. The Objective Heater is designed for stable heating of microscope objectives in order to improve temperature conditions in the observation area. The heatable mounting frame with circular and slotted cut-outs, can be easy installed at the microscope stage insert with an opening of 160x110 mm. The base plate is directly heated from below. The frame has been specifically developed for CO<sub>2</sub>-gassing together with a CO<sub>2</sub>-Cover.

## VS-LMS Flexible Multiple Laser Engine with highly stable Laser Outputs

The new generation of Visitron Systems VS-LMS Laser Merge Systems is now available in a very compact design with up to 8 laser lines, three FC fiber outputs and an optional motorized alignment function.

The unique design combines the beams of up to eight diode or solid state laser to a single collinear laser beam. This beam can be channeled into three different outputs for simultaneous laser application like Confocal / FRAP or TIRF (RingTIRF).

## VisiScope 4Elements

### VS-LMS Laser Merge System

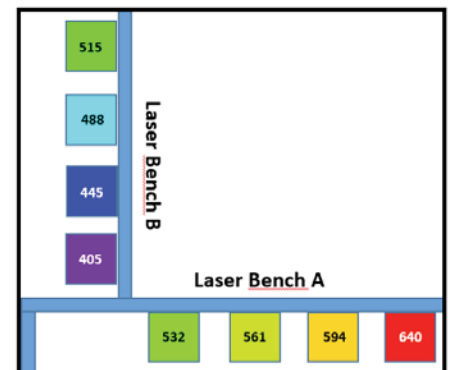


### Features and Benefits:

- » Multi-line laser source including up to 8 lasers
- » Flexible selection of diode laser modules
- » All solid-state lasers for high stability and lifetime
- » FC-coupling design with focus correction
- » Thermally managed system
- » Port Switcher with up to 3 output ports e.g. VisiScope Confocal, VisiFRAP, VisiTIRF including iLAS support

### VS-AOM Acousto Optical Modulator - High Speed Optical Shutter

An acousto-optic-modulator (AOM) is a device which can be used for controlling the power, frequency or spatial direction of a laser beam with an electrical drive signal. It is based on the acousto-optic effect, i.e. the modification of the refractive index by the oscillating mechanical pressure of a sound wave. The AOM is used in at the VS-LMS for high speed switching and intensity control if a solid state laser is used.



Wavelength* (nm)	Maximum Power Rating* (mW)
405	300
445	100
488	200
515	100
532	150
561	200
594	150
640	200



# VisiScope 4Elements

## VS-LMS Laser Merge System

### VS-LMS Option Laser Engine with Motorized Laser Alignment

No more tedious, complex and time consuming laser alignment! The new generation of VisiTron Systems Laser Merge Systems VS-LMS-MOT100 is available with an optional Auto-Calibration (AC) function. After a rough manual laser alignment, in order to reach that the laser light is visible on the fiber output, the VisiView algorithm maximizes the output power and conserves it for a long period of time.



#### Option: External Port Switcher

Port Switcher with up to 3 output ports e.g. VisiScope Confocal, VisiFRAP, VisiTIRF / RingTIRF support

#### Specifications:

Maximum number of lasers	8
Wavelength range (nm)	405-640*
Option: Multiport switch output	up to 3
Blank/TTL Modulation (diode) MHz	5
Blank/TTL Modulation (OPSL) MHz	0.05
Computer control interface	USB
TTL/Analog control interface	D-SUB

## ViRTEx Visitron Realtime Experiment Control Device

The ViRTEx-100/200 provides sophisticated electronics for experiment control, where highly accurate timing is essential. Typically it is used in Confocal, FRAP and TIRF experiments. All of these applications need fast and highly accurate TTL synchronization of scientific interline, frame-transfer or sCMOS cameras with illumination devices like Poly-chromator, LED or laser systems. Furthermore for precise Z-stack 3D image acquisition, highly accurate Z-Focus Piezo control is required and now supported by the ViRTEx-200 device.

## VisiScope 4Elements

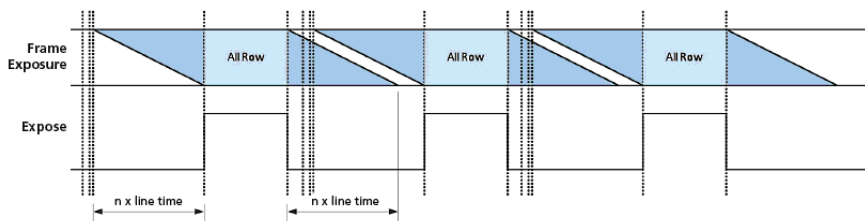
### ViRTEx Synchronization



### Timing sCMOS Cameras

In rolling shutter mode, the rows of an sCMOS camera are continuously exposed and digitized. The pixels of a row are digitized simultaneously, then the next row is digitized. Each row requires about 10µs depending on the digitizer speed. This means that each row is exposed 10µs later than the previous one. With 1000 rows to be digitized, the shift between the first row and the last row adds up to 10ms.

sCMOS sensors are actually digitizing symmetrically to the horizontal center line from top to center and from bottom to center. The following timing diagram thus shows only one half of the chip. As the readout of the two halves takes place simultaneously, the timing diagram on one half describes the timing correctly.

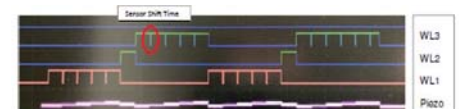


Due to the overlapping exposures, it is not possible to change the illumination or the focus without crosstalk between the exposed frames. So, for fast multiwavelength/ multi-Z sequences, it is necessary to illuminate the sensor only in the time where no overlap with the next/previous frame takes place.

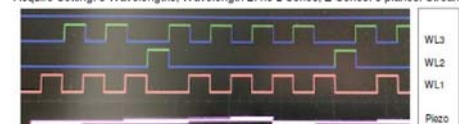
In the figure above, this area is named AllRow. Virtex uses the AllRow output of the sCMOS camera to illuminate the chip with the appropriate timing.

### Features and Benefits:

- » TTL synchronization module for fast experiment control e.g. device streaming
- » camera / illumination device synchronization
- » connection via USB 2.0
- » 16 TTL output lines
- » 4 analog out channels
- » optional up to 8 channels
- » 4 camera inputs connector board (exposure signal for stream mode)
- » available models: ViRTEx -100 / 200 stand-alone devices



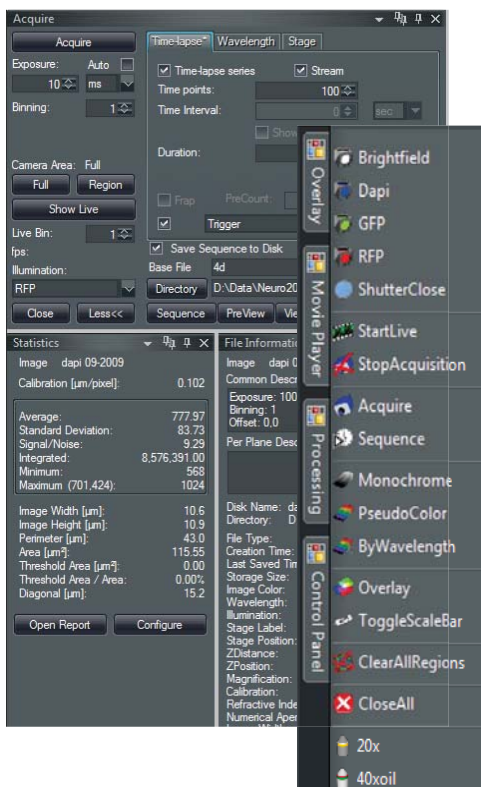
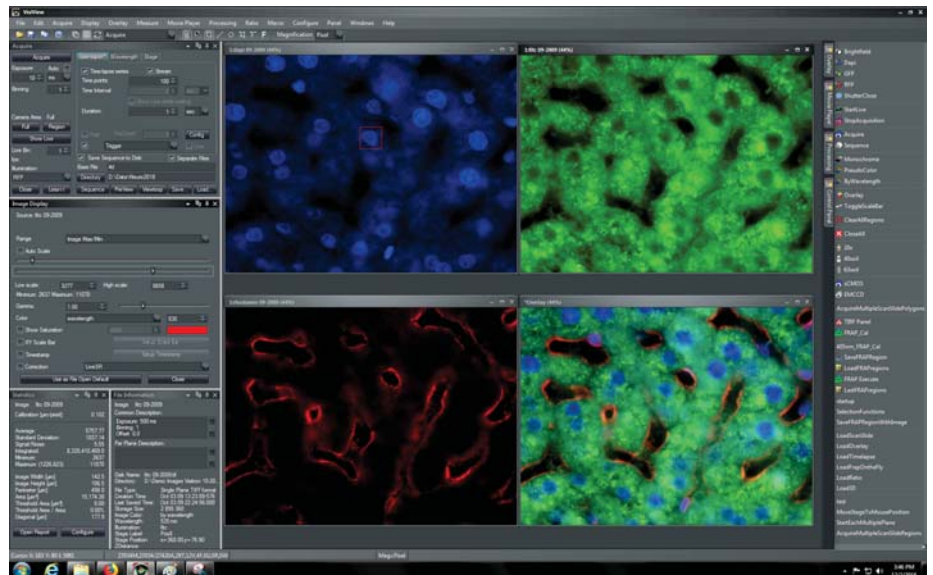
Focus Setting: Do Wavelength for Each Z. Stream ON  
 Acquire Setting: 3 Wavelengths, Wavelength 2: no z Series, Z-Series: 5 planes, Stream C



# VisiScope 4Elements

## VisiView® Imaging Software

VisiView® is a high performance imaging software from Visi-tron Systems GmbH for BioMedical applications. The software is designed as an integrated imaging software which includes comprehensive microscope control, unexpected control of peripheral device, image acquisition, analysis and documentation. Its multitasking ability supports realtime image handling and up to 6D multidimensional acquisition. The VisiView® software represents the philosophy of simple operation and seamless integration of applied standards.



### Time-Lapse Acquisition

Acquire changes in living specimens over time at defined intervals and display the image sequence as a movie to show cellular dynamics. The image sequence will be saved in single TIFF, multiframe stack or .nd format.

### Single or Multichannel Acquisition

The MDA-Multi Dimensional Acquisition gives you a comprehensive view of your multi dimensional experiment. This means a free combination of z-stack (focus), different wavelengths (channel), time points and different xy stage positions in one sequence acquisition (6D-imaging).

### Control of Automated Microscopes

The scope control allows you to control all motorized microscopes from any vendor. We have easy access to any illumination component like filter cube changer, shutter or condenser control. The objectives can be easily selected and calibrated. The focus control allows both the automatic generation of Z-stack images and the software autofocus readjustment to keep your cells in best focus.

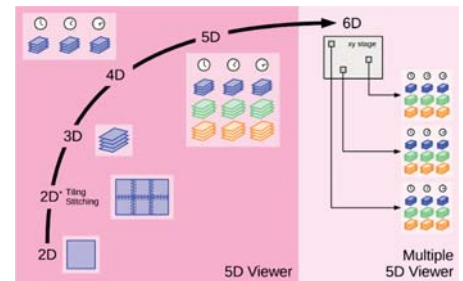
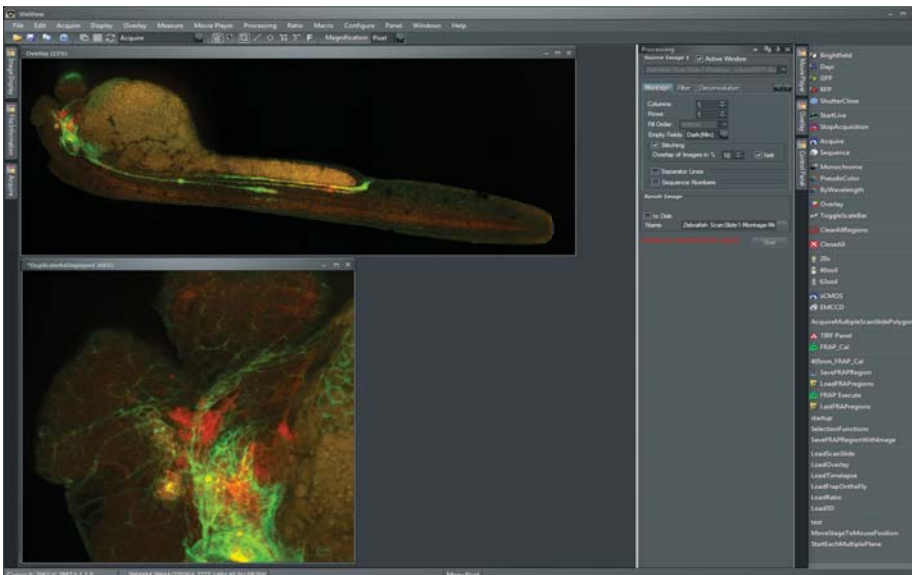


## Scan Slide with Time-Lapse, Multicolor and Z-Stacks

The scanning routine of the Scan Slide Module can be easily used as part of more complex experiments to offer the best flexibility. Watch your specimens grow in time-lapse experiments. Obtain stitched images from multiple fluorescence channels, e.g. showing proteins of interest and tissue-specific markers. And get comprehensive 3-dimensional datasets by combining the XY scan with Z-stacks.

# VisiScope 4Elements

VisiView®  
Imaging Software

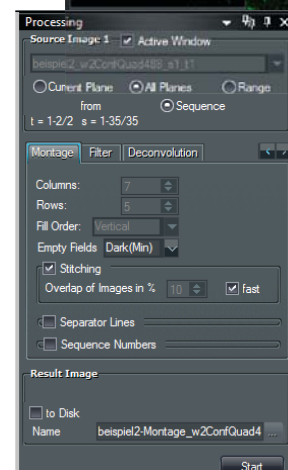
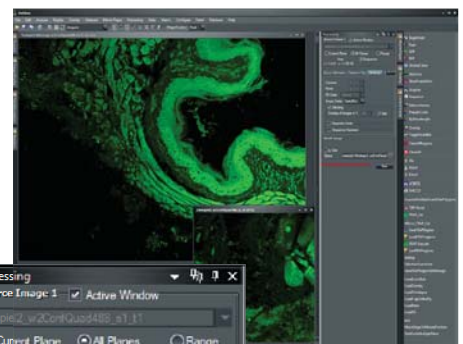


## Scan Slide - Scanning of free surfaces

Some specimens with an irregular shape, e.g. tilted, long and thin, would require very large (rectangular) scanning areas and the resulting stitched image would contain large empty regions. Therefore, VisiView offers a special version of the Scan Slide Module, which allows to scan free-form surfaces and is based on the combination of low and high mag or NA objectives. In a first step, a low-power objective is used to produce a tile-scan overview of the sample. The user can now draw a free-hand region to exactly trace the outlines of the area of interest. In a third step, this region is scanned at high resolution with a high-power objective. Besides improved acquisition speed, this procedure can dramatically reduce the size of stitched images. The XY displacement and the exact magnification factors of the two objectives in use are taken into account during this process.

## Quick overview with Scan Slide technique

A new function of the Scan Slide Module is based on the combination of low- and high-power objectives. First of all, a tile-scan is performed with a low-power objective (5x or 10x) in order to produce an overview of the whole sample. Now, the user can select a ROI for higher res. scan.



## Exceptional Service and Support

### Customized Developments

Do you need customized software features to improve the functionality of your experiment or to implement special equipment in your microscopy setup?

The VisiView® software would be a perfect solution. Our strength are customer oriented software solutions.

### Training Courses

Visitron Systems GmbH has a highly qualified support and application team at its disposal for installations and single or group training, directly at the customer place or at the office in Puchheim Germany. Whether you are new to VisiView® or you are an imaging professional, our courses help you in use of the latest imaging software techniques.

### Technical Support

When you are registered with your copy of VisiView® software, we are offering a 12 month maintenance support by our technical support engineers via phone, email or on-line teamviewer software.

### Online Tutorials and Web-Seminars

View video tutorials of introduction or features in VisiView® software or access to on-line web-seminar by teamviewer software.