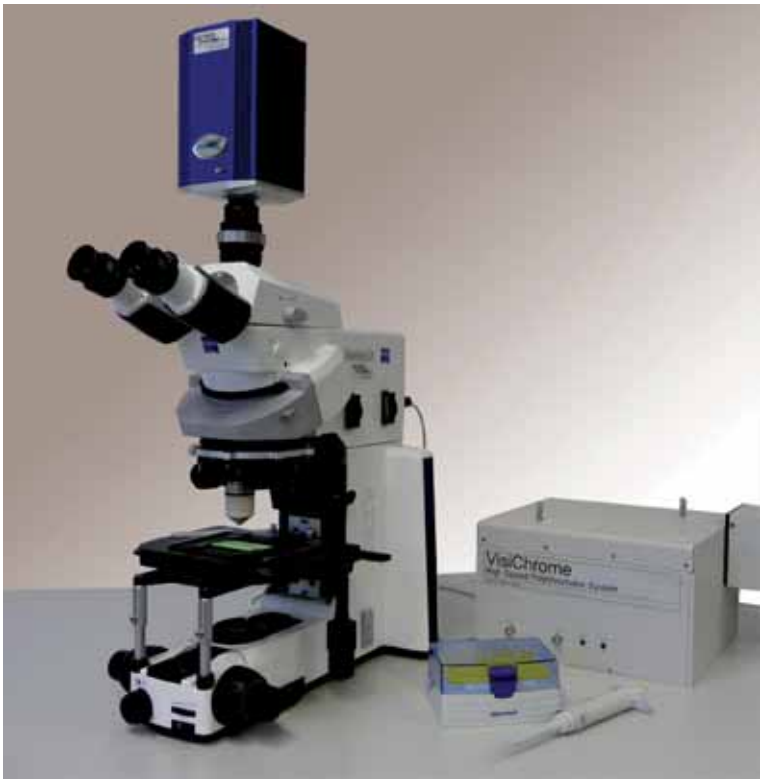
VisiFluor High Performance Calcium, Ion and FRET Imaging

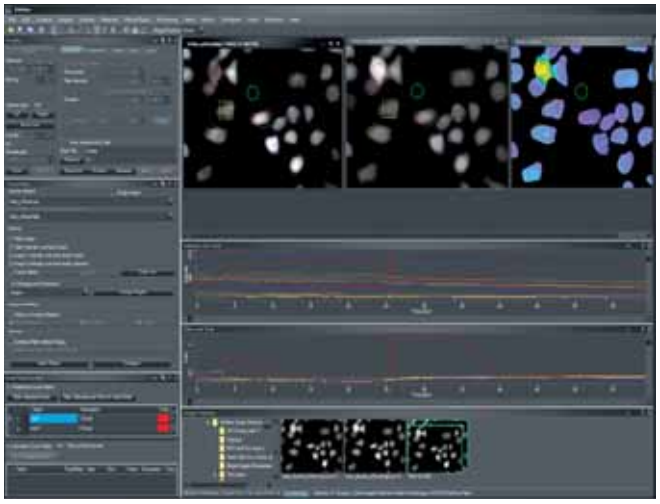
The measurement of intracellular ion concentration, calcium, pH etc. with ratiometric dyes like Fura-2 or BCECF is one of the main applications of the VisiFluor ratio imaging system. But not only high speed ratio images can be acquired, also single wavelength images of dyes like calcium green, fluo-3 etc. are possible for on-line image recording.

VisiFLUOR

Fluorescence Ratio Imaging System

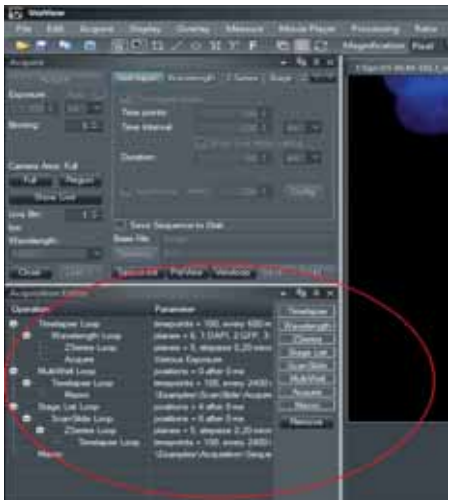


Axio Examiner with VisiChrome illumination and Coolsnap HQ camera.



New Acquisition Editor

To easily create your own arbitrary experiment sequence



High Temporal Resolution

High temporal resolution may be obtained using fast integrating CCD or sCMOS cameras.Typically, measurements over milliseconds up to 500 Hz are required. The VisiFluor imaging system supports a range of scientific grade cameras which approach these specifications.

Dual Excitation Ratiometric Dyes

Ion	Probe	Excitation 01	Excitation 02	Emission
Calcium.	Fura-2	340 nm	380 nm	510 nm
pH	BCECF	440 nm	490 nm	530 nm
pH	SNAFL-1	514 nm	550 nm	600 nm

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The ratiometric imaging requires rapid wavelength switching. Besides of conventional Polychromators, new LED systems are available with a wide variety of LED combinations ranging from UV 340/380nm to VIS wavelengths. Precise and time-accurate synchronization between LED and CCD or sCMOS cameraqs are handled by the VS-ViRTEx experiment control unit.
As an alternative for slower ratio applications, still emission or ecxitation filter wheel systems are used.

CoolLED pe340 LED System

Lumencor RETRA Fura LED System

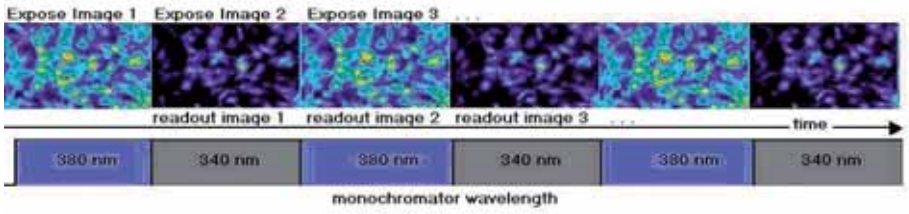
Omicron Fura LEDHub with ViRTEx experiment control unit

Use of new modular LED Systems

The 340 nm and 380 nm LED illumination system provides the optimum excitation wavelengths for Fura-2-based calcium imaging, allowing high-precision, stable, high-throughput imaging with video-rate time resolution.

High-speed acquisition

Until recently, the response time of illumination systems for Fura-2 imaging has been limited to milliseconds due to mechanical switching of the wavelengths in arc lamp and monochromator systems. However, the new pE-340fura can be controlled via convenient BNC TTL connections for precise illumination control in as little as 20 microseconds.



Do Not Waste your Light:

Perform your measurement as long as the signal lasts. The answer to photo-bleaching and toxicity is efficiency and speed. Therefore, we offer a number of high-end solutions focusing on the following features:

Highest Sensitivity:	Maximum quantum efficiency (up to 95%) Minimum system noise (down to 2 e^-) Minimum dark signal
Minimum Dead-Time:	Using of frame transfer, Interline CCD or sCMOS Cameras Leading to duty-cycles up to 100%
Dual Wavelength Stream:	Acquisition runs at full speed, while the excitation wavelength toggles

VisiFRET Imaging System

FRET in Live Cell Imaging

Förster (fluorescence) resonance energy transfer (FRET) is the process of radiation-free 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 induces fluorescence of the FRET acceptor.

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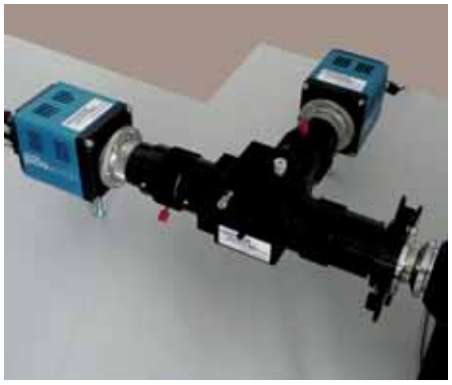
Fluorescence Ratio Imaging System

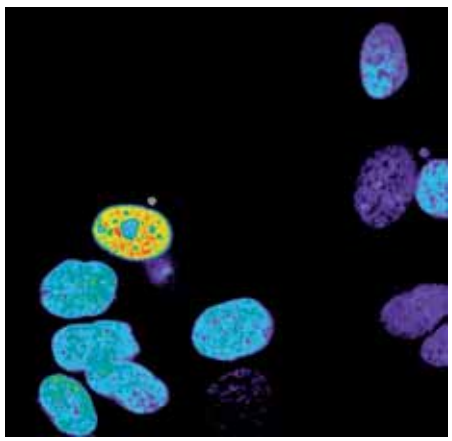
Zeiss Axio Observer with Cairn Optosplit Imager for realtime FRET analysis



Cairn TwinCam with two sCMOS edge cameras.

Simultaneous Image Acquisition with DualView Image or DualCam

For emission ratio applications like FRET, an emission filter wheel system can be used. For simultaneous measurement of two emission wavelengths (CFP/YFP) at the same time, a DualView / QuadView Imager is the better solution. The VisiView SplitView option allows the on-line division of the two or four image sets.



Quantitative Imaging

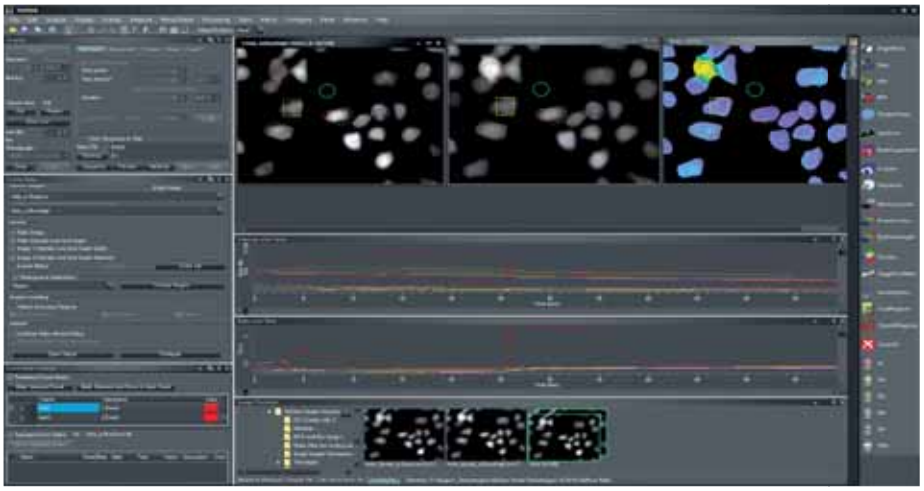
Using our VisiFRET fluorescence imaging system you can obtain quantitative temporal and spatial information about the binding and interaction between proteins, lipids, enzymes, DNA and RNA in vivo.
These processes are usually below the resolution of a light microscope. Because of the development of a number of green fluorescent proteins, it is possible to measure the integration of intracellular molecules.

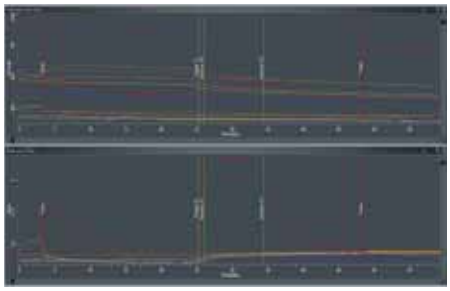
VisiFLUOR

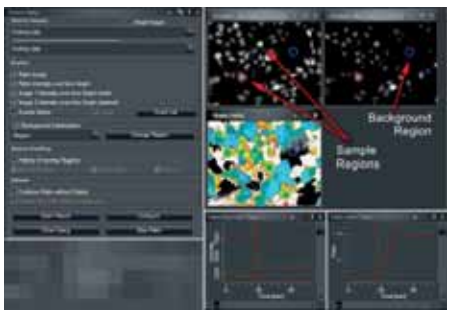
Fluorescence Ratio Imaging Software

VisiView® On-Line Ratio Option

The Ratio option of VisiVIEW® is an application solution. It is designed specially for on-line ratio applications of 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.







Interactive Display and Graphs

A display of multiple graphs gives flexible access to the experimental data or measurements. The VisiView® Ratio option enables interactive replay of image data and graph traces. The display shows the correct image sequence depending on time with correct intensity values.

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 happened.

Background Subtraction

VisiView offers background subtraction using either a constant value as background or adaptive background values using a dedicated region. To select the background region “Background Subtraction” has to be activated and “Region” must be selected.

Visitron Systems GmbH • Benzstraße 36 • D-82178 Puchheim ☎ 0049 89 890 245 0 www.visitron.de