Online STED FLIM with bh - Abberior Combination

The combination of the Abberior STED system with the bh SPC-150 or SPC-160 TCSPC FLIM modules records FLIM data at a spatial resolution of better than 40 nm [1, 2, 3]. The image format can be as large as 2048 x 2048 pixels with 256 time channels per pixel. Since July 2016 the bh the SPCM data acquisition software has an online display function for fluorescence lifetime images [4]. The function can be used to display STED FLIM images during the acquisition of the data.

Online Lifetime Display

Online lifetime display is based on the calculation of the first moment, M1, of the decay data in the individual pixels of a lifetime image [4]. First-moment calculation delivers the fluorescence lifetime at near-ideal photon efficiency. High-quality lifetime images are thus obtained even at a low number of photons per pixel. The implementation of the M1 algorithm in the bh SPCM data acquisition software is able to calculate a megapixel-size lifetime image in less than 100 ms. Importantly, the algorithm is running on normal FLIM data with 256 or 1024 time channels per pixel. Online display therefore does not conflict with precision offline multi-exponential FLIM analysis - the FLIM data are still recorded and saved at full time resolution. An example of a STED FLIM image obtained by the online FLIM function is shown in Fig. 1.

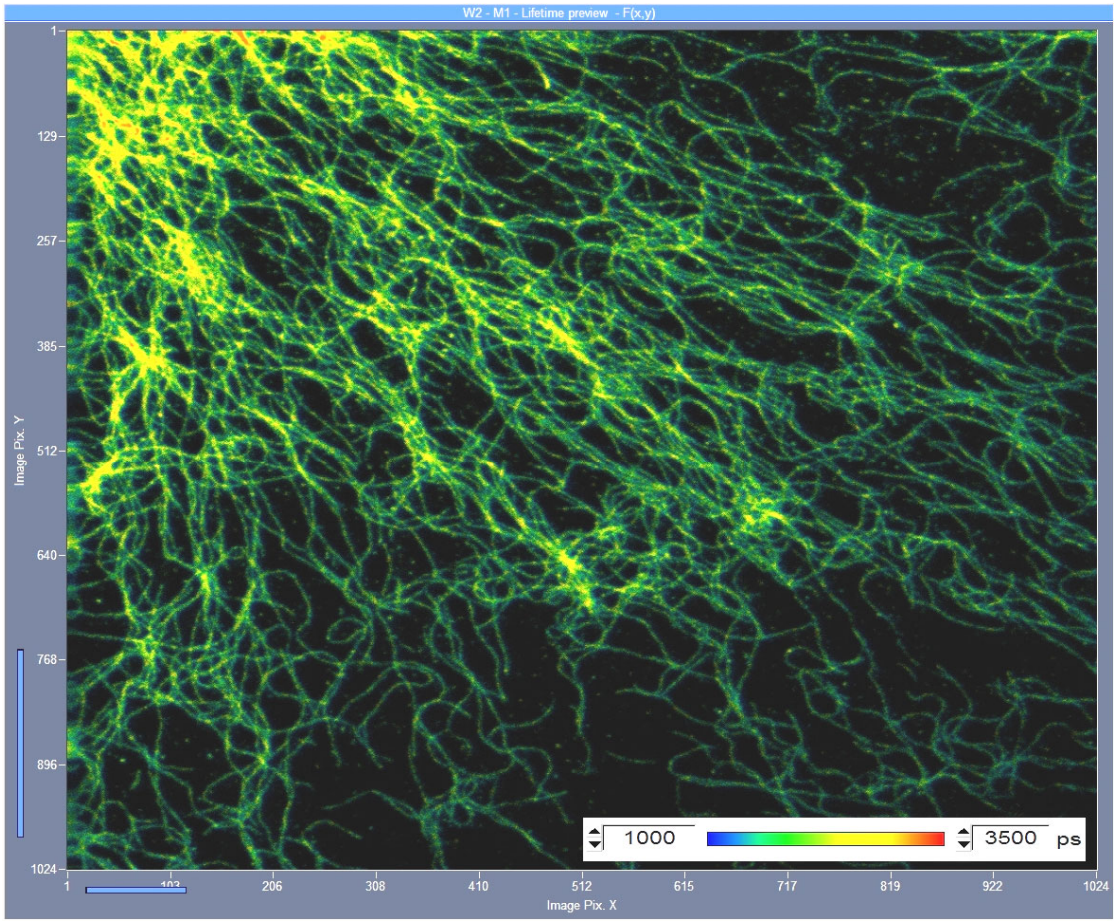

Fig. 1: STED FLIM image displayed by the online-lifetime function of SPCM. Micro-tubules in a bovine pulmonary artery cell.

Discussion

Influence of the depletion component

The decay curves of STED FLIM contain the normal decay function of the fluorophore plus a fast component from the depletion of the fluorescence in the outer part of the Airy disc by the STED laser. The M1 calculation delivers an intensity-weighted sum of the moments of the fluorescence decay and the depletion component. The presence of the depletion component causes a linear shift of the calculated lifetimes towards shorter decay times. A lifetime variation of the fluorophore (due to variation in the molecular environment) is thus correctly represented as a similar variation in the M1 data. If absolute lifetime values are to be displayed by the online FLIM function this can be obtained by using a modified reference moment in the online FLIM display parameters. Please see [4] for details.

Time-Gated STED Images

Time-gating rejects fluorescence from the undepleted part of the fluorescence decay, and thus increases the contrast of STED images [5]. In the Abberior system the STED depletion for most fluorophores works so well that the gain in contrast and resolution is barely visible. Nevertheless, time-gating can be achieved by defining appropriate 'Window Intervals' in the SPCM software and displaying images in the defined time windows [2]. Time-gating acts both on online-intensity images and online-lifetime images. Since the FLIM system records a full set of decay data a over the pixels the gate position can even be adjusted after the acquisition. A comparison of an ungated image with a gated one is shown in Fig. 2.

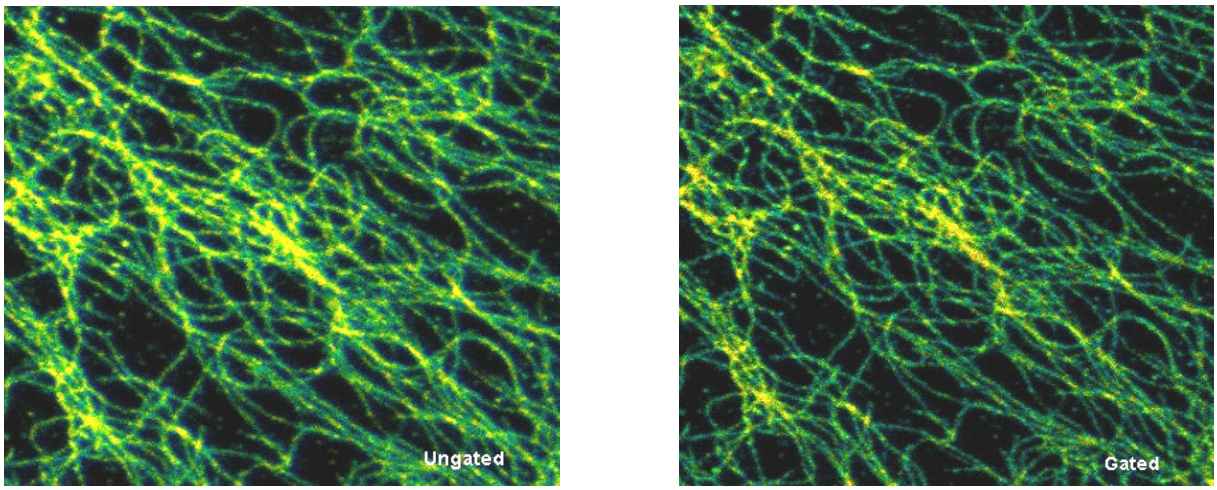

Fig. 2: Ungated (left) and gated online-FLIM image (right). Detail from the data in Fig. 1.

Acknowledgement

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