FLIM of Macroscopic Objects: Imaging in the Primary Image Plane of the DCS-120 Scanner

Abstract: Fluorescence lifetime images of macroscopic sample as large as 15 mm can be recorded in the primary focal plane of the DCS-120 confocal scanner. Optical resolution is in the range of 15 µm, acquisition times range from a few seconds for low pixel numbers to several minutes for high-resolution images.

Principle

Multidimensional TCSPC is a powerful technique to obtain fluorescence lifetime images of microscopic samples in laser scanning microscopes [1, 2]. A laser beam is sent down the beam path of the microscope, and scanned around a pivot point located in the principle plane of the microscope lens. The fluorescence light is collected back through the microscope lens, separated from the excitation by a dichroic mirror, and detected in one or several wavelength intervals or polarisation channels. Depth resolution or optical sectioning is obtained by confocal detection [3] or multiphoton excitation [4, 5].

Images obtained in microscopes usually cover image areas of no more than 1 x 1 mm. Larger objects can, in principle, be scanned by placing them in the intermediate image plane of a confocal scanner. The optical principle is shown in Fig. 1. The figure shows the internal beam path of the bh DCS-120 scanner, but the principle shown essentially applies to other confocal scanners as well.

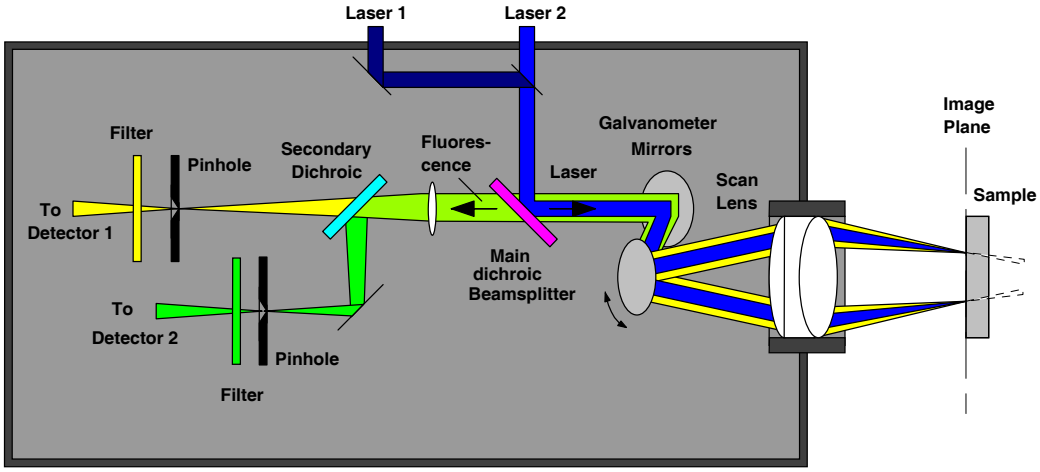

Fig. 1: Principle of the DCS-120 scanner for imaging macroscopic objects (simplified)

The laser beam is scanned by two fast-moving galvanometer mirrors. The scan lens focuses the laser beam into an image plane shortly in front of the scanner. When the scanner is used with a microscope, this image plane coincides with the upper image plane of the microscope. For scanning large samples, the image plane of the scan lens is brought in coincidence with the sample surface. As the galvanometer mirrors change the beam angle the laser focus scans across the sample. Fluorescence light excited in the sample is collimated by the scan lens, de-scanned by the galvanometer mirrors, and separated from the excitation light by the main dichroic beamsplitter. The fluorescence beam is further split into two spectral or polarisation components, and focused into pinholes. Light passing the pinholes is sent to the detectors. For more details please see [3].

Results

Fig. 2 shows two examples of images recorded in the primary image plane of the DCS-120 scanner.

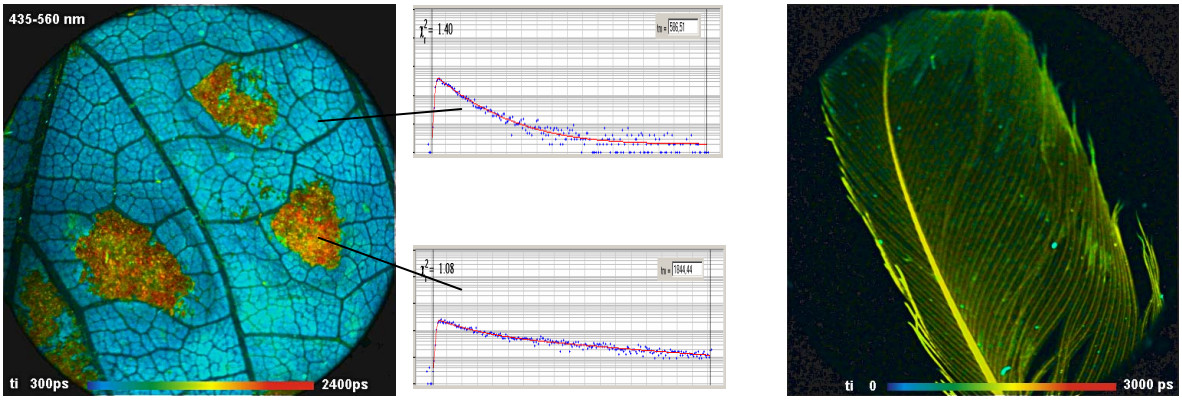

Fig. 2: FLIM in the primary image plane of the DCS-120 scanner, excitation 405nm, scan 512 x 512 pixels. Left: Leaf with a fungus infection. Middle: Decay functions of healthy and infected areas. Right: Feather of a songbird.

Image area and Resolution

The maximum diameter of the image area in the primary image plane of the scanner is about 15 mm. Smaller areas can be scanned by using the 'Zoom' function of the scanner, see [3]. The size of the laser spot in the image plane is about 15 µm. The resolution in the detection path is even higher because the numerical aperture in the focus of the scan lens is larger than for the laser beam. That means, full-size images could, in principle, be reasonably scanned with at least 1024 x 1024 pixels, with moderate oversampling even with 2048 x 2048 pixels.

Collection efficiency

The numerical aperture of the detection beam path is given by the beam path diameter (about 3 mm) and the focal length of the scan lens (40 mm). The collection efficiency is thus considerably lower than in combination with a microscope. However, macroscopic imaging can use much higher laser power, which compensates for low collection efficiency. Even weakly fluorescent samples, such as human skin, or the feather shown in Fig. 2, easily deliver count rates on the order of several 100,000 counts per second.

Acquisition times

The acquisition time depends on the number of pixels in the image and the requirements to the accuracy of the lifetimes [2, 3]. The images shown above were recorded within about one minute. For images with 128 x 128 pixels the same lifetime accuracy is obtained in less than 10 seconds.

References

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