

Recording Z Scans with the DCS-120 Confocal Scanning FLIM System

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Abstract. The bh DCS-120 FLIM system in the Zeiss Axio Observer Z1 version is able to record Z stacks of FLIM images. The sample is scanned at a rate on the order of 1 to 10 frames per second. The TCSPC system runs a series of measurement cycles over specified periods of time while actively controlling the Z position of the Axio Observer. After each measurement cycle the data are saved into a file. The result is a number of FLIM data files for consecutive planes of the sample.

Principle

The bh DCS-120 confocal scanning FLIM system [3] is based on the DCS-120 scan head and bh's multidimensional TCSPC technique [1, 2]. The scanner scans the sample at high pixel rate with a high-repetition rate pulsed laser beam, the returned photons are detected in two detector channels through confocal pinholes, and recorded by two parallel bh SPC-150 TCSPC modules. The TCSPC modules build up photon distributions over the time in the fluorescence decay, the image coordinates [4], and, if the MW-FLIM multi-spectral detectors are used, the wavelength of the photons [5]. The photon distribution can either be built up in the memory of the TCSPC module ('Scan Sync In' mode), or in the memory of the computer ('FIFO Imaging' mode). The recording process does not use any time gating or wavelength scanning and thus delivers a near-ideal detection efficiency. The images can be acquired at scan rates as fast as 1 pixel per microsecond. Subsequent frames of the scan are accumulated; the acquisition is continued over as many frames as necessary to obtain an appropriate signal-to-noise ratio. For bright samples acquisition times of less than one second per image can be obtained [3, 6]. A DCS-120 / Axio Observer system is shown in Fig. 1.

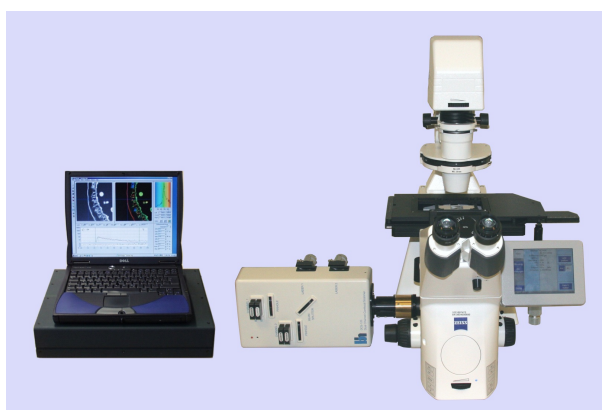


Fig. 1: DCS-120 confocal scanning FLIM system, Axio Observer Z1 version

To obtain Z stacks with the TCSPC imaging technique the measurement has to be repeated for subsequent planes of the sample. Repetitive measurement can be achieved by the 'Cycle' and 'Autosave' features of the SPCM operating software of the bh TCSPC modules [2]. In SPCM version later than Aug. 2008 active control of the Zeiss Axio Observer Z1 microscope has been

implemented [2, 3]. Obtaining Z stacks of FLIM images with the DCS-120 / Axio Observer Z1 systems is thus merely a matter of system parameter setup.

System parameters for Z scanning

The measurement control section of the system parameters is shown in Fig. 2. The setup for the Scan Sync In mode is shown on the left, the setup for the FIFO imaging mode on the right.

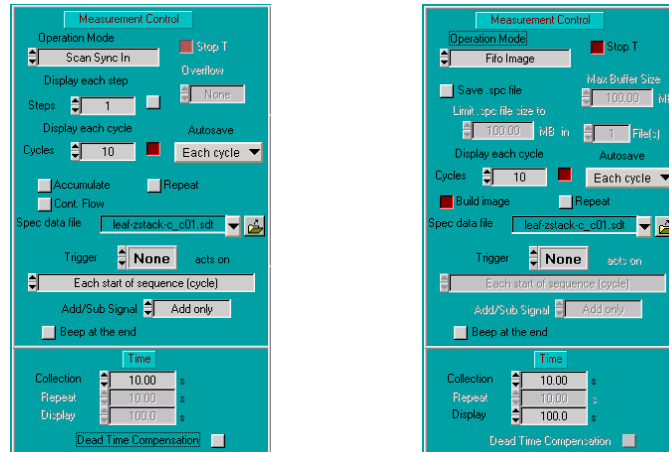


Fig. 2: System parameters for Z-scanning. Left: Scan Sync In mode. Right: FIFO Imaging Mode

Both setups record a series of 10 images at 10 consecutive Z positions. The images are saved into separate files. The general file name is defined under ‘Spec data file’. The SPCM software extends the file names with ‘_C01 ... _C10’ for the subsequent measurement cycles. The details of Z scanning are defined in a separate Z control panel, see Fig. 3, left.

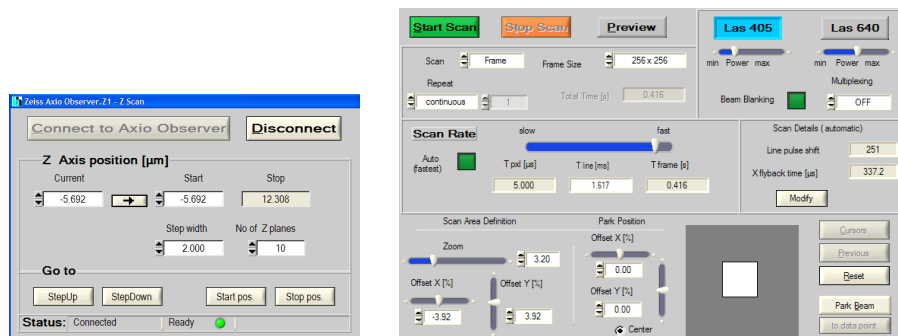




Fig. 3: DCS-120 scanner setup. Left: Z scan parameters. Right: XY Scan parameters

The control panel shows the current Z position. The position can be changed by typing in a new position, by clicking on the  symbols, by clicking on the Step Up or Step Down buttons, or by turning the focusing knob at the microscope. A click on  defines the current position as a start position of the Z scan. ‘Step width’ an ‘No of Z planes’ define the distance and the number of Z steps. ‘No of Z planes’ is automatically connected to the ‘Cycles’ in the system parameters, see Fig. 2.

X-Y scanning of the subsequent Z planes is controlled via the ordinary scan parameters of the DCS-120 scan head. The scanner control panel is shown in Fig. 3, right. The scan format used is 256x256 pixels. Scan formats from 16x16 to 2048x2048 pixels are available; any of these can be used. However, please note that the file sizes for large pixel numbers can be enormous. A single

recording cycle with 256 x 256 pixels and 256 time channels in a dual-channel SPC system delivers 64 Megabytes. A Z stack of 16 such images produces one Gigabyte of data. Increasing the pixel numbers may therefore be inappropriate. It should also be noted that Z scanning causes an enormous load on the sample; photobleaching may therefore preclude the use of extremely large image formats as well. A substantial improvement has been obtained with the introduction of the bh PMH-100-40 hybrid detector modules [3, 7]. These detectors are 5 to 10 times more sensitive than a conventional PMT. Thus, images can be obtained at lower excitation power and correspondingly less photobleaching.

To run a Z scan sequence, first start a scan in the 'Preview' mode. The Preview runs a fast scan and displays images in intervals of one second. After choosing the location and the zoom of the scan, change the Z position until you have found the Z plane you want to start with. You can do this by turning the focusing knob at the microscope, or by changing the position via the Z control panel. When you have found the right position, define it as a start position. Switch off the preview, start the ordinary scan, and start the measurement. The SPCM software will automatically record the defined number of images at consecutive Z positions and save them into files.

Two examples of Z scans are shown in Fig. 4 and Fig. 5. Both data sets were obtained in the FIFO Imaging mode, with 256x256 pixels and 256 time channels. The acquisition time per Z plane was 10 seconds, the average count rate about 10^6 photons/second. The other setup parameters were as shown in Fig. 2 to Fig. 3.

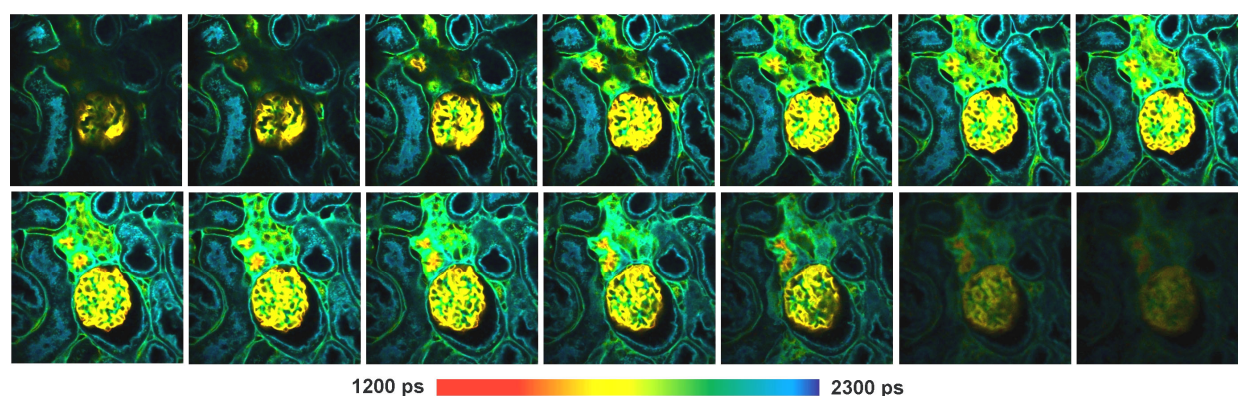


Fig. 4: Z stack recording of a mouse kidney section. Images 256x256 pixels, 256 time channels. 14 steps in Z, step width 1.5 μ m. FIFO Imaging mode, 256x256 pixels, 256 time channels. Setup data shown in Fig. 2 and Fig. 3.

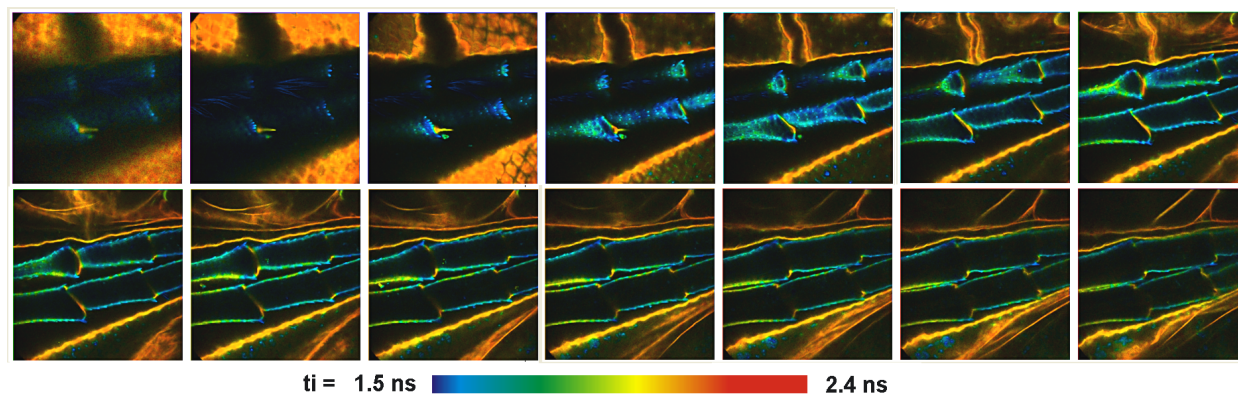


Fig. 5: Z stack recording, part of a water flea, autofluorescence. Images 256x256 pixels, 256 time channels. 15 steps in Z, step width 4 μ m. Zeiss water C Apochromate, bh HPM-100-40 hybrid detector.

Tips and Tricks

Changing between instrument configurations

The DCS-120 system can be used to record single and dual-wavelength FLIM data, multi-wavelength FLIM data, time-series and Z series of FLIM data, and FCS data. To easily change between different modes and configurations we recommend to put the corresponding system setup files into the list of 'Predefined Setups'. Changing between different instrument configurations is then a matter of a single mouse click [2, 3].

Multi-File View

For measurements that deliver a series of FLIM data files it may be required to quickly switch through the corresponding images. For this purpose, the SPCM software has a 'Multi-file View' function implemented. A series of FLIM data files is specified, and individual images are selected and displayed on a mouse click. Moreover, the Multi-File View is able to switch through series of data files consecutively, displaying the images like a movie. Please see [2] for details.

Analysis of multi-dimensional FLIM data

Analysing Z series or time series of FLIM recordings image by image can be troublesome. bh has therefore developed a new FLIM data analysis software. The bh OPTISPEC software especially addresses the sequential and multi-dimensional recording features of the bh TCSPC technique. It not only analyses single FLIM data sets, but also multi-wavelength FLIM data, time series of FLIM measurements, or Z stacks of FLIM measurements. For multidimensional data sets, OPTISPEC allows the user to fit a global model to the entire data array. By defining parameters as 'global', correlations of fit parameters between different pixels, different wavelength intervals, or different steps of a time series can be exploited. The OPTISPEC software is described in a separate handbook [8].

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