

## Mosaic FLIM: New Dimensions in Fluorescence Lifetime Imaging

Mosaic FLIM is based on bh's multi-dimensional TCSPC FLIM technique [1]. In contrast to conventional TCSPC FLIM, Mosaic FLIM acquires several FLIM recordings into a single large FLIM data set. The results are displayed and saved as mosaics of FLIM images [2, 3]. The elements of a FLIM Mosaic can be recordings for

- a) different locations of the scan field in the sample [2, 3]
- b) subsequent Z planes [3, 4]
- c) subsequent times after a stimulation of the sample [2, 3]
- d) different wavelength [2, 3]

The results of Mosaic FLIM can be considered photon distributions over the scan coordinates,  $x, y$ , of the individual mosaic elements, the times,  $t$ , of the photons after the excitation pulse, and additional parameters, such as the location of the scan field in the sample (a), the depth of the image plane in the sample (b), the time after a stimulation of the sample (c), or the wavelength of the photons (d). The advantages of Mosaic FLIM over a conventional record-and-save procedure are:

- no readout and save time between subsequent mosaic elements
- time-series data (c) can be accumulated for repeated stimulation. This way, high lifetime accuracy is achieved even for very fast recordings. Element time can be down to 50 ms for fast scanners.
- the entire data set can be analysed in a single analysis run
- global fitting can be used over the entire array of mosaic elements

### Examples of FLIM Mosaic Recordings

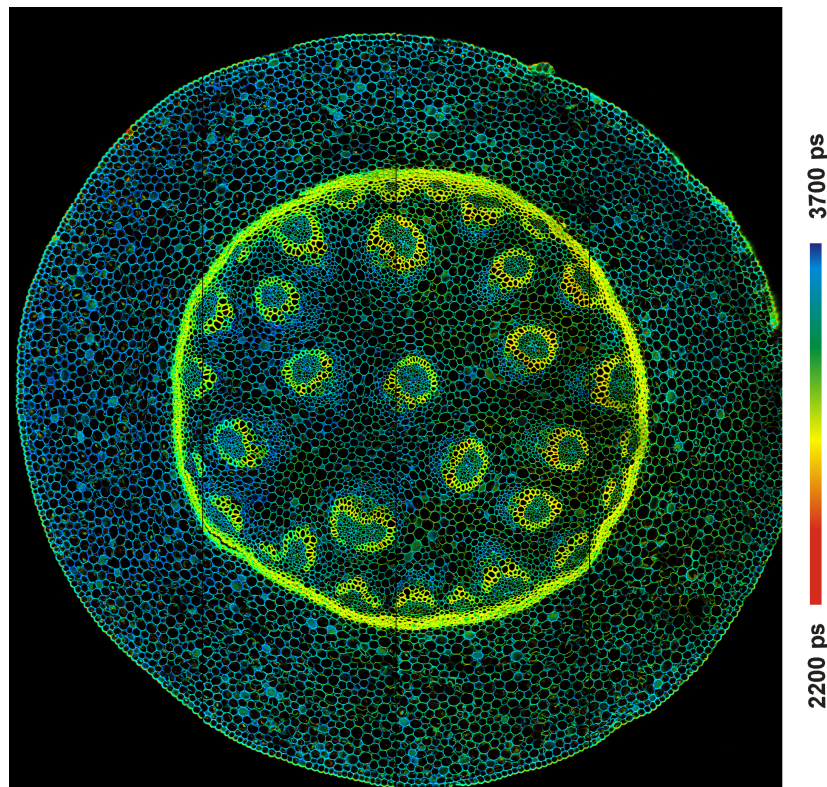


Fig. 1: Spatial Mosaic FLIM of plant tissue. 4x4 elements, each element has 512x512 pixels, with 256 time channels per pixel. Total pixel number 2048x2048, total size of image area 2.5 x 2.5 mm. Recorded with Simple-Tau 150 FLIM system [1, 5] and Zeiss LSM 710 in Tile-Imaging mode. Analysis by bh SPCImage [1, 5].

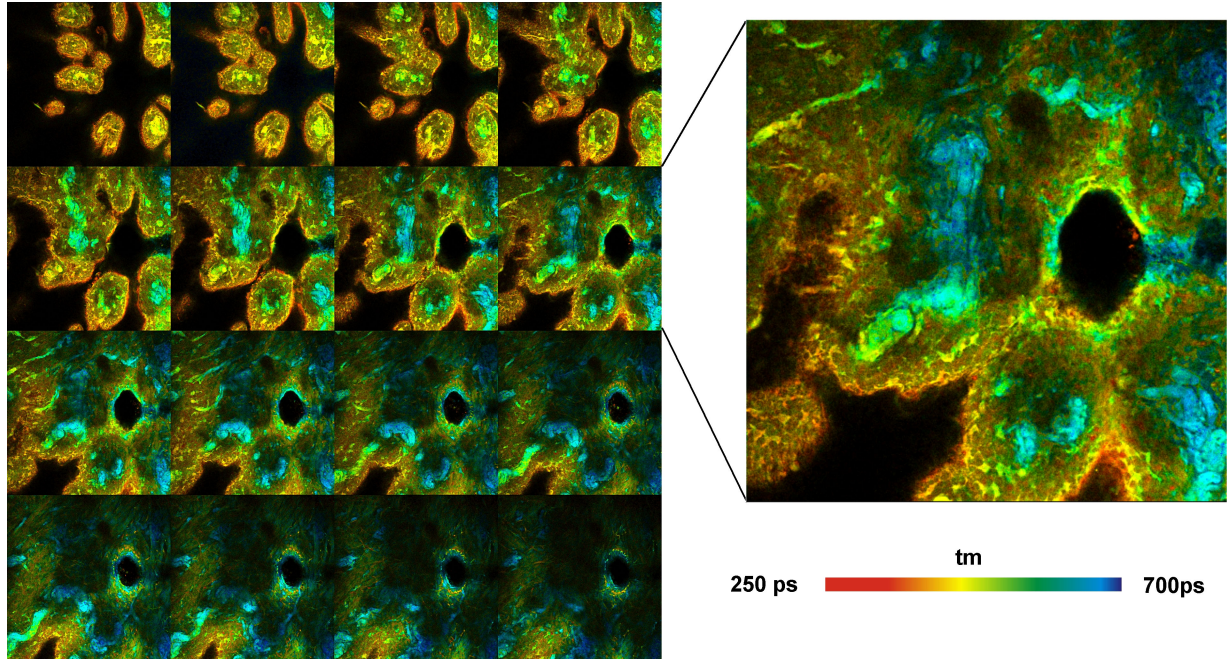


Fig. 2: Z-stack recorded by Mosaic FLIM. Pig skin stained with Indocyanin Green. 16 planes from 0 to 60  $\mu\text{m}$  from top of sample, each plane 512x512 pixels, 256 time channels. Amplitude-weighted lifetime of double-exponential decay. Image of plane 8 shown magnified on the right. bh Simple-Tau 150 FLIM system [1, 5] and Zeiss LSM 7MP OPO system [4].

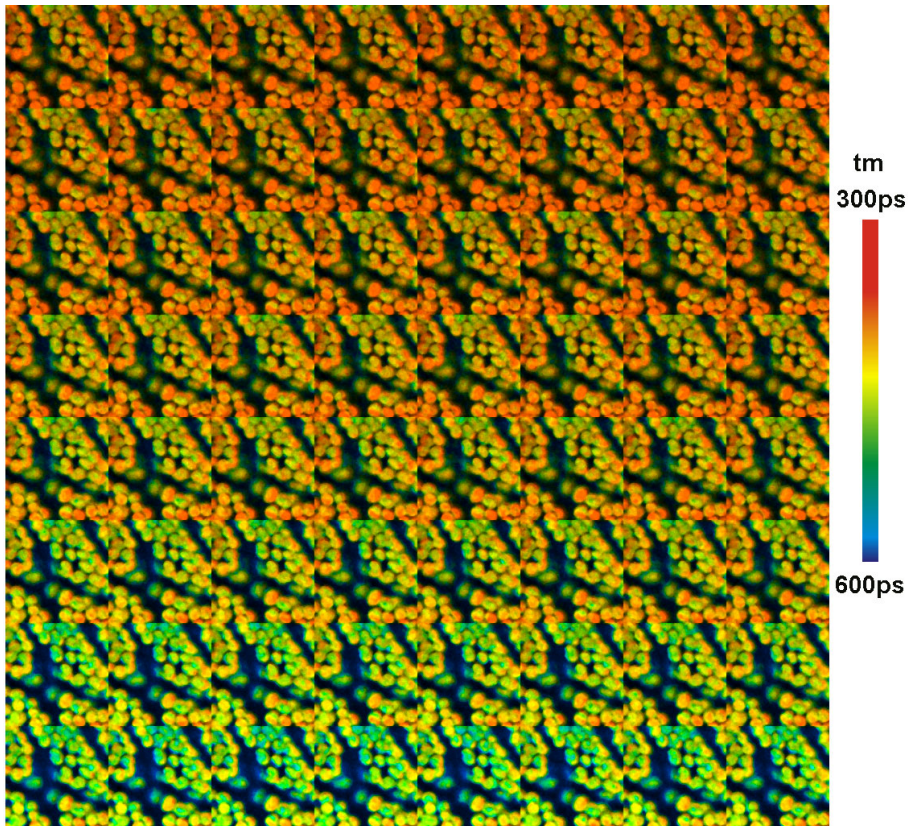


Fig. 3: Time series recorded by temporal Mosaic FLIM. Non-photochemical transients in chloroplasts of a moss leaf. 64 mosaic elements for consecutive times after turn-on of excitation light. Acquisition time per element 1 second, total time of sequence 64 seconds, image size of each element 128 x 128 pixels, 256 time channels. Time runs from lower left to upper right. bh DCS-120 confocal FLIM system [6] with SPC-150 TCSPC modules [1], SPCImage data analysis software.

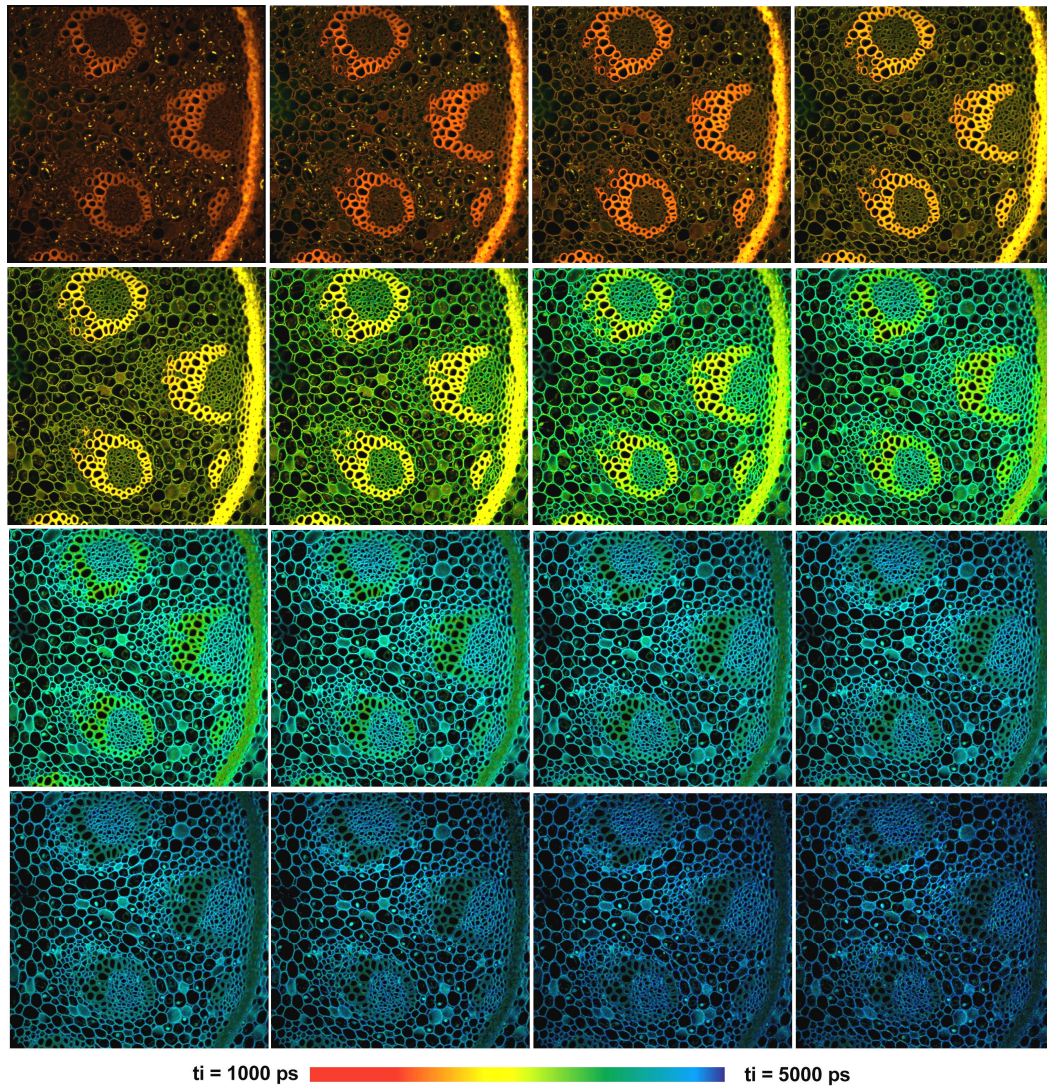


Fig. 4: Multi-wavelength Mosaic FLIM. bh MW-FLIM GaAsP 16-channel detector, SPC-150 TCSPC FLIM module. 16 images with 512 x 512 pixels and 256 time channels were recorded simultaneously [1], results displayed and saved in Mosaic FLIM mode. Wavelength from upper left to lower right, 490 nm to 690 nm, 12.5 nm per image. bh DCS-120 confocal scanning FLIM system [6], Zeiss Axio Observer microscope, x20 NA=0.5 air lens.

## System requirements

Mosaic FLIM requires a system computer with 64-bit architecture running Windows 7 or Windows 8, 64 bit. System memory should be 16 Gigabytes or more. SPCM data acquisition software must be 64 bit, version 9.60 or later.

## References

1. W. Becker, The bh TCSPC Handbook, 5th edition (2012). Available as printed copies or online from [www.becker-hickl.com](http://www.becker-hickl.com)
2. Megapixel FLIM with bh TCSPC Modules. Application note, see [www.becker-hickl.com](http://www.becker-hickl.com)
3. H. Studier, W. Becker, Megapixel FLIM. Proc. SPIE 8948 (2014)
4. Multiphoton NDD FLIM at NIR Detection Wavelengths with the Zeiss LSM 7MP and OPO Excitation. Application note, see [www.becker-hickl.com](http://www.becker-hickl.com)
5. Becker & Hickl GmbH, Modular FLIM systems for Zeiss LSM 510 and LSM 710 family laser scanning microscopes. User handbook, 5th edition. Available on [www.becker-hickl.com](http://www.becker-hickl.com)
6. Becker & Hickl GmbH, DCS-120 Confocal Scanning FLIM Systems, user handbook, edition 2012, available on [www.becker-hickl.com](http://www.becker-hickl.com)