

## **Quantitative Ultra-fast FLIM**

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Increasing the speed of Fluorescence Lifetime Imaging (FLIM) is essential to cementing its importance as a tool in the Life Sciences. This technique is already well established, but imaging dynamic processes requires shorter acquisition times. Our novel rapidFLIM approach dramatically reduces the acquisition time through a combination of fast scanning, hybrid photomultiplier detectors which are capable of handling very high count rates, and TCSPC modules with ultra short dead times. With the new FLIMbee fast scanning add-on for the MicroTime 200, this technique can be used with our microscopy platform as well as being offered as an upgrade kit for conventional Laser Scanning Microscopes (LSMs).

With this hardware combination, excellent photon statistics can be achieved in significantly shorter time spans, allowing fast processes to be measured with the high resolution achievable in confocal microscopy. Depending on the image size, rapidFLIM allows imaging at a rate of several frames per second, enabling dynamic processes, such as protein interactions, FRET dynamics, or chemical reactions to be imaged in a time-resolved manner. With these high frame rates, FLIM can also be used on highly mobile species such as cell organelles and for other live cell imaging applications.

Recently, we have further pushed the limits of this method by systematically reducing the effects of decay distortions at very high count rates, allowing quantitative data analysis to be performed even at count rates  $\gg 10$  Mcps. This technique has been applied to quantitatively analyze FRET measurements using fluorescent proteins.