TCSPC at Wavelengths from 900 nm to 1700 nm

We describe picosecond time-resolved optical signal recording in the spectral range from 900 nm to 1700 nm. The system consists of an id Quantique id220 InGaAs SPAD, a bh SPC-150 TCSPC device, and a bh BDS-SM 1064 nm ps diode laser. In contrast to earlier InGaAs SPADs the id220 works in a continuous (asynchronous) mode. The id 220 / SPC-150 combination can be operated at a pulse repetition rate in the 10 to 100 MHz range. As a result, there is virtually no pile-up distortion, and advanced multi-dimensional TCSPC modes are applicable. The width of the temporal IRF (Instrument Response Function) is about 230 ps, including laser pulse width and pulse dispersion in the optics. We demonstrate the application of the system to the recording of time-of-flight distributions in turbid media, for fluorescence decay measurement, and for fluorescence lifetime imaging (FLIM) in combination with fast galvanometer scanning.

Detectors for the Spectral Range beyond 900 nm

For many years, a problem of TCSPC was the lack of suitable single-photon detectors for the infrared region. PMTs with IR-sensitive cathodes were available but suffered from high dark count rate and low quantum efficiency. Superconducting detectors have good quantum efficiency but extremely small active areas. They are still in the state of prototype development. The situation improved with the availability of single-photon avalanche diodes (SPADs) based on InGaAs material. These detectors achieve a quantum efficiency of about 20% at a wavelength 1300 nm. The useful wavelength range extends from 900 nm to 1700 nm. The problem of early InGaAs SPADs was the high dark count rate, and the strong afterpulsing. To avoid instability, the devices could only be operated in a gated mode: The reverse voltage was increased above the breakdown level for a period of 10 to a few 100 ns, and then decreased below the critical level for several microseconds. Gated operation is acceptable for general photon counting in combination with low-repetition rate light sources. It is, however, a problem in combination with TCSPC: TCSPC works best at high repetition rate of the light signals, and requires the detector to be active continuously.

Standard TCSPC detection in the IR became possible with the introduction of the Id Quantique id220 detector [1]. The id220 is the first InGaAs SPAD that is operated in a continuous mode. It thus can be used for TCSPC without any restrictions. In this article, we demonstrate IR TCSPC by combining an id220 detector [1], a bh Simple-Tau 150 system [3], and a BDS-SM (1064 nm) picosecond diode laser [4]. The components are shown in Fig. 1.

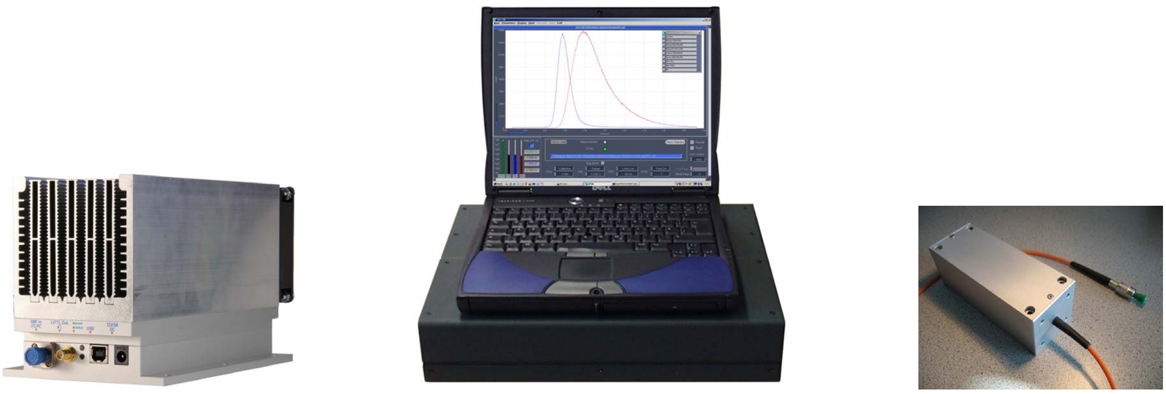

Fig. 1: Left to right: Id 220 InGaAs SPAD detector, Simple-Tau 150 TCSPC system, BDS-SM picosecond diode laser

Basic TCSPC Experiment

For a general test of the id220 in combination with the bh TCSPC modules we used the setup shown in Fig. 2. A bh BDS-SM picosecond diode laser generates a 50 MHz train of picosecond light pulses at a wavelength of 1064 nm. The optical output from the laser is via a single-mode fibre. The end of the fibre was brought in contact with a cuvette containing a dye solution or a scattering medium. The light from the sample was collected by a multi-mode fibre and transferred to the detector. The TCSPC system was a bh Simple-Tau 150, containing an SPC-150 TCSPC module [3]. The detector output pulses were adapted to the input voltage range of the SPC module by an attenuator and an A-PPI-D pulse inverter. The synchronisation of the TCSPC module comes directly from the laser.

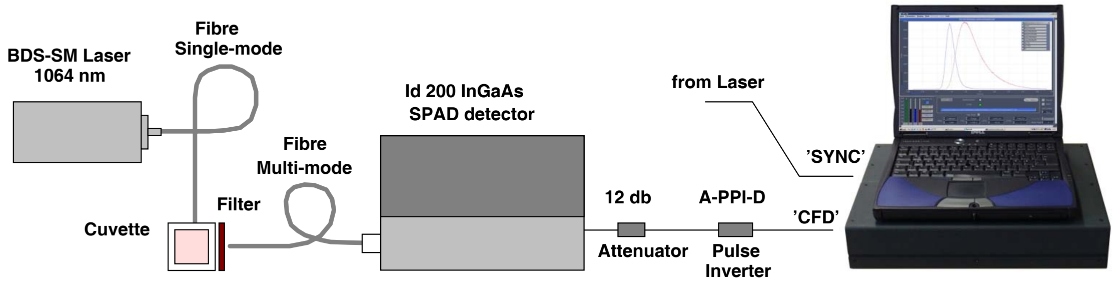

Fig. 2: Standard TCSPC experiment

Compared to a setup with focusing and light-collecting lenses the optics is not the most efficient one. It was used because it is insensitive to alignment and thus less likely to produce artefacts by re-absorption and re-emission.

Typical waveforms recorded with the system are shown in Fig. 3. The curves show signals obtained from diluted milk (black curve), IR1061 (Aldrich, brown curve), and undiluted milk (red curve).

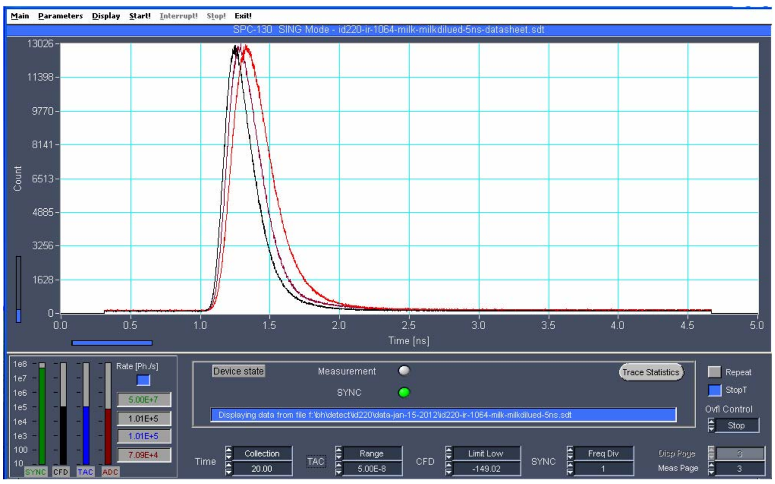

Fig. 3: Waveforms recorded with the setup shown in Fig. 2. Black curve : Diluted Milk. The curve can be expected to reproduce the temporal IRF. Brown curve: Fluorescence decay of IR1061. Red curve: Photon migration in milk, undiluted. Time axis 0 to 5 ns, 500 ps per division, 1.22 ps per data point.

The curve from the diluted milk (black curve) can be expected to reproduce the temporal IRF. It is surprisingly fast: The full width at half maximum (FWHM) is 230 ps. This includes the laser pulse width, the transit time differences in the 1 cm cuvette, and the transit time spread in the detector fibre. A deconvolution of the IR1061 fluorophore from the IRF delivers about 50 ps. This is in agreement with the fluorescence decay time reported for IR1061 in the literature. The photon migration time (shift of first moment) in the milk is about 85 ps. This appears low compared to measurements at wavelengths below 800 nm but can be explained by the fact that the scattering coefficient decreases with the wavelength.

Operating Conditions of the Detector

The id 220 detector comes with software that allows the user to adapt the detector parameters to the current experiment. Both the detection efficiency and the dead time (quenching time after detecting a photon) can be modified. We made test measurement with various parameter combinations. The best TCSPC results were obtained with the detection efficiency set to the maximum of 20%, and a dead time of 5 µs. Reducing the detection efficiency results in lower dark count rate. However, the efficiency drops by the same ratio as the dark count rate. Unless there is abundance of light little is gained in terms of signal-to noise ratio. Moreover, lower detection efficiency results in a broader IRF. Reducing the detection efficiency is therefore not recommended. The dead time of 5 µs was found to be a compromise between counting speed and afterpulsing background. Dead time settings larger than 5 µs unnecessarily reduce the saturated count rate, dead times shorter than 5 µs result in a noticeable increase of the signal-dependent counting background. Please note that a dead time of 5 µs is much longer than the dead time of the bh TCSPC modules, and much longer than the dead time of visible-range detectors. For 5 µs dead time, the saturated count rate is 200 kHz. The recording rate should therefore be kept below 100 kHz.

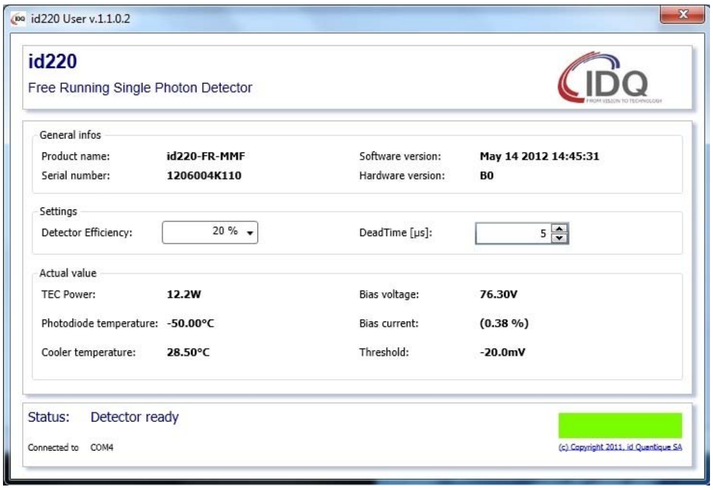

Fig. 4: Setup of the id 200 detector parameters

Fluorescence Lifetime Imaging (FLIM)

We evaluated the performance of the id 220 / SPC-150 combination for TCSPC FLIM at infrared wavelengths.

TCSPC FLIM uses the capability of multi-dimensional TCSPC to record a photon distribution over several parameters [2]. The sample is scanned by a focused laser beam. Single photons of the fluorescence light are detected, and, for every individual photon, the time in the laser pulse period and the location of the laser spot in the image area are determined. Accumulating the photons over a large number of scans, the TCSPC device builds up a photon distribution over the time in the signal period and the coordinates of the scan. The result can be considered an array of pixels, each one containing a full fluorescence decay curve. The advantage of the multi-dimensional recording process is that it does not reject any photons by gating, and that it works at the high scan rates used in modern lasers scanning microscopes.

The experimental setup is shown in Fig. 5. It consists of a Simple-Tau TCSPC system, a BDS-SM 1064 nm picosecond diode laser, a bh DCS-120 confocal scan head [3, 5] attached to a Nikon TE 2000 microscope, and the id220 InGaAS SPAD detector.

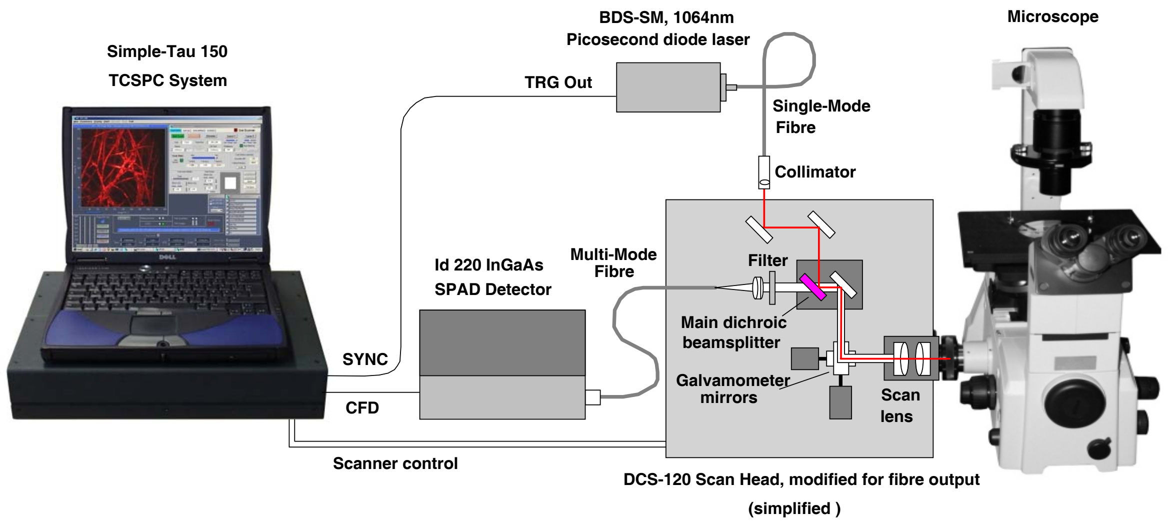

Fig. 5: Principle of FLIM setup

A typical IR FLIM image obtained with the system is shown in Fig. 6. The image shows cellulose fibres stained with IR 1061 from Aldrich. The image format is 512 x 512 pixels, 256 time channels per pixel. An x20, NA=0.6 microscope lens was used. The detector count rate was kept below 50 KHz to avoid saturation of the intensity data. The excitation power for this count rate was about 30 µW in the sample plane.

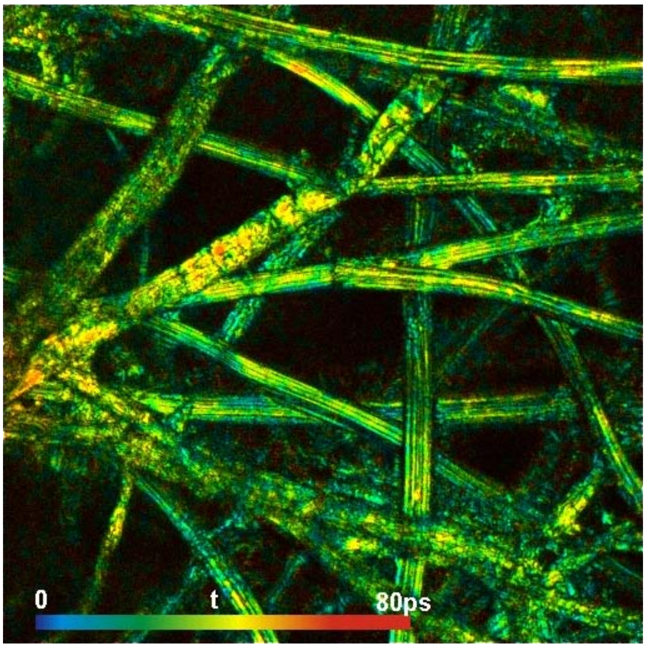

Fig. 6: Cellulose fibres, stained with IR1061 (Aldrich). Excitation 1064 nm, detection 1100nm to 1400nm. 512x512 pixels, 256 time channels per pixel. Colour represents fluorescence lifetime, lifetime range from 0 to 80 ps.

The FLIM data were processed by bh's SPCImage FLIM analysis software [5, 6]. The analysis was performed with a measured IRF, obtained from the reflection of the laser at the cover slip. The IRF and a typical decay curve from a selected pixel are shown in Fig. 7. As expected from the results of the cuvette experiments, the fluorescence lifetimes are very short. The distribution of the lifetime over the pixels peaks at about 50 ps. Despite of the fact that the lifetimes are much shorter than the FWHM of the detector, clear lifetime differences were resolved throughout the image. This shows that the id 220 detector is well applicable to IR FLIM.

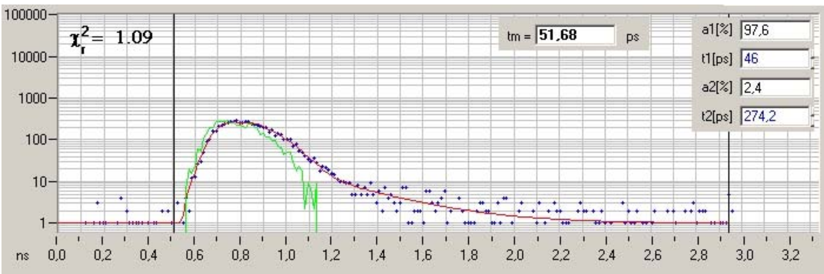

Fig. 7: Decay data (blue spots) and IRF (green) in a selected pixel

Summary

We have shown that the combination of the id 220 InGaAs SPAD and the bh SPC-150 TCSPC card is able to record fluorescence decay curves of IR dyes, time-of-flight distributions of photons in scattering media, and, with a confocal scanner, FLIM images of samples under a microscope. Unfortunately, the fluorescence lifetimes of currently available IR dyes are extremely short. The fluorescence lifetime obtained for IR1061 was about 50 ps. Nevertheless, the lifetime and its variation were well detectable in the FLIM data. Although the results described above were obtained with an SPC-150 card in a Simple-Tau system they can be considered typical of all bh TCSPC devices. Existing TCSPC systems may therefore be upgraded for IR detection with the new detector.

References

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