AN IMAGE-BASED SYSTEM FOR REAL-TIME CORRECTION OF DEFOCUS IN OPTICAL MICROSCOPY

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Defocus severely reduces image sharpness in various optical microscopy techniques. Indeed, in wide-field methods, specimen-induced aberrations or thermal drifts of the microscope hardware can result in a walk-off between the focal plane of the detection objective and the sample. When imaging living specimens in a 3D environment, the sample itself may move along the optical axis, changing its focal position. The same problem can occur in light sheet microscopy, if the illumination light sheet goes slightly off the focal plane [1].

Two main autofocus approaches are used in optical microscopy. In contrast-based techniques, many images are collected at different focus positions: the sharpest one identifies the 'in-focus' position [1,2]. On the other hand, triangulation-based methods exploits the reflection of oblique light from a reference plane (usually the coverslip) to measure the distance between the objective and said plane [2,3]. Although the latter approach can afford real-time correction, it provides only the position of a reference, not the one of the real sample or of the light sheet.

Here, we took an optical principle commonly used in photography, i.e. phase detection, and adapted it to microscopy. In this approach, the rays passing through two distinct parts of the pupil are separated to form different images. Defocus results in a lateral shift between the two images. In a single camera exposure it is thus possible to measure the amount of defocus of the image and use this information to keep the microscope focused with a closed-loop control system.

This method provides a one-shot direct measurement of the focal position of the plane of interest, overcoming the limitations of current approaches. We demonstrate the capabilities of our technique in several applications, from long-term imaging of cell cultures to 3D tracking of living nematodes in a gel to high-resolution light sheet imaging of cleared murine brains. Notably, existing methods simply would not allow some of the experiments we show (as real-time autofocus of 3D moving objects) or would radically increase imaging time (as in light sheet microscopy). We anticipate that this novel autofocus approach will provide a valuable tool in the field of automated microscopy.

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