

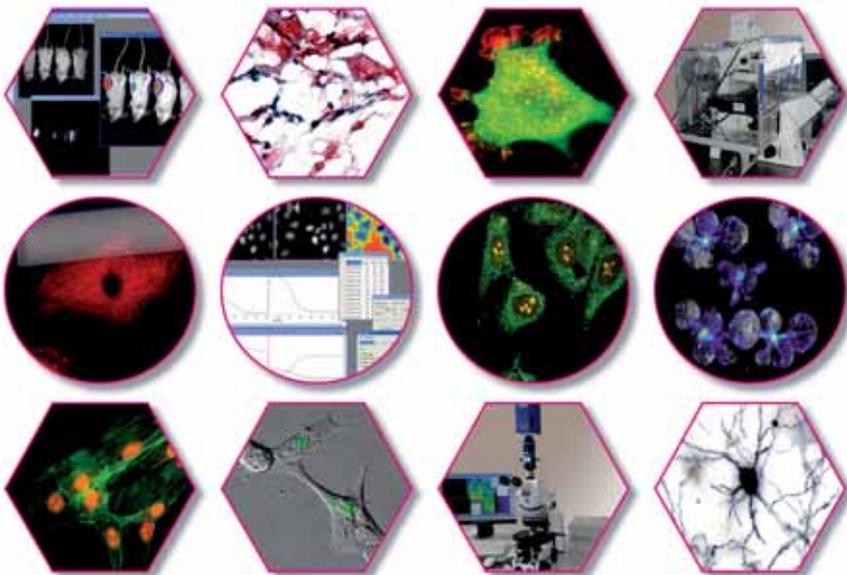


VisiView[®] Version 3

Imaging Software for
Life Science Research

VisiView[®] is a high performance imaging software from Visitron Systems GmbH for Bioluminescence applications. It is specially designed to meet the needs for high-speed image acquisition and processing with ease of use. Our software controls complex automated microscopes and microscope equipment in combination with multidimensional acquisition and analysis. Its multitasking ability supports simultaneous image acquisition and analysis. The VisiView[®] software represents the philosophy of simple operation and seamless integration of applied standards.

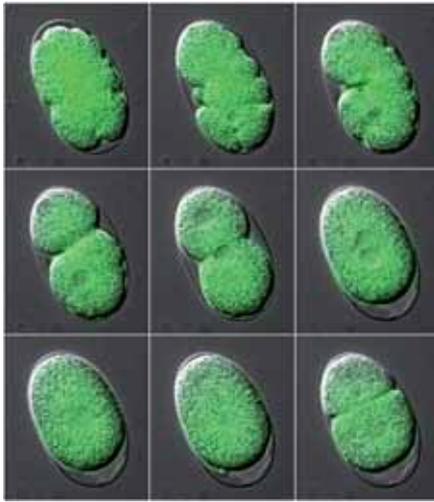
Flexible and
microscope platform
independent software



VICITRON
SYSTEMS GmbH



.....more than 20 years of excellence.



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, multifile stack or .nd format.

Device Stream Acquisition

Give your research a competitive benefit by speeding up your acquisition!

Our advantage is to control illumination devices e.g. high speed illumination systems, AOTF or z-piezo focus systems between the frame transfer or interline shift time of the CCD camera. The image sequences can be continuously acquired without time delay.

Acquisition of images using automated microscopes and peripherals

Image analysis begins with accurate acquisition. The VisiView® software supports a wide range of digital color or black/white scientific grade cameras, automated microscopes and other microscope peripheral devices. Display and scaling of high-dynamic images up to 16bit is possible.



Single or Multichannel Acquisition

Don't miss a detail!

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

No limits! Design your experiment for your needs.

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.

Automate with Macro and Toolbar

Reproducible and easy to use!

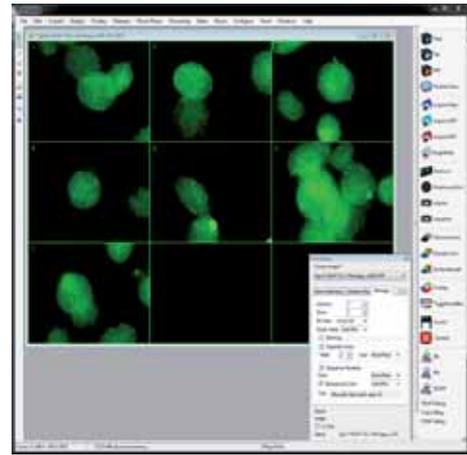
The VisiView® software offers convenient customized toolbars and easy control of system settings and Python macro functions. It is perfect for frequently performed operations. The macro menu covers all functions for automation, commands and sequences.

Control of motorized XY-Stage

In order to acquire at different stage positions you will need a motorized scanning table as well as the VisiView stage control option. You can define multiple stage position and use them for a multi dimensional acquisition experiment in combination with time-lapse, multi-color and Z-stack.

Scanning of Multiwell Plates

The scanning technique can also be used for scanning multiwell culture plates with different sizes like 6-, 12-, 24-, 96 well formats. In addition, high magnification sub-scanning within a single well is supported. To review images, the standard display mode is used with easy hot-key selection of multi-stage-positions, zoom and movie function.



Scan certain positions of your specimen or multiwell culture plates in just one process. Use highly precise motorized XY-stages synchronized with the acquisition cycle at maximum speed. Observe multicolour fluorescence with up to 4 cameras at the same time to get maximum time resolution. VisiView grows with your demands.

Acquisition of images developed for analyzing large areas

Simultaneous Image Acquisition: Up to four Cameras in Multi-Camera Mode

Four Cameras – in live image displays, who can do this? Beside the control of multiple cameras from different vendors or models within one PC, VisiView supports up to four cameras from the same model in simultaneous mode. This allows the observation of e.g. four different fluorochromes at the same time. As a result the negative effects related to sequential image acquisition, like time delay between colors, are avoided.

This function is perfectly suited for performing highly reliable ion measurements with emission ratio dyes (e.g. indo-1, cameleon), FRET or performing colocalisation studies.

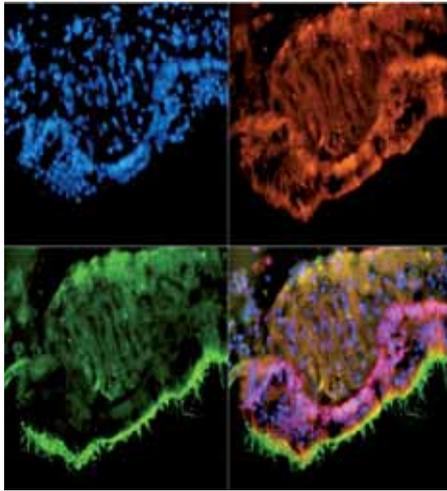
SplitView Analysis

Allows the on-line division of images acquired with an optical image splitter, which is mounted in front of the CCD camera. This replaces time consuming post-processing and enables on-line analysis of emission ratio experiments.

Create AVI Movies

Easily convert sequences files e.g. stack image format into AVI video format for easy presentation.





Merge Color Channels

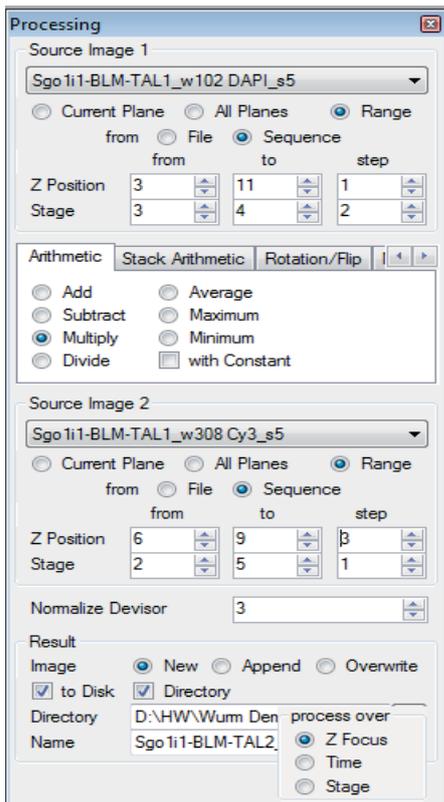
Combine several color images to get the complete picture. The overlay module allows to merge multiple fluorescent, bright-field, phase contrast or DIC images into a color composite image. Up to 7 channels are available. If a channel is optically shifted, the xy pixel alignment compensates the shift.

Colocalization

Colocalization refers to the observation of the spatial overlap between two different fluorescent labels, each fluorescence image having a separate emission wavelength. To see the differences located in the same area or very near to one another, VisiView® calculates the area, gray value etc. of the image and reports it into Microsoft® Excel or text file.

Measure, process and analyze your images

Get more information from your image with the measurement, processing and analysis tools of our VisiView® software. Calibrate the size and measure the brightness over time for individual regions. Align and calculate sequences of images to improve the result. Report the image values into a customizable spreadsheet format for further analysis.



Pseudo-Color or Wavelength Calibration

Each acquired image can be automatically assigned to a wavelength via the illumination control dialog. For the selected fluorescence filter, an emission wavelength e.g. for EGFP - 525nm will be chosen. The image will be automatically displayed in green and the overlay option will recognize the correct color.

Statistic

The statistics menu indicates image or region information within the active window. Features comprise calibration size $\mu\text{m}/\text{pixel}$, average, standard deviation, signal/noise, integral, min/max, perimeter, area μm^2 and diagonal μm .

Measurement

The measurement option offers comfortable evaluation of image data by statistical processing, line scan graph, intensity over time graph and histogram display.

Processing and Analysis

The processing and analysis option offers comfortable processing of single or complex MDA - Multi-Dimensional image data, analysis and archiving of image sequences. All analysis values can be reported into a text file or directly into Microsoft® Excel.

Line-Scan Analysis

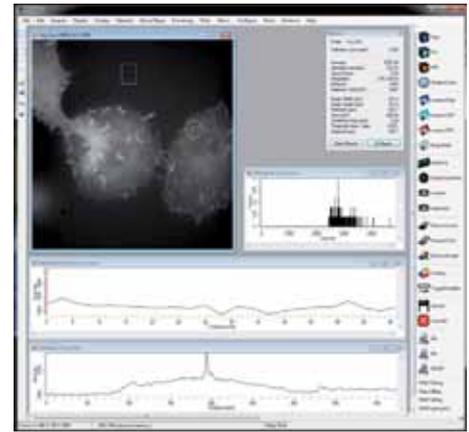
The Line Scan feature gives you a one-dimensional intensity distribution for the line region that is shown within your image. The y-axis shows the intensity values and the x-axis shows the position values. The x-axis values represent calibrated distance.

Histogram Analysis

The Histogram feature gives you graphical distribution of the frequency of the intensity values. The total area of the histogram is equal to the image brightness.

Montage - Tiling Display

For display of acquired sequences e.g. time-lapse or z-axis stacks, usually a montage display is used. This option is included in the processing menu. Free selection of columns, rows, fill order, separator lines etc. can be chosen for best overview.



Quantitative image analysis usually starts with image acquisition and ends with the presentation of measured results. VisiView® offers a comprehensive set of tools including image scaling, rotation, calibration, background subtraction, shading correction, color changing and adding a scale bar.

Measure, calibrate,
stitch, rotate and
threshold your images

Calibrate Distance within Images

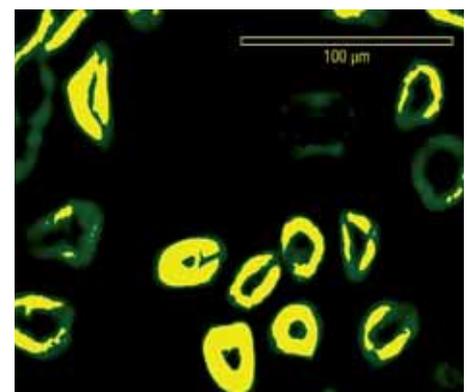
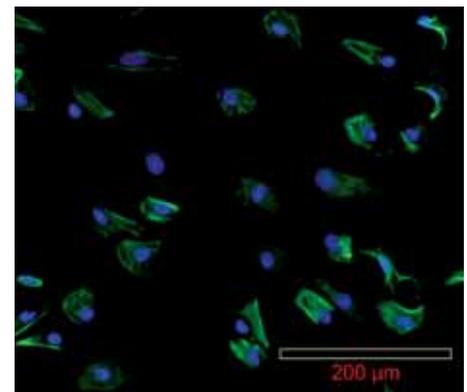
With the calibrate dialog you can calibrate existing images or the acquisition system, which means that future acquired images will be calibrated automatically. The objective calibration is done via the magnification setup. If an automated microscope is used, the system will change automatically depending on the objective lens in use.

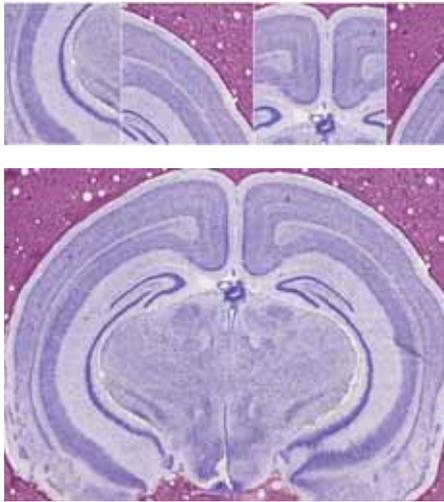
Dynamic Scale Bars

Use the scale bar during a running experiment. Even if you zoom or bin the image, the scale bar displays the correct calibrated values. The scale bar is variable in size, color, description and position and can be easily stamped into the final documented image.

Region Measurements

The region measurements function is an easy tool for manual region measurements by combining multiple parameters. Regions are available as line, circle, square and polygon. To document your work, regions can be stored and easily transferred to other images or stacks.





Scan Slide Module

Scanning your specimen with VisiView® is a very easy three-step procedure. The first step of the acquisition process (which only needs to be performed once) is the auto-calibration of the setup. The second step is the definition of the scanning area and the third step is the scan itself.

Already during the scan the result is directly displayed in an overview image.

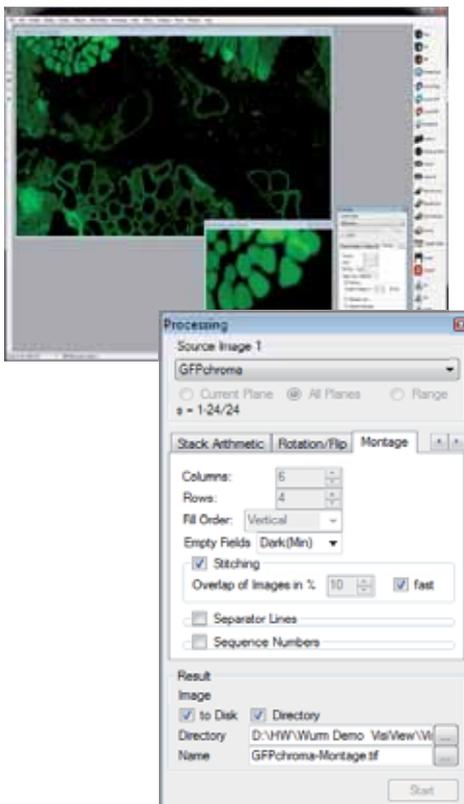
The scan area can be defined by marking the upper left and the lower right corner. VisiView®'s Live View Mode helps you to find and set the positions of interest in your sample.

Upon starting the scan, single images are acquired and tiled to a high-resolution image of the entire specimen.

Automatic scanning of the sample improve throughput, extend overview, allows repeating analysis

The Scan Slide Module generates a comprehensive view of specimens, which exceed the conventional field of view. This is achieved by automatic scanning of a user-defined sample area and subsequent image stitching. Precise stitching algorithms assure maximum accuracy of these high resolution images.

Scan the whole area of your specimen or multi-well culture plate in just one process. Use highly precise motorized XY-stages synchronized with the acquisition cycle for maximum speed.



Auto-Calibration

In order to obtain the best results, the system needs to be perfectly calibrated. This procedure is usually performed once during the installation of the microscopy setup and does not need to be repeated unless integral parts are exchanged.

In VisiView® the calibration process is quiet, simple and fast. One needs to mark an object in the middle of a Live image and start the calibration. VisiView® then automatically measures the camera angle and the system magnification in order to determine the precise image xy calibration.

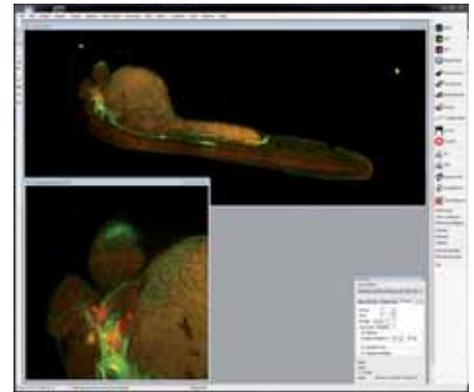
Stitching of Multiple Images

Analysis of large surfaces requires to scan the area of the specimen. The acquired multiple images can then be automatically stitched to an ultra high resolution image during acquisition or afterwards in the post-processing. Two different algorithms are available: A fast and a slow high-precision one. Typically, a 10% overlap of the scanned images is used to achieve best results.

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. In a first step a tile-scan is performed with a low-power objective (5x or 10x) in order to produce an overview of the whole sample. The user is now requested to select an area of interest on this image, which is subsequently scanned at higher resolution with a high-power objective.

Importantly, the XY displacement and the exact magnification factors of the two objectives in use are automatically measured and taken into account during this process.



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.

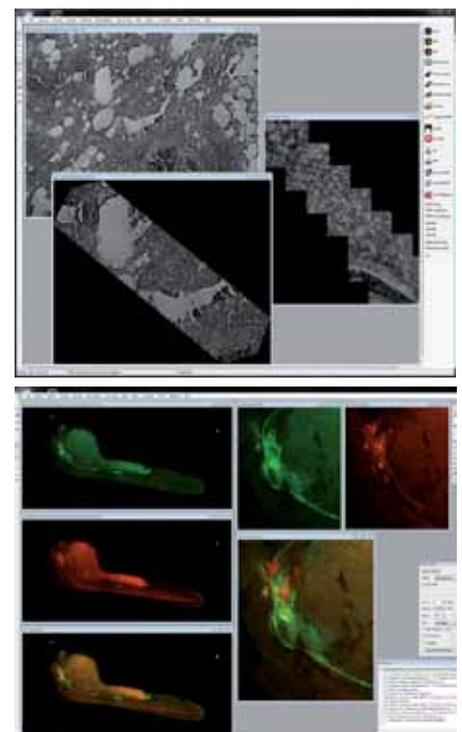
Scan Slide in all dimensions
XY combined with time-lapse, multicolor and Z-Stack

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-power 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.





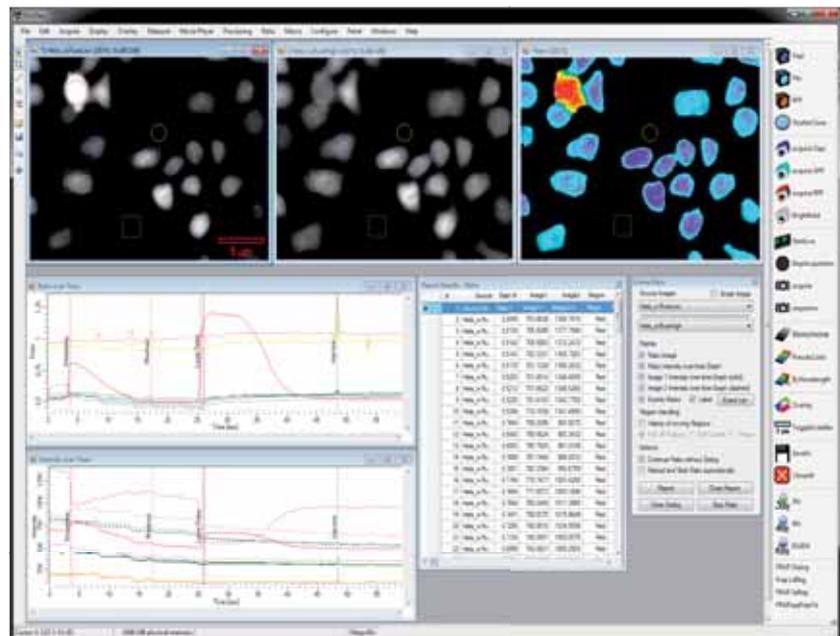
Online Ratio Module

All in one

The requirements of biomedical research in cell biology are continuously growing and getting more complex. This process is challenging the development of imaging software in microscopy. The VisiView® software designed by microscopy specialists offers seamless integration of hardware. The flexibility and clear menu structure helps for easy operation. A modular architecture allows the extension with new or additional application modules like Ratio, FRAP, FRET, structured light Confocal etc.. A simple solution with powerful functions to meet your demands for the application.

On-Line Ratio
Investigate changes in
fluorescence intensities
in your cells

The Ratio Option of the VisiView® is specially designed for on-line ratio applications like dual wavelength intra-cellular measurements such as Fura-2, BCECF, FRET or single wavelength dyes, calcium green, fluo-3, etc.. The ratio option provides a simultaneous display of the original wavelength, e.g. 340nm, 380nm, ratio and the corresponding intensity graphs. Either intensities over single or multiple regions of interest are selectable. The flexible architecture of VisiView® in combination with application modules allows researchers to pick the software solution that best suits their needs.

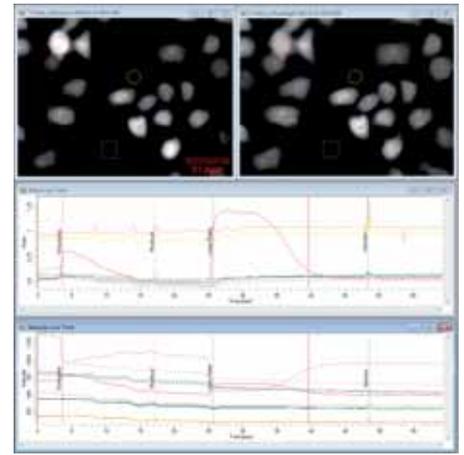


Making Measurements, History Function

Regions of interest or arbitrary shapes can be placed on every raw source or ratio image to display the intensity value or ion concentration. The measurements can be done during on-line acquisition. Regions can be repositioned using the history function if the cell has moved or the stage was touched. Individual regions can be frozen or changed by editing the current region or even all regions at once.

Trigger Protocol

For flexible experiment control, complex user defined trigger sequences can be important. The trigger protocol option offers variable on/off switching of external devices during all kinds of experiment sequences.



FRET Ratio in living Cells

Förster resonance energy transfer (FRET) is a radiationless mechanism of energy transfer between two spatially close fluorophores called donor and acceptor. In FRET condition, photoexcitation of the FRET-donor molecule leads to a decreased donor fluorescence and an increased fluorescence of the FRET acceptor.

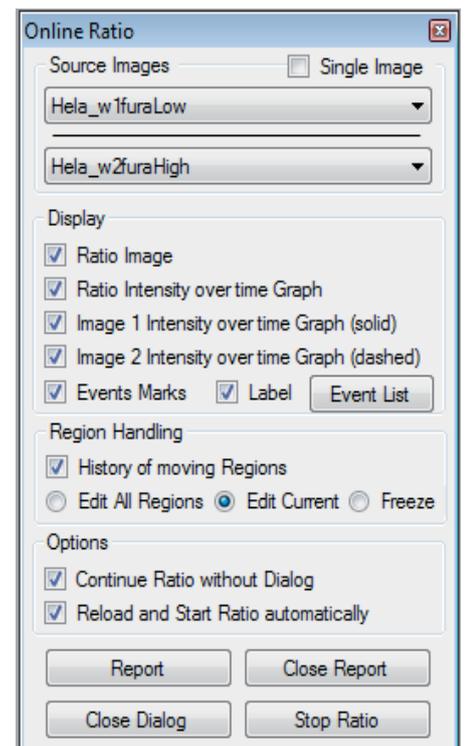
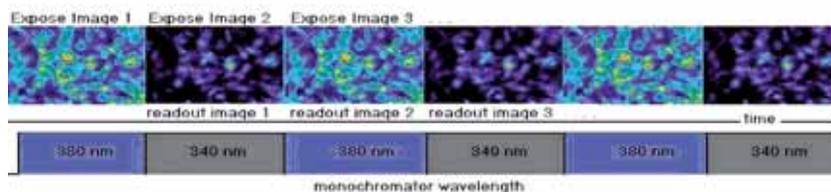
Measure calcium concentration and pH value and visualize intra- and intercellular processes

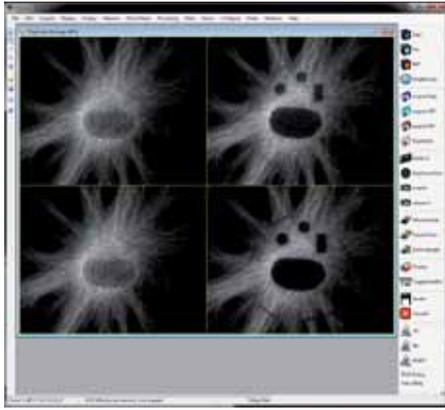
Online Time Interval Sequences

A novel feature of the ratio option is the capability to change the time interval without interrupting the measurement or stopping the experiment. For instance, you can have the system acquire images with a long interval. After e.g. injection you can switch to short intervals or streaming acquisition mode.

Further Functionality

- » High Speed Wavelength Changers
- » CCD Camera, Binning and Super Pixel
- » SplitView Ratio
- » Threshold Measurement
- » Data correction, e.g. background, shading
- » Event Marks
- » Logging of Data to Microsoft® Excel or Text File





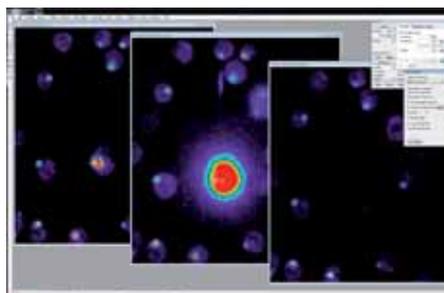
FRAP - Ablation Module

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.

Analyze FRAP
Fluorescence Recovery
by using 1D or 2D
VisiFRAP scanner

FRAP - Fluorescence recovery after photo bleaching is commonly used to measure the lateral mobility of molecules in a lipid bilayer. For this, the molecule of interest is linked to a fluorescent dye, which is at the first step bleached by high intensity light in a specific area of the sample. The lateral diffusion of intact fluorophores into the bleached area is called fluorescence recovery and can be measured by immediate and sequential image capture after the bleaching step.



FRAP Acquisition Dialog

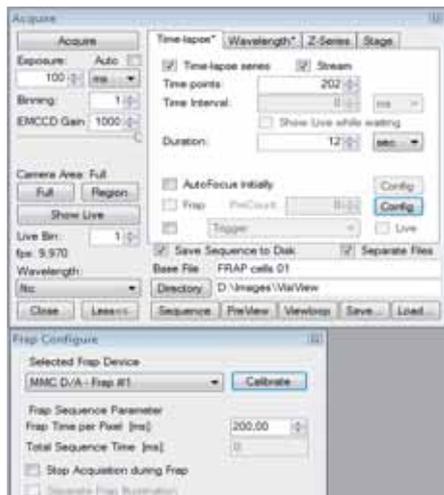
The FRAP configuration dialog is directly accessible from the time lapse tab of the clear-cut VisiView acquire dialog. It gives you control over FRAP parameters as well as access to the simple auto-calibration procedure. Further, you can easily test the FRAP parameters using a live preview before you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



Screening Module

Acquisition Dialog and Setup

In the stage tab of the acquisition menu, the screening conditions can be defined. Either the standard xy stage position control or the control of multiwell plates can be selected. Because of the magnification calibration, the VisiView® software knows automatically the correct scanning area of the selected multiwell format. With a single mouse click all wells or individual wells can be selected. Only selected wells are scanned and acquired. Before each acquisition, the autofocus can be used to find the best optical section. The whole settings can be stored and reloaded for later usage.



Recent advances in imaging technology and live-cell fluorescent probes like GFP / RFP, allowing cell biologists to quantitatively examine cell structure and cell functions at higher spatial and temporal resolution than years ago.

The Screening option of the VisiVIEW® imaging software helps scientists to acquire automatically a high throughput of cells growing in dishes or multiwell plates. The sample area can be extended by scanning multiple sites per well. VisiView's unparalleled autofocus always helps to find the best focus.

Using automated fluorescence microscopy with XYZ-stage and hardware autofocus for research in cell based screening

Configure Well Dialog

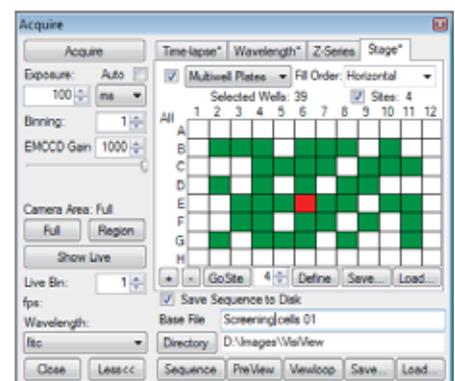
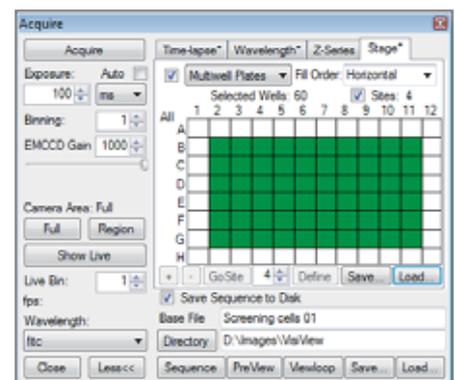
There are different sizes of multiwell plates available. The VisiView® screening option supports 6, 12, 24, 48, 96, 384, 1536 and custom formats. For the selected plate type a plate layout is created with calibration values for offset, distance between columns and rows, well size and well shape. In addition, multiple sites for each single well can be selected, e.g. 16x16, if higher objective magnification is used for high resolution imaging mode. The specimen settle time optionally prevents from taking blurred images of samples wobbling due to inertia.

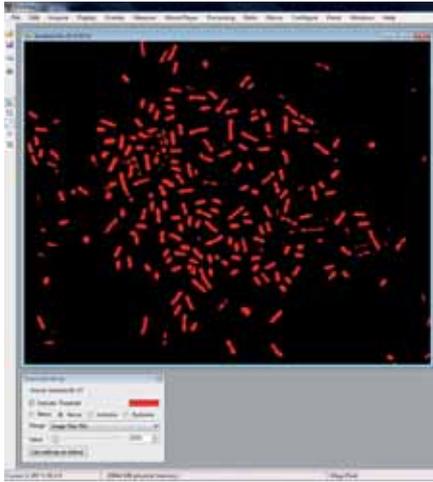
Autofocus

The autofocus module calculates the optimal focal section for each multi-well sample in reflected-light, transmitted-light and fluorescence. For images that are recorded as time lapse or at different well positions, the cells are automatically refocused if a focus shift appears.

Calibration

The screening system is easy to use because of the predefined stage and magnification calibration. The user only has to select the objective and the current xy-stage position.





Object Analysis Module

Segmentation of Image Information

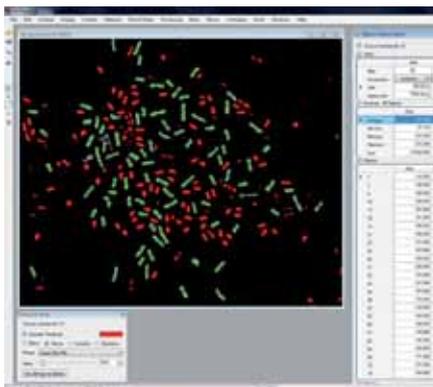
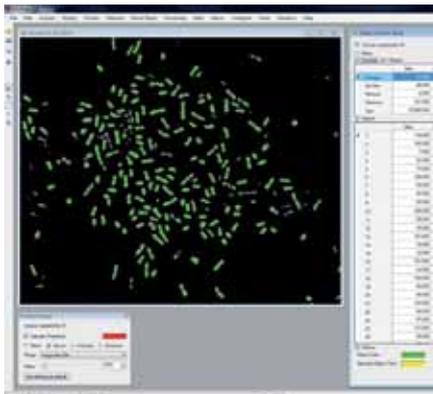
The segmentation of cell images is a necessary step of many automated biomedical image processing procedures. The function offers intelligent threshold operations for images to identify your objects or image information. The threshold function offers various methods like below-, above-, inclusive-, exclusive to segment the image.

Image Enhancement

Because of shading or background effects, correction and enhancement of images is required before segmentation can be performed. The processing tool of VisiView® helps to prepare your image for optimal analysis.

Object analysis
count, classify and
measure multiple cell
parameters

Automated object identification and counting allows the user to choose different parameters for morphometric measurement or classification of cells. Measure all objects or define filters to restrict your measurements to objects which meet specific customized criteria.



Object Analysis - Cell Counting

Measure or count cells automatically with a wide range of object classifiers. The object analysis tool makes it possible to determine morphometric parameters from the specimen and to report them automatically into Microsoft® Excel or text format.

The well arranged object analysis dialog displays selectively filter functions, sum of object statistic or single object values. Again, VisiView's unmatched on-line functionality offers simple on-line adjustment of threshold to improve the object segmentation and analysis result.

Certain options can be selected to improve the display and analysis like object color, display object border, fill holes, create object mask etc.. Artifacts are removed by the individual filter functions and displayed in a different color. This function makes the VisiView® imaging software a powerful analysis tool.

On-Line Object Counting

The object counting of VisiView allows both the analysis of stored images and the on-line counting at live acquisition. Depending on the selected filter criteria all images and results can be stored or rejected.

Python Programming

VisiView 3 major version upgrade new features

After an extensive development period we are proud to introduce our new VisiView 3 major version upgrade. It provides improved usability, device handling and macro programming right from the start of installation.

Installation of VisiView on new computers is now a one step process. All VisiView associated third party software components are installed automatically (except imaging device drivers). By the newly designed VisiView Updater only a single file is needed for automatic patch update. Next to more general changes on device controlling VisiView 3.0 now allows to assign specific hardware autofocus offset values for individual stage positions.



VisiView 3.0 provides a real step forward to improved usability, device handling and macro programming. The VisiView setup process becomes a one-step procedure!

Python programming supports the freedom of experiment design and image post-processing!

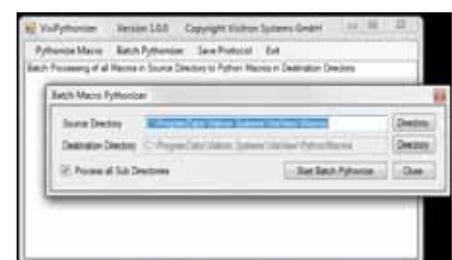
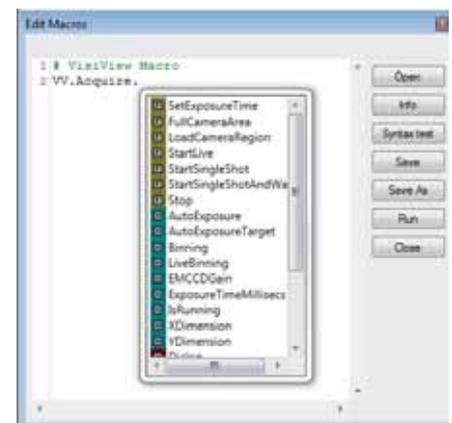
New features in the VisiView 3 major version upgrade.

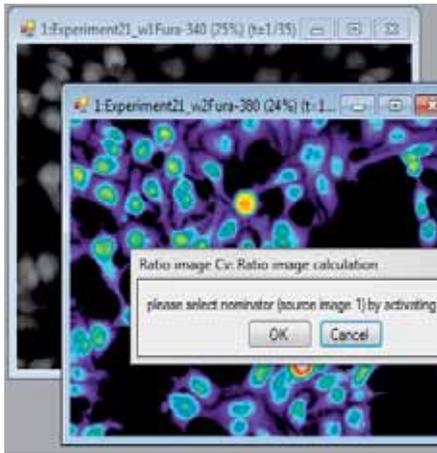
Unprecedented Flexibility

Unprecedented flexibility of the software is achieved by integration of the widely known Python programming language. Due to its free accessibility, it is beyond the limit of proprietary macro languages. Upon update to VisiView 3 all old-style VisiView macros are translated into python macros by the VisiView Pythonizer.

Although Python is a very powerful programming language the syntax is easy and very clear. In conjunction with the intelligent macro editor with auto-complete function the user can start writing macros from scratch without any programming experience.

Python macros are the basis for automation of booth simple but frequently used and of more complex imaging tasks. Macros can be assigned to toolbar buttons in order to have direct access from the user interface.





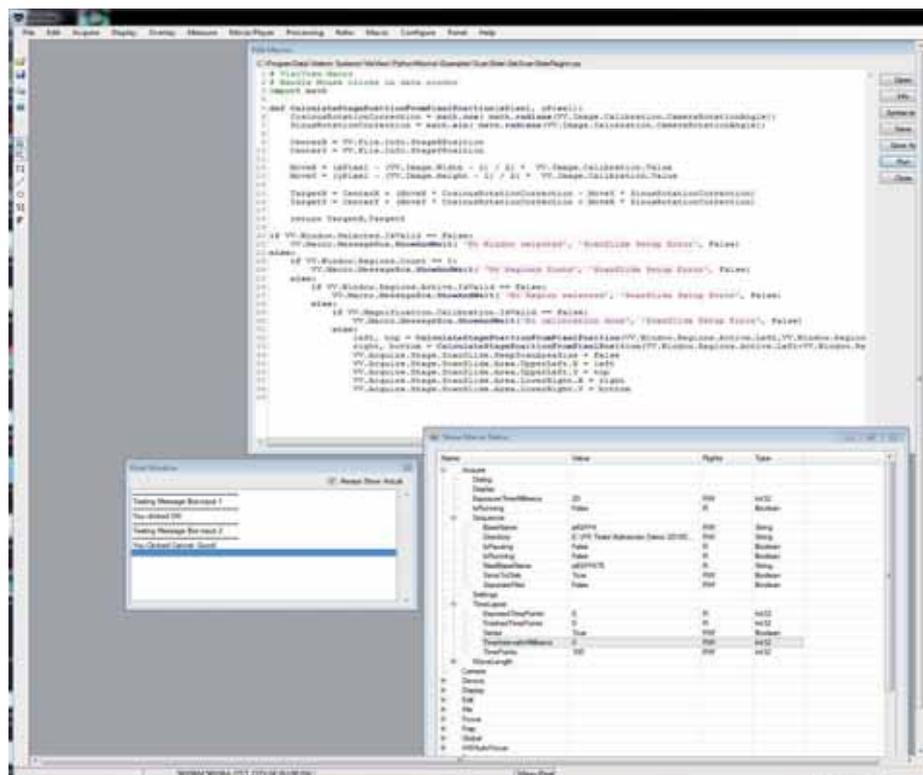
Easy to use customized scripting

Use Python macros to make your workflow interactive. Users can be guided through otherwise complex imaging tasks by message boxes, print-outs, and input boxes. This saves time, minimizes the risk of mistakes, and supports higher reproducibility. More experienced users will be able to expand the VisiView Python functions by implementing Python standard libraries and freely available third party libraries like the "OpenCV" one. Thus dynamic data exchange between VV and third party software as well as advanced image processing becomes feasible. Macro examples on how to include additional libraries are given and individual training on advanced python programming can be offered to interested customers.

VisiView® Python Features

Features

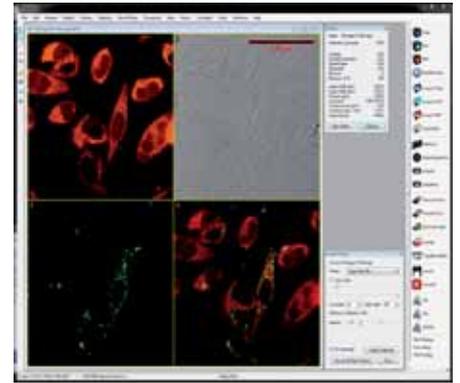
- » Clean and short syntax
- » Well documented scripting language
- » Allows user input, message boxes and print outs
- » Allows more complex operations and interleaving
- » VisiPythonizer translates existing VisiView macros to Python
- » Comfortable auto-complete function for VisiView-specific commands



VisiView® Overview

VisiView® Optional Plug-Ins:

- Illumination option
- Z Focus option including SW Autofocus
- Hardware Autofocus option
- XY or XYZ Stage Control option
- Dual Camera or SplitView option
- Ratio option
- Measurement, Magnification, Processing option
- Object Analysis, Colocalization Analysis option
- Automatic Scan Slide option - XYZ-time and multi color
- Scanning of Multiwell Plates
- Remote control from external programs



Basic VisiView® Functions:

- Realtime image acquisition under MS-Windows
- True color and up to 16 bit monochrome display
- Time-lapse function and fast streaming
- Handling of large movie sequences
- On-line overlay of multichannel fluorescence
- Merge up to 7 different images
- TTL shutter control
- One camera driver support
- Customized toolbar and macro programming
- Movie player and digital recorder
- Context sensitive help function

Modular VisiView® software with various plug-ins and device drivers for advanced microscopy imaging

VisiView® Hardware Support:

- Camera driver: Photometrics, QImaging, Hamamatsu, PCO, Diagnostic Instruments, Andor, VisiCAM
- Automated microscope: Zeiss, Olympus, Leica, Nikon
- Shutter / filterwheel: Vincent, Ludl, Prior, Sutter and ASI
- XYZ stage: ASI, Märzhäuser, Ludl, Prior, Leica and Nikon
- Z-stage: Piezo z-focus control
- Microscope hardware autofocus: Zeiss, Leica, Nikon, Olympus and ASI
- Illumination system: X-Cite, HXP and PhotoFluor
- LED illumination: Lumen, CoolLED, Zeiss Colibri, Lumencor
- High speed illumination: DG4, Lambda-XL, Polychromators
- Optical image splitter
- Multi camera control: e.g. up to 4 CoolSnap cameras
- Laser illumination: diode and solid state lasers
- AOM and AOTF: intensity and channel control
- Confocal: CSU W1, CSU 22/X1, CARV-II, X-Light, Infinity
- FRAP: 1D and 2D VisiFRAP; iLAS
- Motorized VisiTIRF condenser and iLAS
- SPIM Light Sheet microscopy - on request





Supported File Formats

Read and write support of 8/16bit TIFF, single and multiple TIFF, BMP, SPE, STK-MetaMorph, nd-MetaMorph.

AVI Movies

Easily convert sequence files into AVI video format.

System Requirements

Windows® 7 Pro
Ultimate 32bit and 64bit support

Computer,- RAM,- Harddisc:
fast Intel CPU min 2,1 GHz, 4.0 GB RAM, 500 GB HDD disk

Specifications are subject to change. Please contact Visitron Systems GmbH or your local reseller for the latest features.

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. With a dynamic team of software engineers we are listening to our customers and with our experience we try to help to suit your specific needs.

Training

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

Technical Support

Once 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.

On-line Tutorials and Web Seminars

View video tutorials to see an introduction and learn about features of VisiView® software or join our on-line web seminar by Teamviewer software.

Find free information and application notes at www.visitron.de

