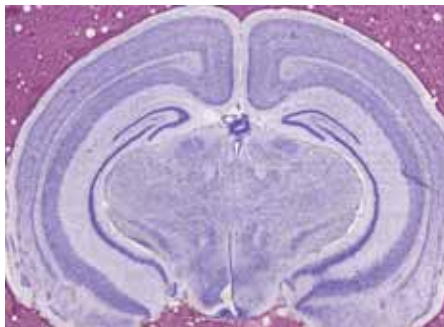
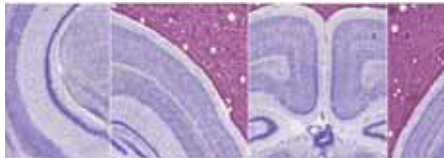


VisiView®

Imaging Software

Acquisition of images: Automated microscope control for analyzing of large surface

Scan the whole area of your specimens 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.



Scan Slide Module

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 maximal accuracy of these high resolution images.

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-position, zoom and movie function.

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 of 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) or performing co-localisation studies.

SplitView Analysis

The 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 sequence files e.g. stck image format to AVI video format for easy presentation.



Simultaneous camera control e.g. up to four Coolsnap cameras.

Object Analysis: Count, classify and measurement of 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.

VisiView®

Imaging
Software

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. Direct access to the pixel value allows particular selection of the boundaries. Individual colors can be selected to display clearly the marked area.

Image Enhancement

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

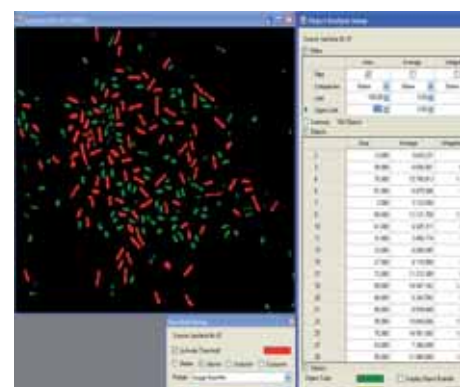
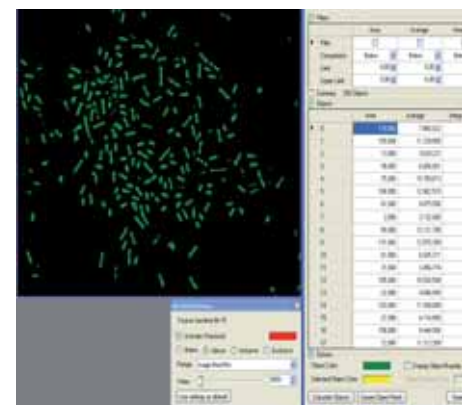
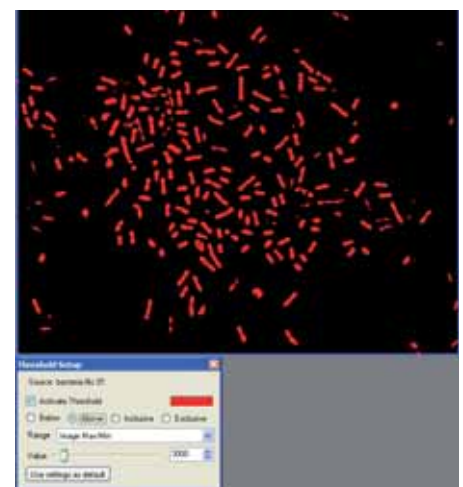
Object Analysis - Cell Counting

Measure or count cells automatically with a wide range of object classification. The object analysis tool makes it possible to determine morphometric parameters from the specimen and to report it 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 different color. This function makes the VisiView® imaging software a complex 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.

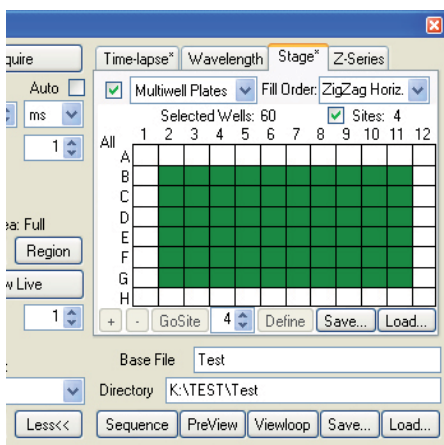


VisiView®

Imaging Software Screening

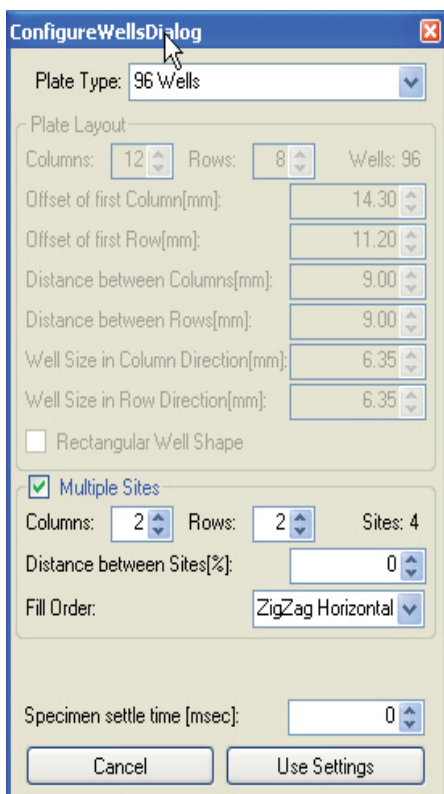
VisiView® Screening option:

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 helps always to find the best focus of the cells.



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 Fill Order selection, offers the user to define the scan direction as ZigZag horizontal / vertical or only horizontal / vertical. The whole settings can be stored and reloaded for later usage.



Configure Well Dialog

There are different sizes of multiwell plates available. The VisiView® screening option supports 6, 12, 24, 48, 96, 384, 1536 multiwell 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 moving due to inertia.

Autofocus

The autofocus module calculates the optimal focal section for each multiwell 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 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.

VisiView® Screening option:

Multi color imaging with automatic color detection and overlay of up to seven fluorescence channels per well gives the user a high flexibility in cellular research. The VisiView® software helps with easy device control and intuitive handling of the software. The macro interpreter language covers all functions for automation of custom demands.

VisiView®

Imaging Software Screening

Microscope Setup

For screening applications, we recommend the usage of a motorized microscope with XYZ-stage. But it is also possible to use a manual microscope with external motorized components like filter wheels, focus devices and XY-stages. Multi-band filter sets with single excitation and emission filters have to be selected carefully according to the used fluorochromes.

For long term time lapse applications cells need perfect incubation conditions. The VisiScope large incubation chamber with CO₂ control is required. It stabilizes the temperature of all internal components like sample holders or objectives during the entire observation.

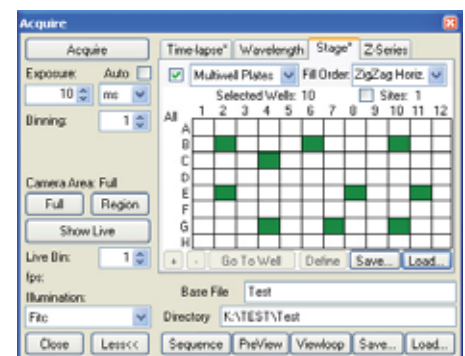
Montage - Tiling Display

There are different ways to display the acquired sequence of multiple wells. Single display as a sequence of image stacks which can be converted to AVI movie format, or overview image of the used multiwell positions as a montage display with columns and rows. The latter function is also known as tiling.

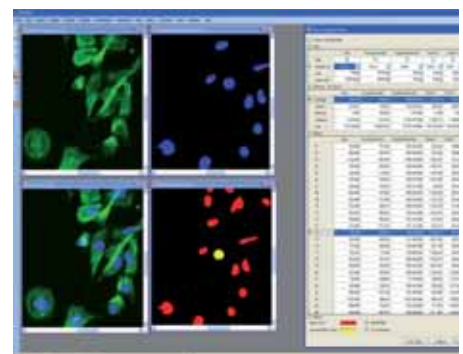
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Axio-Observer with motorized XYZ-stage for multiwell scanning.

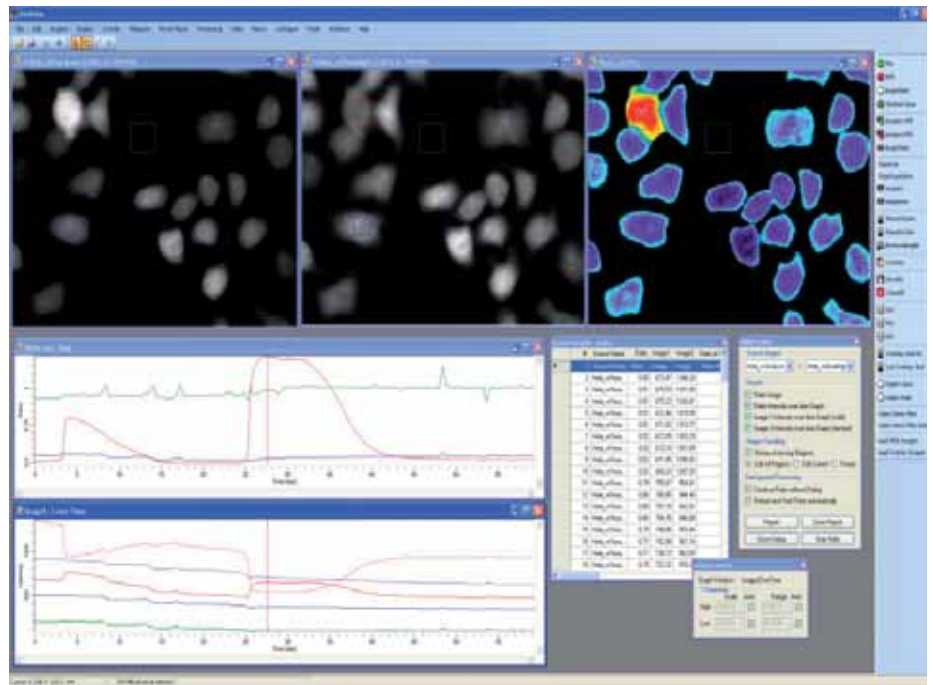


VisiView®

Imaging Software Ratio

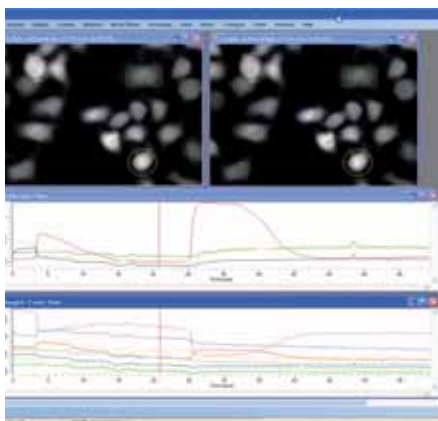
VisiView® Ratio option:

The Ratio option of the VisiView® is an application solution. It is designed specially for on-line ratio applications for single or dual wavelength intracellular measurements such as Fura-2, BCECF, FRET or single wavelength dyes. The Ratio option provides a simultaneous display of the original wavelength e.g. 340 nm, 380 nm, ratio image and the graphs for intensities. Intensity vs time graph can be plotted for multiple regions at once.



Making Measurements

Regions of interest, circular, rectangular or polygon shape can be placed on every raw source or ratio images to display the intensity value or ion concentration. The measurements can be done simultaneously. Regions can be repositioned using the history function if the cell has moved or the stage was touched. Individual region position can be frozen or changed by editing the current region or even all regions.



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.

VisiView® Ratio option:

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.

On-line 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 let the system acquire images with a long time lapse. After e.g. injection you can switch to fast time lapse or streaming acquisition mode.

Wavelength Changers

The ratio option supports device drivers for numerous available illumination systems. From standard filter wheels and shutters up to tunable illumination systems e.g. polychromators (VisiChrome) and high speed filter changers e.g. DG4. The devices are controlled automatically during image acquisition. High speed stream acquisition supports maximum frame rates of the CCD camera changing wavelengths between frame or interline transfer.

CCD Camera, Binning and Super Pixel

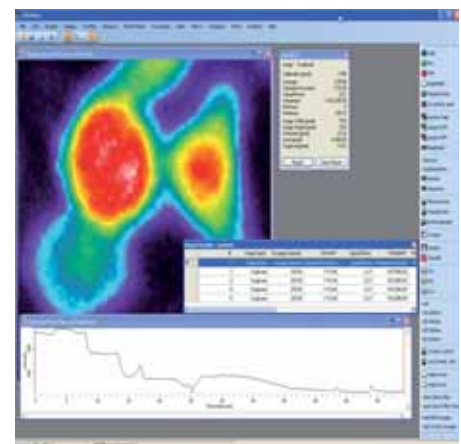
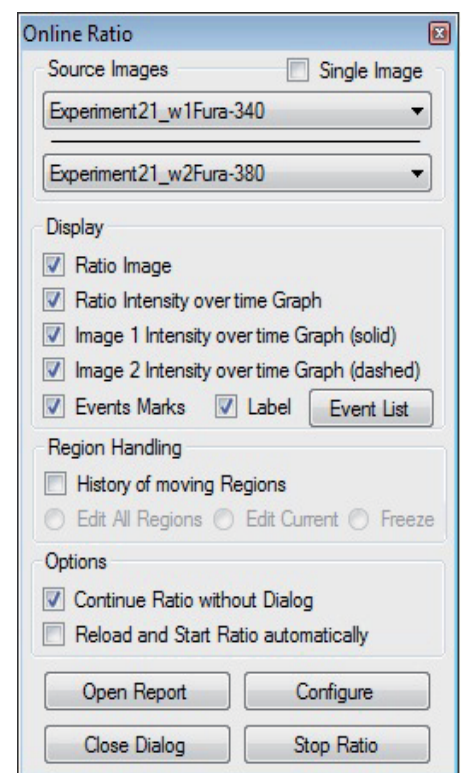
If the signal is too weak or noisy, the binning technique of the CCD camera can be used to improve the signal-to-noise ratio. Binning groups neighbored pixels to create a super pixel. Typically up to 8x8 pixels can be selected. In addition binning speeds up the frame rate up to e.g. 300 frames/sec..

SplitView Ratio

It allows the on-line separation of images acquired with an optical image splitter, which is mounted between the microscope and the CCD camera for simultaneous multichannel imaging of FRET or emission ratiometric applications.

VisiView®

Imaging Software Ratio



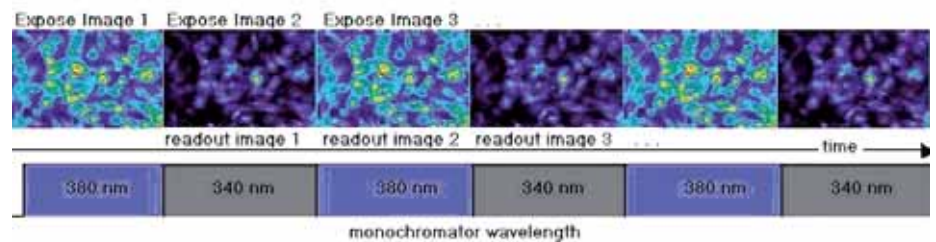
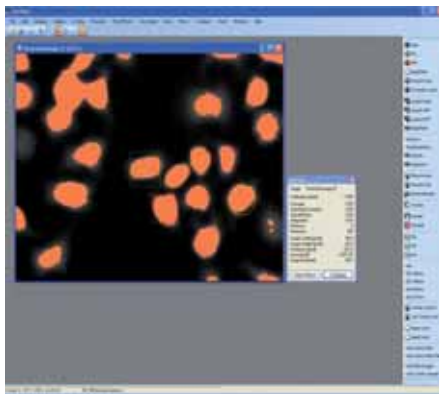
VisiView®

Imaging Software Ratio

VisiView® Ratio option:

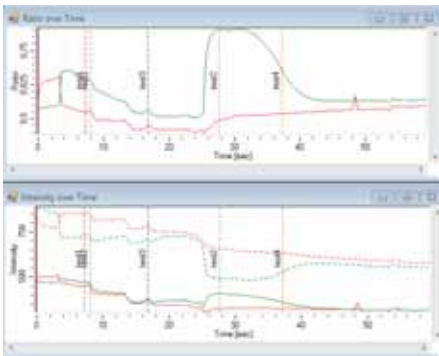
Interactive Display and Graphs

By clicking on graph traces, you can display a readout of time and data value for the region. When playing back by scrolling or movie mode, an experiment on the graph will show the image that corresponds to the location on the graph.



History of Moving Regions

The history function "edit or move regions" is ideal tool for situations in which objects that are measuring have moved simultaneously and in the same direction. For example, if you add a solution to a dish, the influx of solution may shift the field of view by a number of pixels.



Threshold Measurement

To provide clear isolation of labeled cells, the VisiView® Ratio can apply a gray level threshold to each collected image. Use of this threshold helps to reduce the distracting effect of low level signal like background fluorescence. This process improves the accuracy of collected data by excluding the threshold region from the ratio calculation.

Event Marks

During the experiment, the event mark function can be used to store the injection time, changes in experiment conditions or applied triggers. The mark in the time scale shows the exact time when the event happend.

Logging of Data to Excel or Text file

The ratio option logs all measurements to either a text file or a Microsoft Excel spreadsheet program. The data file stores the timestamp of each measurement and e.g. user defined selection of the wavelength average intensity.

	A	B	C	D	E	F	G	H
1	Source Name	Ratio	Image1 Intensity	Image2 Intensity	Time	Time Units		
2	Meta_w/first_low	0.52	805	1181.94	0	sec		
3	Meta_w/first_low	0.53	807.13	1149.81	0.4	sec		
4	Meta_w/first_low	0.53	806.96	1146.75	0.8	sec		
5	Meta_w/first_low	0.53	804.27	1140.72	1.2	sec		
6	Meta_w/first_low	0.53	803.13	1132.51	1.6	sec		
7	Meta_w/first_low	0.54	803.4	1126.53	2	sec		
8	Meta_w/first_low	0.54	803.84	1125.13	2.4	sec		
9	Meta_w/first_low	0.54	803.48	1123.15	2.8	sec		
10	Meta_w/first_low	0.54	811.74	1122.75	3.2	sec		
11	Meta_w/first_low	0.8	873.21	846.45	3.6	sec		
12	Meta_w/first_low	0.81	874.42	837.38	4	sec		
13	Meta_w/first_low	0.81	874.3	836.88	4.4	sec		
14	Meta_w/first_low	0.8	872.06	838.47	4.8	sec		
15	Meta_w/first_low	0.79	877.81	844.88	4.8	sec		
16	Meta_w/first_low	0.79	877.81	844.88	4.8	sec		

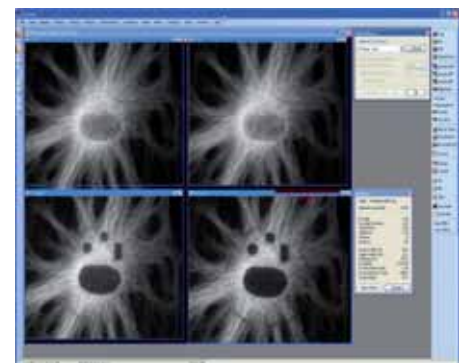
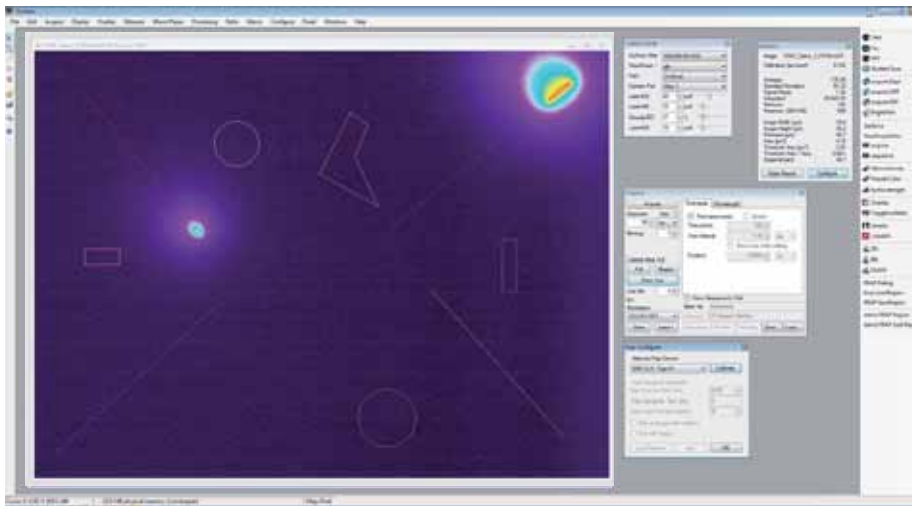
VisiView® FRAP option:

Bleaching or Photoactivation

Photobleaching or photoactivation is achieved by scanning with a laser beam controlled by a 2D galvanometer over a user defined region. The VisiView FRAP option is controlling the 2D galvanometer module, the laser illumination and the synchronization. The laser intensity can be adjusted by the user. Regions to be covered by the laser beam can be a single point, a line or a sequence of lines. Areas can be defined as circles, rectangles or polygon shapes.

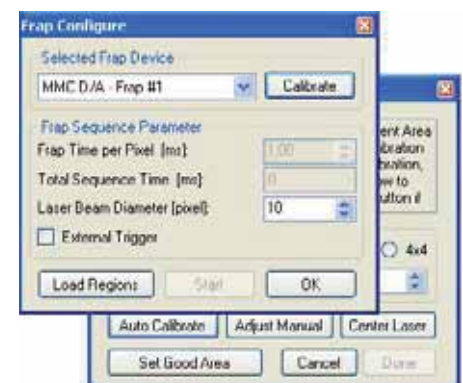
VisiView®

Imaging Software FRAP



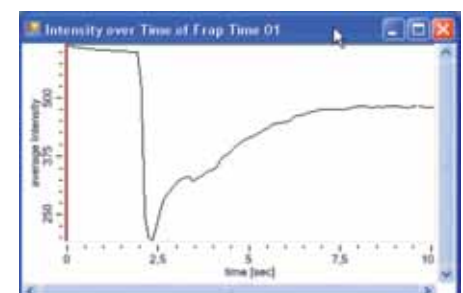
Laser Control and Calibration

The VisiView® laser control dialog allows the direct programming of a single laser regarding intensity or shutter mode. If multiple laserlines are used, a high speed VS-AOTF100 is selecting the necessary laserline. A calibration which matches the camera coordinates with the laser scanner coordinates needs to be done once for each microscope objective used for FRAP. Subsequently, the appropriate calibration is selected automatically when the objective is changed by the VisiView® software.



Region Analysis

To define the region or points to be bleached or activated, you have to draw one or more regions in an image of the specimen. A control dialog allows the selection of the time which the laser beam stays at one point and the number of bleaching cycles to be performed. The intensity over time graph shows the intensity information for each region. All analysis values can be reported into a text file or directly into Microsoft Excel spreadsheet program.

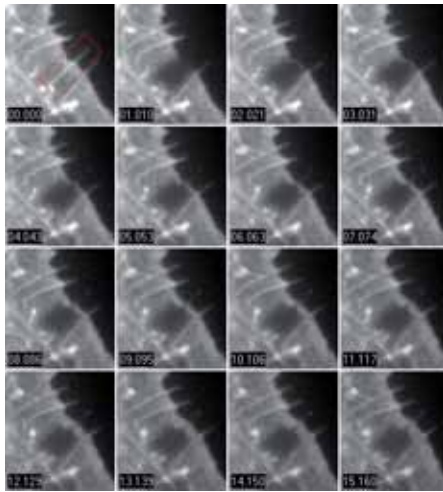


VisiView®

Imaging Software Overview

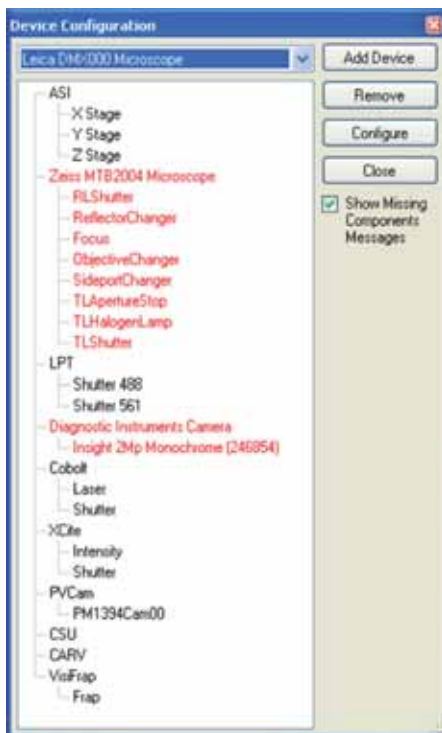
Basic VisiView® Functions:

- » 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 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
- » Create AVI movies



VisiView® Optional Plug-Ins:

- » Measurement
- » Processing
- » Illumination
- » Magnification
- » Z-axis / autofocus
- » XYZ-stage control
- » Device streaming
- » Trigger protocol
- » Threshold measurement
- » Object analysis
- » Splitview (splitting of optical images)
- » On-line ratio (Fura-2 and FRET analysis)
- » Screening
- » Scan slides



VisiView® Hardware Support:

- » Camera driver: Photometrics, QImaging, Hamamatsu, PCO, Diagnostic Instruments, Andor (on request)
- » Automated microscope: Zeiss, Olympus, Leica, Nikon
- » Shutter / filterwheel: Vincent, Ludl, Sutter and ASI
- » XYZ-stage: ASI, Märzhäuser and Ludl
- » Z-stage: Piezo z-focus control
- » Autofocus hardware support: Nikon perfect focus, Zeiss definite focus, Olympus ZDC/ZDC2 and Leica autofocus
- » Illumination system: Xcite, HXP and PhotoFluor
- » LED illumination: CoolLED, Colibri, Lumencor
- » High speed illumination: DG4, Polychromators
- » Optical image splitter
- » ILser illumination: diode and solid state lasers
- » AOTF / AOM intensity and channel control
- » Confocal: CSU 22/X1/W1, X-Light, CARV-II and Infinity
- » FRAP: 1D and 2D scanner
- » VisiTIRF-mot control

System Requirements

Windows® XP Pro, or Windows® 7 Pro; 32bit and 64bit support