TCSPC Fibre-Probe System with an Exchangeable Tip

Abstract: This application note describes a fluorescence-lifetime detection system based on a fibre-optical probe with an exchangeable tip. The excitation light is delivered to the tip via a single-mode fibre, the emission light is transferred to the detector by a multi-mode fibre. The electronic part of the system consist of a bh BDL-SMN picosecond diode laser, a bh PMH-100 hybrid detector or MW-FLIM GaAsP multi-wavelength detector, and a Simple-Tau 150 TCSPC system. The system features high sensitivity and short acquisition time. Clean fluorescence decay curves from a 10^{-7} mol/l fluorescein solution were recorded within an acquisition time of 0.5 seconds, time-series of autofluorescence decay curves were recorded at a speed of 100 ms per step.

Fibre-optical probes in combination with TCSPC have been described for a variety of tasks in spectroscopy of biological tissue [1, 8, 9, 12]. Recently, Cui et al. [10] have implanted optical fibres in the brains of mice to record behaviour-related Ca^{++} signals. A limitation in these applications had been motion artefacts by variable speckle patterns in the excitation fibres. Cui et al. solved this problem by using single-mode fibres for excitation. The use of single-mode fibres, however, leads to a problem with fibre-to-fibre coupling. The animals therefore could not be freely connected and disconnected to or from the measurement system. A solution was presented in [6, 11] in form of a novel miniature fibre-to-fibre connection for single-mode fibres. A second way to solve the problem is to use an implantable fibre tip that contains a short piece of multi-mode fibre [7]. It is connected to the single-mode source fibre and the multi-mode detection fibre of the fibre-probe system by a single miniature fibre connector.

System Architecture

The instrument consists of the fibre probe, the exchangeable tip, the excitation laser, the detector, and the TCSPC system. The setup is shown in Fig. 1.

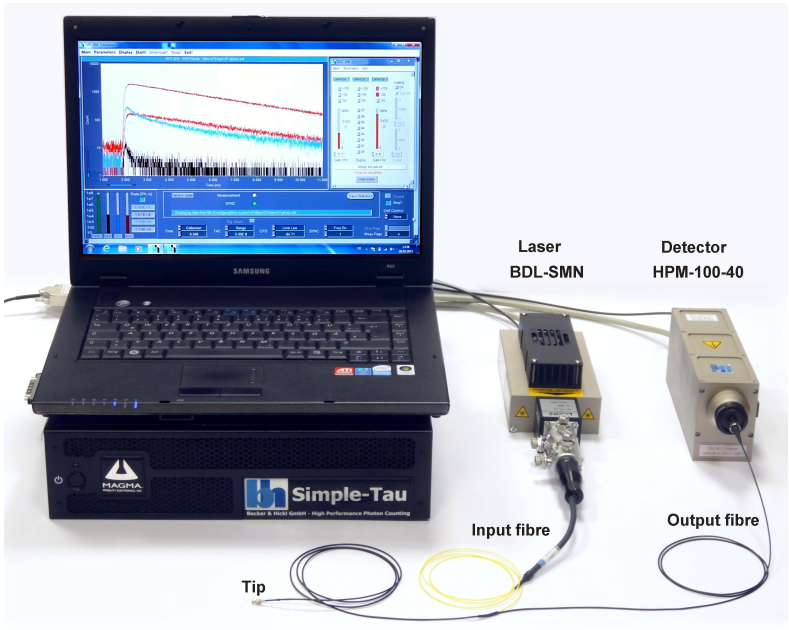

Fig. 1: Fibre-optical TCSPC system with fibre probe, BDL-SMN laser, HPM-100-40 hybrid detector, and Simple-Tau 150 TCSPC system

The excitation light is delivered by a BDL-SMN picosecond diode laser [5]. It is injected into the input fibre of the fibre probe. The fluorescence light returned from the measurement object is transferred to an HPM-100-40 detector [2, 3] by the output fibre of the probe. The input fibre is single-mode to minimise motion artefacts. The output fibre is multi-mode to obtain a high collection efficiency. The fluorescence decay curves are recorded by a bh Simple-Tau 150 TCSPC system [2].

The principle of the fibre probe [7] is shown in Fig. 2. The probe consists of the input fibre (single mode) with a Qioptiq compatible fibre connector, the output fibre (multi-mode) with an FC connector, a miniature fibre connector, and the exchangeable tip. The tip contains of a short piece of multi-mode fibre. The tip is the only part of the system that is common for the excitation and the detection beam. Background signals from fluorescence an Raman light generation in the glass of the probe are therefore kept at an acceptably low level. Photos of the tip and the miniature fibre connector are shown in Fig. 3.

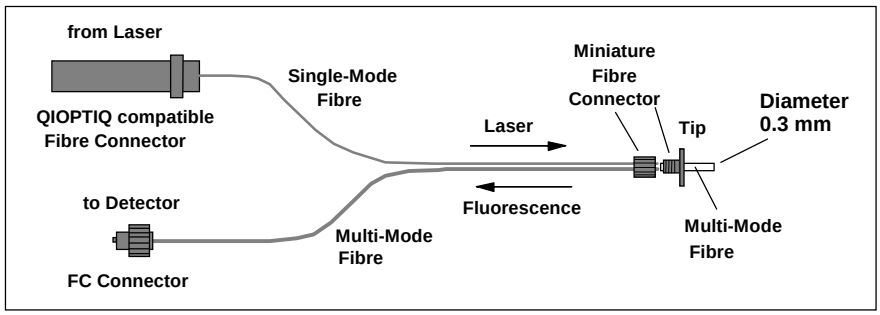

Fig. 2: Principle of the fibre probe

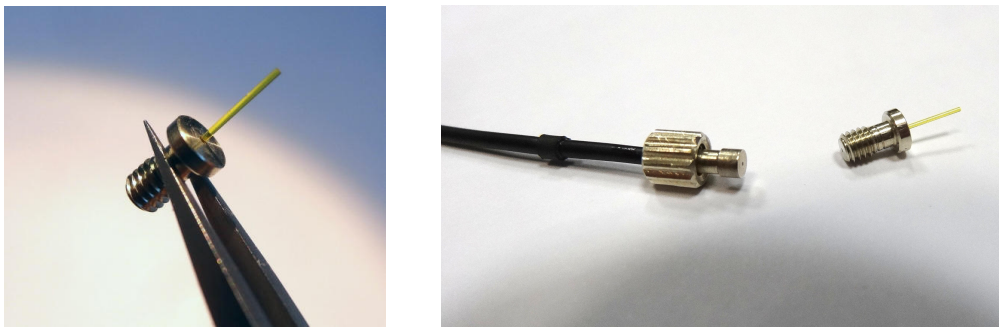

Fig. 3: Left: Exchangeable tip. Right: Connection of the tip to the fibre system by miniature fibre connector

Test Results

HPM-100-40 Hybrid Detector

The system shown in Fig. 1 was tested with fluorescein solutions of different concentration. For comparison, we also recorded the autofluorescence signal from mammalian skin. Fig. 4, left and right, show fluorescence decay curves of Fluorescein-Na 10^{-6} mol/l, Fluorescein-Na 10^{-7} mol/l, autofluorescence of mammalian skin, and the background fluorescence from the fibre probe. The excitation wavelength was 473 nm, the excitation power at the probe output was adjusted to 20 µW. The acquisition time was 0.5 seconds in Fig. 4, left, and 5 seconds in Fig. 4, right.

The results show that the probe background is low enough to record clean data from a 10^{-7} mol/l fluorescein solution. The signal obtained from the solution was approximately at the level of the autofluorescence of mammalian skin. Both the autofluorescence signal and the fluorescein signal were more than an order of magnitude stronger than the background signal from the probe.

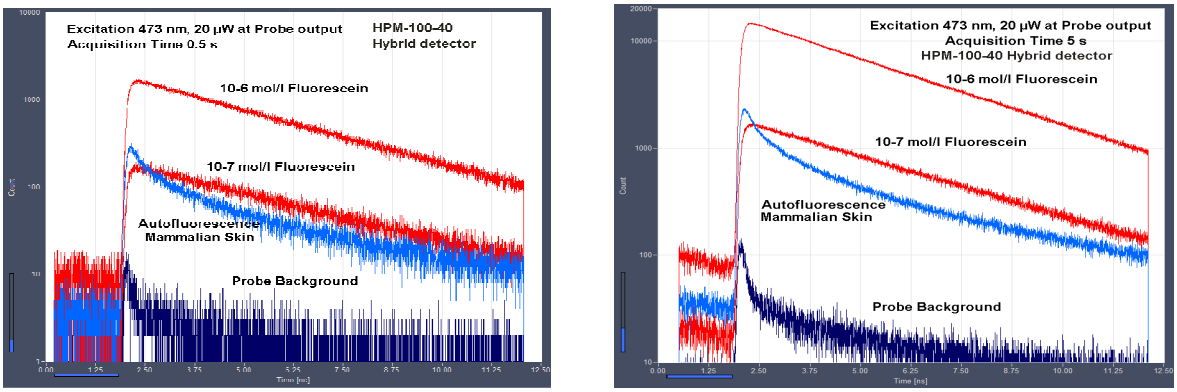

Fig. 4: Decay curves recorded with a HPM-100-40 hybrid detector. Fluorescein-Na 10^{-6} mol/l, Fluorescein-Na 10^{-7} mol/l, autofluorescence of mammalian skin, background fluorescence from the fibre probe. Excitation power 20 µW at probe output. Left: Acquisition time 0.5 seconds. Right: Acquisition time 5 seconds.

It should be noted that these results were obtained at an extremely low excitation power of 20 µW. This is a level which is considered safe for in vivo measurements in biological systems. With the BDL-SMN diode laser the excitation power can, in principle, be increased to more than 1 mW at the probe output.

MW FLIM GaAsP Multi-Wavelength Detector

The bh MW-FLIM family detectors (based on PML-16 16-channel detectors) record fluorescence decay curves in 16 wavelength intervals simultaneously [2, 4]. For multi-wavelength recording with the fibre probe we used the MW FLIM GaAsP version with a Gallium-Arsenide-Phosphid cathode. It has an efficiency about 5 time higher than the versions with multi-alkali cathodes [2].

A 0.5-second recording from a 10^{-7} mol/l fluorescein solution is shown in Fig. 5, left, a recording of autofluorescence from mammalian skin in Fig. 5, right. The excitation power was 20 µW at the tip output. The results show that even multi-spectral decay data can be obtained at low excitation power and within a surprisingly short acquisition time.

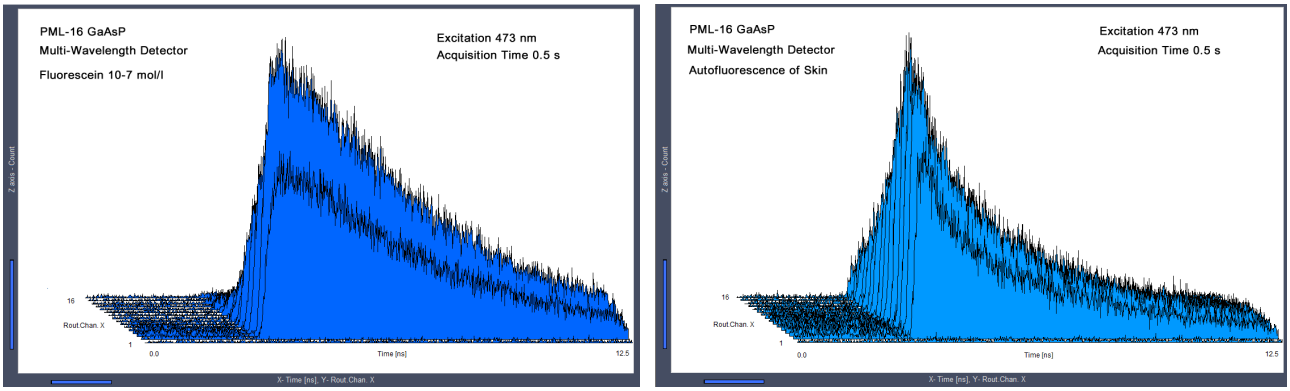

Fig. 5: Multi-wavelength decay data recorded with the MW FLIM GaAsP detector. Left: Fluorescein-Na, 10^{-7} mol/l, acquisition time 0.5 seconds. Right: Autofluorescence of mammalian skin, acquisition time 0.5 seconds. Excitation 473 nm, 20 µW.

Time-Series Recording

The acquisition times achieved with the system are short enough to record physiological changes in live objects at the millisecond time scale. To demonstrate the feasibility of time-laps recording we recorded a time-series of autofluorescence decays from human skin. Temporal changes were induced by scanning the tip over the surface of the skin. The excitation power was adjusted to

100 µW. Under these conditions, the TCSPC system recorded about 10^6 photons per second. A recording rate of 10 measurements per second, i.e. a time per curve of 100 ms was used. A typical result is shown in Fig. 6.

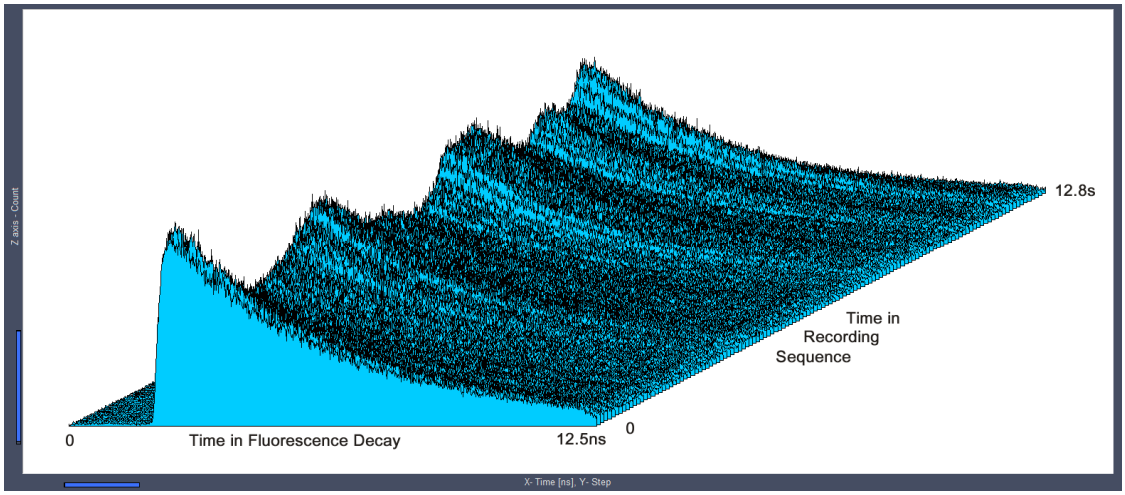

Fig. 6: Time-series recorded at a rate of 100 ms per curve. Autofluorescence of skin, HPM-100-40 hybrid detector, excitation power 100 µW.

The data shown in Fig. 6 contain about 100,000 photons per decay curve. The relative accuracy at which a fluorescence lifetime can be derived from 100,000 photons is about 0.3 % [1, 2]. This is much better than required for most bio-medical applications. For an accuracy of 1%, only 10,000 photons per curve would be needed. That means, the speed of the sequence can be increased to 100 curves per second without introducing unacceptable variance in the recorded fluorescence lifetimes.

References

1. W. Becker, Advanced time-correlated single-photon counting techniques. Springer (2005)
2. W. Becker, The bh TCSPC handbook. 6th edition. Becker & Hickl GmbH (2014), www.becker-hickl.com.
3. Becker, W., Su, B., Weisshart, K. & Holub, O. (2011) FLIM and FCS Detection in Laser-Scanning Microscopes: Increased Efficiency by GaAsP Hybrid Detectors. Micr. Res. Tech. 74, 804-811
4. Becker & Hickl GmbH, 16 channel detector head for time-correlated single photon counting. User handbook, www.becker-hickl.com (2006)
5. Becker & Hickl GmbH, BDL-SMN series picosecond diode lasers. User handbook, www.becker-hickl.com
6. Becker & Hickl GmbH, Implantable fibre-optical fluorescence-lifetime detection system for in-vivo applications. Application note, www.becker-hickl.com
7. IFP-201 Implantable Fibre Probe for *in vivo* Fluorescence Decay Measurements. Data sheet, www.becker-hickl.com
8. P. A. A. De Beule, C. Dunsby, N. P. Galletly, G. W. Stamp, A. C. Chu, U. Anand, P. Anand, C. D. Benham A. Naylor, P. M. W. French, A hyperspectral fluorescence lifetime probe for skin cancer diagnosis. Rev. Sci. Instrum. 78, 123101 (2007)
9. S. Coda, A. J. Thompson,1,5 G. T. Kennedy, K. L. Roche, L. Ayaru, D. S. Bansi, G. W. Stamp, A. V. Thillainayagam, P. M. W. French, C. Dunsby, Fluorescence lifetime spectroscopy of tissue autofluorescence in normal and diseased colon measured ex vivo using a fiber-optic probe. Biomed. Opt. Expr. 5, 515-538 (2014)
10. G. Cui, S.B.Jun, X. Jin, M.D. Pham, S.S. Vogel, D.M. Lovinger, R.M. Costa, Concurrent activation of strial direct and indirect pathways during action initiation. Nature 494, 238-242 (2013)
11. G. Cui, S.B.Jun, X. Jin, G. Luo, M.D. Pham, D.M. Lovinger, S.S. Vogel, R.M. Costa, Deep brain optical measurement of cell type-specific neural activity in behaving mice. Nature Protocols, 9(6) 1213-1228 (2014)
12. C. Dunsby, P.M.W. French, Single-point probes for lifetime spectroscopy: Time-correlated single-photon counting technique. In: L. Marcu. P.M.W. French, D.S. Elson, (eds.), Fluorecence lifetime spectroscopy and imaging. Principles and applications in biomedical diagnostics. CRC Press, Taylor & Francis Group (2015)