ConfoCor 3
Detection Module for
Fluorescence Fluctuation Analysis

Focusing on Single Molecules

Microscopy from Carl Zeiss

We make it visible.
Explaining diffusion processes and molecular interactions and their relation to cellular compartments and structures is essential in the detailed understanding of cell functions. In many cases it is not the average signal that is of interest but the behaviour of single molecules acting in an individual manner. Fluorescence-based assays at a single molecule level have revolutionised the way we can observe molecules at work in their natural surroundings. Many of these technologies are based on fluorescence fluctuation analysis (FFA) and include fluorescence correlation spectroscopy (FCS).

The new ConfoCor 3 Detection Module is much more than an imaging device. It not only allows you to observe single molecules non-invasively but also to resolve fast dynamic processes with great accuracy. To cap it all, fully automatic, computer-controlled routines mean it is also easy to use.
Milestones of the FCS Technology in Bioscience

Carl Zeiss has played a pioneering role in FCS. By working closely with leading scientists, we have repeatedly achieved significant breakthroughs and continue to devote significant resources to maintaining our market leadership in this field. Just some of many milestones in FCS:

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

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

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

1996 Carl Zeiss introduces the world’s first automated fluorescence correlation spectrometer, the ConfoCor 1.

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

2000 The age of biophysics dawns in cell biology with the ConfoCor 2/LSM 510 META, an innovative module that makes an ingenious technology available to a wide range of users.

2005 Carl Zeiss introduces the ConfoCor 3, a module tailor-made for observing live cell processes through its direct attachment to a channel of the LSM detection module.
Ideal for Sensitive Imaging and Fluctuation Analysis

Combine the ConfoCor 3 with a microscope from LSM 5 Family and you have the ideal symbiosis for sensitive imaging and fluctuation analysis. This combination of fast detectors and online data analysis enables you to resolve ultra-fast dynamic processes. The choice of APD or GaAsP gives you the greatest possible flexibility in balancing the requirements of maximum sensitivity and time resolution. Sites of interest can be easily spotted, marked and approached with great precision by the laser beam in a sequential manner. These are just some of the features that make the ConfoCor 3 a unique and fully automated sensor for studying mobile molecules, even at a single-molecule level.

The ConfoCor 3 from Carl Zeiss – a new benchmark in fluorescence fluctuation analysis.
Features
You Care about Your Specimen, We Take Care of the Technology

The ConfoCor 3 works like an extended arm of the LSM 510 in quantitatively investigating cellular processes. Just image your cell, select your measurement position and the system will provide you with all the parameters of interest in real time. Nothing could be easier.

Great precision
The scanning mirrors of the LSM 510 are used for precise positioning. Just take your image and point to your site of interest. Pixel-precise positioning allows you to target even sub-cellular structures.

Ultra-sensitivity
No need to compromise on sensitivity with the ConfoCor 3. You can follow the fate of single molecules in time and space.

Spectral resolution
Two well-balanced channels make efficient dual-colour experiments possible. Experience cross-correlation at its best in combination with the new family of C-Apochromat objectives.

Convenient reproducibility
Since all measurement tasks can be automated, you can define repeated measurements at different locations. As a result, you can look forward to significantly improved statistics.
Selection of measurement positions inside the nuclei of two HepG2 cells expressing an EGFP fusion protein of HNP1/H9252. Up to 99 consecutively approached positions can be defined in three dimensions. Multiple measurements can be triggered at each selected position.

Specimen: P. Henmerich, IMB Jena, Germany

Improving statistical significance. Consecutive correlation measurements can be easily averaged to obtain correlation functions with a better signal-to-noise ratio. Note that the averaged curve in yellow, in the example the average of 10 measurements, bears less noise than one single measurement displayed in red.
Fluorescence correlation spectroscopy (FCS) is a proven and powerful technology that enables you to gain new insights into dynamic cellular processes. The intrinsic read-out parameters – diffusion time, coincidence and concentration of molecules – and calibration-free measurements make FCS the ideal tool for quantitative analysis of the concentration, location, interaction and mobility of molecules.

**Investigating molecule distribution in cells**

The local concentration of proteins is responsible for triggering a variety of cellular events. The uptake efficiency of carrier molecules determines their usefulness in the delivery of cargo. Protein gradients represent growth clues. FCS can give you all the numbers you need for a quantitative description of such systems.

**Looking at diffusion in different ways**

The underlying principles of diffusion processes in cells may vary a lot. FRAP (Fluorescence Redistribution After Photobleaching) and FCS give you different perspectives on these processes. FRAP reveals long-distance relationships while FCS supplies you with the diffusion at the specific position of observation and can provide insights into underlying principles.

**Studying protein complex formations**

Integrating proteins into complexes may inhibit or activate protein functions. If you are interested in quantitatively linking changes in complex formation with activity, FCS is the ideal method.

**Detecting common movement**

Vesicles are the transport entities in cells delivering proteins into different compartments. Toxins, however, may take advantage of these pathways. If you want to determine the state at which sub-units separate and wreak havoc in the cell, just follow their fate with FCS.

**Quantifying receptor ligand interactions**

Binding ligands to their cognate receptors triggers off signal transduction pathways. Pharmaceutical drugs are often antagonists or agonists that interfere with or boost cellular responses. If you want to find out more about binding strengths, FCS is the technology you need.
Fluorescence recovery after photobleaching of eGFP (green), eGFP-WTRB (red), eGFP-MutRB (blue), and eGFP-H2B (lilac) in the nucleus of Rat-1 cells. The mean of at least 20 experiments +/- standard deviation is always shown. Relative fluorescent density shown in per cent.

Intracellular cross-correlation between A and B sub-units of endosomally localised cholera toxin in live cells.

Loss of intracellular cross-correlation between A and B sub-units of cholera toxin on separation in the GOLGI – also note their different diffusion characteristics.

FRAP time series in the nucleus of Rat-1 cells expressing GFP, H2B-GFP (histone H2B), and GFP-RB. A 2.9 µm x 2.9 µm area of the nucleus was photobleached for 0.688 sec with 100% transmission of 488 nm light from an argon laser. Images shown on the figure: pre-bleach (A and B, the white box in A represents the bleach region), immediately following the bleach (C), and at 1, 2, and 3 seconds post-bleach (D, E, and F, respectively). The colour code in images B-F indicates pixel intensity.

Fluorescence recovery after photobleaching of eGFP (green), eGFP-WTRB (red), eGFP-MutRB (blue), and eGFP-H2B (lilac) in the nucleus of Rat-1 cells. The mean of at least 20 experiments +/- standard deviation is always shown. Relative fluorescent density shown in per cent.

Specimen: S.P. Angus, D.A. Solomon, R.F. Henigan, E.S. Knidsen, University of Cincinnati, US

Specimen: K. Bacia, P. Schwille, Technical University Dresden, Germany
The ConfoCor 3 comes with a software that not only enables you to control the detection module but also makes a convenient analysis of measurement data possible. You can choose single measurements or software-controlled multi-measurements. Auto-correlation and cross-correlation are calculated in real time – at the same time as the current measurement. And you can opt to have the raw data for individual analysis. All these functions are distinguished by an automated ease of use that takes the time-consuming effort out of data analysis.

The basic software module provides you with all the necessary tools to analyse freely moving molecules. But you can also opt for advanced software modules that:

- Incorporate the most common methods (free diffusion, anomalous diffusion and flow) and provide for user-defined models
- Allow for global and interactive fitting with the possibility of defining start values and boundaries
- Provide for photo counting histograms.

Automated routines ensure you enjoy maximum reproducibility and statistical significance. And if all this is not enough, you can simply take the raw data and analyse them in any way you want.

Graphical interfaces for defining models (above) and programming routine tasks (right)
Data analysis
All data are acquired simultaneously.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Acquisition</th>
<th>Calculation</th>
<th>Display</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto-correlation</td>
<td>Analysis of interactions</td>
<td>1 or 2 channels</td>
<td>Multiple tau</td>
<td>Autocorrelation</td>
<td>Number of molecules, 2 diffusion times, 2 fractions, triplet time / fraction</td>
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<tr>
<td></td>
<td>One partner labelled</td>
<td></td>
<td>algorithm</td>
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<td></td>
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<td></td>
<td>Differentiation by diffusion properties</td>
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<td>Cross-correlation</td>
<td>Analysis of interactions</td>
<td>2 channels</td>
<td>Multiple tau</td>
<td>Cross-correlation</td>
<td>Number of molecules, diffusion times</td>
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<tr>
<td></td>
<td>Two partners labelled</td>
<td></td>
<td>algorithm</td>
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<tr>
<td></td>
<td>Differentiation by diffusion properties</td>
<td></td>
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</tr>
</tbody>
</table>

Biophysical models
Free diffusion model including triplet transitions and up to three components:

\[
G(\tau) = \frac{1 + \frac{T}{(1 - T)}e^{-\frac{\tau}{T_T}}}{N} \left( \sum_{i=1}^{M} \frac{y_i}{\left(1 + \frac{\tau}{\tau_{D_i}}\right)} \right) + 1
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>User-selectable values</th>
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<tbody>
<tr>
<td>( G(\tau) )</td>
<td>Correlation function</td>
<td>-</td>
</tr>
<tr>
<td>( \tau )</td>
<td>Correlation time</td>
<td>-</td>
</tr>
<tr>
<td>( T )</td>
<td>Fractional population of triplet state</td>
<td>Included / not included</td>
</tr>
<tr>
<td>( \tau_T )</td>
<td>Decay time of triplet state</td>
<td>Yes / no, depending on ( T )</td>
</tr>
<tr>
<td>( N )</td>
<td>Average number of fluorescent molecules in the detection volume</td>
<td>Result of fit</td>
</tr>
<tr>
<td>( M )</td>
<td>Number of fluorescent components</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>( y_i )</td>
<td>Contribution of i-th fluorescent component</td>
<td>( \sum_{i=1}^{M} y_i = 1 )</td>
</tr>
<tr>
<td>( \tau_{D_i} )</td>
<td>Translational diffusion time of i-th fluorescent component</td>
<td>Result of fit</td>
</tr>
<tr>
<td>( S )</td>
<td>Structure parameter describing length vs. diameter of confocal volume</td>
<td>( S = \frac{\omega_2}{\omega_1} )</td>
</tr>
<tr>
<td>( \omega_1, \omega_2 )</td>
<td>Distance from centre of beam focus in radial and axial directions at which the collected fluorescence intensity has dropped by a factor of ( e^2 ) compared to its peak value for Gaussian beam profile.</td>
<td>Result of fit</td>
</tr>
</tbody>
</table>

Fitting routine
Marquardt algorithm, a non-linear least-square algorithm using \( X^2 \) criterion.
The flexible modular design of the ConfoCor 3 allows you to adapt the system to your specific needs. Perfect interplay between the ConfoCor 3 and the LSM 510 turns the system into a fully integrated imaging and spectroscopy platform. An objective of unmatched performance delivers high resolution images. And the system’s compact design minimises light paths for even greater sensitivity.

The ConfoCor 3 module is directly attached to the channel 4 port of the LSM 510 (META) detection head. There are two detector types to choose from: APDs (avalanche photodiodes) or GaAsP PMTs (gallium arsenide phosphide photomultiplier tubes) for highest sensitivity and time resolution. New real-time electronics allows for fast data transport and processing. And in combination with the new generation of C-Apochromat objectives, the system is very suitable for dual-colour cross-correlation measurements.

### Filter wheels for advanced configuration

<table>
<thead>
<tr>
<th>Major beam splitter 24</th>
<th>IR-block filter wheel</th>
<th>Secondary beam splitter</th>
<th>Emission filter 1</th>
<th>Emission filter 2</th>
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</thead>
<tbody>
<tr>
<td><strong>Position</strong></td>
<td><strong>Type</strong></td>
<td><strong>Position</strong></td>
<td><strong>Type</strong></td>
<td><strong>Position</strong></td>
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<tr>
<td>1</td>
<td>NT 80/20</td>
<td>1</td>
<td>BG 39*</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>HFT 488/561/633</td>
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<td>none</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>HFT KP 700/488</td>
<td>3</td>
<td>KP 680 IR*</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>HFT KP 700/561</td>
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<td>Block</td>
<td>4</td>
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<tr>
<td>5</td>
<td>HFT 458/514</td>
<td>5</td>
<td>KP 610 IR*</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>HFT 458/561/633</td>
<td>6</td>
<td>Block</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>HFT 488/561</td>
<td>7</td>
<td>KP 575 IR*</td>
<td>7</td>
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<tr>
<td>8</td>
<td>HFT KP 650</td>
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<td>Block</td>
<td>8</td>
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<tr>
<td>9</td>
<td>NFT 490*</td>
<td>9</td>
<td>NFT 635</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>NFT 500</td>
<td>10</td>
<td>NFT KP 545*</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>NFT 515*</td>
<td>11</td>
<td>NT 50/50</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>NFT 545</td>
<td>12</td>
<td>AP (plate)</td>
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</tr>
<tr>
<td>13</td>
<td>NFT 565</td>
<td>13</td>
<td>Mirror</td>
<td>13</td>
</tr>
<tr>
<td>14</td>
<td>NFT 590*</td>
<td>14</td>
<td>KP 610 IR*</td>
<td>14</td>
</tr>
<tr>
<td>15</td>
<td>NFT 600</td>
<td>15</td>
<td>KP 575 IR*</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>Block</td>
<td>16</td>
<td>Block</td>
<td>16</td>
</tr>
</tbody>
</table>

*Light shaded filters are not included in the basic configuration*
**Microscope**

**Stand**
Inverted Microscope Axiovert 200 M

**Z drive nosepiece**
Coarse & fine z-focus; minimal step size of 50 nm; travel range 10 mm

**Z drive Piezo**
Travel range closed loop 250 µm; 70 steps / sec.; resolution 0.75 nm; step size & repeatability 5 nm

**Laser module**

**Lasers**
- Ar-Laser (458/477/488/514 nm, 30 mW); HeNe Laser (633 nm, 5 mW); HeNe Laser (543 nm, 1 mW), or a DSSP Laser (561 nm, 10 mW).
- Optional: HeNe Laser (594 nm, 2 mW); NLO, UV Ar-Laser (80 mW) oder 405 nm Diode Laser (30 mW)

**Dampening**
10-, 100- and 1,000-fold higher suppression of laser light by AOTF

**Shutters and Filters**
Line filters in front of the Argon laser and shutters in front of the HeNe and DSSP lasers efficiently suppress any unused laser light

**Electronics module**

**System control**
Control of Microscope, laser modules, scan modules and other accessory equipment; control of data exchange and synchronisation by real-time system.

**System computer**
High-end PC with extensive RAM and HD space, ergonomic high-resolution TFT flat screens, numerous accessories, OS Windows XP, multi-user environment

**Detectors**

**APD**
- Fibre-coupled, actively quenched avalanche photodiodes;
- sensitivity of detector for VIS range: 50 – 75% (dependent on wavelength);
- dark count rate: < 250 kHz; dead time: 50 ns (corresponding to 20 MHz time resolution for counted photons);
- pulse width: 35 ns; total After-pulse (100 – 500 ns): 0.3%

**GaAsP detectors**
- Direct coupled, cooled gallium arsenide phosphide photomultiplier tubes;
- sensitivity of detector for VIS range: 35 – 50% (depending on wavelength);
- transit time spread (TTS) 280 ps; FWHM of pulse < 25 ps

**Pinhole**
One pinhole motorised from channel 4, adjustable in x, y; z-position can be optimised by collimator setting; xy travelling range as projected into sample space: 7.57 µm; xy step size as projected to sample space: 15 nm

**Software**

**LSM 510**
Basic software plus options including Physiology, Multiple Time Series, Image VisArt, Deconvolution, 3D for LSM, Kinetics, FRET, Visual Macro Editor

**ConfoCor 3**
Basic software plus options including Extended Models, Global and Interactive Fit, and Photon Counting Histogram

**Lasers**

<table>
<thead>
<tr>
<th>Type</th>
<th>Wavelength</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV Ar Ion</td>
<td>351/364 nm</td>
<td>80 mW</td>
</tr>
<tr>
<td>Diode</td>
<td>405 nm</td>
<td>30 mW</td>
</tr>
<tr>
<td>Ar Ion, multiline</td>
<td>458/477/488/514 nm</td>
<td>30 mW</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 mW</td>
</tr>
<tr>
<td>He-Ne</td>
<td>543 nm</td>
<td>1 mW</td>
</tr>
<tr>
<td>DSSP</td>
<td>561 nm</td>
<td>10 mW</td>
</tr>
<tr>
<td>HeNe</td>
<td>594 nm</td>
<td>3 mW</td>
</tr>
<tr>
<td>HeNe</td>
<td>633 nm</td>
<td>5 mW</td>
</tr>
</tbody>
</table>
**System Overview ConfoCor 3**

- Laser module RGB (458, 477, 488, 514, 543/561, 594, 633 nm)
- Laser Enterprise II 653 (80 mW, 351 nm, 364 nm) UV Laser module
- 16:10 flat screen monitor 24"
- Control computer
- LCD TFT flat screen monitor 19"
- Upgrade kits LSM 510 to LSM 510 META
- System electronic rack
- VIS, VIS/UV, VIS/NLO or VIS/405 scan module LSM 510
- NLO kit for direct coupling
- Laser module V (with output coupling)
- VIS, VIS/UV, VIS/NLO or VIS/405 scan module LSM 510 META
- Option: Fiber decoupling channel 4
- Fluorescence Correlation Spectroscopy (FCS)
- Plug-in unit for external laser
- ConfoCor 3
- System table with breadboard Wide: 1000x750mm (1200x950 overall)
- System table with active absorption width 1800 mm, height 750 mm, depth 1400 mm
- System table with active absorption width 1200 mm, height 750 mm, depth 1000 mm
- Control computer
Several solutions for incubation will be offered.

- **Axiovert 200 M SP**
- **PIEZO objective focus**
- **2-axes control panel**
- **Scanning stage DC 120x100 with mounting frame for inverted stand**
- **Motor control MCU 28**
- **ConfoCor 3**
- **VIS, VIS/UV, VIS/NLO or VIS/405 scan module LSM 510 or LSM 510 META**
- **AxioCam HRm**
- **AxioCam HRc**
- **AxioCam MRm**

- **100 HAL illuminator with collector**
  - Halogen lamp 12 V 100 W
- **Switching mirror mot**
- **Transmitted-light channel for LSM 5**
- **Detection module external PMT for Non Descanned Detection**
- **Non Descanned Detection kit motorized NDD module with shutter**
- **Detection module external PMT for Non Descanned Detection**
- **HBO 100 illuminator with lamp mount and collector**
  - HBO 100 illuminator self-adjusting, with lamp mount and collector
  - Power supply unit for HBO 100
  - FluoArc variable intensity lamp control for HBO 100
  - X-Cite 120 fiber coupled illuminator
- **Detection module external PMT for Non Descanned Detection**
- **Non Descanned Detection kit motorized NDD module with shutter**