Definite Focus from Carl Zeiss

How to Avoid Drifting

Particularly, long-term experiments in Live Cell Imaging are often impaired or only possible to limited extent as a result of drifting in z accompanied by the loss of the original observation plane. The primary reason for this is the mechanical expansion of the components in the heat-up phase. Definite Focus from Carl Zeiss is able to rapidly compensate for this drifting. This is achieved by continuous monitoring of the distance between the objective and the culture vessel by means of infrared light and a corresponding correction in case of deviations. Definite Focus overcomes the disadvantages of previous approaches to drift reduction and additionally allows novel experiments, which e.g., require rapid temperature change.

Live Cell Imaging And Drifting in Z

The high resolution display of structures in the cell down to observation of individual molecules is at the focus of Live Cell Imaging today. Different contrast methods are used; fluorescence contrast is of primary importance. In this context LSM and TIRF microscopy extend the available options via improved resolution or a better signal-to-noise ratio, respectively. Multichannel time-lapse experiments, in which several different fluorophores in the cell are observed for a longer period of time, are typical. Incubation components for optimal live cell conditions, highly sensitive cameras for the detection of extremely weak signals, and, in part, very complex software modules for analysis of the data round out such an imaging station.

Conventional Methods of Optimisation

Relief is possible in some cases by means of extensive waiting periods prior to performance of the actual experiment, which take the thermal processes described into consideration. Other approaches provide for constant room temperature e.g. by shifting the experiments into disturbance-free periods of time, because repeated opening of laboratory doors can cause temperature variations and thus drifting.

Software-based autofocus systems use a completely different strategy. In this case image parameters such as contrast are assessed. Different z-planes are approached and the evaluation results compared. Subsequently, the plane that corresponds best to the evaluation criteria is brought into focus. However, two aspects of this are disadvantageous. The selected plane is not always identical with the desired z-plane. It is also problematical.

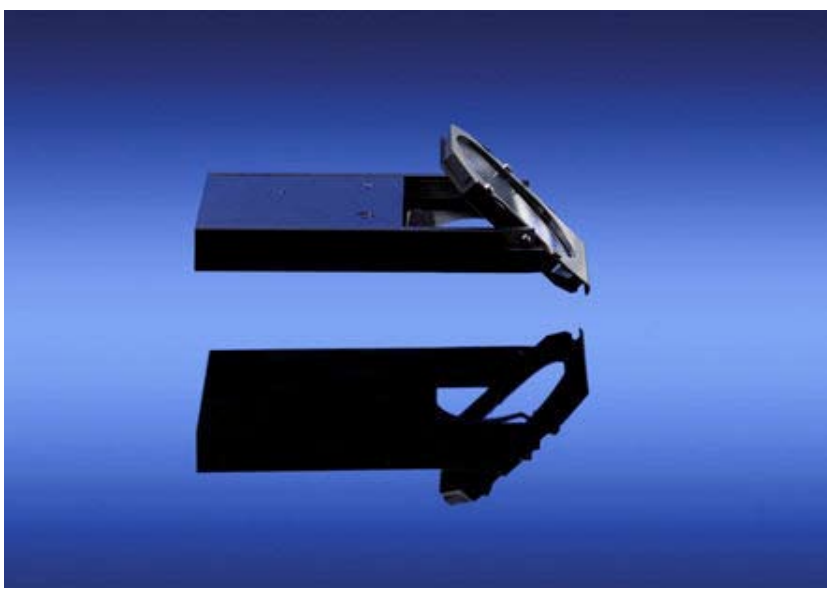

Fig.1: The beam combiner for Definite Focus is located in the nosepiece carrier.

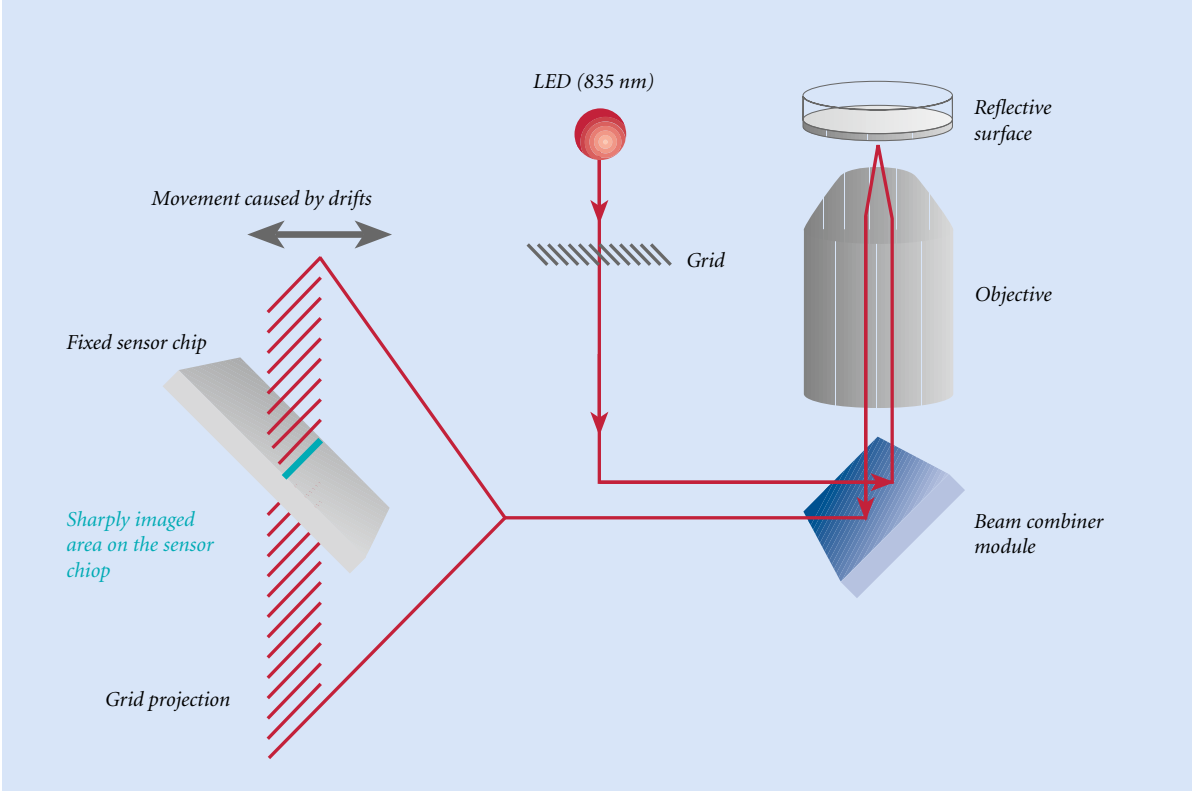

Fig.2: Definite Focus projects a grid onto the bottom of the culture vessel, where it is reflected onto a camera chip that is positioned at a slant. If there is a drift, Definite Focus returns the relative position of the grid projection back to the original state and thus counteracts drift effectively.

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Via the infrared light of an LED, which is coupled into the nosepiece carrier, a grid is projected onto the bottom of the culture vessel. The bottom acts as a reflective surface and reflects the image back onto a slanted camera chip. Due to the slanted position of the camera chip only a small area of the grid is well focused (fig. 2). In cases, involving drifting, the relative position of the grid projection to the camera chip changes. Definite Focus controls the Axio Observer.Z1's z-drive and corrects the position until the original grid area is again sharply depicted on the camera chip, i.e. until the original distance between the objective and that the bottom of the culture dish is again achieved. This occurs continuously or at defined intervals.

Instances of drifting are thus eliminated. Waiting for a temperature-stable state of all microscope components is no longer necessary. Definite Focus additionally exhibits an advantage in the evaluation of the infrared signal. Due to the large grid area observed, the algorithm can counteract possible disturbances due to scattered light, for example, from the culture vessel. The system is thus less sensitive than other, similar approaches to the correction of drifting in z.

Definite Focus is compatible with nearly all objectives and allows the use of all contrast methods. Plastic dishes and LD objectives can also be employed. Standard filter sets can be utilised for fluorescence. All the established fluorophores can be used. The wavelength of 835 nm, which is used, lies well outside of the range of excitation and emission spectra. Definite Focus is optimally integrated in the Axio Observer's control concept. It can be controlled via the TFT touch screen display and be used in time-lapse experiments by taking it into consideration in the experimental planning.

If the culture vessel is changed, Definite Focus can also be used as a focus finder. In this context a single activation of this function automatically positions the z-drive and thus the objective in the previous observation plane. Searching for the right z-plane, which is frequently very time consuming, is not required.

Fig 8: Definite Focus on the Axio Observer.Z1 from Carl Zeiss ensures focus stability and is compatible with all options of Live Cell Imaging.

Typical Applications and Perspective

Definite Focus can be used in nearly all applications involving Live Cell Imaging and overcomes the disadvantages of previous approaches in many situations. In particular, Definite Focus is advisable in long-term experiments which run for several hours because drifting then becomes increasingly noticeable. Applications with high power objectives are typical candidates because the image's shallow depth of field makes drifting more rapidly apparent.

It opens additional opportunities for novel experiments which were previously nearly impossible. Studies based on protein-folding mutants or on heat-shock experiments require, e.g., two different temperatures in the same experiment, whereby a rapid change is desirable. Naturally, rapid drifting in z occurs in the process. Definite Focus is able to react quickly to this.

Because of Definite Focus, incubation systems can focus more on sample conditioning than temperature conditioning of the microscope. Incubation setups which encompass the microscope will often be replaced by solutions which find adequate space on the microscope stage. The manual access to the entire system and its control are optimised.

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