Instrumentation

Time-resolved fluorescence intensities are measured via time-correlated single photon counting (TCSPC) using a pulsed excitation light source and a single-photon sensitive detector. The arrival time of the emitted fluorescence photon is recorded with picosecond accuracy concerning the incoming excitation light (Figure 1).

Repeating the excitation-emission process many times will give a decay profile (Figure 2). Pulsed lasers or LEDs can be used as a source of excitation. The excitation light is passed through a polarizer before it hits the sample in the cuvette. Part of the light passes through the sample, the other to the electronics as “sync” signal. The light emitted by the sample molecule is passed first through a polarizer and then through a monochromator to select a specific wavelength (~range). The light is then detected and amplified by a photomultiplier tube (PMT). The emitted light signal, as well as reference light signal, is processed through a constant fraction discriminator (CFD) which eliminates timing jitter. After passing through the CFD, the reference pulse activates a time-to-amplitude converter (TAC) circuit. The TAC charges a capacitor which will hold the signal until the next electrical pulse. In reverse TAC mode the signal of “sync” stops the TAC. This data is then further processed by an analog to digital converter (ADC) and multi-channel analyzer (MCA) to get a data output. To make sure that the decay is not biased to early arriving photons (pile-up), the photon count rate is kept low (usually less than ~ 1.5 % of excitation rate (repetition rate of the light source).

The maximum – and usually used – repetition rate of the here available NanoLED is 1 MHz. Thus, the count rate on the detector should not exceed 15’000 counts/sec. Laser and LEDs can be operated at much higher repetition rates and are rather limited by the fluorescence lifetime of the samples.

The time interval between two laser pulses should be ~ 10x as long as the fluorescence lifetime time τ. For Alexa488, τ = 4 ns resulting in $\Delta t >= 40$ ns and maximum repetition rate of 1/40 ns = 25 MHz.

For the MCA, the longer a molecule takes to emit a photon, the higher the voltage of the resulting pulse. The central concept of this technique is that only a single photon is needed to discharge the capacitor. Thus, this experiment must be repeated many times to gather the full range of delays between excitation and emission of a photon (Figure 2). After each trial, a pre-calibrated computer converts the voltage sent out by the TAC into a time and records the event in a histogram of time since excitation.

This histogram has a limited number of bins (for our setup 1024), which are equally

distributed between two subsequent light pulses. In our setup for a repetition rate of 1 MHz, the time between two pulses is 1/1 MHz = 100 ns.

Since the probability that no molecule will have relaxed decreases with time, a decay curve emerges that can then be analyzed to find out the decay rate of the event.

Since the emission of the photons is a stochastic process, it can be described by an exponential decay F(t). The inverse of the characteristic decay time constant kF is called the fluorescence lifetime τ of the respective fluorophore.