Implantable Fibre-Optical Fluorescence-Lifetime Detection System for in-vivo Applications

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This application note describes a fluorescence-lifetime detection system based on picosecond diode laser excitation, a single-mode excitation fibre, one or several multi-mode detection fibres, high-efficiency single-photon detectors, and a portable time-correlated single photon counting (TCSPC) system. The excitation and detection system can easily be connected and disconnected from the measurement object via small-size, low-weight fibre connectors. The system records fluorescence decay curves, phosphorescence decay curves, time-series of fluorescence decay curves, intensity-traces, and fluorescence correlation data. By triggering the acquisition with an external stimulation of the measurement object, dynamic effects in the fluorescence lifetime and intensity down to millisecond range can be recorded.

Picosecond Diode Lasers

Fluorescence excitation is performed by a bh BDL-SMN or BDL-SMC picosecond diode laser. Available wavelengths are 405 nm, 445 nm, 473 nm, 488 nm, 515 nm, 640 nm, 685 nm, and 785 nm. The pulse width is on the order of 50 to 90 ps. The pulse repetition rate is switchable between 20 MHz, 50 MHz, and 80 MHz. Depending on the wavelength and the selected repetition rate, the maximum available (average) laser power is from about 0.5 to several mW. The laser is shown in Fig. 1, left, typical pulse shapes in Fig. 1, right . The laser is operated from a simple +12V power supply; the driver electronics is integrated in the laser head.

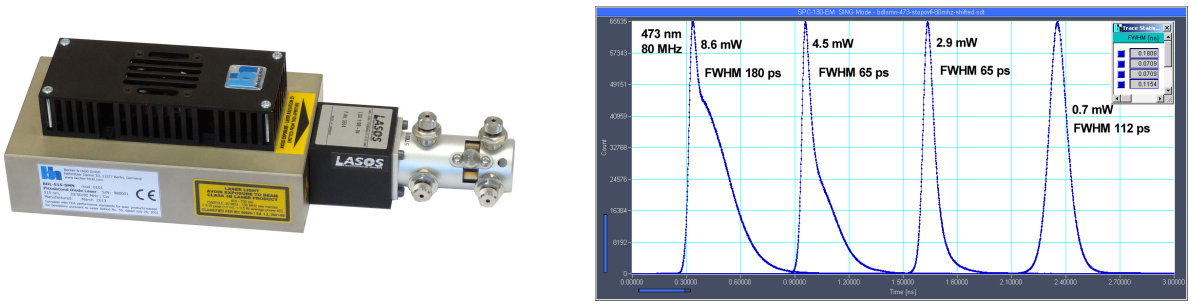

Fig. 1: Left: BDL-SMN picosecond diode laser. Right: Pulse shapes for different laser power. 473 nm version, 80 MHz.

Fibre coupling system

Excitation and detection fibres are shown in Fig. 2. Typical fibre core diameters are 3 µm for the excitation fibres, and 25 µm for the detection fibres. The excitation fibres are single-mode fibre fibres. This avoids motion artefacts by mode fluctuations when the fibres are bent. The detection fibres are multi-mode to increase the light-collection efficiency. Intensity changes by mode fluctuations do not occur on the detection side because the fluorescence signal is diffusely coupled and has a wide optical spectrum. Due to the small diameter the fibres can be implanted into live animals, see [6, 7].

The excitation and detection fibres are either separate (Fig. 2, left), or the fibres are mounted in a single excitation/detection probe (Fig. 2, right).

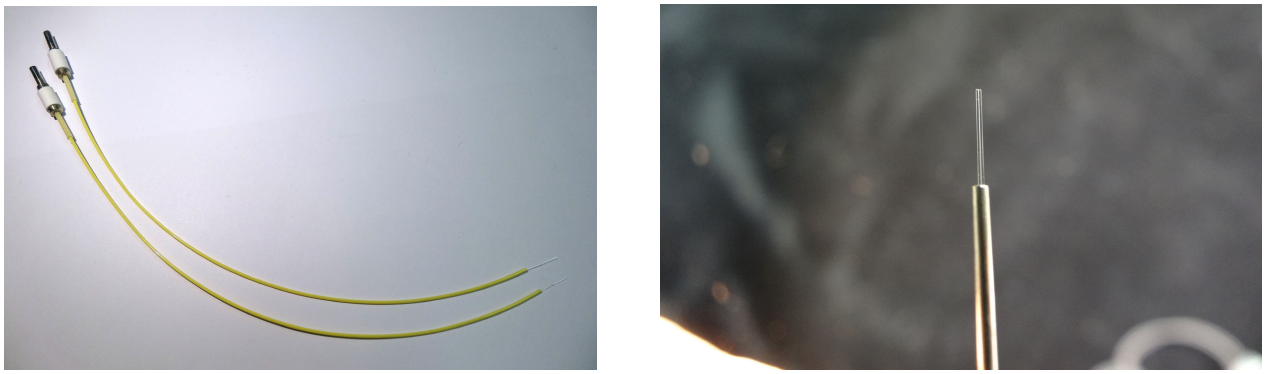

Fig. 2: Excitation and detection fibre (left), combined excitation/detection fibres (right)

For in-vivo applications it is important that the excitation and detection system can be connected and disconnected from the measurement object. This is achieved by small-size light-weight fibre connectors, Fig. 2, left, upper left. Details of the connectors are shown in Fig. 3. The total weight of the fibre connection is about 2 g.

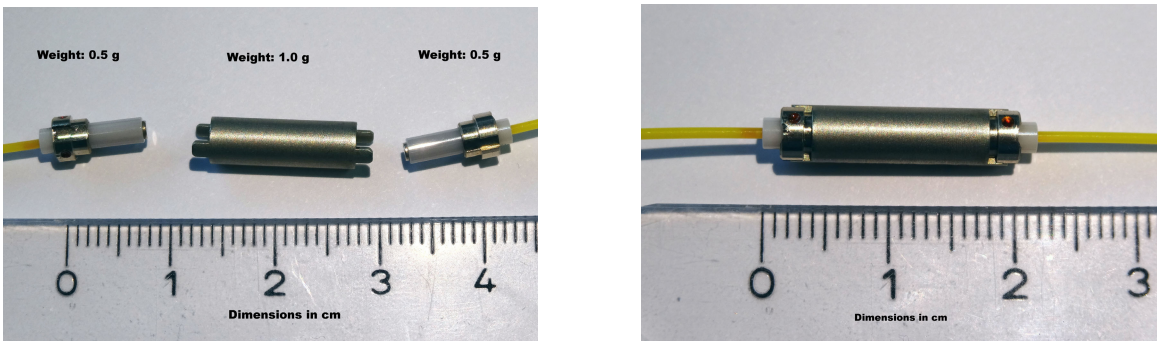

Fig. 3: Fibre mini connectors for excitation and detection fibres. Unconnected (left) and connected (right)

The principle of the fibre-based excitation and detection system is shown in Fig. 4.

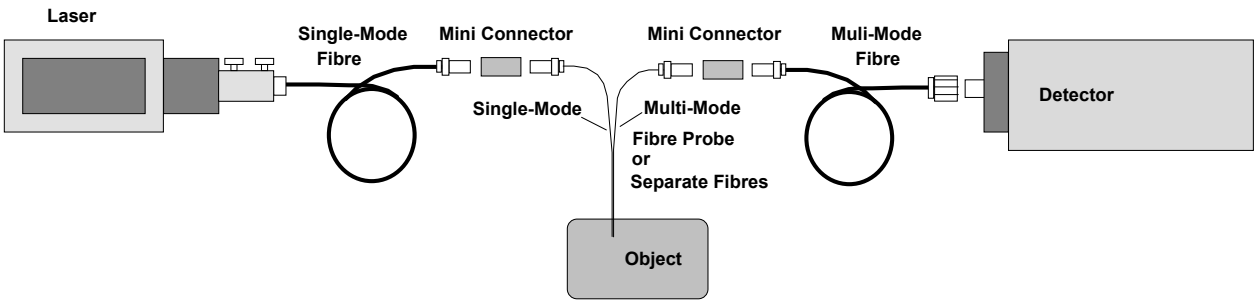

Fig. 4: Principle of fibre excitation and detection system

Detectors

The fluorescence signals are detected by bh HPM-100-40 hybrid detector modules [2]. The detectors combine excellent detection efficiency up to about 700 nm, extremely clean and short instrument-response function (IRF), and exceptionally low background signals. For the near-

infrared range up to 900 nm the HPM-100-50 detectors can be used. Emission filters can be inserted in the fibre adapters of the detectors. To detect in two wavelength intervals simultaneously two HPM detectors can by coupled to an optical beamsplitter assembly, see Fig. 5, right.

Fig. 5: Left: HPM-100 hybrid detector. Right: Two HPM-100 detectors coupled to a beamsplitter assembly.

Multi-spectral detection is obtained by using the bh PML-SPEC multi-spectral TCSPC detector, see Fig. 6. This detector is based on a PML-16 16-channel PMT array and a polychromator. It records simultaneously in 16 wavelength intervals [2, 3]. Since 2014, the PML-SPEC detector is available with a GaAsP cathode [4]. This cathode has about 5 times the efficiency of the bialkali cathodes of the older detector versions. The PML-SPEC connects directly to the TCSPC system described below. The detector uses bh's routing technique, therefore only one TCSPC module is needed to record the data of the 16 channels. Compared to the HPM-100 detectors, the PML-SPEC assembly has the advantage of spectral resolution. The disadvantage is that some light is lost in the polychromator, and that the IFR is wider [2]. The use of the PML-SPEC is mainly recommended for autofluorescence applications where spectrally resolved decay data provide essential information on the measurement object.

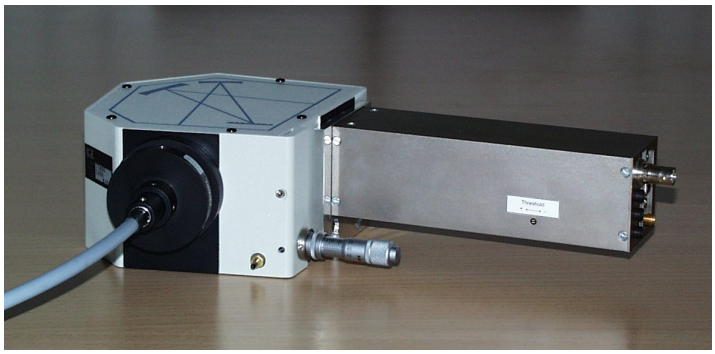

Fig. 6: PML-SPEC spectrally resolved TCSPC detector

TCSPC System

The complete TCSPC electronics is contained in an extension box connected to a laptop computer, see Fig. 7. The system is modular. Additional experiment control modules or TCSPC modules can be added to the system. Thus, depending on the type and the number of detectors used, systems with one, two, three, or four parallel TCSPC channels are available.

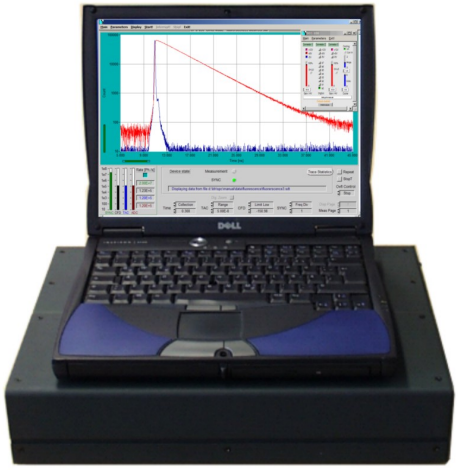

Fig. 7: Simple-Tau TCSPC system. Up to four TCSPC modules are contained in an extension box connected to a laptop computer.

The TCSPC system uses bh's multi-dimensional TCSPC technique [1, 2]. It is able to record fluorescence decay curves, time-series of fluorescence decay curves, intensity-traces, and fluorescence correlation data. Phosphorescence decay curves can be recorded simultaneously with fluorescence data [2, 5]. By triggering the acquisition with an external stimulation of the measurement object, dynamic effects in the fluorescence lifetime and intensity down to microsecond range can be recorded. With a multi-wavelength detector, such data can be recorded simultaneously in 16 wavelength intervals. Please see [2] for details.

References

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