

Zeiss BiG-2 GaAsP Detector is Compatible with bh FLIM Systems

Abstract: We tested the Zeiss BiG-2 GaAsP detector in combination with the bh TCSPC FLIM systems. The outputs of both detector channels were connected to the inputs of a Simple-Tau 152 (SPC-152) dual-channel TCSPC FLIM system via A-PPI-D pulse inverters. The BiG-2 detector was used both at the NDD port of a multiphoton microscope and at the confocal port of a one-photon microscope. In both cases FLIM data were obtained at a sensitivity comparable to the bh HPM-100-40 GaAsP hybrid detectors. The IRF width was 250 ps (FWHM). The decay data were clean, without optical or reflections or leakage of excitation light. The high detection efficiency and the absence of laser background was confirmed by FCS experiments.

The BiG-2 Detector

The Zeiss BiG-2 detector contains two PMTs with GaAsP photocathodes. The GaAsP photocathode has an efficiency about 5 times higher than a conventional photocathode. It is sensitive from about 350 nm to 700 nm. The BiG-2 detector can be used both at the confocal ports and at the non-descanned ports of the LSM 710/780/880 family confocal or multiphoton microscopes. The light is split on the two detectors by commonly used Zeiss-type microscope beamsplitter cubes. The cubes can be equipped with user-specific dichroic beamsplitters and filters to match the spectral properties of the fluorophores to be observed. The BiG-2 detector has an electrical output for the detector signal. It can therefore be used as a detector for TCSPC FLIM. The output amplitude for a single photon is on the order of 300 mV, perfectly compatible with the bh TCSPC FLIM systems.

Interfacing the BiG-2 with the bh FLIM system

For the tests of the BiG-2 detector we used a standard bh dual-channel Simple-Tau FLIM system for the Zeiss microscopes [2, 3]. The two output signals of the BiG-2 were connected to the 'CFD' inputs of the two SPC-150 modules via A-PPI-D pulse inverters. The 'SYNC' signals for the TCSPC modules came from the laser(s) as described in [2]. Typical CFD parameters for the BiG-2 detectors are Threshold = -200 mV, and Zero Cross = -10 mV to +10 mV. The electronic transit time in the BiG detector is about 5 ns, compared to <1 ns for the bh hybrid detectors. The different delay matters only if a BiG detector is frequently swapped with HPM-100 hybrid detectors. In that case, we recommend to use the bh DEL-32 USB-controlled delay switch box in the SYNC path. The delay settings for the DEL-32 are contained in the predefined setups of the TCSPC system [1, 2] and automatically recalled with the TCSPC setup data for the different detectors.

Instrument Response Function

We tested the BiG-2 detector at an LSM-710 NLO microscope that had an OPO as an excitation source, see also [4]. The BiG-2 detector was attached to the non-descanned port [2] of the LSM 710 NLO. Fig. 1, top shows the IRF of the BiG-2 detector. It was recorded by detecting the second-harmonic (SHG) signal from finely powdered sugar.

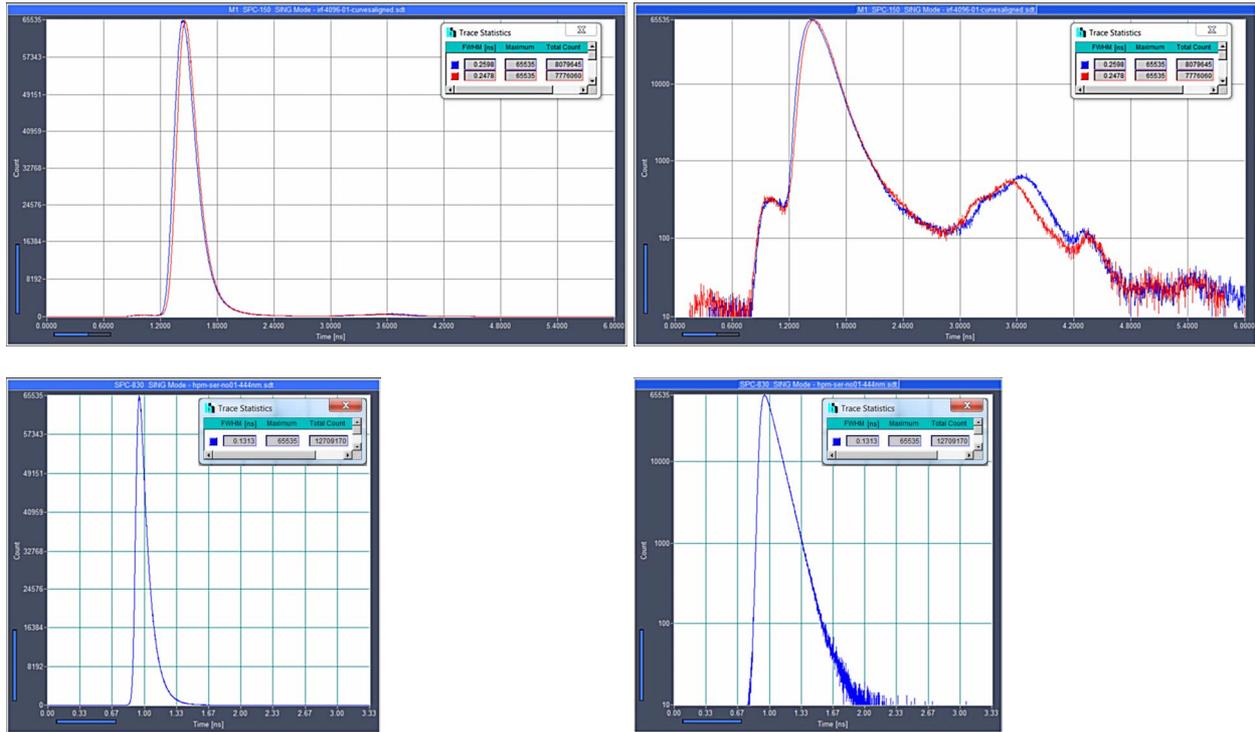


Fig. 1: Top: IRF of the two channels of the BiG-2 detector recorded by the SPC-150 modules of the Simple-Tau system. Left: Linear scale. Right: Logarithmic scale. Horizontal scale 0 to 6 ns. The IRF width is about 250 ps. Bottom: IRF of the HPM-100-40 detector for comparison. Horizontal scale 0 to 3.3 ns, diagrams are at same time scale as Fig. 1, top. The IRF of the HPM is 130 ps.

The full-width at half maximum (FWHM) is about 250 ps. The logarithmic display (top right) shows that there is a slight shoulder before the main peak, and a secondary peak about 1.5 ns later. Such secondary IRF structures are a common feature of conventional PMTs [1]. Their presence in the BiG-2 detector is therefore not surprising. The amplitude of the sub-structures is moderate and has little influence on the accuracy of fluorescence lifetime analysis. It may, however, complicate the extraction of with low-amplitude decay components from double- or triple-exponential decay profiles.

Fig. 1, bottom shows the IRF of an HPM-100-40 hybrid detector at the same time scale. The HPM-100-40 is the standard detector for the bh FLIM systems, and still the gold standard in IRF shape, time-resolution and efficiency [5]. A comparison of the curves shows that the HPM has a narrower IRF (130 ps FWHM) and no secondary peaks.

NDD FLIM with two-Photon Excitation

NDD FLIM images were recorded with an LSM710/780 OPO system [4]. Lifetime images recorded from a *covallaria* sample are shown in Fig. 2, left. The photons were detected in a wavelength interval from 575 to 610 nm. The image is free of optical reflections or other artefacts. The decay data are clean, without any traces of optical reflections or leakage of laser light, see Fig. 2, right.

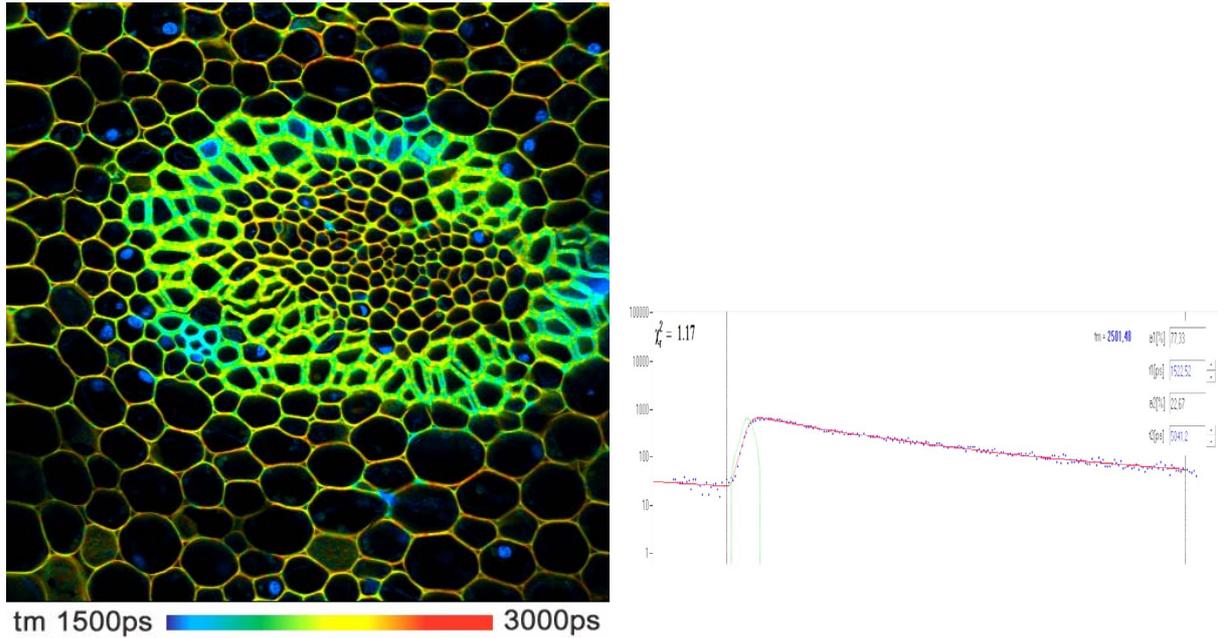


Fig. 2: Left: NDD FLIM image of a convallaria sample. Right: Decay data in selected pixel of the image. Excitation by OPO at 1100 nm, detection at 575 to 610 nm. Data format 512x512 pixels, 256 time channels. Amplitude-weighted lifetime of double-exponential decay, incomplete decay model [1,2].

A more challenging test for any laser scanning microscope is imaging of thick samples of high internal scattering. We therefore stained a sample of fresh pig skin with methylen blue, and recorded images with 2-photon excitation at 1100 nm. The BiG-2 detector passed this test without problems. The result is shown in Fig. 3. The lifetime image is shown on the left, decay curves from two selected areas on the right.

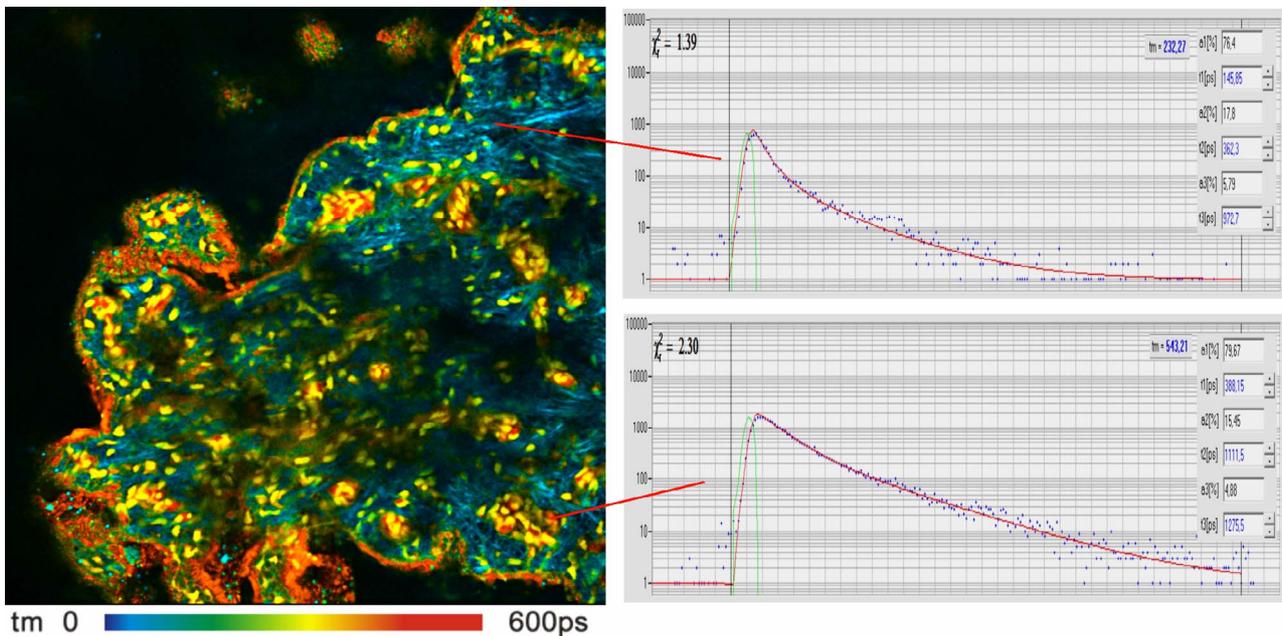


Fig. 3: Fresh pig skin stained with methylen blue. Left: FLIM image, 512x512 pixels, amplitude-weighted lifetime of triple-exponential decay. Right: Fluorescence decay curves in two selected spots of the image. Two-photon excitation by OPO at 1100 nm, non-descanned detection by BiG-2 detector.

Confocal FLIM with One-Photon Excitation

Confocal FLIM was tested in an LSM 880 with a 405 nm picosecond diode laser. In the LSM 880 the confocal output from the scan head is used for the ‘Airy-Scan’ detector. It is therefore not directly accessible. For additional confocal detectors Zeiss offers a beam switch that is inserted between the output of the scan head and the Airy Scan detector, see Fig. 4, left. The 90° position of the switch directs the beam to a vertical (BiG-type) port. This port can be used both for the BiG-2 detector and for the bh hybrid detectors, see Fig. 4, right.

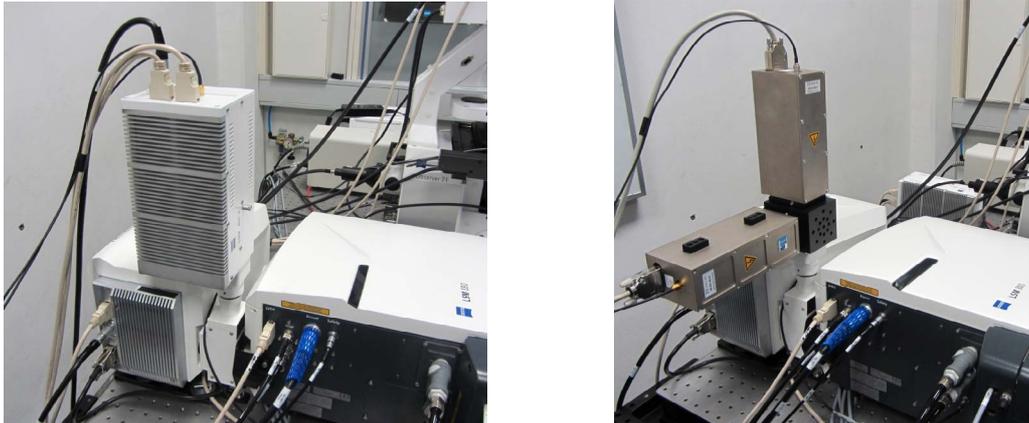


Fig. 4: LSM 880 scan head with BiG-2 detector (left) and with two bh HPM-100 hybrid detectors (right). The detectors are connected to a beam switch between the Airy Scan detector and the scan head.

Fig. 5 shows FLIM images of a convallaria sample excited at 405 nm. The images were detected by the two spectral channels of the BiG-2 detector, and recorded by the two parallel TCSPC channels of the Simple-Tau 152 FLIM system.

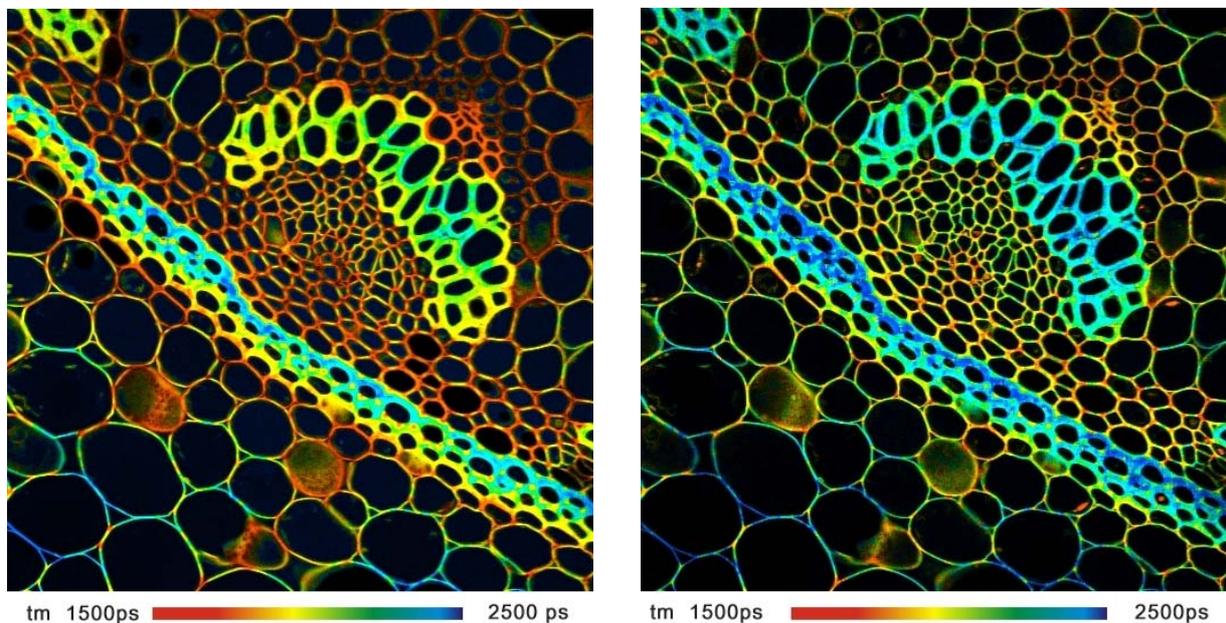


Fig. 5: Confocal FLIM images recorded by BiG-2 detector and LSM 880. Left: Channel from 500 to 550 nm. Right: Channel from 575 nm to 610 nm. Convallaria sample, excitation by 405 nm ps diode laser, 512x512 pixels, 256 time channels. bh Simple-Tau 152 dual-channel TCSPC FLIM system.

Images recorded in this setup were found free of any artefacts. The sensitivity of the system was excellent. A comparison of the BiG-2 with the HPM-100-40 hybrid detectors (setup shown in Fig. 4) showed no difference in efficiency.

FCS

FCS recording is a sensitive test for detection efficiency. We therefore used the FLIM system to record FCS from a number of dye solutions with the BiG-2 detector. The results were excellent. Fig. 6 shows decay curves and FCS curves of Atto 425 excited with the 405 nm ps diode laser. FCS with 405 nm excitation is not trivial. Problems can especially occur by fluorescence of optical components, by leakage of laser spectral background, and by poor transmission of the optics. Not so with the LSM 880 - both decay curves and FCS curves were recorded at high efficiency and without any noticeable artefacts.

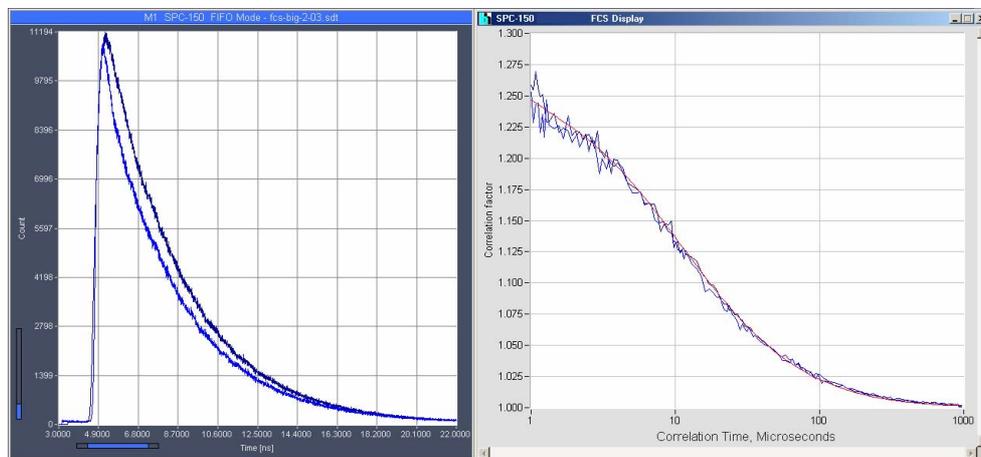


Fig. 6: Fluorescence decay functions and FCS curves detected in the two channels of the BiG-2 detector. Atto 425, excitation by 405 nm ps diode laser. bh Simple-Tau 152 dual-channel FLIM system. The red curve is a fit with two diffusion terms and a triplet term.

The bh TCSPC systems are able to record FCS also CW lasers [1]. Fig. 7 shows an example. It shows an FCS curve of Rhodamine 110, excited by the 488 nm CW laser of the LSM 880.

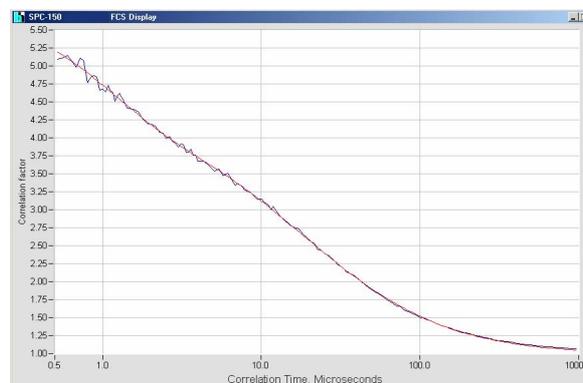


Fig. 7: FCS of Alexa 488 excited by 488 nm CW laser. Blue: FCS data. Red: fit with two diffusion terms and a triplet term.

Conclusions

The BiG-2 detector in combination with the bh FLIM systems delivers lifetime data with excellent sensitivity and reasonably good time resolution. We did not find a noticeable sensitivity difference to the bh HPM-100 hybrid detectors [5]. Compared to the HPM detectors the IRF is about a factor of 2 wider, and not entirely free of secondary peaks. For fluorescence decay time longer than 400 ps this has little influence on the lifetime accuracy. The secondary peak may, however complicate the analysis of multi-exponential decay profiles with low-amplitude components.

Due to its excellent efficiency the BiG detector works well also in FCS applications. The afterpulsing of the BiG-2 we tested was surprisingly low. It was difficult to discern from the triplet component of the FCS data.

In summary, it can be concluded that the BiG-2 is an excellent detector for steady-state imaging and a very useful detector for FLIM. The advantage of the BiG-2 is that detectors need no be swapped when a microscope port is used both for FLIM and for conventional imaging. However, for high-end FLIM, such as double-exponential FRET measurements or metabolic imaging via the fluorescence NADH the hybrid detectors still deliver superior results. If such experiments have to be performed we recommend to keep a set of hybrid detectors in reserve and attach them instead of the BiG-2 when needed. The mechanical adapters of the hybrid PMTs and the BiG-2 are identical. The detectors can thus be swapped by just loosening a single screw.

References

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