

SPC Modules Record FLIM with Bidirectional Scanning

Abstract: With Version 9.73 SPCM Software, the bh TCSPC / FLIM systems are able to record FLIM with bidirectional scanning. As usual, data recording is synchronised with the scanning by frame clock, line clock, and pixel clock pulses from the scanner. Each first line clock pulse indicates the beginning of a forward scan, each second one the beginning of a backward scan. The recording procedure automatically reverses the data from the backward scan and compensates for the line shift caused by the dynamic behaviour of the scanner. The FLIM data structure is the same as for unidirectional scanning. Thus, standard online intensity and lifetime display functions of the SPCM software are available, and data can be analysed by SPCImage as usual.

Bidirectional Versus Unidirectional Scanning

There are two different ways to raster-scan a sample in a laser scanning microscope. Unidirectional scanning uses a linear forwards scan and a fast ‘flyback’. Data are recorded only during the forward scan. Bidirectional scanning uses a linear scan in both directions, and records data both during the forward and the backward scan. The differences are shown in Fig. 1.

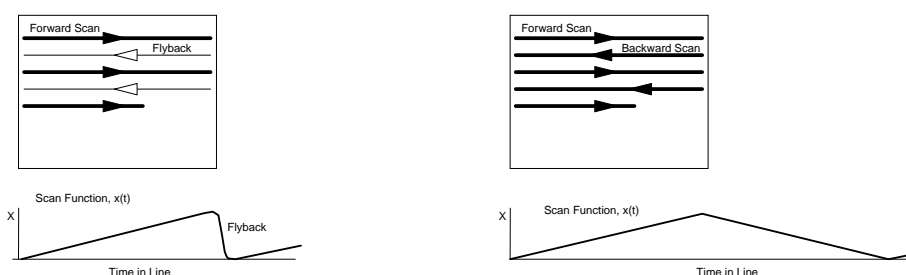


Fig. 1: Unidirectional scan (left) and bidirectional scan (right)

At first glance, a bidirectional scanning appears far more efficient than a unidirectional one. No time has to be wasted for the flyback, the acceleration forces acting on the scan mirrors are lower, and there is no need to turn off the excitation laser during the beam flyback. However, these advantages come at a price. Due to dynamic effects, there is a lag (or phase shift) of the scanner position behind the scanner control voltage. The result is a horizontal shift of the image data between the forward scan and the backward scan. The size of the shift depends on the scan speed and is thus not easy to correct. Moreover, the horizontal scan must be linear (or at least symmetrical) within 0.1% of the image size to avoid a mismatch of the forward and backward scan data. This is not easy to achieve, especially if high scan rates are desired. A unidirectional scan does not have these problems. The scanner lag does not matter and a much larger nonlinearity can be tolerated. The beam flyback can be made much faster than the forward scan. Even for the fastest scan rates, it normally requires no more than 10% to 20% of the line time [2]. The speed advantage of a bidirectional scan is therefore smaller than commonly believed. Nevertheless, almost all laser scanning microscopes and a number of clinical scanning systems have a bidirectional scan implemented, or offer it at least as an option. A FLIM system attached to these systems should therefore be able to record data with a bidirectional scan.

Implementation in the SPCM Software

FLIM recording by bidirectional scanning has been implemented in SPCM software, version 7.93. It is available in the 'FIFO Imaging' mode, see Fig. 2, left. The scan parameter definitions are shown in Fig. 2, right.



Fig. 2: SPCM system parameters for bidirectional scanning. Left: Measurement control section of system parameters, 'FIFO Imaging' Mode is selected. Middle: Page Control section, Image pixels X and Y should be set according to the pixel format of the scanner. Right: More Parameters panel, accessible by clicking on 'More Parameters' in the SPCM system parameters. Bidirectional scanning is selected by 'Scan Type' = Bidir, the line shift is corrected by 'Left Border' and 'Right Border'

Operation Mode must be 'FIFO Imaging' [1, 4], see Fig. 2, left. 'Image Pixels X' and 'Image Pixels Y' (Fig. 2, middle) should be set to the number of pixels in the microscope scan. The parameters which synchronise the FLIM recording with the scanning are defined in the 'More-Parameters' panel (click on the 'More Parameters' button to open it). To activate bidirectional recording, set 'Scan Type' to 'Bidir'. 'Second Line Clock' must be set to 'Use'. The line clock at the beginning of the backward scan then starts the recording of each second line, and reverses the direction of recording from 'forward' to 'backward'. The 'Left Border' and 'Right Border' parameters delay the recording of the data within each line by the defined number of pixels. The borders are used to correct for the line shift caused by the lag of the scanner. The correct values have to be determined experimentally. Please note that the scanner lag (and thus the values of the parameters) depend on the scan speed. You may thus need different scan parameter sets for different scan speeds. Please remember that you can put these setups in the 'Predefined Setups' panel, from where they can be loaded by a single mouse click.

The other parameters are the same as for unidirectional scanning. 'Polarity' defines the polarity of the scan clocks, the line and pixel clock predividers bin several lines and pixels into a single line or pixels of the result. Use divider settings of 1 for bidirectional scanning. Please refer to [1] for details.

Results

FLIM data recorded by bidirectional scanning are shown in Fig. 3, left and right. The data were recorded by a bh Simple-Tau 152 FLIM system [1], with two SPC-150 TCSPC FLIM modules, in combination with a Zeiss LSM 880 [3]. The sample was excited by the 445 nm ps diode laser of the LSM880, the fluorescence was recorded via the confocal port of the scan head. The image on the left shows a convallaria sample scanned with 512x512 pixels, the image on the right a BPAE cell sample scanned with 1024x1024 pixels. Both were calculated by the online-lifetime function of the

SPCM software [5]. No double structure or blurring of details is visible, showing that the data from the forward scan perfectly match those from the backward scan.

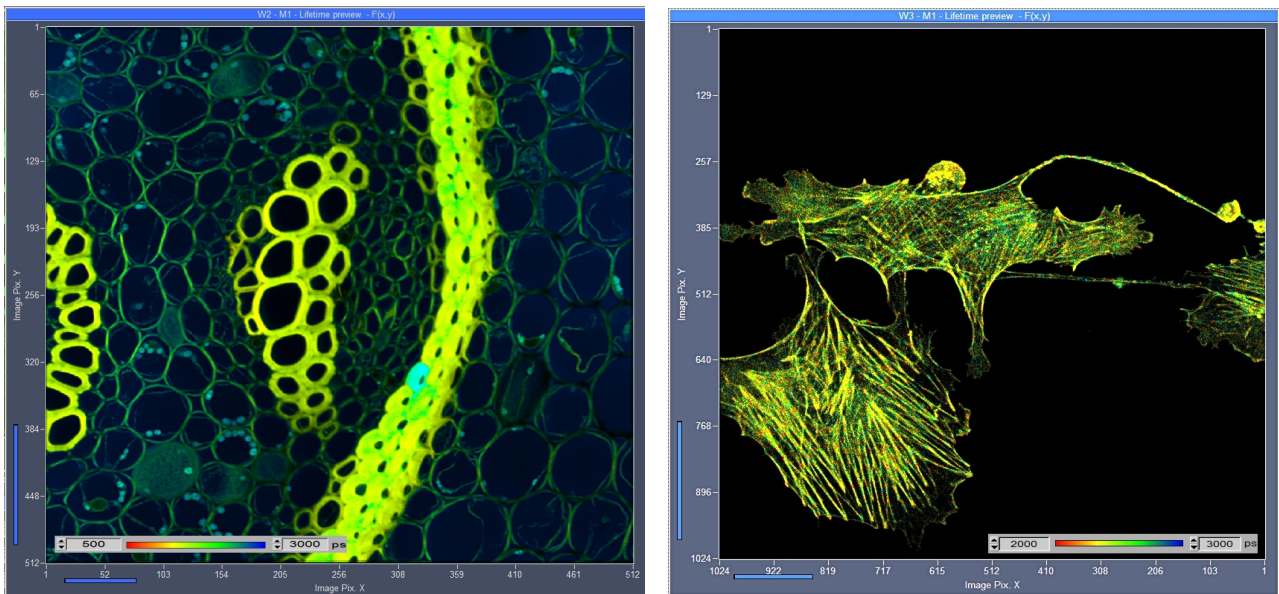


Fig. 3: Convallaria sample (left, 512x512 pixels) and BPAE Cell sample (right, 1024x1024 pixels), recorded with bidirectional scanning. SPC-150 TCSPC module, Zeiss LSM 880 in bidirectional scan mode. One-photon excitation at 445 nm. Images from online-lifetime function of SPCM software [5].

Fig. 4 shows the Convallaria data of Fig. 3, left, analysed by bh SPCImage FLIM data analysis software. No special adjustment was used, the data were just analysed as described in [1, 2, 3].

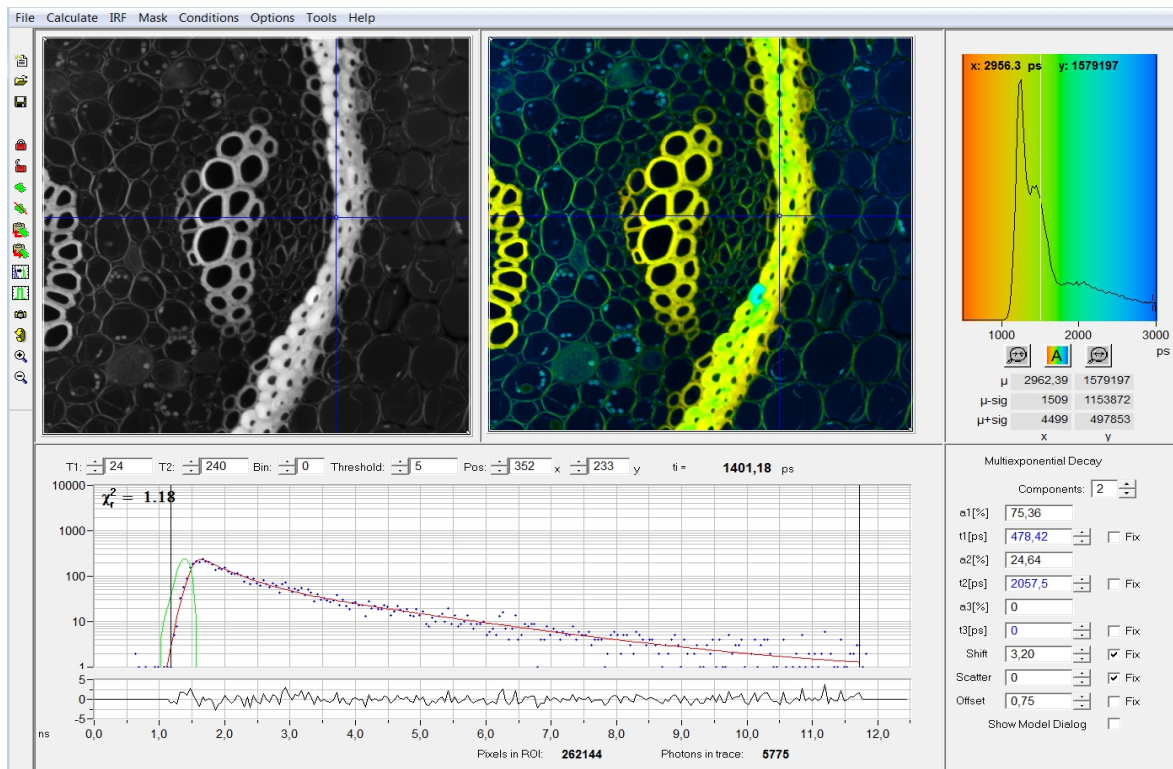


Fig. 4: Convallaria sample data as in Fig. 3, left, analysed with SPCImage. Double-exponential decay model, intensity-weighted lifetime of decay components.

Summary

FLIM data recording in combination with bidirectional scanning became available with Version 9.73 of the bh SPCM software. The data are recorded in the FIFO imaging mode [1, 4], the synchronisation of the recording with the scanning is defined in the 'More Parameters' panel of the SPCM system parameters [1]. Bidirectional recording works for all bh TCSPC modules which have the FIFO Imaging (software accumulation) mode implemented. That means the function is available for all SPC-150, SPC-150N, SPC-160 and SPC-160pcie modules, and for SPC-830 modules manufactured later than May 2007 [4]. The structure of the data recorded is the same as for unidirectional scanning, and the same pixel numbers and time channel numbers can be achieved [6]. The online intensity [1] and lifetime image [5] display functions of the SPCM software are available, and the recorded data can be analysed by bh SPCImage FLIM data analysis software as usual [1, 2, 3].

References

1. W. Becker, The bh TCSPC handbook. 6th edition, Becker & Hickl GmbH (2015), available on www.becker-hickl.com
2. Becker & Hickl GmbH, DCS-120 Confocal Scanning FLIM Systems, 6th ed. (2015), user handbook. www.becker-hickl.com
3. Becker & Hickl GmbH, Modular FLIM systems for Zeiss LSM 710 / 780 / 880 family laser scanning microscopes. 6th ed. (2015), user handbook. available on www.becker-hickl.com
4. Becker & Hickl GmbH, FLIM in the FIFO Imaging Mode: Large Images with Small TCSPC Modules. Application note, available on www.becker-hickl.com
5. Becker & Hickl GmbH, SPCM Software Runs Online-FLIM at 10 Images per Second. Application note, available on www.becker-hickl.com
6. H. Studier, W. Becker, Megapixel FLIM. Proc. SPIE 8948 (2014)