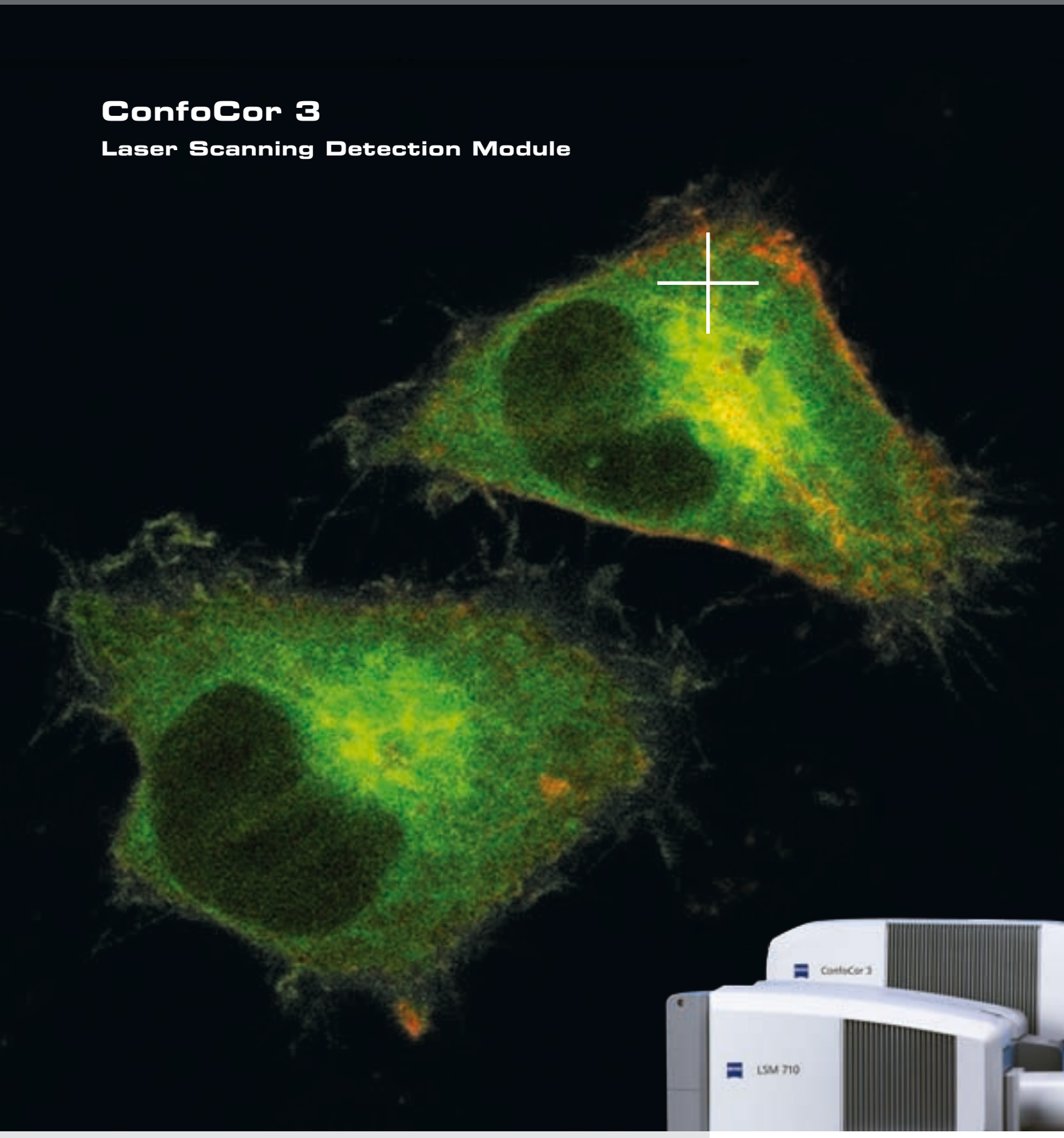
ConfoCor 3

Laser Scanning Detection Module



Quantitative Image and Fluctuation Analysis –
Every Photon Counts

ZEISS

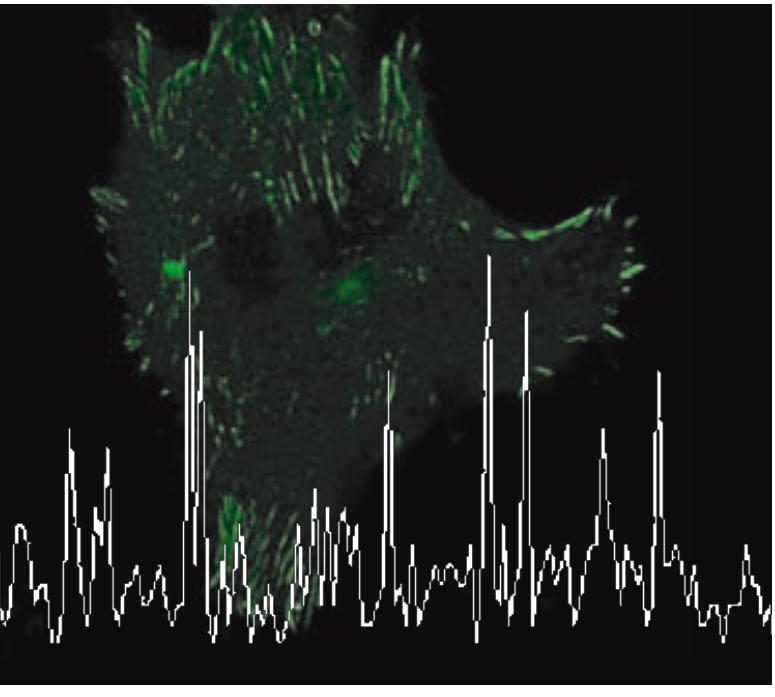
We make it visible.

Unraveling Cellular Processes

Unraveling cellular processes requires describing them in quantitative terms – a task requiring supremely sensitive detection methods capable of detecting single molecules.
This is the task where the combination of the LSM 710 and the ConfoCor 3 displays its strengths: Imaging with the very best signal-to-noise ratio for every requirement.

Sensitivity however is not the only feature that counts. Another essential contribution to optimum results is intelligent data analysis that can compute dynamic information from intensity images.
With its special modules for fluctuation analysis, the ZEN software fulfills your needs.

ConfoCor 3 on the LSM 710 –
your key to quantifiable success.



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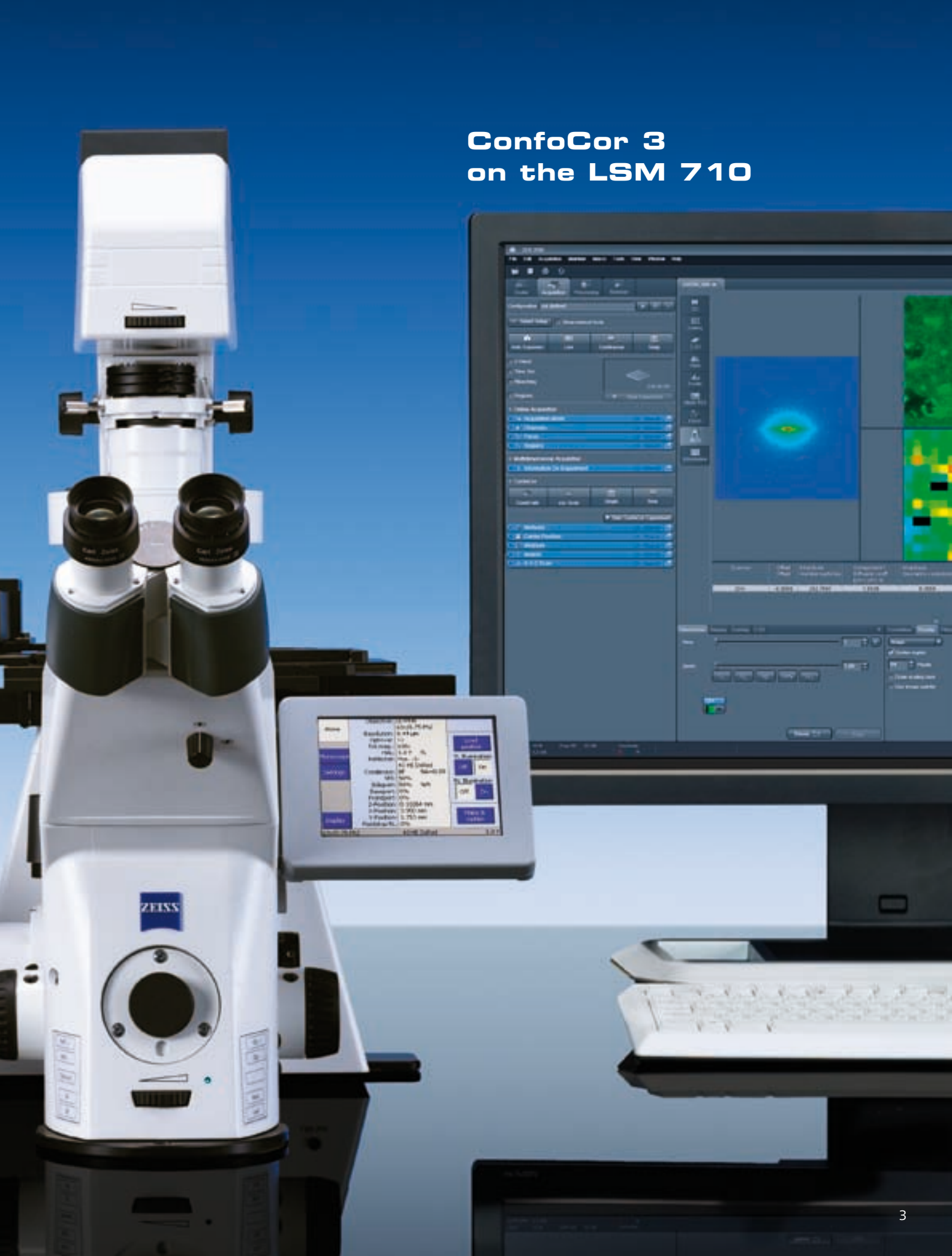


ConfoCor 3 on the LSM 710

A Clever Combination that Taps Synergies

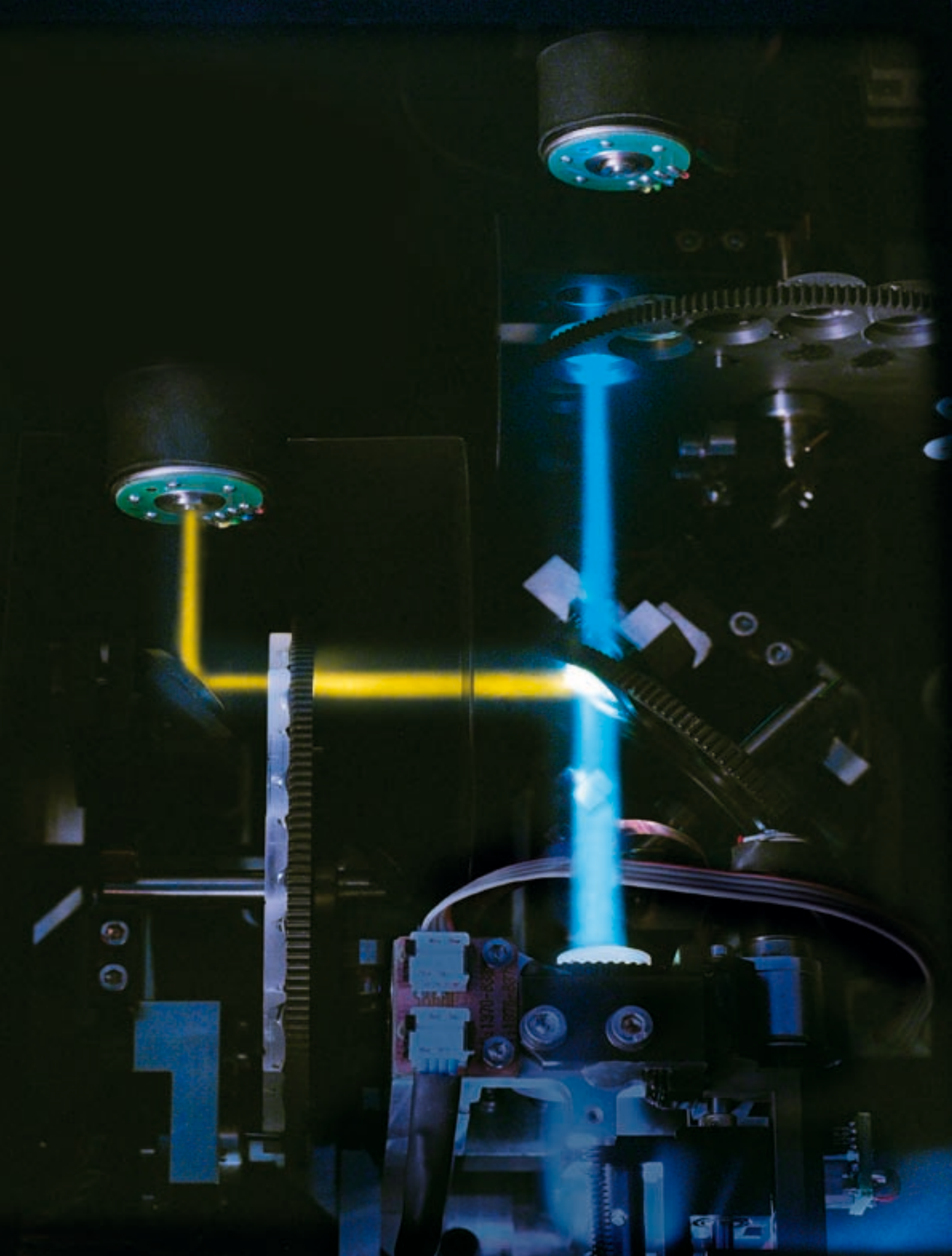
An expert duo of supreme sensitivity and dynamics.
The very combination that makes this microscope
for the most exacting requirements.
The ultimate in sensitive detection – simply perfect.

Flexibility in image acquisition
Continuous or single photon counting provides the broad basis for sensitive detection. The most efficient acquisition mode is always available, whether expression is high or low.

Images with quantifiable data
The ZEN software offers powerful algorithms to display the dynamics derived from the fluctuations in the image.

The ConfoCor 3 is coupled to the external channel output of the LSM 710 via a free parallel beam. Best optical quality and stability are guaranteed.

The inner workings of the ConfoCor 3 shows the high degree of automation. All optical elements can be controlled through the software, to ensure high reproducibility and precision.



APD Imaging

Sensitive Imaging and Photon Counting

Seeing where there is hardly anything to be seen: an assignment that calls for the power of an avalanche photodiode (APD). Counting every single photon, APDs provide the ultimate in photon counting sensitivity. The dark noise of these detectors is extremely low, resulting in an exceptionally high signal-to-noise ratio.

Specimen-preserving procedures
The use of APDs allows laser outputs to be substantially reduced where delicate specimens are involved. With less unnecessary stress on the specimens, cells can be observed over a much longer period.

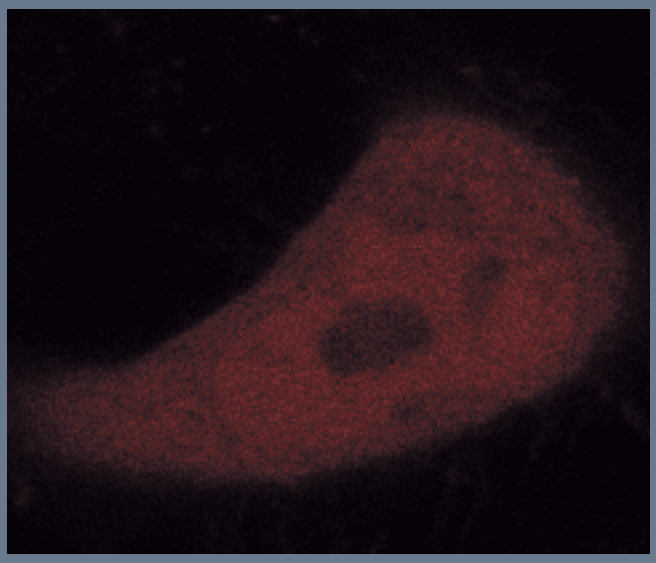
Particularly strong in the red wavelength range
The APDs used in the ConfoCor 3 are highly efficient especially in the red wavelength range, just where fluorochromes can typically be very weak. Combination with the Quasar detector easily allows the balancing of different intensity fluorochromes.

Particularly strong with weak expression
Thanks to their low dark noise, APDs have an excellent signal-to-noise ratio. So you can safely sum up the interesting signals from several images, whilst the background remains nicely dark.

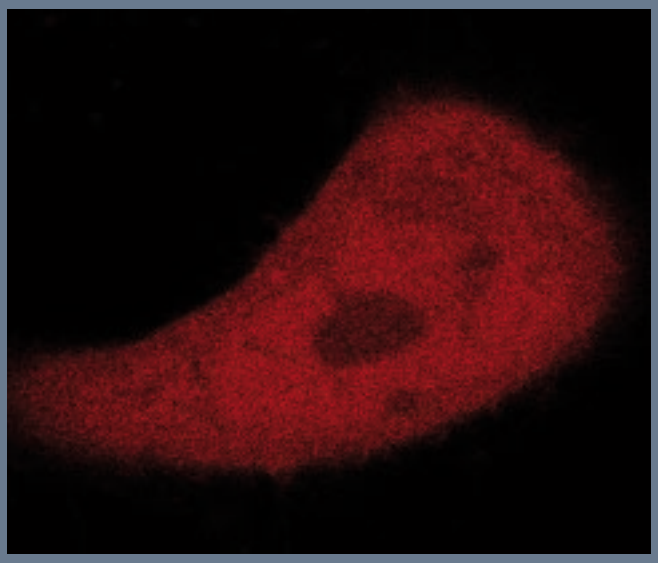
Detect subtle differences
The extremely sensitive APDs detect the tiniest and fastest changes in the fluorescent signal.

C-Apochromat 40x / 1.2 W Korr UV-VIS-IR from Carl Zeiss.
Excellent color correction plus high transmittance –
from IR to VIS to UV.

Avalanche photodiode from Perkin Elmer.
Extreme sensitivity in the red range, low dark noise.

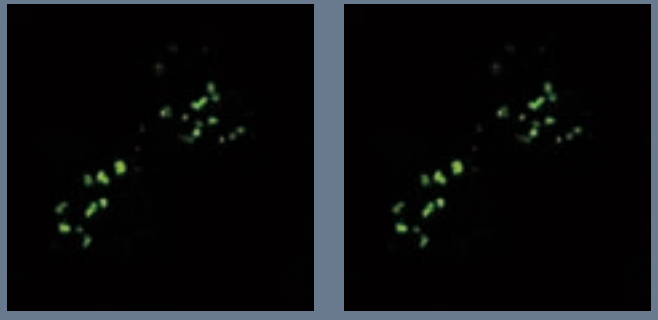


HeLa cell expressing DsRed1.



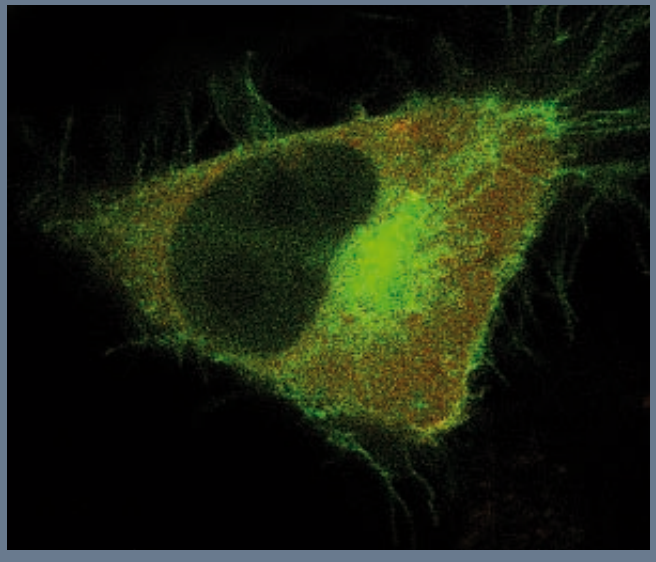
Despite laser output reduced approximately four times, the increased sensitivity of the APD is clearly visible. Specimen: Takako Kogure and Atushi Miyawaki, RIKEN Brain Institute, Wako, Japan

HepG2 cells expressing EGFP-CENP I.

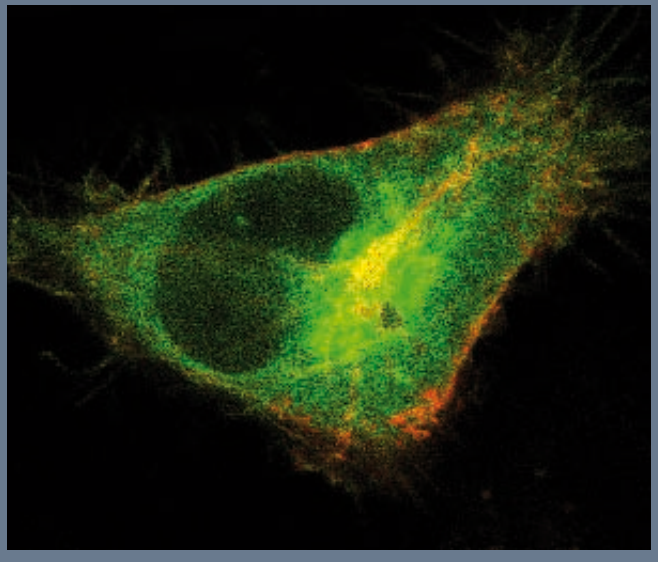


A time series of 500 images acquired with laser power reduced five times clearly displays less bleaching due to the use of APDs. Specimen: Stefanie Weidtkamp-Peters and Peter Hemmerich, Fritz Lipmann Institute, Jena, Germany

Distribution of the proteins Keima-PKC (red channel) and EGFP-MARCKS (green channel) in HeLa cells at rest (left), and after stimulation with PMA (right).



Subtlest changes can be followed extremely well with APD detectors. Specimen: Takako Kogure and Atushi Miyawaki, RIKEN Brain Institute, Wako, Japan



FCS

Fluorescence Correlation Spectroscopy

Determining the diffusion, concentration, localization and interaction of molecules with single-pixel accuracy is the function of FCS. All this information is directly obtained and analyzed from the fluctuation of the fluorescent signal, with no need to calibrate. Performing an analysis is as easy as this:

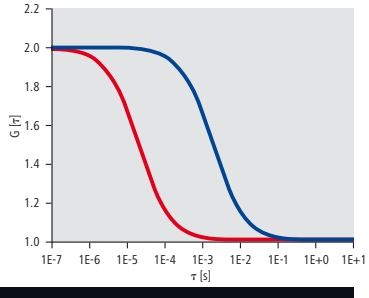
Define the site of measurement
Define the measuring site within an LSM image and simply mark it. Start the measurement, and the LSM scanners will precisely target the marked site. This way you can examine several sites of interest in automatic succession and with high resolution.

Start fluctuation analysis
A mouse click starts the recording of the fluctuations caused by molecule movement against time. The APD detectors will collect almost all of the photons captured by the objective.

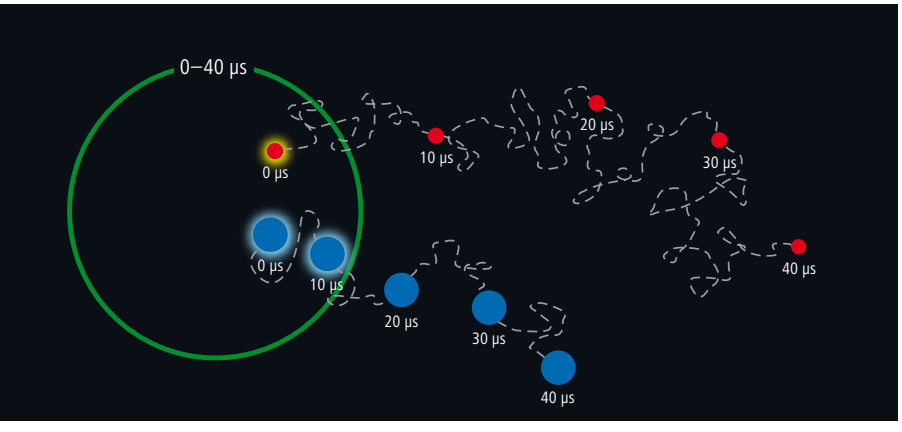
Determine correlation
From the fluctuation, the system determines time correlation in real time during the measurement. The signal is compared either with itself (autocorrelation) or with another signal (cross-correlation), to show similarity to itself or another signal, respectively.

Adapting to the model
Now you can ascertain parameters of interest by adapting the correlation to a particular model. The correlation amplitude provides information on the number of particles, while the correlation decay time indicates their speed.

In the temporal correlation diagram the correlation G(τ) is plotted versus the correlation or lag time τ_D. The number of particles and the their diffusion time can be computed from the non-normalized amplitude G(0) and the characteristic decay time, respectively. Please note that the diagram displays normalized correlation functions top emphasize time the different decay times.

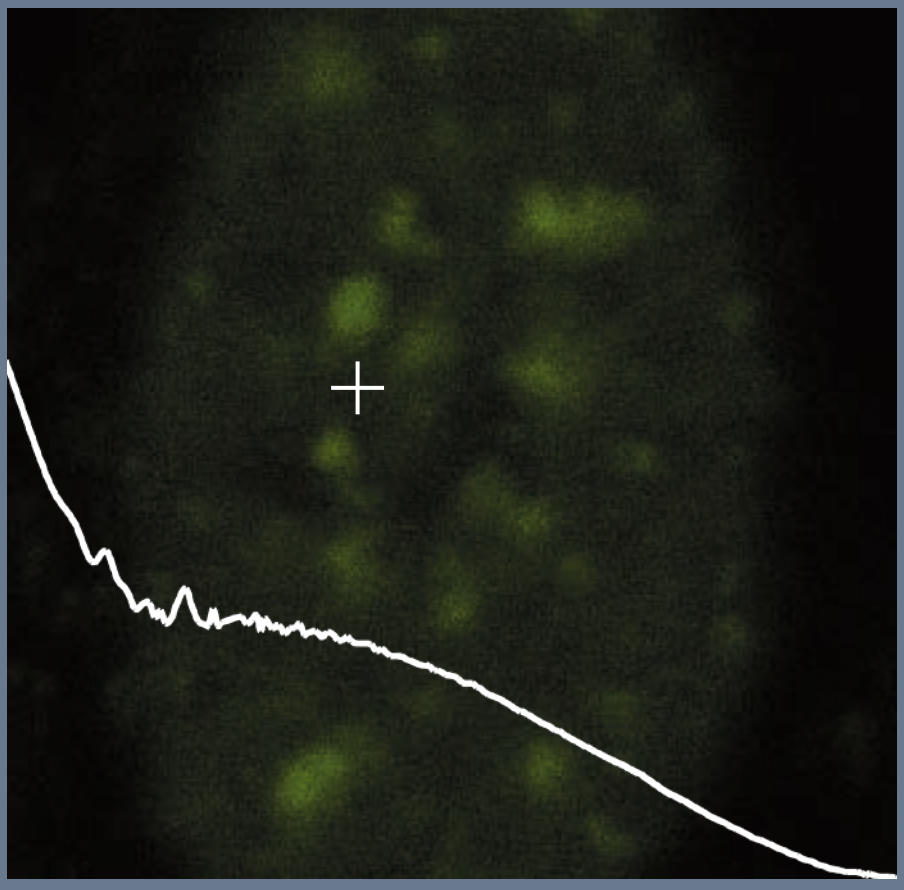


Assessing conformity
How well the end predictions describe the system examined can be assessed from the deviations between correlation and the theoretical model derived. The smaller the deviations, the greater the probability that the theory and reality match.



Snapshots of diffusion of particles of different speeds during a point measurement. The faster molecule (red) will have left the stationary observation volume after a smaller time delay, as it covers a greater distance per unit of time. Therefore, its time correlation decays faster.

In the examples, the measuring positions in the LSM image are marked by crosses; the resulting cross-correlation functions are superimposed on the images.

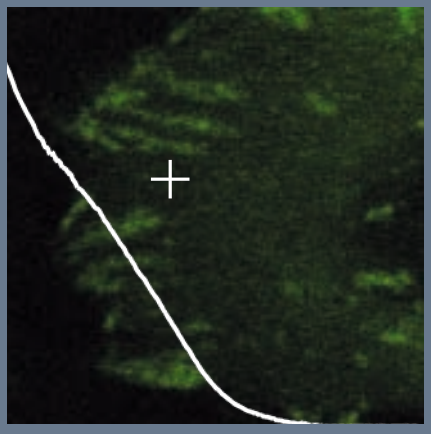


HepG2 cell expressing EGFP-HP1. The protein binds chromatin. Correlation analysis shows two molecule classes having diffusion coefficients of 5.8 µm²/s (unbound) and 0.2 µm²/s (transiently bound to chromatin).

Specimen: Karolin Klement and Peter Hemmerich, Fritz-Lipmann-Institute, Jena, Germany

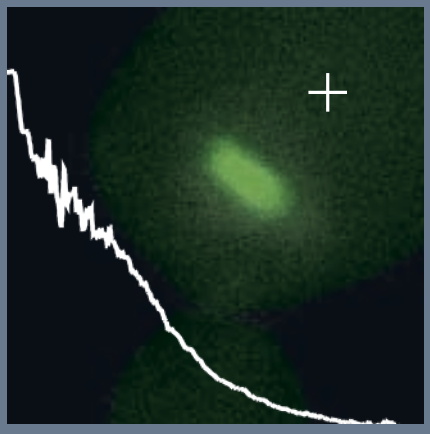
tsEoS-Paxillin expressed in an HFF-1 cell. Paxillin is involved in the formation of foval adhesion sites in the cell. Due to transient binding to the structures, the motility compared to the free molecules decreases from 2.5 to 0.18 µm²/s.

Specimen: Harald Hess, Howard Hughes Medical Institute, Janelia Farms, USA



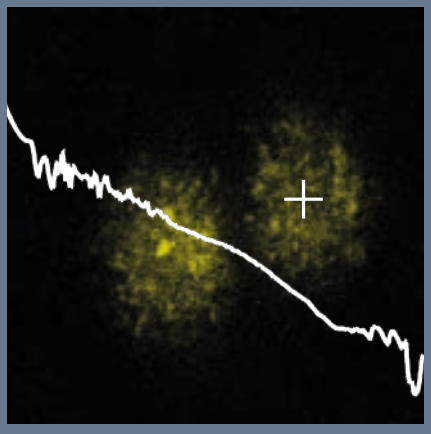
GFP-Bim1 expressed in baker's yeast. In proliferation, the protein accumulates in spindle pole bodies. In the cytosol, it remains motile, with a diffusion coefficient of 1.4 µm²/s.

Specimen: Susanne Trautman, Eidgenössische Technische Hochschule, Zurich, Switzerland



Topaz A1 adenosine receptor expressed on the membranes of CHO cells. In the membrane, the receptor has a diffusion coefficient of 1.3 µm²/s.

Specimen: Steve Briddon, Queens Medical Institute, University of Nottingham, UK



RICS

Raster Image Correlation Spectroscopy

If an image is generated by scanning, it contains not only spatial but also temporal information. From such an image you can determine information on molecule speeds and concentrations without upsetting its cellular equilibrium.

Select the measuring window
Use a suitable zoom to select the area of interest from an overview image.

Start the measurement
Record a time series stack of intensity images at a suitable scanning speed.

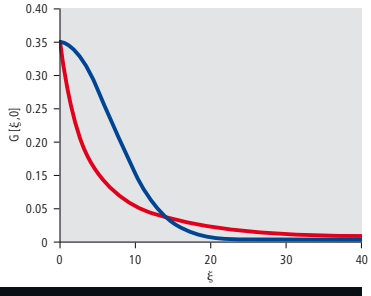
Filter the data
Disturbing structures in the image, such as stationary objects or slow drifts, are eliminated by subtraction of a moving average.

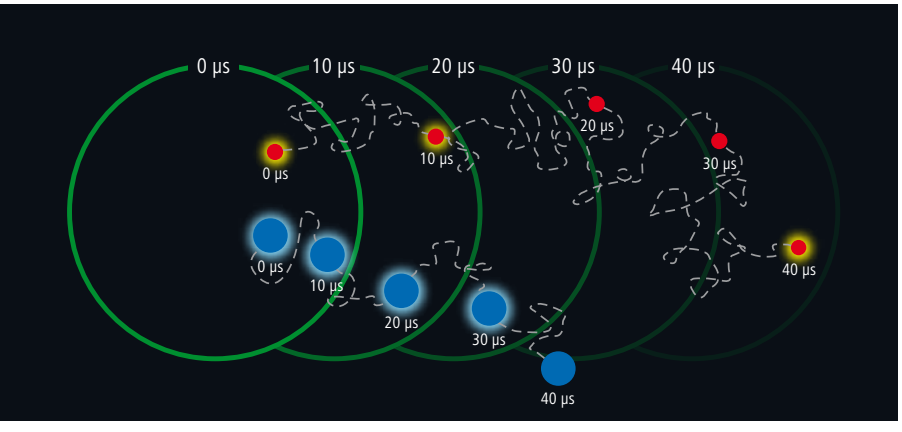
Determine correlation
The system computes spatial correlation from the pixel fluctuations, comparing the image either with itself (auto-correlation) or with another image (cross-correlation).

Adapting to the model
Allowing for the settings used for image acquisition, you can adapt the correlation to a particular model, and thus ascertain parameters of interest, such as the relative number of particles and their speed.

Assessing conformity
How well the model employed describes the measurement data can be seen from the deviations between correlation and fit. The better the model fits, the smaller the deviations.

In the spatial correlation diagram (depicted is a cut through the x-axis) the correlation G(ξ,0) is plotted versus the pixel shift ξ. The relative molecule number can be computed from the amplitude G(0,0). The decay shape contains the information on the diffusion time τ_D of the molecule.

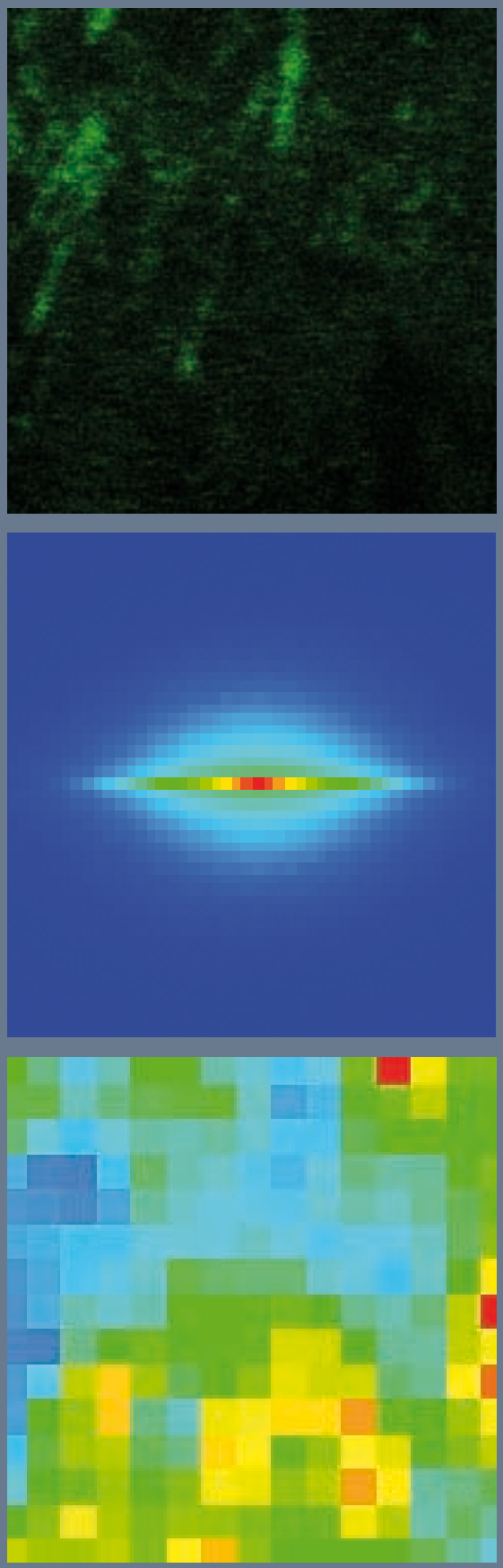




Snapshots of diffusion of particles of different speeds with moving beam. The faster molecule (red) will have left the moving observation volume faster with smaller spatial distances, but may re-enter the volume with long distances, as it covers greater distances per unit of time. Therefore, its spatial correlation first diminishes faster but remains to exist longer.

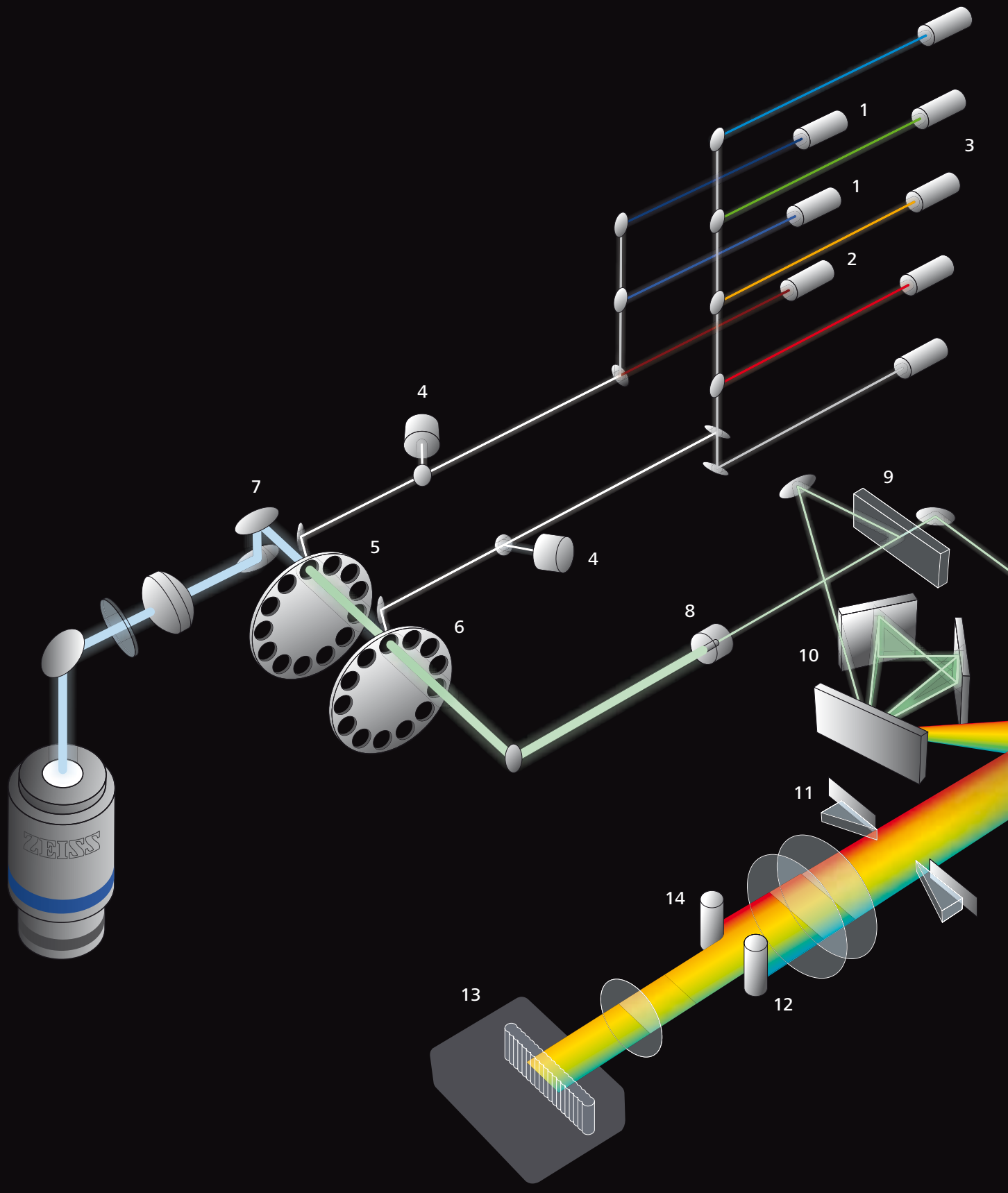
Intensity image of spherules sized 20 nm in solution (top). The resulting correlation diagram (bottom) contains information on the number (N=0.8) and speed (D=8.9 µm²/s) of the spherules.

Specimen: InVitrogen – Molecular Probes



Time series of an HFF-1 cell expressing tdEoS-Paxillin (the first of 100 pictures). Correlation analysis shows a mean diffusion coefficient of 0.78 µm²/s (middle). Mapping reveals the local difference in the dynamics of Paxillin (bottom). The protein is distinctly slower in the region of the focal adhesion structures.

Specimen: Harald Hess, Howard Hughes Medical Institute (HHMI), Janelia Farms, USA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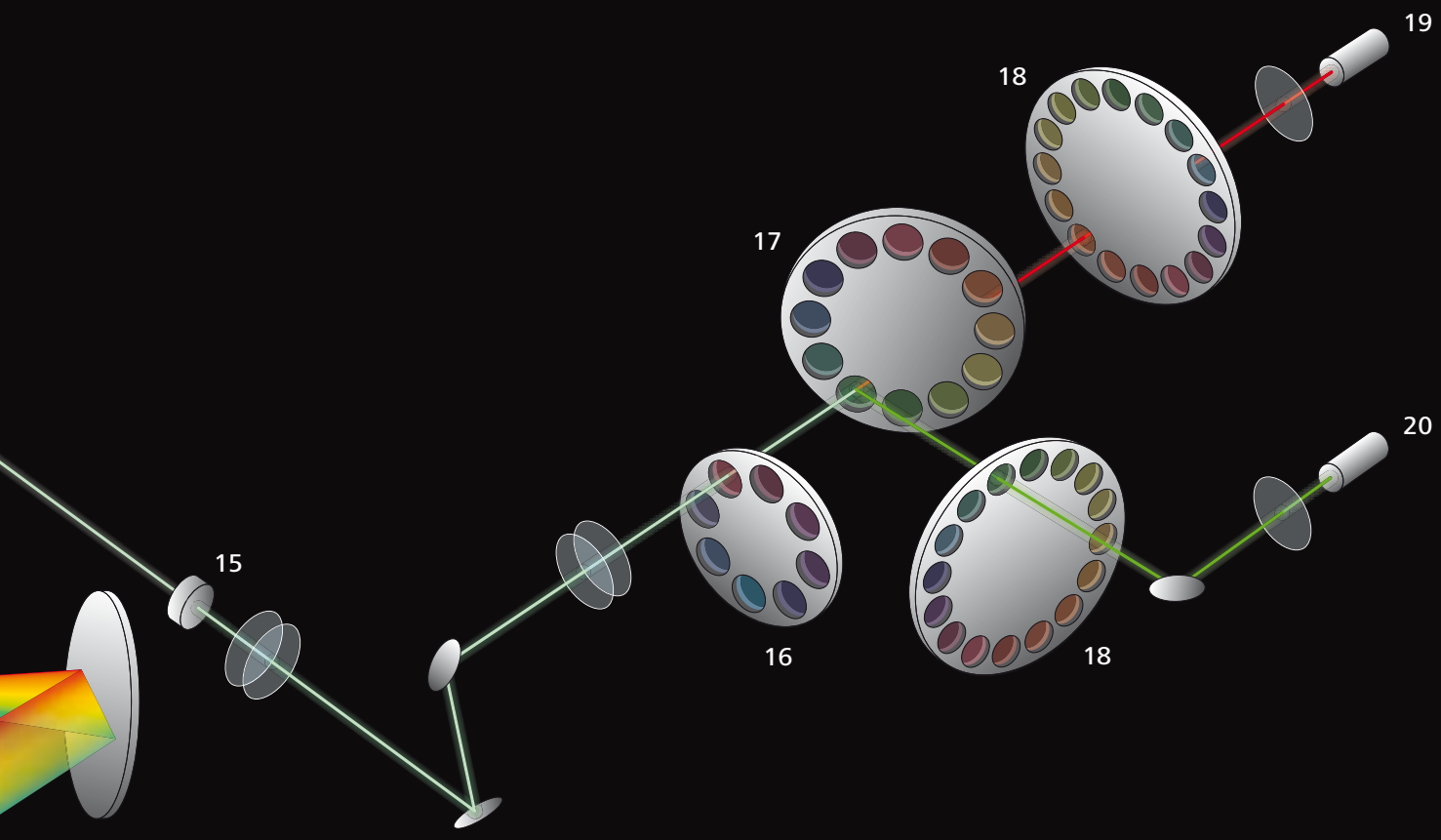
ZEISS

The beam path of the LSM 710 / ConfoCor 3 setup combines spectral detection and single photon counting.



1	V PCT laser coupling ports
2	IR PCT laser coupling port (IR laser not usable in combination with ConfoCor 3)
3	VIS PCT laser coupling ports with VIS AOTF
4	Monitor diode
5	InVis TwinGate color beamsplitter (retrofit)
6	Vis TwinGate color beamsplitter (user-exchangeable)
7	Scanning mirror (SF 20, 6k x 6k)
8	Central pinhole
9	Beamsplitter for external channel
10	Spectral beamsplitter and recycling loop
11	Spectral beam guide
12	QUASAR PMT, spectral channel # 1
13	QUASAR PMT, spectral channels # 2-33 (or # 2)
14	QUASAR PMT, spectral channel # 34 (or # 3)
15	External channel
16	Blocking filter wheel
17	Secondary beamsplitter wheel
18	Emission filter wheels
19	APD detector (# 1)
20	APD detector (# 2)

ZEN Software

Efficient Setting, Recording, Analysis and Cataloguing

The LSM 710 and the ConfoCor 3 are controlled via the ZEISS ZEN software.
The easy-to-understand user interface is an ideal environment for microscopy.

Control window,
with ConfoCor 3 group of controls

Perfect integration
The ConfoCor 3 is operated through special controls that perfectly integrate with the existing software structure. Interplay with the LSM takes place in the background, unnoticed by the user.

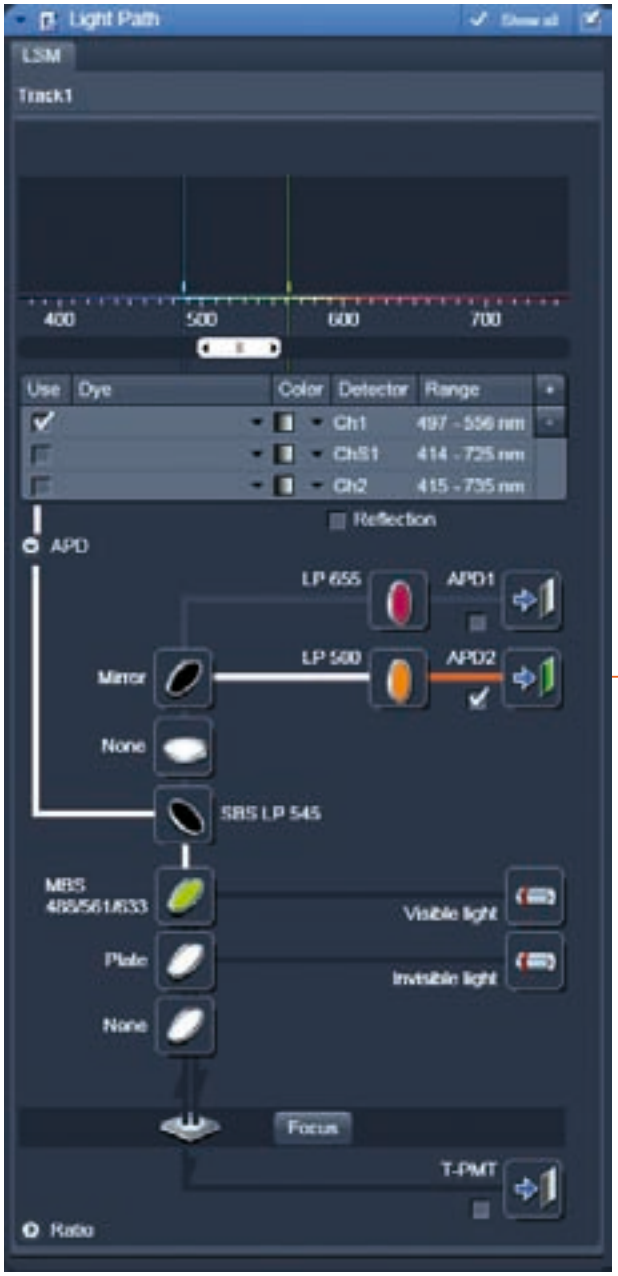
Everything important available at a click
Checking whether the experiment is really worth while, starting the measurement, and a lot more: everything is available at a click in the software. That way you save time and can concentrate on the essentials.

Count rate window opened by one
of the ConfoCor 3 action buttons

ZEN for APD Imaging

Ultimate Sensitivity in Imaging

Like photomultiplier tubes (PMTs), avalanche photodiodes (APDs) are point detectors, but of higher quantum efficiency. The detectors used in the ConfoCor 3 are twice as sensitive in the green spectral range, and even three to four times as sensitive in the red one. Thanks to their low dark noise, APDs have an excellent signal-to-noise ratio.



Group of beam path controls

Emission filter group in the ConfoCor 3

Photon counting at its best

Avalanche photodiodes count individual photons – a process that is far more sensitive than analog detection. This also means less specimen stress with the same image intensity.

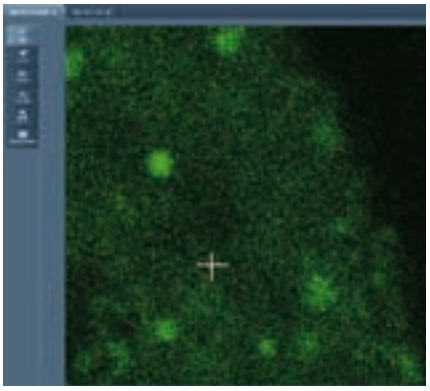
Optimum combinability and flexibility

APD detectors can be freely combined with the Quasar detector, so that weak and strong fluorescence can be simultaneously captured and balanced. A large number of filters in the ConfoCor 3 module permits optimization for almost every fluorescent dye.

ZEN for FCS

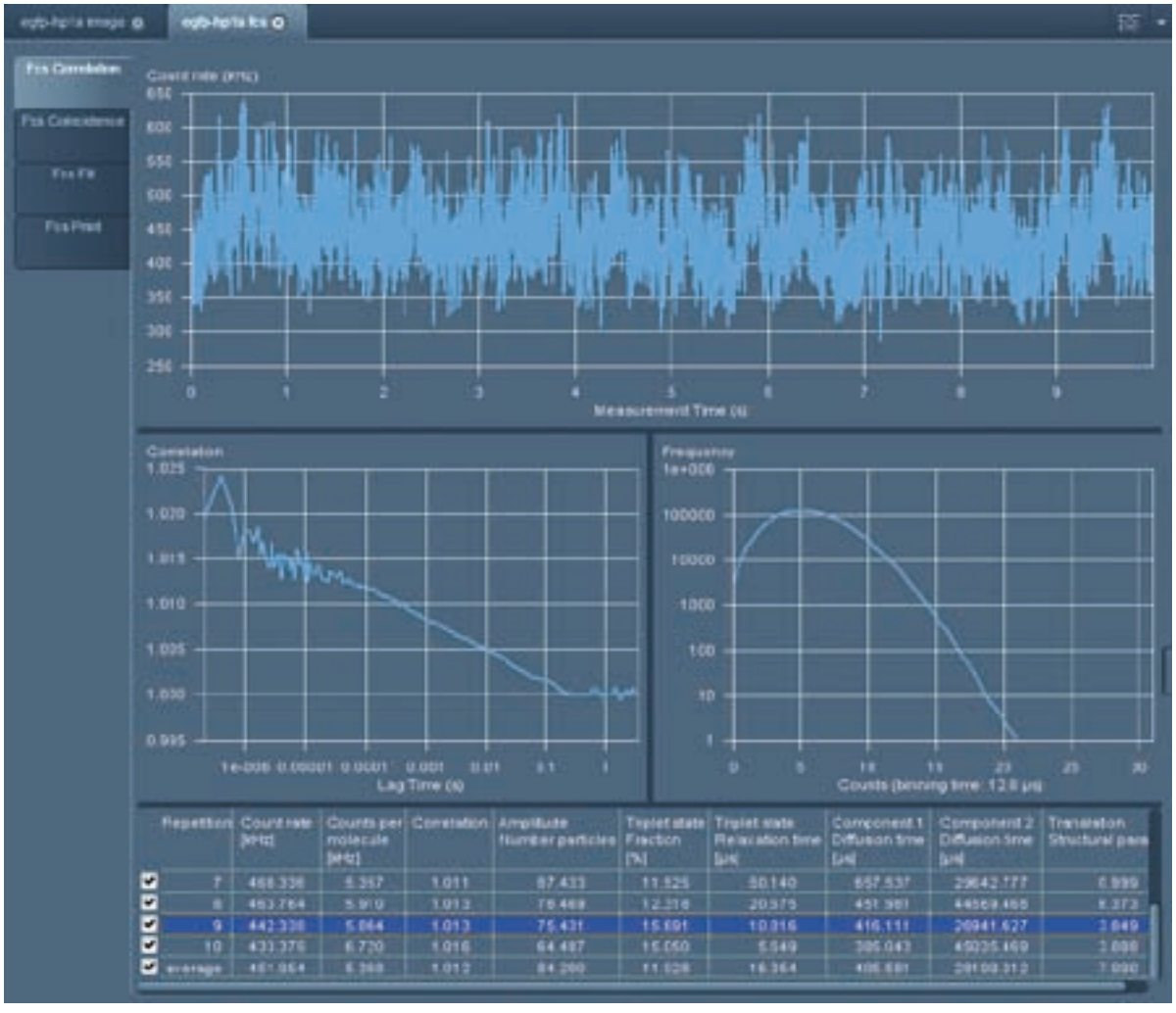
Measuring Dynamics with Pinpoint Accuracy

The ZEN software renders the best possible support to the precise, high-resolution measurement of molecule concentrations, speeds and brightnesses.



Selecting the measuring position in the intensity image

FCS window displaying the count rate track (top), the correlation function (centre left), the photon count histogram (center right), and the result table with the adapted parameters (bottom).



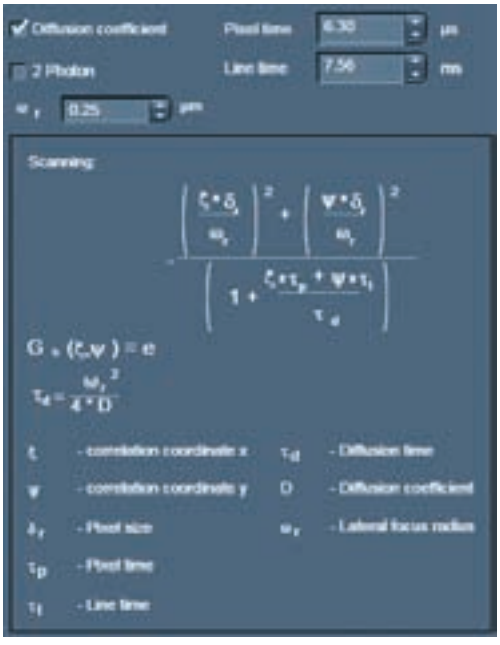
ZEN for RICS

Discovering Temporal Information Hidden in the Image

In the RCS module you determine the number and speed of moving molecules from images. The ZEN software offers many setting options facilitating the interpretation and display of the data.

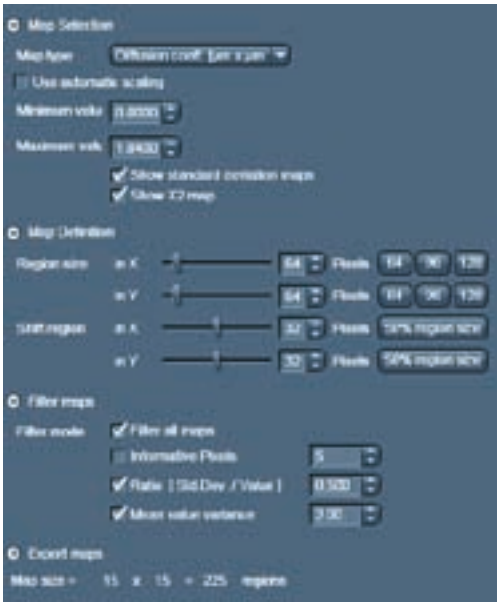
Determining correlation
Select the RICS register tab, and ZEN automatically computes correlation and adapts it to easy-to-assemble models. Moreover, the ZEN software helps you answer the question how well the model fits the recorded data.

Fast detection of heterogeneities
ZEN automatically computes the number and speeds of molecules from overlapping regions. Display by molecule number and diffusion maps permits differences to be assigned to cell regions and to be realized at a glance.



Menu for setting the model function that describes the scanning pattern of the laser beam.

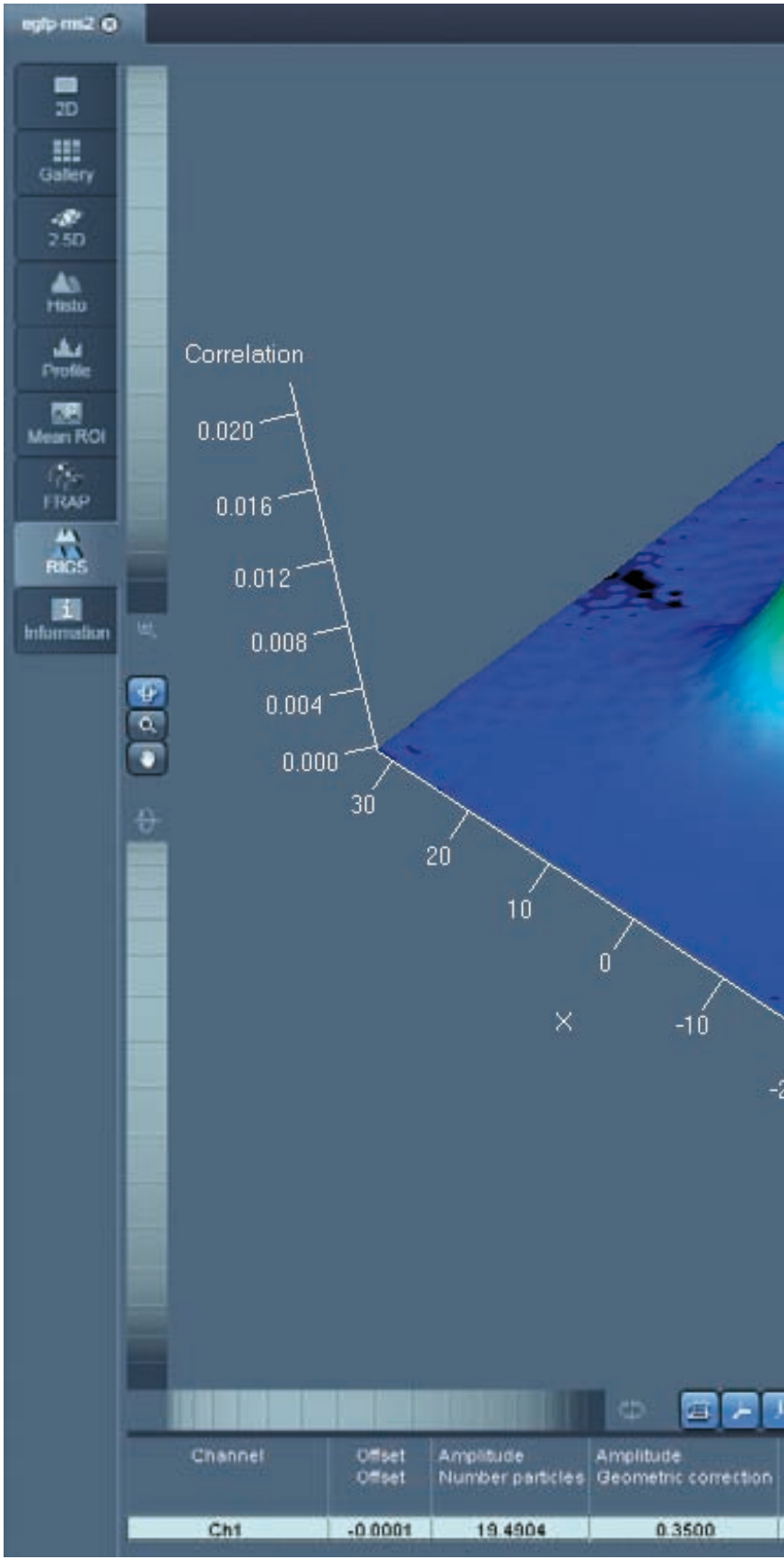
Menu for setting and filtering the molecule number and diffusion maps.



Example of a molecule number map. This is composed of the displacement of a 64 x 64 pixel region by 32 pixels each per X and Y increment in an image sized 512 x 512 pixels. The number per pixel is color-coded according to the scale on the left.
(specimen description see pages 18/19)

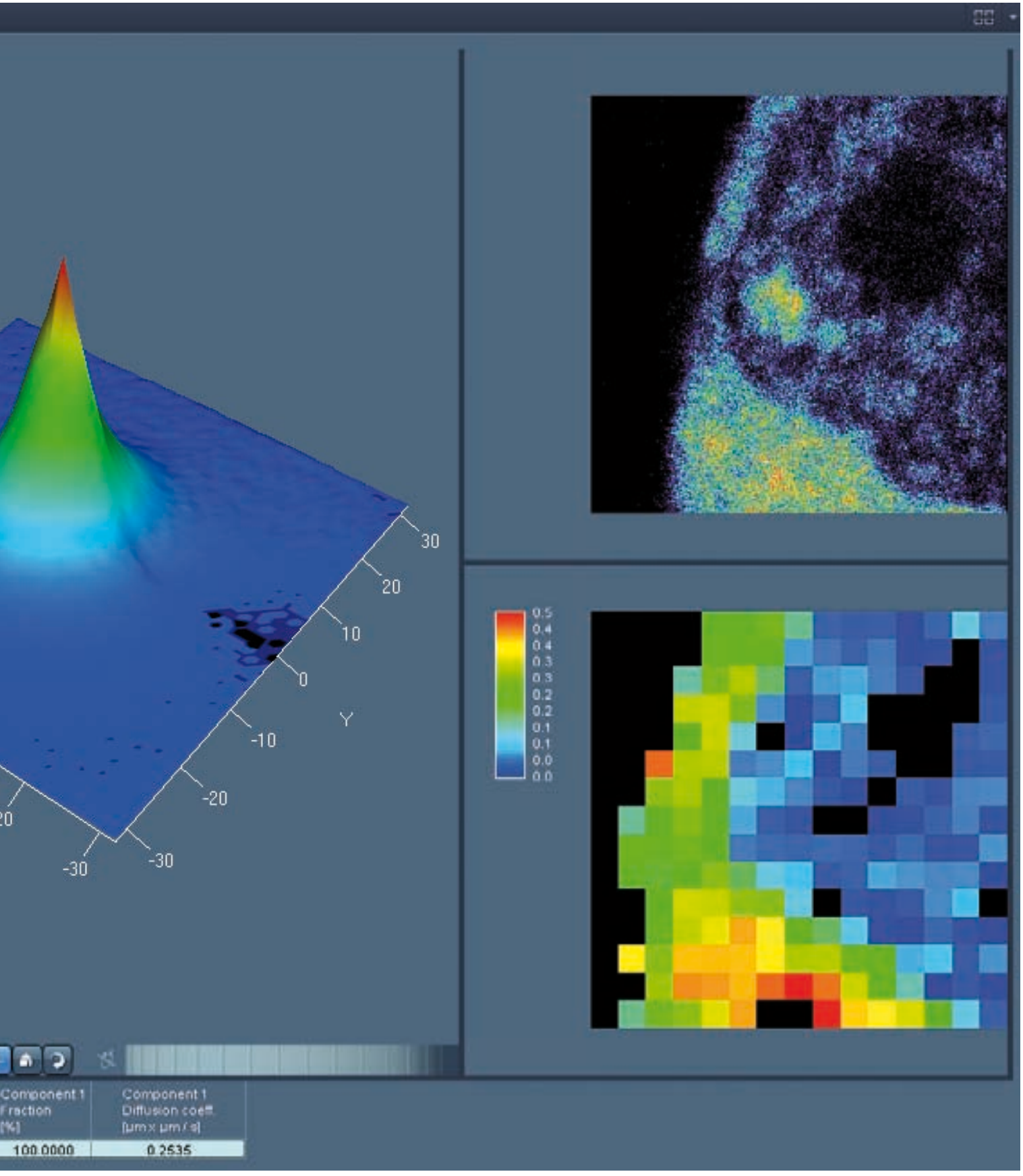
ZEN for RICS

See everything at a glance:
fluctuation,
correlation,
mapping and
parameters –
for efficient data analysis and
the resulting scientific findings.



RICS image window with display of correlation in 2.5 D (left), the intensity image (top right), the diffusion map (bottom right), and the results table with adapted data.

Specimen: EGFP-MS2 protein expressed in U2OS cells. The cells produce an HIV reporter transcript containing MS2 binding sites.
Ute Schmidt and Edouard Bertrand, IGMM-CNRS, Montpellier, France


30
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0.4
0.3
0.3
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0.2
0.1
0.1
0.0
0.0
Component 1
Fraction
[%]
Component 1
Diffusion coeff
[µm x µm / s]
100.0000
0.2535

System Properties

The ConfoCor 3 used on the LSM 710 makes up a system with a wide range of dynamics and sensitivity, adapted to widely varied expression rates and kinetic behaviors. Correlation algorithms of the ZEN software permit molecule concentration and speed to be quantified.

Integration
ConfoCor functions are integrated in the existing ZEN software for easy system handling

Ergonomics
Automated changing between configurations

Stability
The ConfoCor3 connects to a free parallel beam of the LSM 710

Positioning modes
Positioning by means of the scanning mirrors or the scanning stage

Positioning accuracy
Positioning by the scanning mirrors with nm precision

Imaging
APDs can be freely combined with the Quasar detector for simultaneous use

Spatial correlation
Linear scanning is excellently suitable for raster image correlation spectroscopy (RICS)

Statistics
Average correlation computed from several measurements

Reproducibility
Call up previous settings for re-use

Optics
Objective specially adapted to FCS and corrected for UV-VIS-IR, ideal for cross-correlation

Sensitivity
The objective is just overfilled to create a diffraction-limited excitation volume for the maximum signal-to-noise ratio

Laser suppression
TwinGate beamsplitter arranged at 10 degrees relative to the incident beam, with excellent laser suppression over optical density OD 4.
An optional filter wheel blocks unwanted secondary lines of the argon laser

Flexibility
Optional iris diaphragm in the excitation beam permits controlled variation of the confocal volume

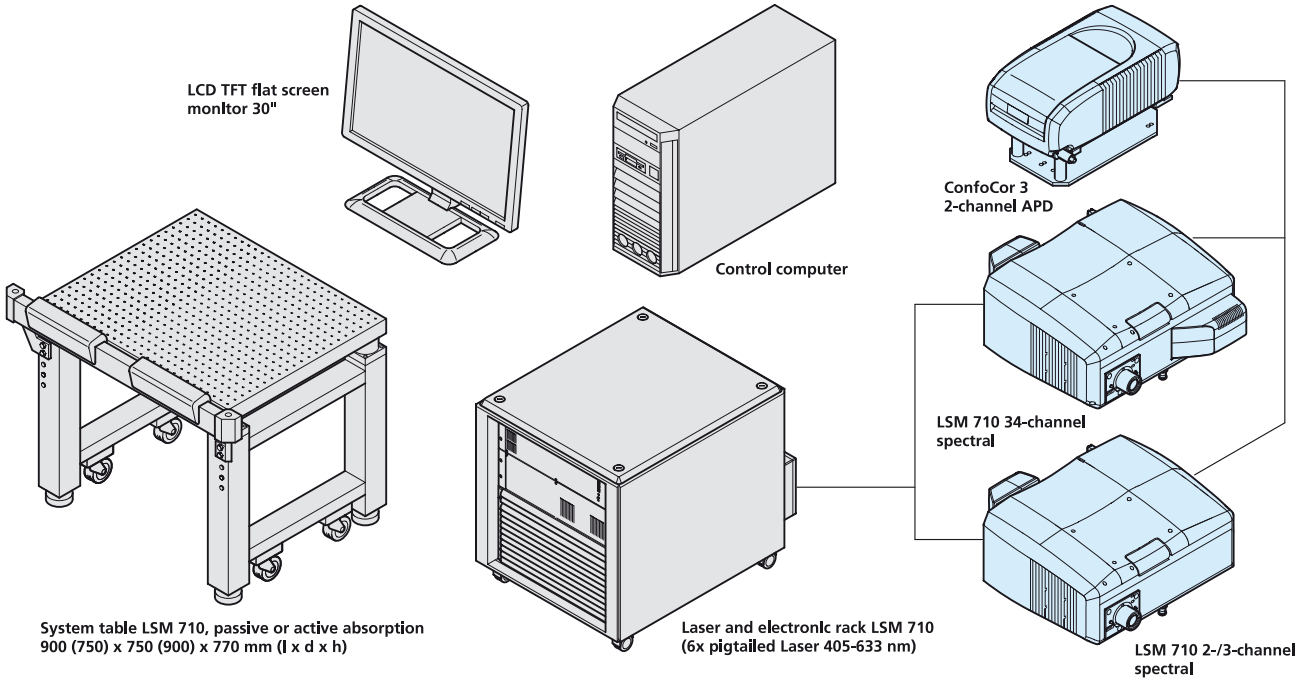
Pinhole setting
Automatic adjustment of the pinhole relative to the beam path in X and Y

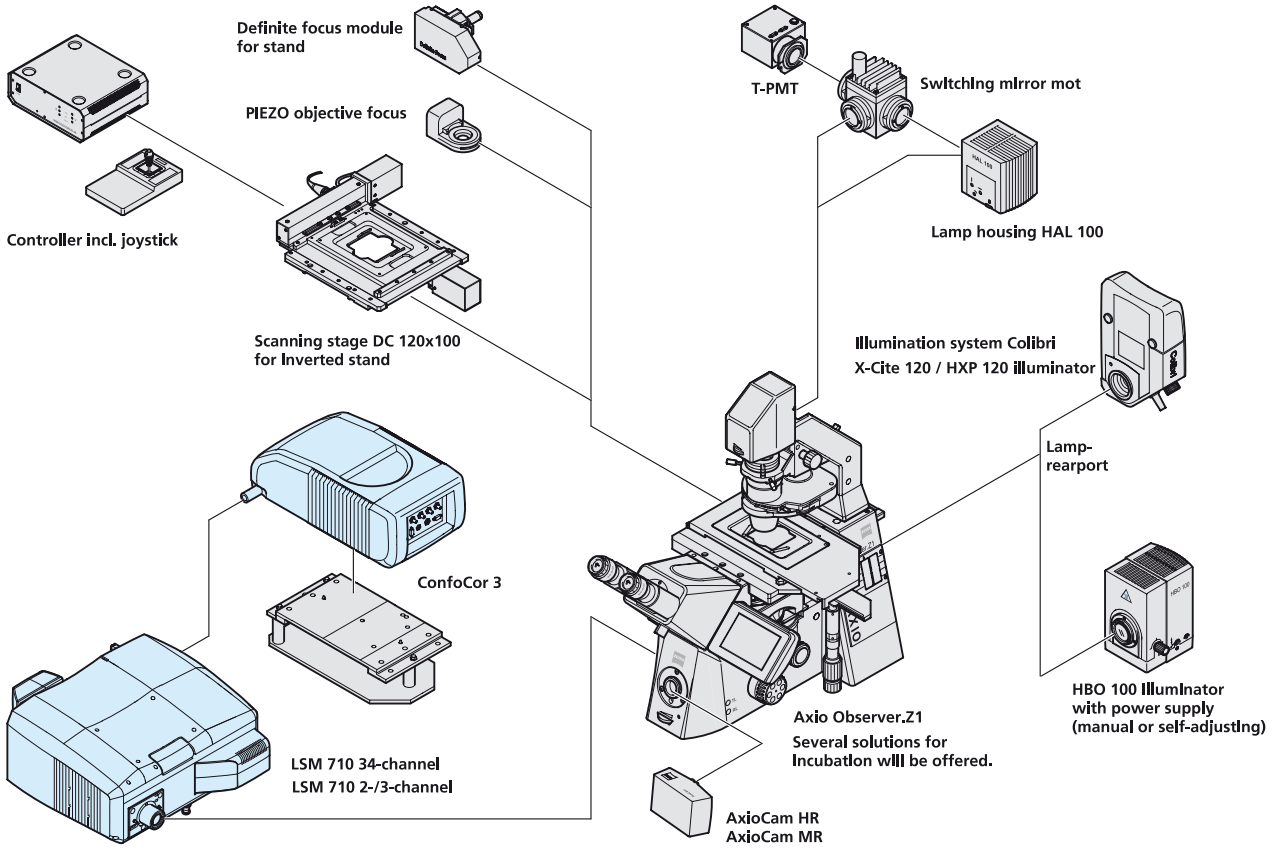
Automation
Selection of multiple measurement positions, which are targeted in succession

Specification

Microscope	
Stand	Inverted: Axio Observer.Z1 with sideport
Z drive	Smallest increment: <25 nm (Axio Observer.Z1). Options: fast piezo objective focus or stage focus; Definite Focus for stands
XY stage	Motorized XY scanning stage; smallest increment 1 µm (Axio Observer.Z1)
Accessories	Digital microscope camera AxioCam, integration of incubation chambers, micromanipulators, etc.
LSM scan module	
Detectors	2, 3 or 34 spectral detection channels, high QE, low dark noise, setting of up to 10 digital gains; prepared for violet light lasers (405, 440 nm)
ConfoCor 3 detection module	
Detectors	2 fiber-coupled, actively suppressed avalanche photodiodes (APDs). Detector sensitivity in the visible range: 40–75 % (depending on wavelength). Dark count rate <250 Hz, dead time 40 ns, time resolution 50 ns (corresponding to 20 MHz time resolution for photon counting). Pulse width: 15 ns, afterpulsing (100–500 ns): 0.5 %
Filter	2 filter configurations: Basic and extended filter sets
Pinhole	Central pinhole
Beam path	Changeable TwinGate major beamsplitter with up to 50 combinations of excitation wavelengths, excellent laser line suppression; optional laser notch filters for fluorescence imaging on reflecting substrates (on request); outcoupling for external ConfoCor 3 detection module
Iris diaphragm	Optional iris diaphragm in the excitation beam path for adjustable filling of the objective's rear aperture (for ConfoCor 3 on request)
Data depth	Selectable: 8, 12 or 16 bit; simultaneous detection in up to 37 channels
Lasers	
Laser modules (VIS, V)	Pigtail-coupled lasers with polarization-preserving single-mode fiber; stabilized VIS-AOTF for simultaneous intensity control, switching period <5 µs, or direct modulation; up to 6 V/VIS lasers directly connectable to the scanning head; 30mW diode laser (405 nm, CW/pulsed), 25mW diode laser (440 nm, CW+pulsed), 25 or 35mW argon laser (458, 488, 514 nm), 1 mW HeNe laser (543 nm), 20mW DPSS laser (561 nm), 2mW HeNe laser (594 nm), 5mW HeNe laser (633 nm) (manufacturers' pre-fiber specs.)
External lasers (NLO)	Prepared laser ports for the extension of all systems
Electronics module	
Real-time electronics	Control of microscope, lasers, scanning module and accessories; monitoring of data acquisition and synchronization by real-time electronics. Oversampling read-out logic for best sensitivity and excellent SNR; data exchange between real-time electronics and user PC via gigabit Ethernet, with option of online data analysis right during image acquisition
User PC	Workstation PC with ample RAM and HD memory space; ergonomic, high-resolution 16:10 TFT flat-panel display, many accessories, Windows VISTA OS (as available), multi-user capability
ZEN software	
LSM 710	Basic software, plus options: LSM Image VisArt plus, 3D Deconvolution, Physiology, FRET plus, FRAP, Visual Macro Editor, VBA Macro Editor, RICS Raster Image Correlation Spectroscopy (PMT & APD)
ConfoCor 3	Basic software, plus options: Extended models, global and interactive fit, and photon count histogram (PCH)

System Overview: ConfoCor 3 on LSM 710





Milestones of FCS Technology in Bioscience

1903 M. von Smoluchovski explains the interrelation between auto-correlation and Brownian movement.

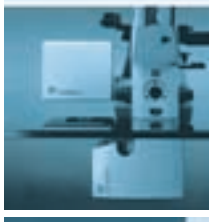
1972 First fluorescence correlation spectrometers are developed in the labs of Cornell University, Ithaca, USA and Max Planck Institute, Germany

1988 First confocal microscope set up for FCS measurements developed at Karolinska Institute in Stockholm, Sweden

1996 Carl Zeiss launches the world's first automated fluorescence correlation spectrometer, the ConfoCor 1.

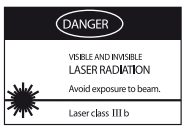
1999 Carl Zeiss designs the ConfoCor 2 for fully automated dual-channel cross-correlation spectrometry.

2000 Carl Zeiss combines the ConfoCor 2 with the LSM 510 to make up a platform which, for the first time, makes biophysical methods in cellular biology available to a wide range of users.



2005 Carl Zeiss introduces the ConfoCor 3, a module tailor-made for observing live cell processes.

2008 Carl Zeiss combines ConfoCor 3 and LSM 710 into a platform.



Working jointly with the LSM 710, the ConfoCor 3 now can bring its strengths to bear better than before.
Highest sensitivity without compromising dynamics. Intelligent algorithms for new interpretations of images. Tried-and-tested quality, continually redefined as technology advances. Quality by Carl Zeiss.

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