bh FLIM Systems Record Calcium Transients in Live Neurons

Abstract: We demonstrate the measurement of transient changes of the Ca^{2+} concentration in live neurons by Fluorescence Transient Lifetime Scanning (FLITS) and by temporal Mosaic FLIM. FLITS is based on the build-up of a photon distribution over the distance along a line scan, the times of the photons after the laser pulse, and the times of the photons after a periodic stimulation of the sample, temporal mosaic FLIM on the buildup of a photon distribution over the coordinates of a fast repetitive x-y scan, and the photon times after the laser pulses and the stimulation pulses. For the commonly used scanners the time resolution is about 1 ms for FLITS and about 40 ms for temporal mosaic FLIM.

Calcium Imaging

Ca^{2+} ions are involved in a large number of cell functions, such as intracellular transport, membrane potential, muscle contraction, gene expression, and cell differentiation. There is a wide variety of Ca^{2+} sensors [4, 5, 6] which change their fluorescence lifetimes with the Ca^{2+} concentration in their local environment. Most likely, the mechanism of the Ca^{2+}-dependent lifetime change is that the fluorophore has a Ca-bound and a Ca-unbound form of different fluorescence quantum efficiency and thus different fluorescence lifetime. The fluorescence lifetime of the bound form is higher than that of the unbound form. Consequently, the net fluorescence lifetime depends on the Ca^{2+} concentration. It can, however, happen that the fluorescence quantum efficiency of the unbound form is so low that the corresponding lifetime component is no longer observed. In that case, an intensity change but no lifetime change is observed [5]. This is the case for the Fluo sensors, as has been shown for Fluo-4 [2]. However, the traditional Ca^{2+} dyes, such as Calcium Green and Oregon Green, display large lifetime changes and work beautifully for lifetime-based Ca^{2+} measurement. An example of a Ca^{2+} image is shown is Fig. 1.

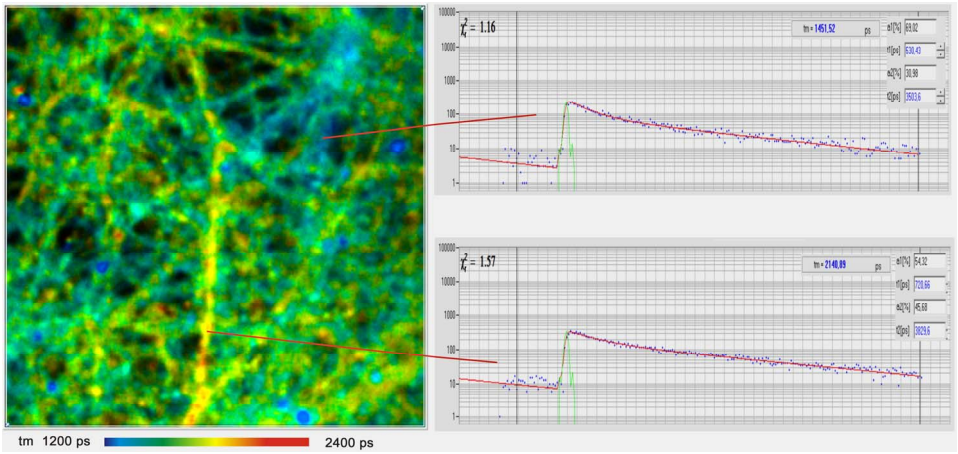

Fig. 1: FLIM image of cultured neurons stained with Oregon green OGB-1 AM. Colour range from τm = 1200 ps (blue) to 2400 ps (red). Decay curves of regions with low Ca (top) and high Ca (bottom) shown on the right. Data courtesy of Inna Slutsky and Samuel Frere, Tel Aviv University, Sackler School of Medicine.

The advantage of FLIM over intensity-based Ca^{2+} imaging is that absolute values of the Ca^{2+} concentration are obtained. This has been used to quantify calcium concentrations in astrocytes of live mice with cortical plaques by multiphoton NDD FLIM [3].

Recording Ca^{++} Transients in Live Neurons

The Ca^{2+} concentration in cells can change within remarkably short periods of time. Recording these 'Ca^{2+} transients' requires a time resolution in the range of less than 50 ms. It is thus usually considered impossible to record Ca^{2+} transients by fluorescence lifetime detection. However, Ca^{2+} transients can easily be recorded by using FLITS (fluorescence lifetime-transient scanning) or temporal Mosaic FLIM [1].

FLITS builds up a photon distribution over the distance within a line scan, the times of the photons in the fluorescence decay, and the time after a stimulation of the sample. The application to the recording of Ca^{2+} transients in live neurons on electrical stimulation has been described in [2]. A typical result is shown in Fig. 2. Hippocamal cultures were prepared from newborn rats and kept under physiological conditions for 12 to 18 days. The cultures were then loaded with OGB-1 AM. A Zeiss LSM 7 MP multiphoton microscope with a normal bh Simple-Tau 150 FLIM system was used to run the FLITS experiments. The cells were stimulated periodically at a fraction of the line clock frequency. To run the experiments, an intensity image was taken by the LSM 7 MP, and an appropriate location for the line scan selected. Then the LSM 7 MP was switched into the line scanning mode. The data acquisition in the FLIM system, the scanning in the LSM 7 MP, and the stimulation were started. The stimulation pulses of 1 ms duration were applied to the cell culture in intervals of 3 seconds. Data acquisition was continued over about 300 seconds, i.e. photons from about 100 stimulation periods were accumulated. The result is shown in Fig. 2, left.

To verify that the FLITS experiment did not cause cell damage or photobleaching a FLIM image was recorded after the FLITS experiment. It is shown in Fig. 2, right. It does not show any cell damage or photobleaching effects along the scanned line. It also shows that the Ca^{2+} concentration returned to the resting level, compare bottom of FLITS image on the left.

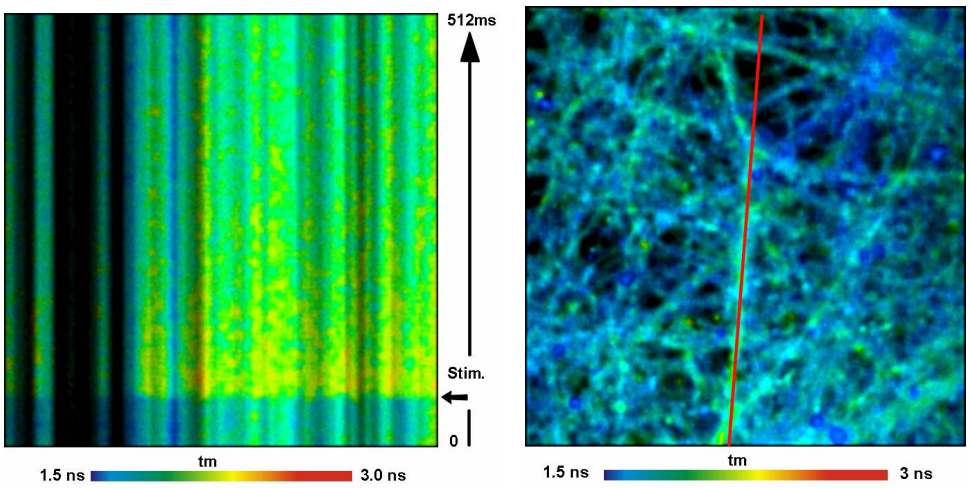

Fig. 2: FLITS of Ca^{2+} transients in live neurons. Left: FLITS image. Right: FLIM image taken after the FLITS recording. Red lines indicates position of FLITS scan. Data courtesy of Inna Slutsky and Samuel Frere, Tel Aviv University, Sackler School of Medicine.

A second way to record fast temporal changes in the fluorescence behaviour of a sample is temporal Mosaic FLIM. The technique records a photon distribution over the coordinates of a fast repetitive x-y scan, the times of the photons after the laser pulse, and the times of the photons after a stimulation of the sample [1]. The result is an extremely fast time series the signal-to-noise ratio of which depends only on the total acquisition time but not on the speed of the x-y scan. The technique

became possible by bh's 64-bit Megapixel technology which is able to record extremely large photon distributions [1, 7].

Ca^{2+} recording by temporal mosaic FLIM is shown in Fig. 3. OGB-1 AM was used as a Calcium sensor. The sample was stimulated electrically every 3 seconds, and 100 stimulation cycles were accumulated. A Zeiss LSM 7 MP was used for the experiment. With 64 x 64 pixels and a zoom factor of 5, the LSM 7 MP reaches a frame time of 38 ms. 150 milliseconds before every stimulation a recording through the entire 64-element mosaic was started. With the frame time of 38 ms, the acquisition thus runs through the entire mosaic in 2.43 seconds. The result shows clearly the increase in the fluorescence lifetime of the Ca^{2+} sensor in the mosaic elements 4 to 6, and a return to the resting state over the next 10 to 15 mosaic elements (380 to 570 ms).

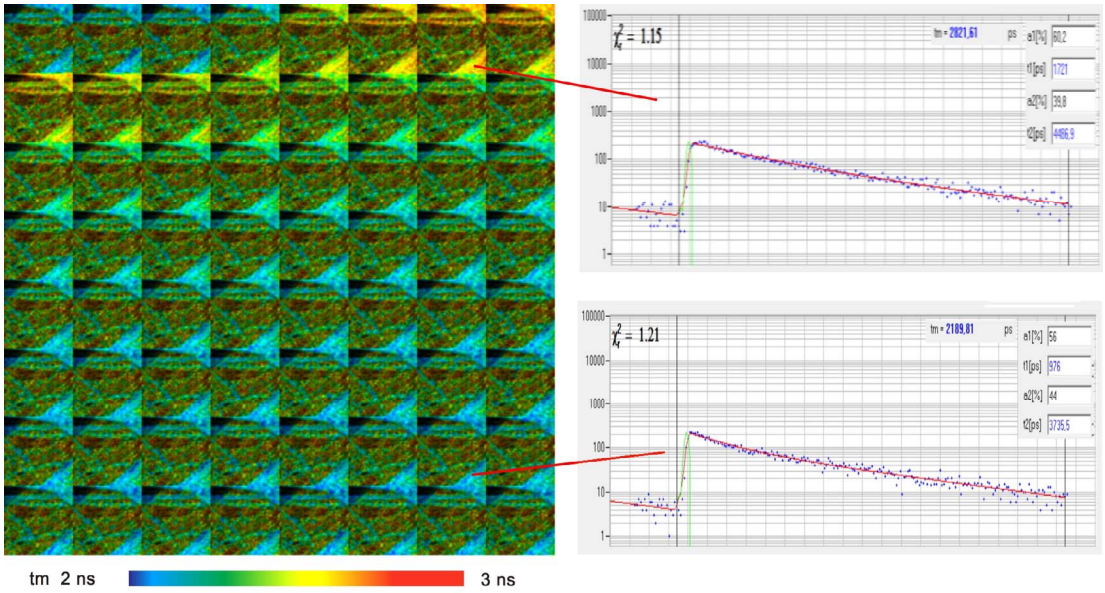

Fig. 3: Temporal mosaic FLIM of the Ca^{2+} transient in cultured neurons after stimulation with an electrical signal. The time per mosaic element is 38 milliseconds, the entire mosaic covers 2.43 seconds. Experiment time runs from upper left to lower right. Photons were accumulated over 100 stimulation periods. Recorded by Zeiss LSM 7 MP and bh SPC-150 TCSPC module. Data courtesy of Inna Slutsky and Samuel Frere, Tel Aviv University, Sackler Faculty of Medicine.

Conclusion

Ca^{2+} transients can be recorded by using the FLITS or the Mosaic FLIM functions of the bh TCSPC FLIM systems. The results are indendent of the spatially variable concentration of the Ca^{2+} sensor dye. Temporal changes in the Ca^{2+} concentration are recorded at a resolution of about 1 ms by FLITS and about 40 ms by Mosaic FLIM.

References

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