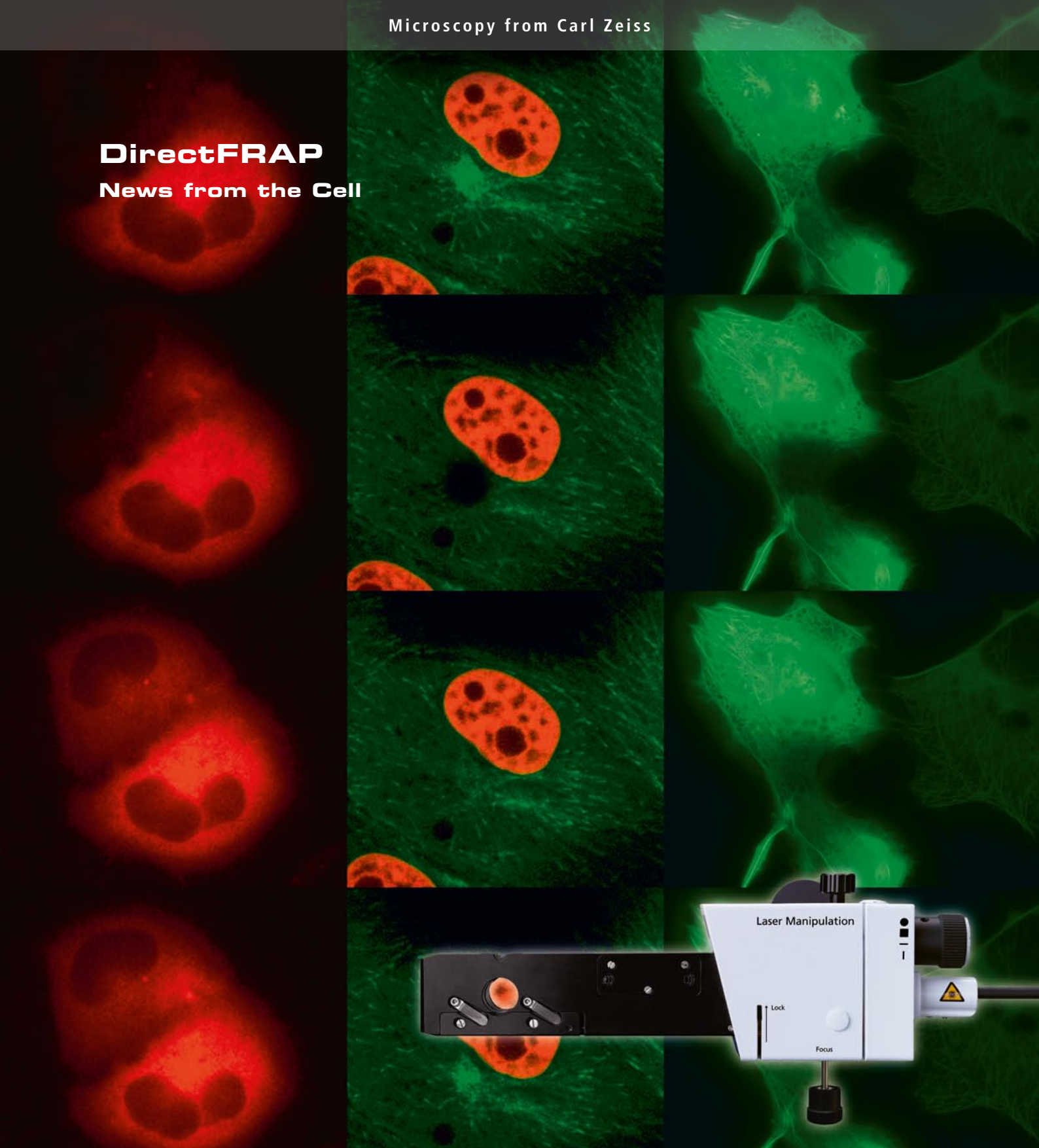


DirectFRAP
News from the Cell



The New Class of Laser Manipulation for the Analysis
of Cell Dynamics



We make it visible.

DirectFRAP. New Insights into Cell Dynamics.

Fluorescence breaks new ground: DirectFRAP from Carl Zeiss allows the manipulation of chromophores in living cells for the analysis of dynamic processes. In this context, highly dynamic processes can now be temporally resolved as a result of the advanced system design. This innovative technology provides scientists with more information on how cells function.

A new standard in the analysis of cell dynamics

With DirectFRAP, Live Cell Imaging has been extended by an important module. In addition to morphological examination, highly dynamic processes can now be analysed. DirectFRAP is the ideal solution for any type of laser manipulation in the living cell – regardless of whether one uses FRAP, FLIP, photoactivation or conversion. The advantage: DirectFRAP solves your problem precisely, economically and with a perfectly tuned workflow.

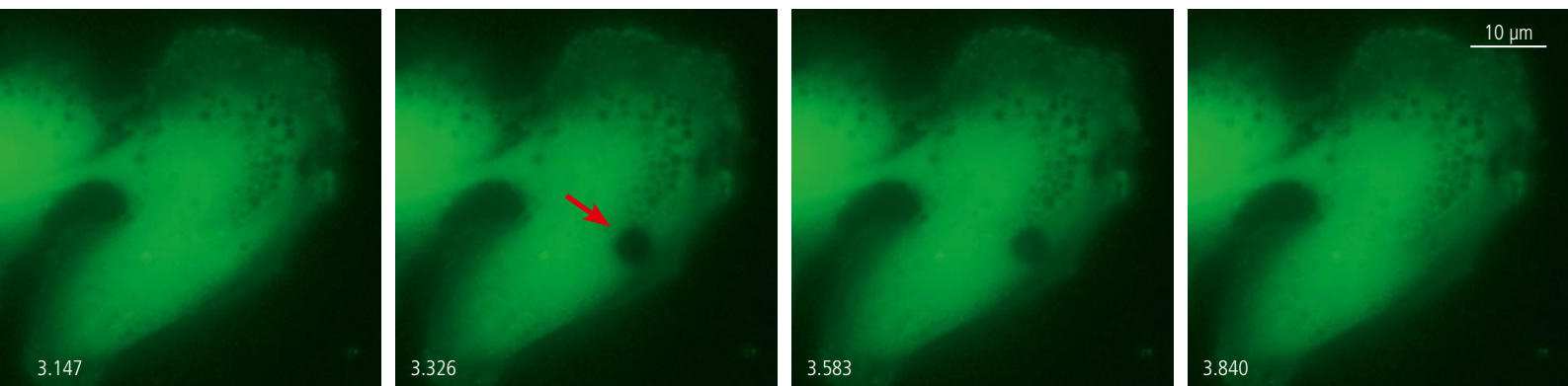
Maximum efficiency: simultaneous manipulation

The innovative aspect of this technique: the fluorophores are switched on or off at exactly the same time across the entire ROI by means of diaphragm imaging. For the shortest possible manipulation times in this context a system design has been implemented which stands out because it has the highest possible laser light yield in the selected sampling area. The diaphragms are perfectly tailored for all issues concerning dynamics including the

manipulation of small cell structures. An AOTF ensures the required precise switching of the laser pulse in the millisecond range.



The laser manipulation slider is easy to use: simply insert it in the laser port of Axio Observer.D1 or Axio Observer. Z1 (see image above right). Except for the integration of the laser safety kit, no additional changes to the microscope are required.



Bleaching of mutated cannabinoid receptor 1-GFP, freely diffusing in the cytoplasm of human astrocytes (U138MG). Pulse duration 50 ms with laser line 488, ROI diameter 5 µm, Plan-APOCHROMAT 63x/1.40 oil. Birgit Kraus, Institute for Pharmaceutical Biology, Regensburg, Germany

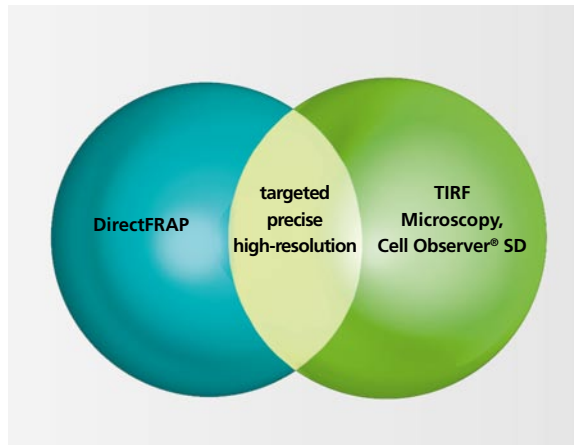


DirectFRAP Imaging System with incubation based on the Incubator PM S1.

Synergies for maximum cell information:

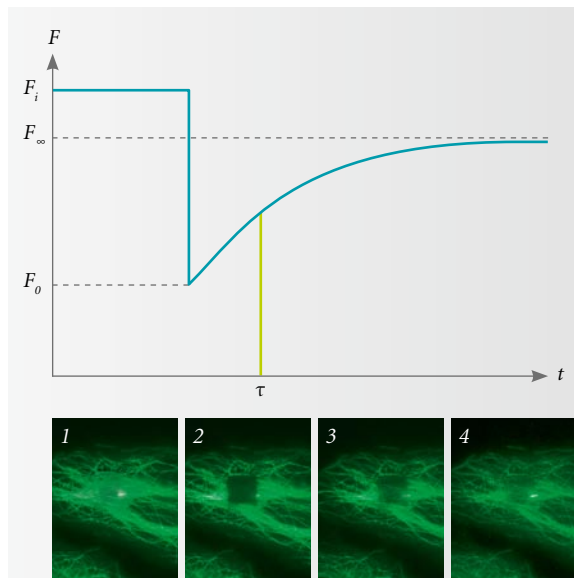
DirectFRAP in combination

A new performance dimension in Live Cell Imaging: the combination of DirectFRAP with sophisticated imaging techniques such as Spinning Disc or TIRF Microscopy. This combination allows an even more specific analysis of the processes, such as the observation of the intensity change in a single optical plane. In addition, one benefits from the higher image resolution in slower experiments by using combination systems. The results of both DirectFRAP as a single system and in combination allow comparison of the diffusion analyses – and so new insights.



DirectFRAP in system combination provides additional specific insights into the functions of the cell.

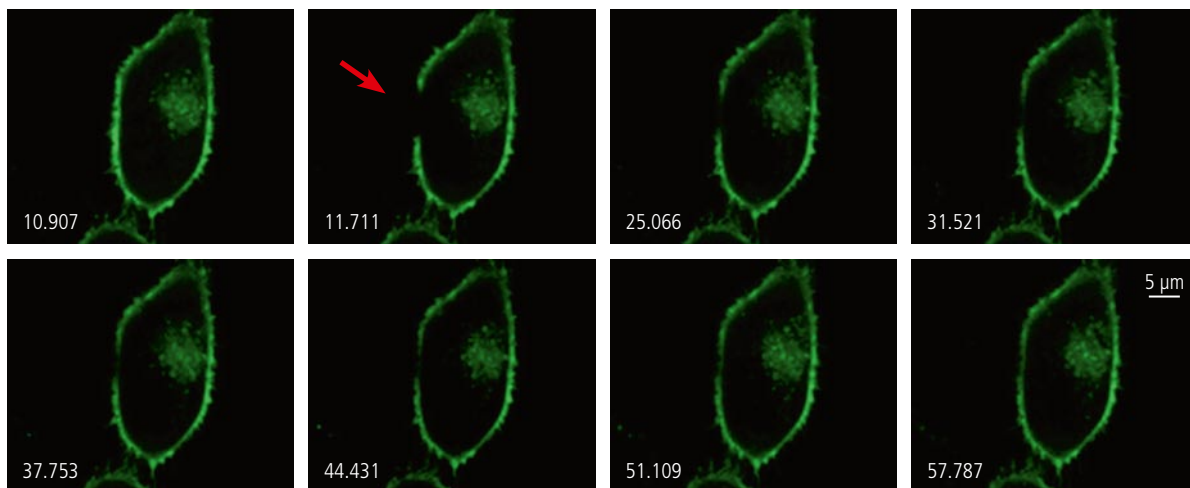
- Economical solution: DirectFRAP, Spinning Disc and TIRF system share the same laser, the same laser module and the same laser safety kit.
- Modular architecture: system combinations can be implemented successively.
- Extremely space-saving solution: the laser manipulation slider is simply inserted laterally into Axio Observer.
- Many options: there is a wide spectrum of incubators available. Additionally, different laser safety incubators can be selected for TIRF Microscopy.



Theoretical intensity curve of a FRAP experiment and the calculation of τ , the time for half-maximal recovery. The AxioVision Physiology module already displays the intensity curve online while the measurement is being made.

More Information from the Cell. DirectFRAP in Detail.

The requirements determine the technical standard. Because it is equipped with many intelligent functions, DirectFRAP provides highly reliable information rapidly so that any changes in the cell will be clearly visible.



DirectFRAP in combination with Cell Observer® SD (spinning disc). Bleaching of flotillin-2 EGFP in HeLa cells. Pulse duration 200 ms with laser line 488, ROI diameter 7 µm, Plan-APOCHROMAT 63x/1.40 oil. E. May, Bioimaging Center, University of Constance, Germany

Highly dynamic and precise:

A new standard for manipulation

Extremely brief manipulation times, simultaneous manipulation across the entire selected area, highly precise control of the laser pulses in the millisecond range by AOTFs, and the short acquisition times of widefield microscopy are the performance characteristic of DirectFRAP. The result is a system which can meet high dynamic requirements. It is even more convincing because of its brilliant image formation at high acquisition rates and a wide observation

field in fast experiments – typical for a widefield system. With its advanced technology it dispenses with many of the dynamic compromises of other systems, e.g. dependence of the laser intensity on the ROI size, iterations within the pulse, or slowed scanning due to efficiency elevation. The acquisition of the first image begins as early as 2 ms after the laser pulse. Thus, precise data and meaningful conclusions are ensured.

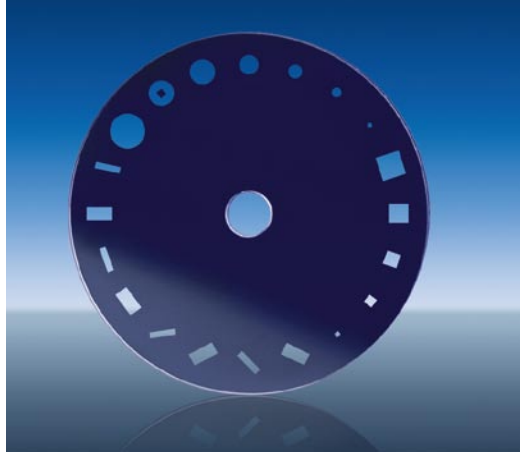
Combination options in overview

	Speed/Dynamics	Image resolution*	Application information
DirectFRAP	•••	•	ROI observation across the entire cell thickness
DirectFRAP + TIRF 3	••	•••	Targeted observation of the ROI in evanescent field using TIRF
DirectFRAP + Cell Observer® SD	••	•••	Targeted observation of the ROI in the selected z-plane by Spinning Disc

* Maximum resolutions are shown in comparison. In extremely fast experiments the resolution can be reduced.



At the front of the laser manipulation slider there is a beam combiner, e.g. 20/80 (epifluorescence/laser). This can be easily exchanged, e.g. for a special beam combiner of the user or a 100% mirror.



The diaphragm wheel in the laser manipulation slider provides exceptionally high flexibility. A decisive advantage: if another diaphragm is selected, the laser intensity per unit remains constant.



The operation of the laser manipulation slider is simple, even when the TIRF 3 slider is being concurrently used (at the front).

Flexible and unique: individual adaptation to your application

The system is designed for all standard fluorophores (see table). The beam combiner in the laser manipulation slider can be easily exchanged. DirectFRAP can thus also be flexibly used for future or very special applications. Imaging is optionally also possible during the laser pulse. A great advantage is the combination of DirectFRAP with other imaging systems such as TIRF or Spinning Disc microscopy. In this case only the desired intensity changes can be selectively analyzed or the manipulation of small structures such as vesicles can be observed individually. Overall, this system is a good investment with a large application spectrum.

More options: the diaphragm wheel

The diaphragm options were specially developed for dynamic experiments. The diaphragm wheel has 19 defined diaphragm positions and an open position. Circular and square ROIs can be photomanipulated from 1.5 to 20 μm . In this context, fine size graduations facilitate an optimum adaptation to the experiment. Two different rectangular diaphragms are available in four different orientations. An inverse diaphragm rounds out the selection.

Intelligent control with Axio Vision: comfortable and efficient

The module Physiology (from version 4.8) is available for DirectFRAP with the AxioVision system software. In this context, a configurable image of the DirectFRAP mask aids the user in positioning the specimen for manipulation and also assists in precise detection of the intensity changes in the bleach ROI. Pulse duration, pulse number, laser intensity are only a few of the many planning parameters. When required the laser pulse can also be triggered interactively. In the Physiology module the intensity changes in the ROI are already displayed online during the experiment. The advantage: you have complete control over your experiment and can rapidly intervene or optimize the conditions at any time as required.

Highest precision requires highest protection: the laser safety kit

Crucial for your safety: protection against laser beams. Carl Zeiss has developed an advanced laser safety which allows you to work without any impediments. The laser is ready for use only when all interlock switches respond positively. Complete laser safety is also guaranteed in the system with TIRF or Cell Observer® SD. A wide selection of laser safety incubators is available for TIRF applications.

Laser lines, filter sets and beam combiners

Application	Laser line ^{a)}	DirectFRAP ^{b)}	Combination with TIRF 3	Combination with Cell Observer® SD
Bleaching of GFP or Emerald	488	FS 74	FS 74	
Conversion of Dendra or Eos	405, 488, 561	FS 38, FS 82	FS 76	
Reversible on-/off switching of Dronpa	405 (on), 488 (off)	FS 83	FS 83	Beam path switch fast or beam combiner 80/20
Activation/conversion of PA-GFP/PS-CFP/Phamret	405, 488	FS 18 ^{c)} , FS 83	FS 83	

^{a)} Standard laser lines for manipulation are printed in bold. Alternative lasers for manipulation and imaging in system combinations are printed regularly

^{b)} An excitation filter wheel is necessary, except for bleaching of GFP or Emerald

^{c)} FS 18 is not necessary for PA-GFP

The Decisive Progress: New Applications for Cell Research.

Cell dynamics in detail: in photo manipulation DirectFRAP provides the decisive advantage in Live Cell Imaging. New insights and more information at all experimental levels allow even more solid scientific statements.

Observation, manipulation and analysis complement one another: precise registration of cell processes

With DirectFRAP you benefit from three factors: from the advantages of the widefield approach for fast processes and combination options, from the innovative concept for new insights in manipulation particularly for highly dynamic processes, and from short acquisition times for extensive data in the modelling of the underlying processes. Typical questions which can be clarified with DirectFRAP:

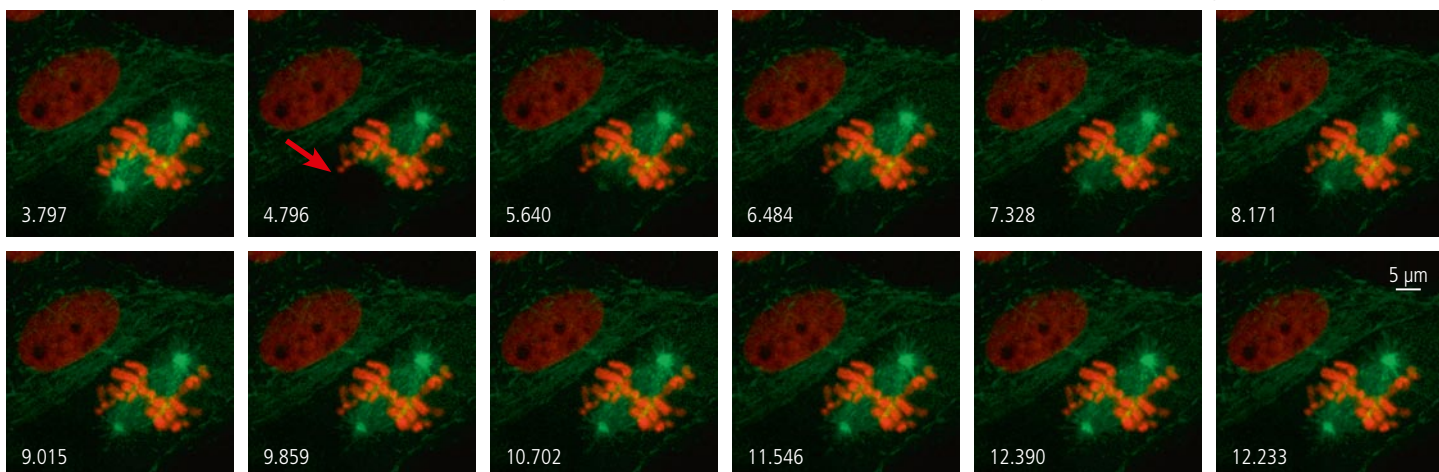
- Are there velocity limiting factors in diffusion?
Are there interactions of the target protein with macromolecules?
- Are there local and temporal variations in the architecture/composition of the cell compartment under consideration (spatial/temporal mapping)?
- What happens subsequent to photo manipulation of small cell structures? Does recovery occur at all; is there a directional dependence?

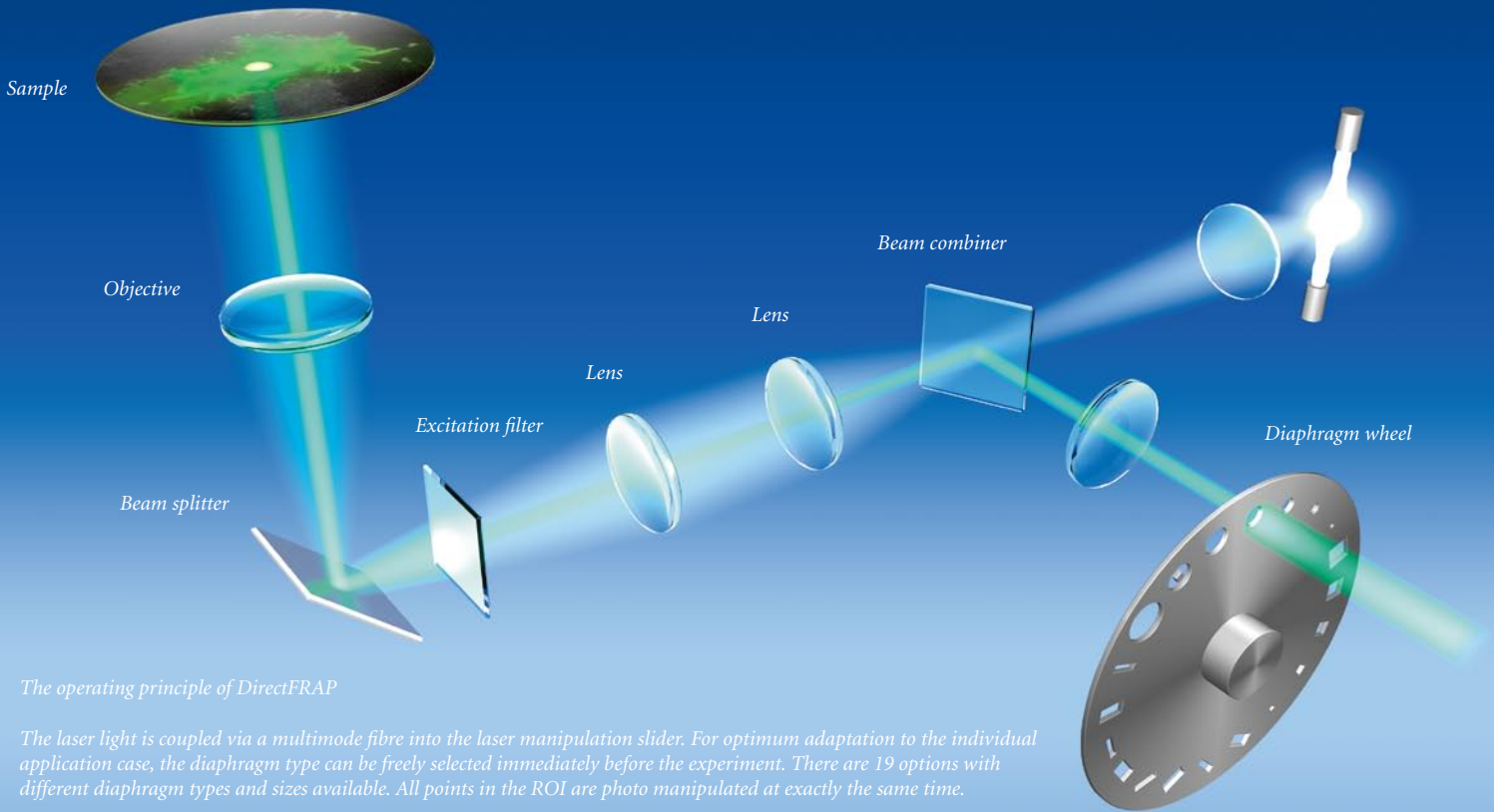
- From which compartment do the fusion proteins which are responsible for recovery come from?
- What are the diffusion coefficients and what is the size of protein complexes? What is the viscosity?

New perspectives: research areas for DirectFRAP

Dynamic processes can be considered differentially. In the next step the user can draw conclusions as to whether and how the cell intervenes in processes. At the same time, he or she recognizes the temporal dimension of the process. Furthermore, the reactions of the cell to altered situations can be studied. Thus, for example, the understanding of the signal cascade can also be extended. All together, the system makes important contributions to the investigation of life.

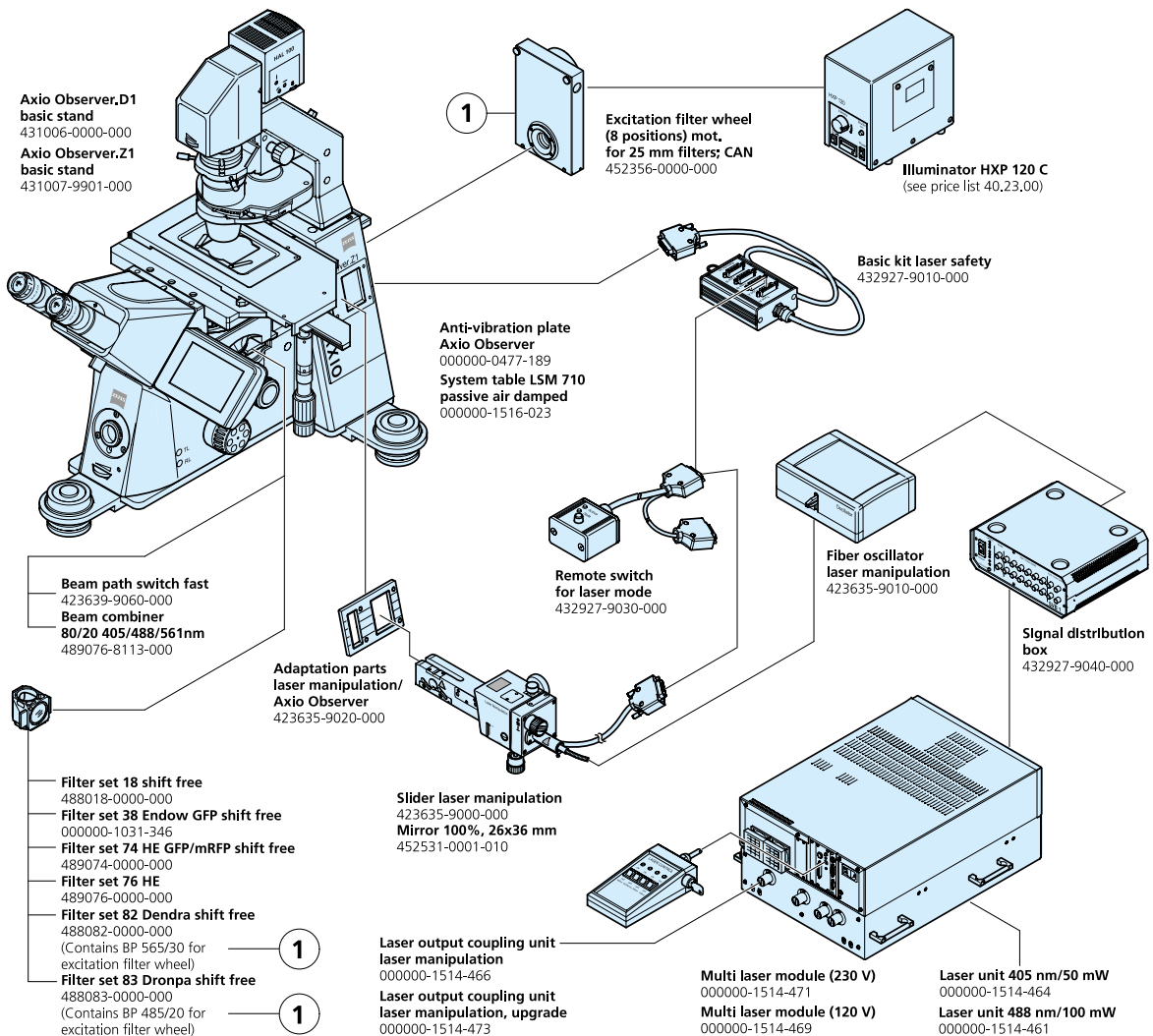
DirectFRAP in combination with Cell Observer® SD (Spinning Disc). Bleaching of EB3-Emerald (green) in LLC-PK1 cells, second staining: H2B-Cherry (red). Pulse duration 300 ms with laser line 488, ROI diameter 9,6 µm, C-APOCHROMAT 63x/1.20 W Korr UV-VIS-IR. Scott Olenych, DHMRI, Kannapolis, USA.





The operating principle of DirectFRAP

The laser light is coupled via a multimode fibre into the laser manipulation slider. For optimum adaptation to the individual application case, the diaphragm type can be freely selected immediately before the experiment. There are 19 options with different diaphragm types and sizes available. All points in the ROI are photo manipulated at exactly the same time.





DirectFRAP from Carl Zeiss. Performance at a Glance.

- Selective photo manipulation particularly for dynamic questions
- Simultaneous manipulation of all points in the ROI
- Exact time control of the laser pulse in millisecond range
- 19 different diaphragm options
- Combinable with Cell Observer® SD, Laser TIRF 3
- Ease of operation
- Intelligent laser safety for maximum protection
- Applications
 - Analysis of fusion protein diffusion
 - Interaction of a target protein with macromolecules
 - Isolated photo manipulation of small structures
 - Determination of dissociation constants
 - Analysis of protein synthesis, transport or compartmentalization
 - Determination of the size of protein complexes
 - Determination of viscosity

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