Measurement of protein mobility and interactions in living cells by 3PEA (P-991)

Facts

- Method suitable for all confocal laser scanning microscopes
- No additional hardware needed
- One method for accurate mobility measurements of fast and immobile proteins
- Fast determination of effective diffusion coefficients

Abstract
Fluorescence recovery after photobleaching (FRAP) is a widely used technique for measuring the mobility and interactions of fluorescently labeled proteins in living cells.
However, several limitations restrict its application: First, conventional FRAP has a rather low temporal resolution of ~100 ms, which prohibits measurements of faster processes. Second, current FRAP evaluation schemes cannot include spatial constraints imposed by the cellular environment on protein mobility. Third, FRAP in its present form ignores the sequential nature of the bleaching and image acquisition process.
In order to overcome the above-mentioned limitations of conventional FRAP, DKFZ researchers have developed a novel FRAP-based method called **3PEA** (**P**ixel-wise **P**hotobleaching **P**rofile **E**volution **A**nalysis).

Development Stage
The method is ready to use following successful testing. The diffusion coefficient of the red fluorescent protein (RFP) and the chromatin remodeling protein Snf2H was determined by 3PEA.

The Technology
Because of its low temporal resolution, FRAP can be used only for the determination of diffusion coefficients of rather slow particles and proteins. Techniques like FCS (fluorescence correlation spectroscopy) are needed for faster particles. Since the use of two different methods (e.g. FCS and FRAP) for one experimental setup limits the comparison and cross-validation of the generated results, it is desirable to determine fast and slow dynamics simultaneously in a single bleaching or correlation measurement.
3PEA extends the strength of FRAP in identifying slowly moving or immobilized particles by additionally including small length and time scales as well as mobility constraints imposed by the cellular environment. 3PEA exploits the inherent time structure of confocal images caused by the sequential nature of the pixel-by-pixel bleaching and imaging process. Protein mobility parameters are obtained by evaluating particle translocations occurring during the acquisition of a single image frame (Fig. 1).
The 3PEA results already acquired rely on the evaluation of a single bleach frame. Thus, the measurement can be conducted within less than one second.

Applications and Commercial Opportunity
The presented technology is thought to be suitable for use in all confocal laser scanning microscopes and would allow automated high throughput FRAP experiments due to its fast measurement time.

Inventors
The inventors are Fabian Erdel and PD Dr. Karsten Rippe, DKFZ Heidelberg, Germany.

Intellectual Property
Priority patent application “A method, system and computer program product for determining the mobility of fluorescent particles” EP2592412 as well as the PCT published as WO2013068113.

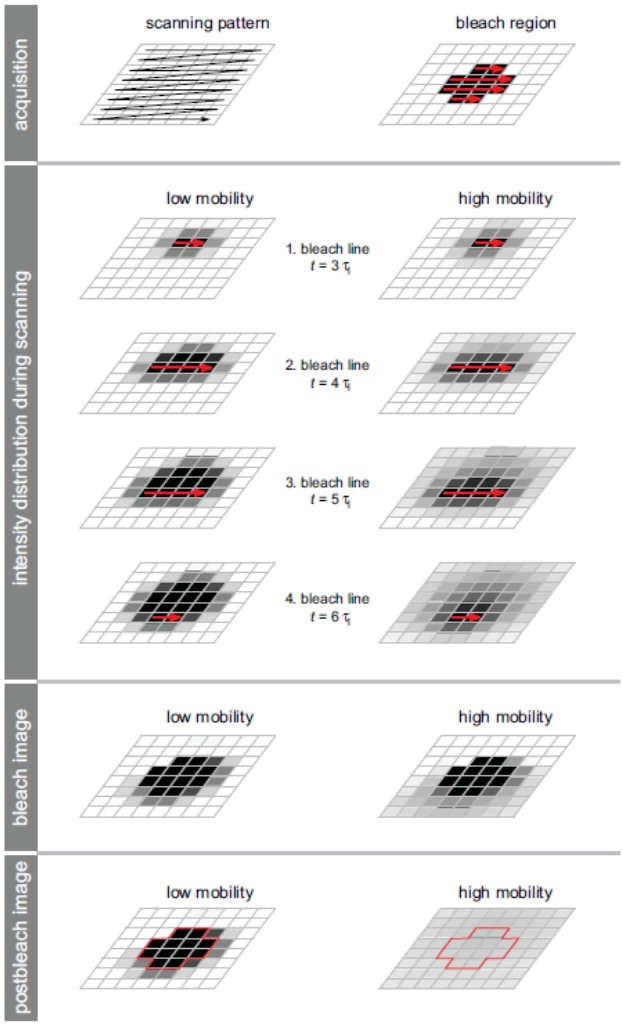

Figure 1: The 3PEA concept
The CSLM acquires and bleaches images via a pixel-by-pixel scanning process in a sequential manner. In the classical FRAP theory this is neglected. Typically, only the integrated intensity in the bleach spot is analyzed over time, starting with the first postbleach image shown at the bottom panel. In contrast, 3PEA explicitly calculates and fits the spatiotemporal intensity distribution generated by the sequential bleach process.

Scientific Reference
“Quantifying transient binding of ISWI chromatin remodelers in living cells by pixel-wise photobleaching profile evolution analysis.” by Erdel F, Rippe K. in PNAS 109(47):E32, 21-30 (2012).

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