

Combining 4-D Microscopy, TIRF and FRAP in a Fast Livecell Imaging Microscope

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KEY WORDS: living cells, 4-D imaging, structured illumination, TIRF, FRAP

Structured illumination [1] has become a popular tool for 3-D sectioning of live biological tissue, not only because it provides an inexpensive, robust alternative to much more expensive laser-scanning-confocals, but also because of its significantly lower photo-damage compared to point-scanning techniques. Applied to thin or not heavily stained samples it would be the optimal choice if it could be made fast enough to turn a 3-D into a real 4-D tool.

Total Internal reflection Microscopy (**TIRF**), on the other hand, extends the range of 3-D sectioning into the nanometer range by allowing the study of processes occurring within nanometers of the substrate-membrane interface. Penetration-depth is wavelength-dependent and being able to easily control TIRF-angle and penetration depth would greatly facilitate TIRF experiments.

Fluorescence Recovery after Photobleaching (**FRAP**) is another important tool for probing live-cell function. It usually comes in conjunction with laser-scanning confocal microscopes.

We have combined all three functions into a single instrument, which allows **millisecond-switching between TIRF, FRAP and structured illumination** [2]. It uses the same mode-switching galvanometer for rapidly moving the grid-pattern over the sample. Unlike in commercial instruments, the grid is positioned in a apochromatically corrected plane of the microscope, hence no refocussing is required and the high speed of image acquisition (seven 3-D fps @ 1340 x 1040 pixel) is maintained in a multicolor-imaging mode, too.

The same (fiber-coupled) laser-sources can be used for TIRF and FRAP. A novel scan-head design allows positioning the laser-focus anywhere in the field (FRAP-mode) or in the backfocal-plane of the objective (TIRF). In TIRF-mode the sample can be illuminated with laser-light at any angle between 0° and the critical angle, and beyond the critical angle the (wavelength-dependent) TIRF-penetration depth can be adjusted.

REFERENCES

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- [2] Uhl, R., US-patent 11/558,122 „microscope device (Polytrope), (2007).