DCS-120 FLIM System Records X-Y Mosaics

Abstract: Starting from software version 9.76, the bh SPCM data acquisition software controls a motorised sample stage. In combination with the bh DCS-120 FLIM scanning system, the stage can be used to record mosaics of FLIM images. The system scans an image at one position of the sample, then offsets the sample by the size of the scan area, and scans a new image. The process is repeated, combining the data of the individual scans into a single, large x-y-t data set. Images covering an area of several mm diameter can be obtained without the need of using low-magnification and low-NA objective lenses.

Principle

With software version 9.76, the control of a motorised sample stage has been integrated in the bh SPCM TCSPC/FLIM data acquisition software. In combination with the Mosaic FLIM function of SPCM, the sample stage can be used to record arrays of FLIM images with the bh DCS-120 confocal and multiphoton FLIM systems [3]. The recording process of Mosaic FLIM is illustrated in Fig. 1. The basic principle is similar to the normal TCSPC FLIM procedure [1, 2]. However, memory space is provided not only for the photon distribution of a single image but for the elements of the entire mosaic. Recording starts in the data space of the first mosaic element. After a defined number of frames, the sample is shifted by the size of the scan area, and the recording is continued in the next data element. The result is a FLIM data array that contains all elements of the mosaic. The data structure is the same as for a single FLIM image with a pixel number similar to the total pixel number of the mosaic.

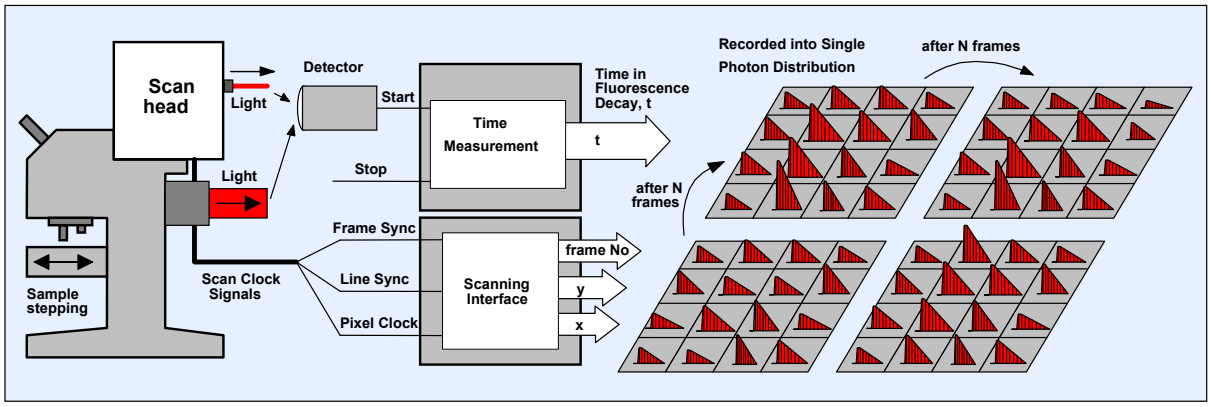

Fig. 1: Mosaic FLIM, recording of a X-Y mosaic

Example

An example of a Mosaic-FLIM image is shown in Fig. 2. The image was recorded by a DCS-120 MP (multiphoton) system in combination with a bh SPC-160 TCSPC system [1]. The mosaic has 4 x 4 elements, each element has 512 x 512 pixels with 256 time channels. The complete mosaic has thus 2048 x 2048 pixels, each pixel containing 256 time channels. The sample area covered by the mosaic is 0.8 mm x 0.8 mm.

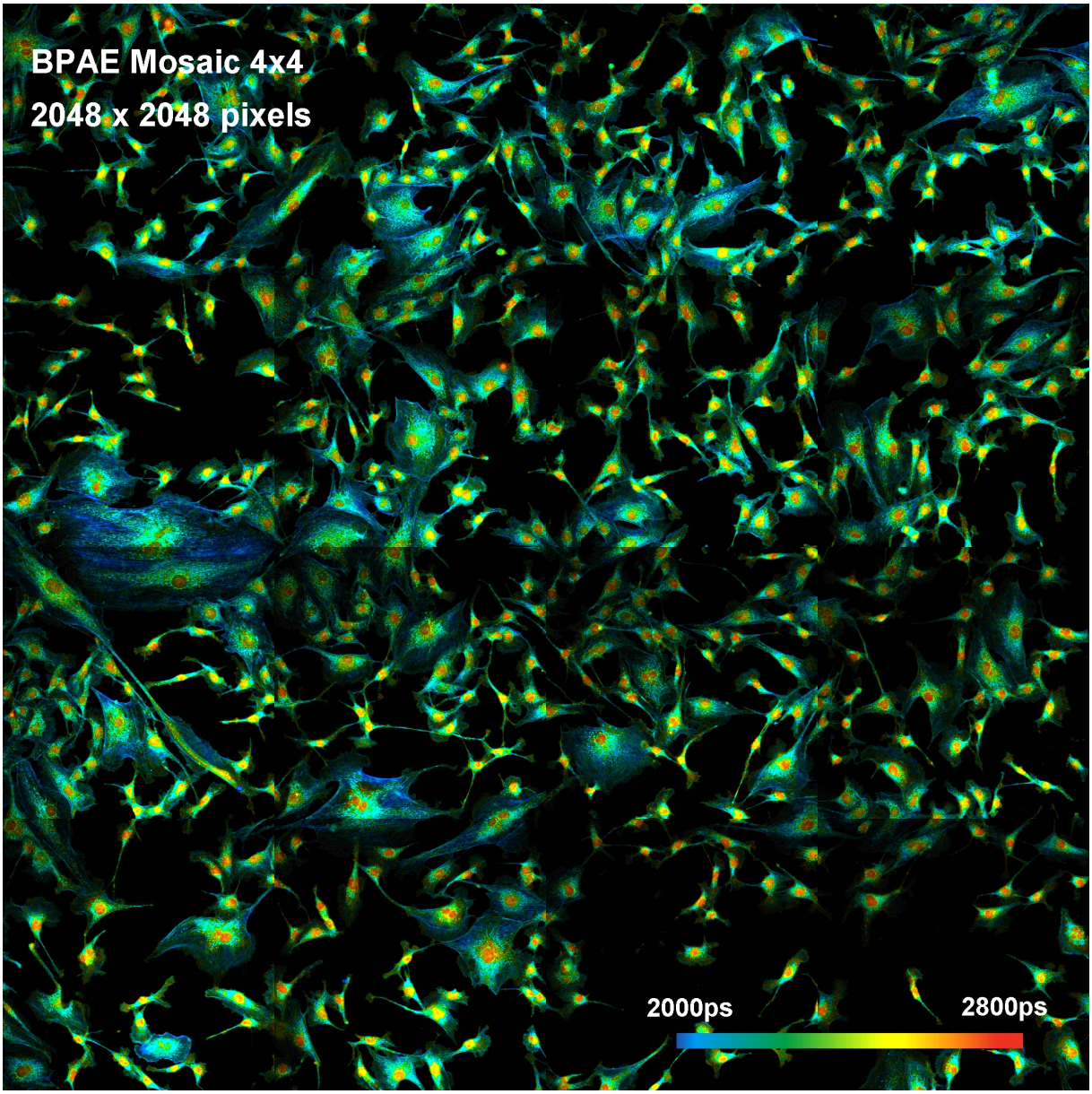

Fig. 2: Mosaic FLIM of a BPAE cell sample. The mosaic has 4x4 elements, each element has 512x512 pixels, each pixel has 256 time channels. DCS-120 MP (multiphoton) system. Data analysis by bh SPCImage. Use Adobe zoom function to see image at higher resolution.

Integration in SPCM

Fig. 3 shows how the optical scanner interacts with the motor stage. When Mosaic (Tile) Imaging is enabled the step width of the motor stage automatically adjusts to the scan area (Zoom factor) selected in the DCS-120 scanner panel. When the measurement is started the SPC system records a mosaic of FLIM images the elements of which have the same x and y size as the step width of the motor stage. The data of the individual elements of the mosaic (or tiles) can be accumulated over a selectable number of frames (20 in Fig. 3). The number of frames per mosaic element can be selected both in the motor stage panel and in the scanner panel.

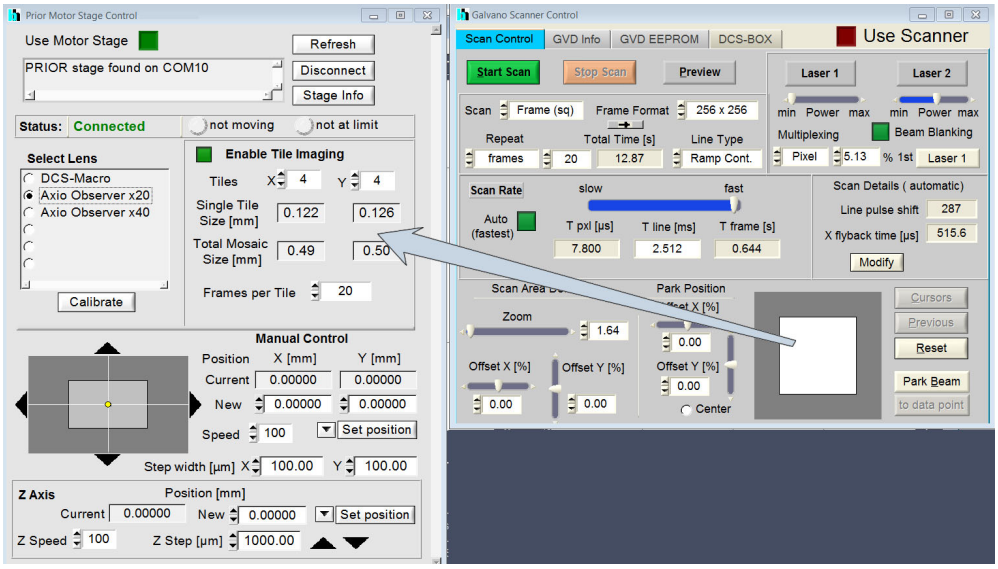

Fig. 3: Interaction of the motor stage with the DCS-120 scanner

The size of the DCS scan area differs for different optical systems and for different microscope lenses. To guarantee that the individual tiles fit together seamlessly the step width of the motor stage can be calibrated. The calibration panel is shown in Fig. 4. The calibration table contains the size, X and Y, of the field of view (scan area) of the optical scanner for Zoom = 1. The calibration factors can (but need not) be made different in x and y to account for possible tolerances in the drivers of the galvanometer mirrors. Up to six calibration values can be defined for different microscope configurations or objective lenses.

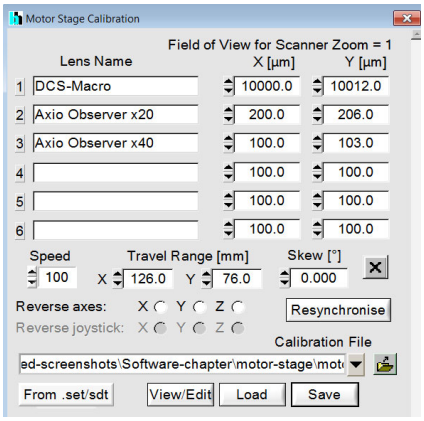

Fig. 4: Calibration of the motor stage

Advantages

Large-area FLIM images are normally recorded by simply using low-magnification microscope lenses. However, such lenses have low NA (numerical aperture). Low NA results in low light collection efficiency, low excitation efficiency for multiphoton excitation, and poor optical resolution. With mosaic FLIM, large image areas can be covered with high-NA objective lenses. The result is high efficiency, both for excitation and collection, and high spatial resolution. Since the entire mosaic is recorded into a single, large FLIM photon distribution, standard SPCImage FLIM data analysis [1] can be applied to the data.

References

1. W. Becker, The bh TCSPC handbook. 7th edition. Becker & Hickl GmbH (2017), www.becker-hickl.com
2. W. Becker, Introduction to Multi-Dimensional TCSPC. In W. Becker (ed.) Advanced time-correlated single photon counting applications. Springer, Berlin, Heidelberg, New York (2015)
3. Becker & Hickl GmbH, DCS-120 Confocal Scanning FLIM Systems, user handbook. Available on www.becker-hickl.com

Contact:

Becker & Hickl GmbH
Berlin, Germany
info@becker-hickl.com