Living up to Life

Leica MICROSYSTEMS

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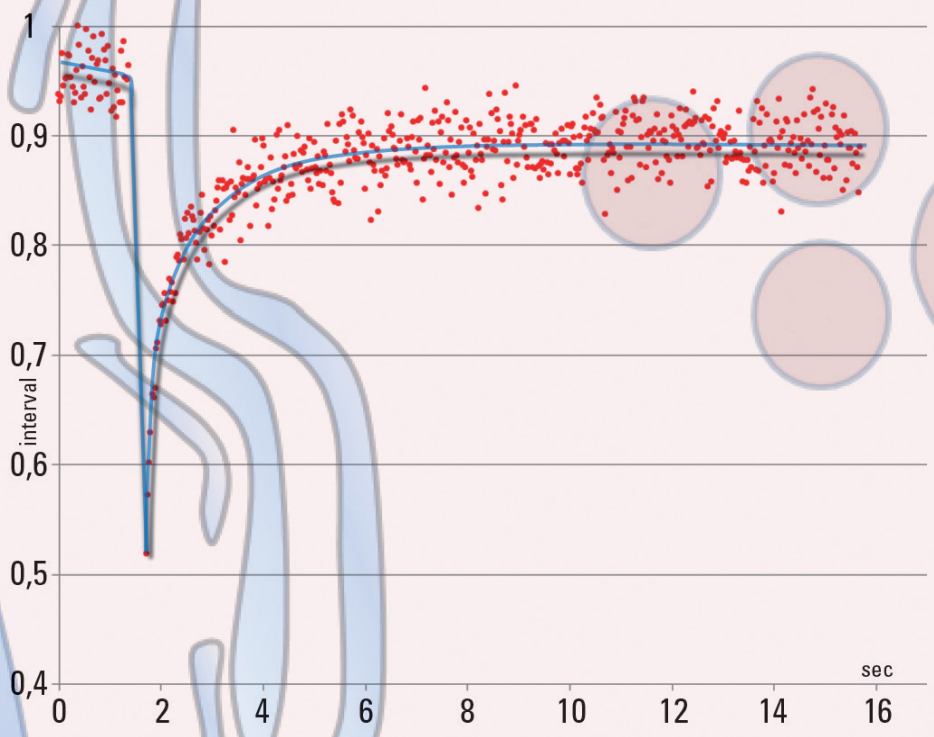
CONFOCAL APPLICATION LETTER

reSOLUTION

FRAP with TCS SP8 Resonant Scanner



General Safety Notes
The system and LAS AF may only be used by persons who have been trained in the use of the system and about the potential hazards of laser radiation.

Observe the user manual
Follow the safety notes and instructions in the user manual.

WARNING

Permanent eye and skin damage from laser radiation
Skin and eye damage can occur while using lasers if safety precautions are not taken. Pay particular attention to the laser safety.

FRAP with TCS SP8 Resonant Scanner

Fast FRAP experiments need a sufficient number of measurement points for meaningful interpretation and fitting analysis. To study very fast translocational processes, the use of a resonant scanner (RS) is preferred. The advantage in using FRAP with the RS is that statistics are much better in experiments that require fast acquisition: If the half time of recovery is about 0.5 sec you may have only about 3 to 4 data points using the conventional scanner, whereas with the resonant scanner you can get about 20 data points.

As a consequence, the time to apply the needed laser power is too short to bleach the region of interest efficiently, as the following numbers show: The resonant scanner acquires the images 20 times faster than the conventional scanner. The pixel dwell time in a 512 format at 400 Hz is about 1.4 µsec (0.4 µsec @1400 Hz) whereas the pixel dwell time at 8000 Hz is about 0.072 µsec (0.048 µsec @ 12 KHz). Comparing a typical FRAP experiment with 1400 Hz vs. 8000 Hz, there is approximately a factor of 5.5 (8 @ 12 KHz) less time to deliver the light to the ROI.

To compensate for the very short pixel time more laser power can be applied. But in almost all standard FRAP experiments which are performed with the conventional scanner, full laser power is already applied during the bleach interval. Hence alternative adjustments are needed. There are two variables affecting the effective light dose in the region of interest besides the actual laser power: The first is the zoom factor of the scanner and the second is the fill factor of the back aperture of the given objective.

Effect of Zoom In

Fig. 1 shows a FRAP experiment performed with a 63x lens at 1400 Hz and a circular ROI with a diameter of about 5µm. The zoom is increased from zoom 6 to zoom 30 to match the field of view to the 5 µm ROI for bleaching. A zoom factor of 5 delivers about 25 more power per area. With the conventional scanner, the full effect of Zoom In can be used. In the case of the resonant scanner, one has to take into consideration that the resonant x scanner cannot zoom in and out very fast. This is the reason why the Zoom In for the x scanner is blocked in the FRAP wizard. In consequence, even the maximum available laser power is often insufficient to bleach effectively. To compensate for the x scanner's inability to zoom fast enough we introduced the FRAP Zoomer for the SP8. The FRAP Zoomer uses the y scanner for zooming in, while the x scanner retains its scanning angle. Thus, Zoom In can be applied for bleaching within FRAP wizard and RS at least for one dimension.

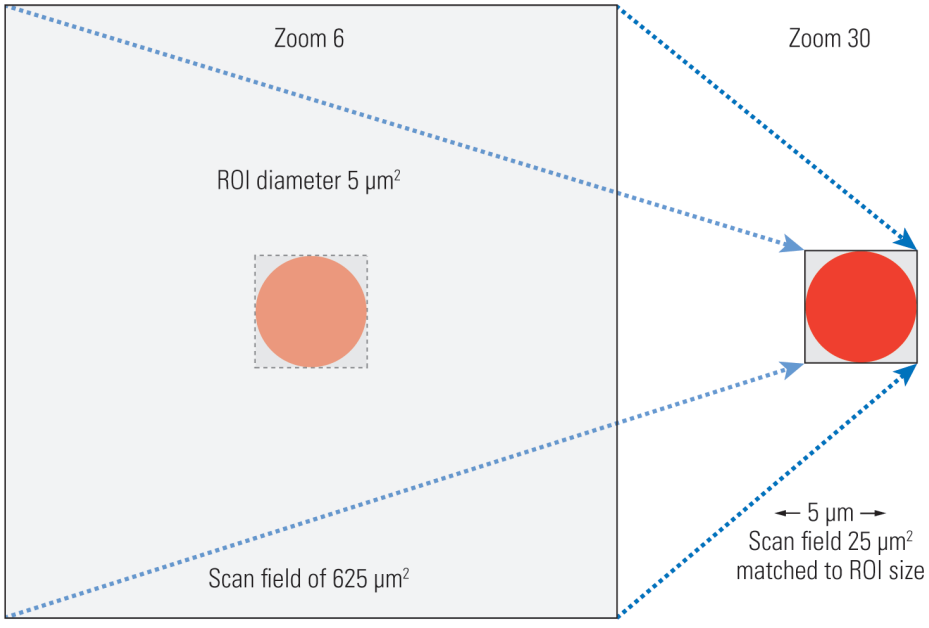


Fig. 1 The diagram illustrates how the combination of ROI with zoom delivers more light to the ROI.

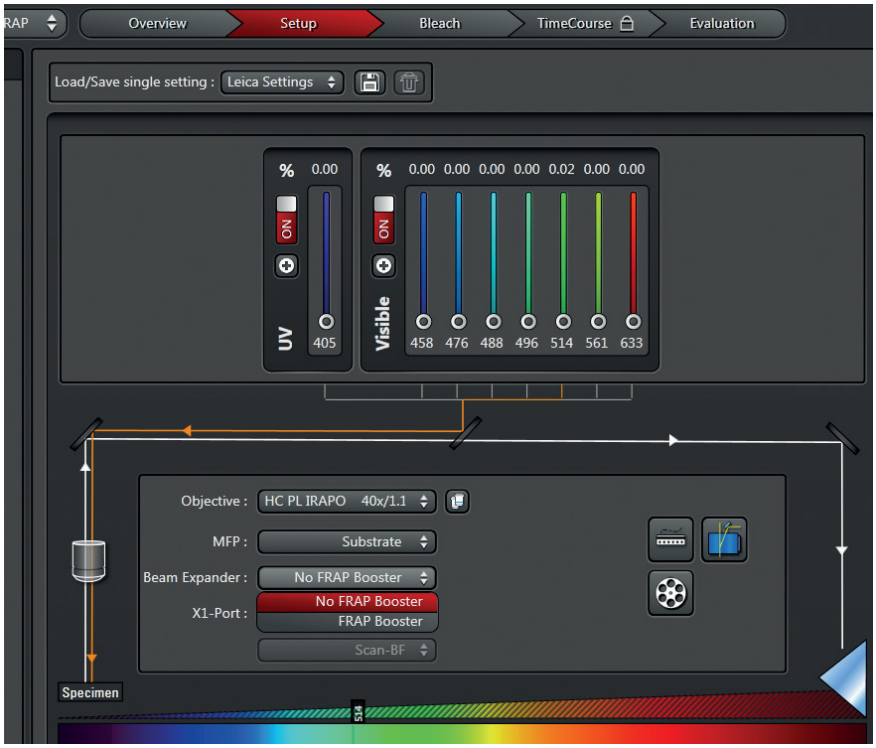


Fig. 2 User interface showing how to retract the beam expander (switch on the FRAP Booster) in the "Setup" step.

Impact of the fill factor of the back aperture of the objective

To compensate for the low bleach efficiency caused by very fast scanning it is helpful to change the fill factor of the back aperture of the objective. This can be done by retracting the beam expander optics (FRAP Booster) within the FRAP wizard. In the first step the beam expander can be retracted for the whole FRAP sequence (**Fig.2**).

If this option is active, the beam expander is retracted from the beam path. As a result, the back aperture of the objective is not completely filled with light anymore: The amount of light is the same but concentrated to a spot in the center, and about 2 to 5 times more light is available, depending on the objective (**Fig. 3 – 5**).

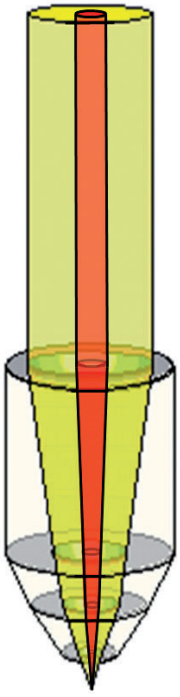


Fig. 3 The diagram illustrates how the pupil of the objective is filled in yellow, beam expander in position. The red color shows the more concentrated light with the retracted beam expander (FRAP Booster active).

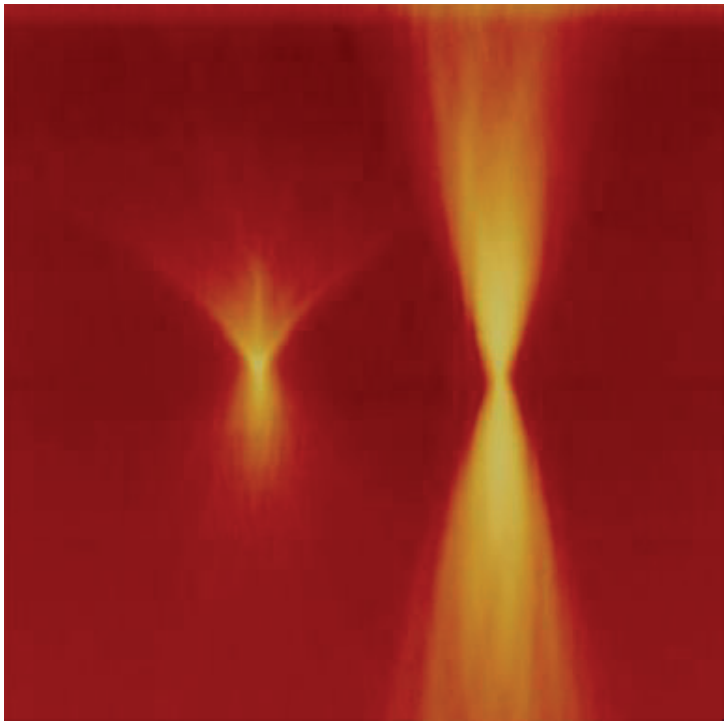


Fig. 4 Maximum projection of an xzy scan after beam park, Chroma fluorescent slide. Left: without FRAP Booster. Right: with FRAP Booster

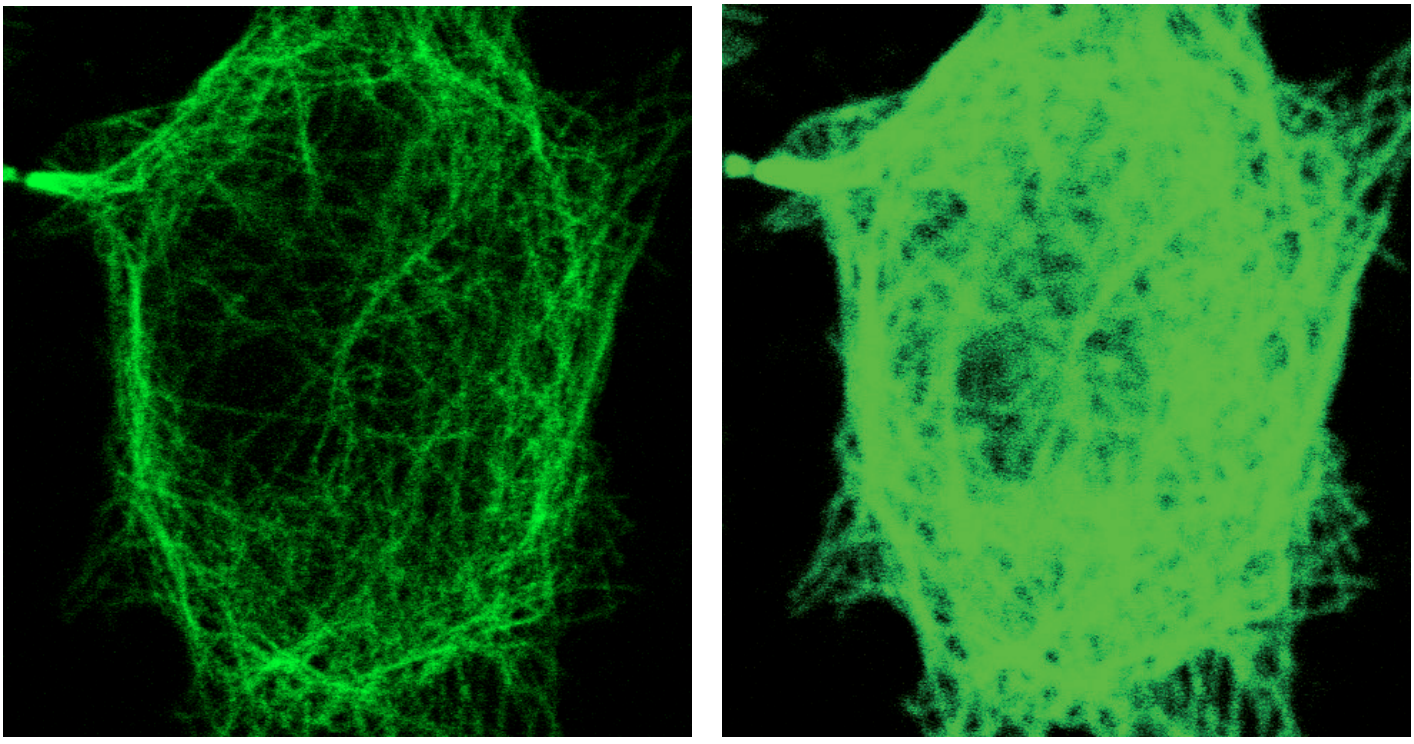


Fig. 5 Left: without FRAP Booster. Right: with FRAP Booster. The same laser settings were used for both images . GFP-Tubulin in HeLa cells, EMBL Heidelberg.

Fig. 6 to 8 is showing the results of FRAP experiments with the resonant scanner.

Fig. 6 Without FRAP booster, without FRAP Zoomer
Fig. 7 Without FRAP booster, with FRAP Zoomer
Fig. 8 With FRAP Booster and with FRAP Zoomer

HeLa cells with free YFP were used for the experiments. The FRAP series were taken with a 40x 1.1 lens and zoom factor of 8 was used during the whole experiment. The frame rate was about 36 fps, the bleaching time about 200 msec. If you need to bleach even faster, you may use all Ar-laser lines for bleaching.

It can be summarized that during FRAP experiments with RS it may happen that the time to apply the needed laser power is too short to bleach the region of interest efficiently. Then appropriate adjustments are needed to concentrate the available amount of light to a smaller area: The best bleaching results can be achieved by combining the FRAP Zoomer and the FRAP Booster.

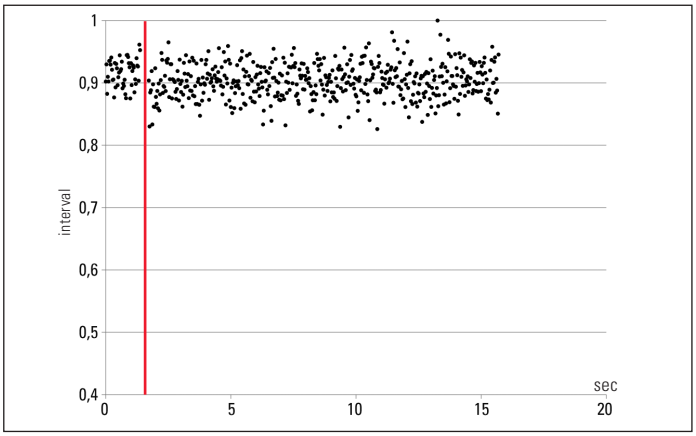


Fig. 6 FRAP experiment without FRAP Booster, without FRAP Zoomer. The red line indicates the bleach pulse.

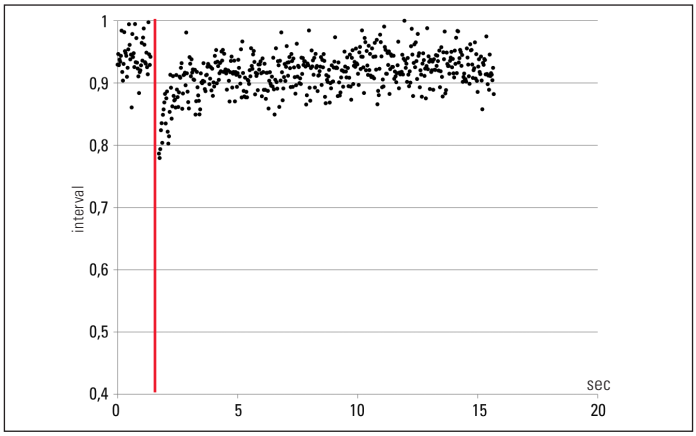


Fig. 7 FRAP experiment without FRAP Booster, with FRAP Zoomer. The red line indicates the bleach interval

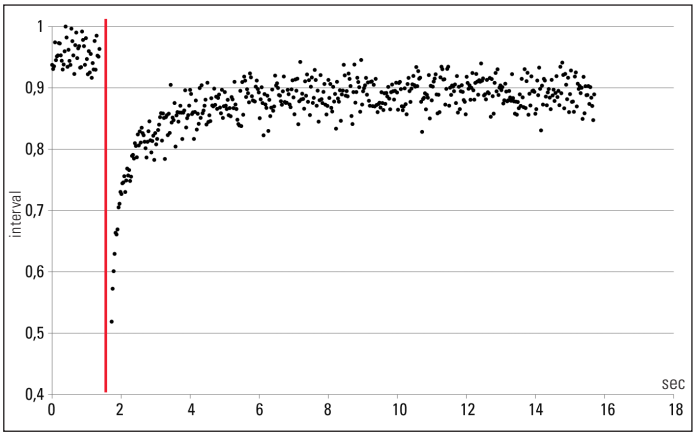


Fig. 8 FRAP experiment with FRAP Booster and with FRAP Zoomer. The red line indicates the bleach interval.

Your notes

Your notes

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