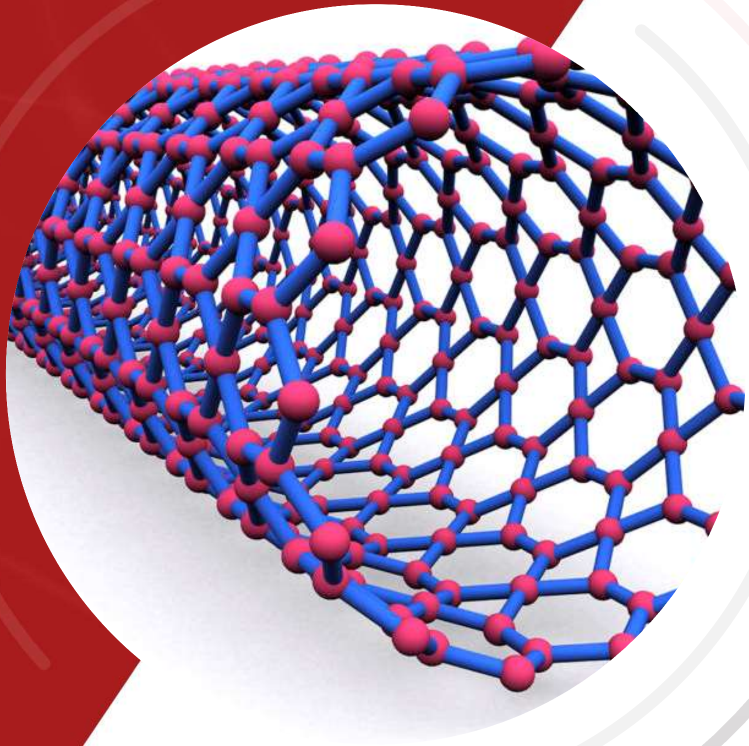
TCSPC and NIR Fluorescence Lifetime Spectroscopy for Nano-Materials Characterization and Biology

ID Quantique

Rik van Gorsel, Ph.D.

VP Quantum Sensing - Americas

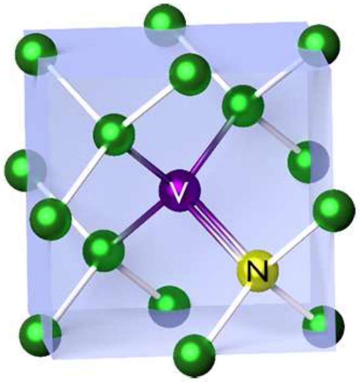


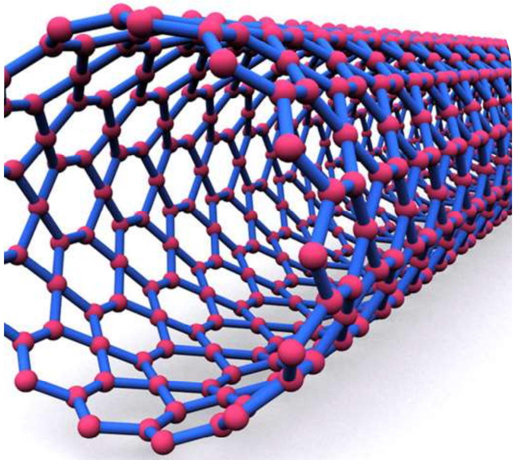
13 October 2020

Fluorescence measurements

- Why use Fluorescence?
  - sensitive
  - selective
  - non-destructive

- Novel Fluorescence measurements
  - diamond vacancy centers
  - low-dimensional nanomaterials





- Applications of NIR Fluorescence Lifetime to
  - Material Science
  - Life Sciences
  - Point-of-Care medicine

01

Fluorescence Lifetime and TCSPC concepts

- What is fluorescence?
    - Intensity, spectrum, lifetime, polarization
    - Jablonski diagram
    - Stokes shift
    - Instrument Response Function (IRF)
- What is fluorescence lifetime?
- How can we measure fluorescence lifetime?
- Time-Correlated Single Photon Counting (TCSPC)
- Förster Resonance Energy Transfer (FRET)

Fluorescence at the single-photon level

Why

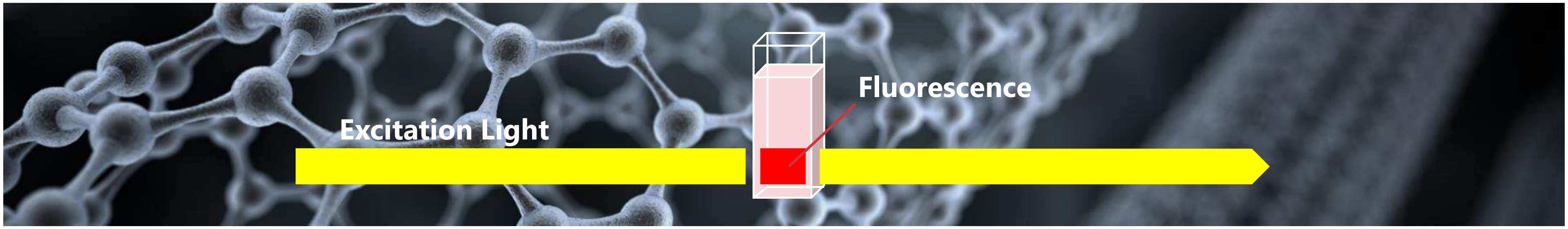
- Sensitive
- Selective
- Non-destructive

- Can do things that one can not do using analogue techniques
- Highest sensitivity
- High time resolution

How

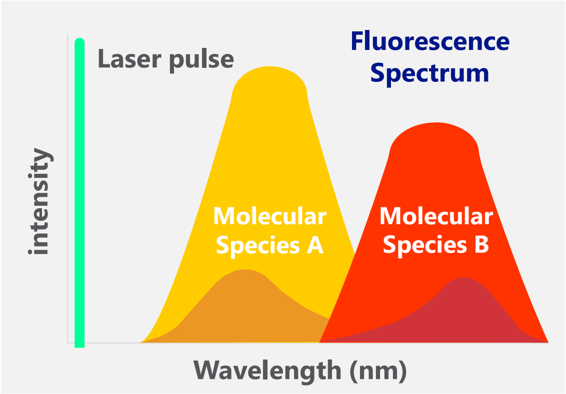
Single Photon Detectors and Electronics
High performance for Best science

Fluorescence

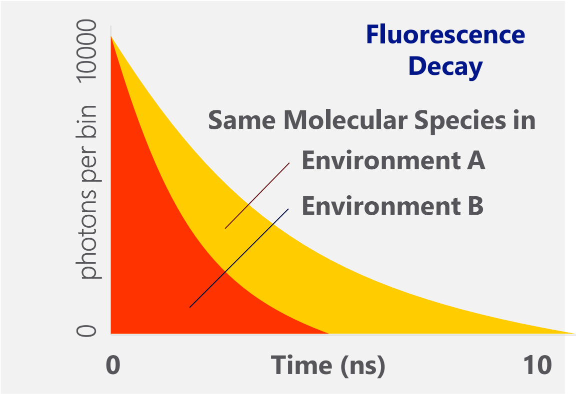


Fluorescence is characterised by

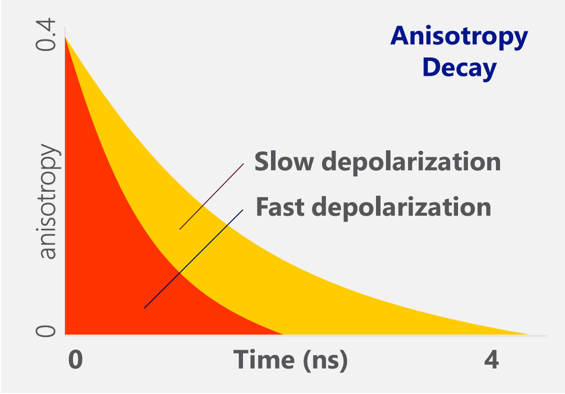
Spectrum



Lifetime

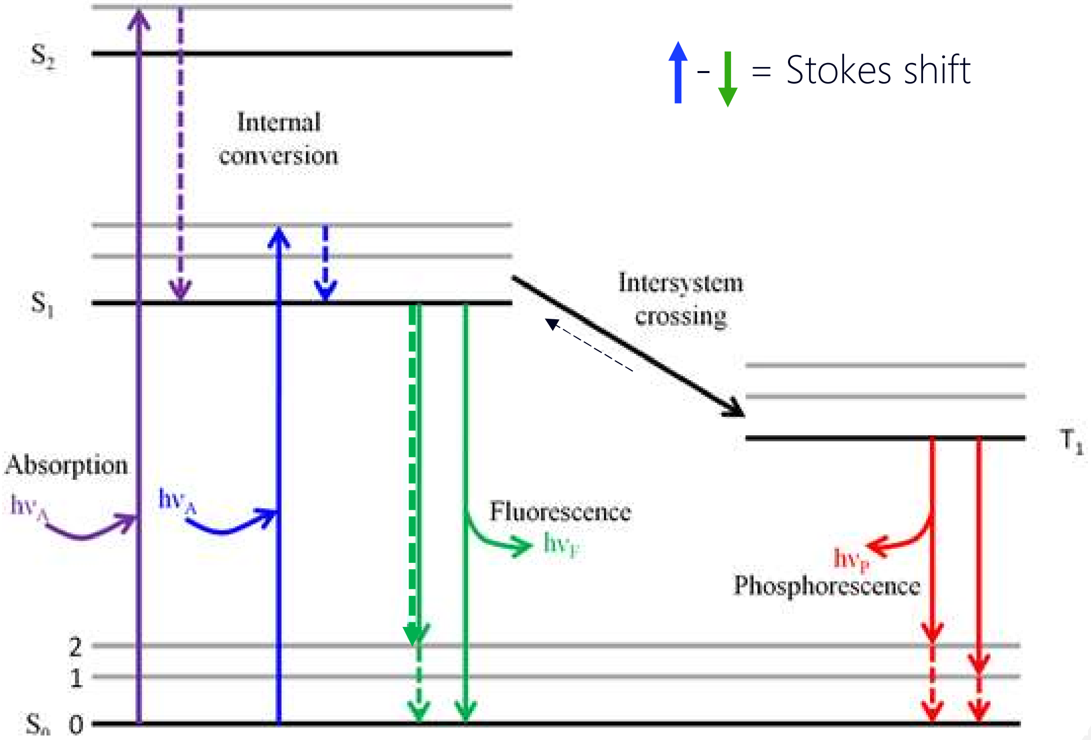


Polarization



Jablonski diagram – what one molecule can do

- Excitation leads to absorption, followed by
- *heat* dissipation ⇣
- fluorescence emission
- or InterSystem Crossing (ISC)
- with delayed fluorescence
- or phosphorescence
- Quenching: e.g. 'stealing' of the energy by another molecule that is close by and *rocks in the same way*: FRET
- FRET –> Dimming and Shortening of the lifetime (τ)
- Stokes shift reflects the energy difference

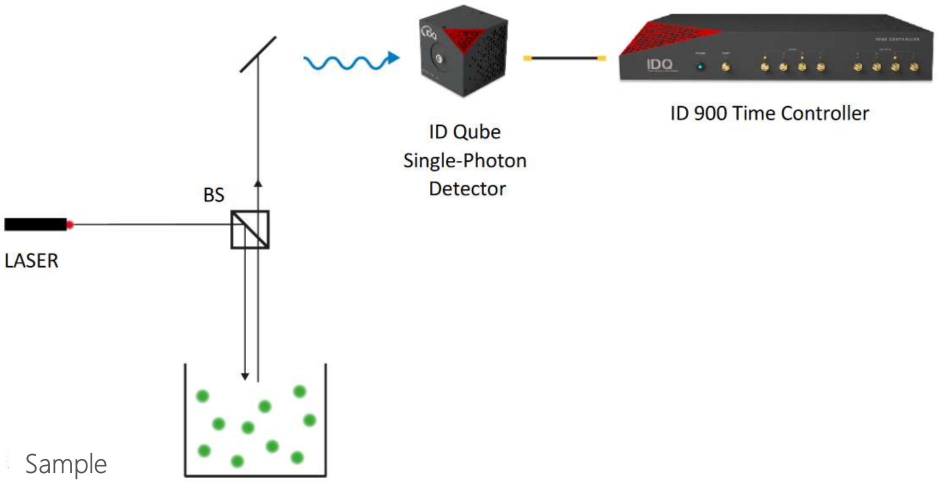


↑ - ↓ = Stokes shift

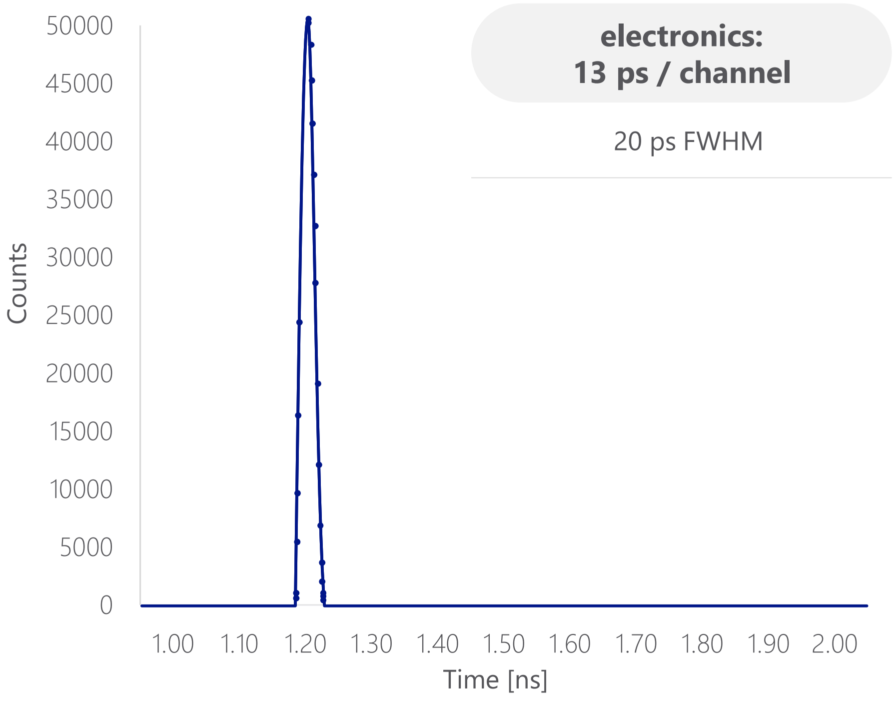
Fluorescence measurement accuracy

A key parameter in measurement accuracy is the IRF, the Instrument Response Function

- The overall **IRF** quantifies the timing uncertainty in a system
- The overall IRF is an important parameter to evaluate fluorescence measurement accuracy, and the ability to discriminate between samples, treatments or conditions.
- The overall IRF is determined by the timing uncertainty, the timing jitter, in a setup that generally includes:
  - laser
  - passive optical components. comprising the central part of the setup
  - detector
  - recording electronics

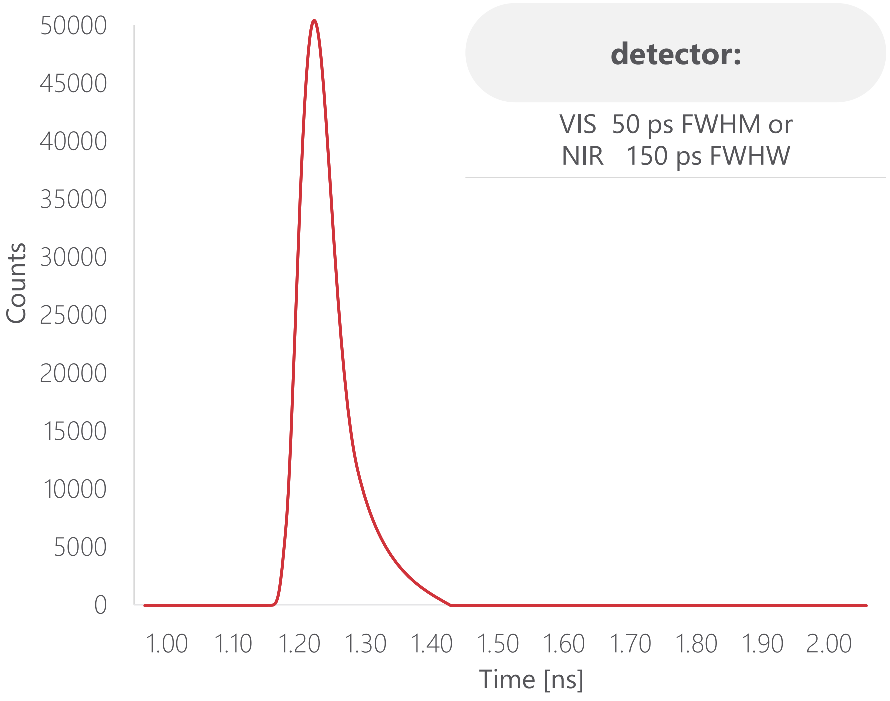


IRF – INSTRUMENT RESPONSE FUNCTION - typical instrument values



electronics:
13 ps / channel

20 ps FWHM



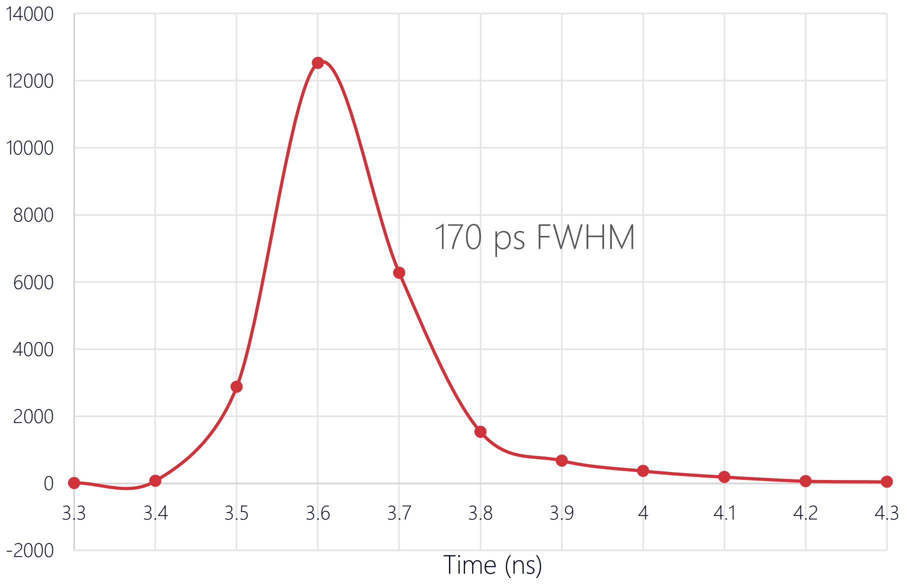
detector:

VIS 50 ps FWHM or
NIR 150 ps FWHW

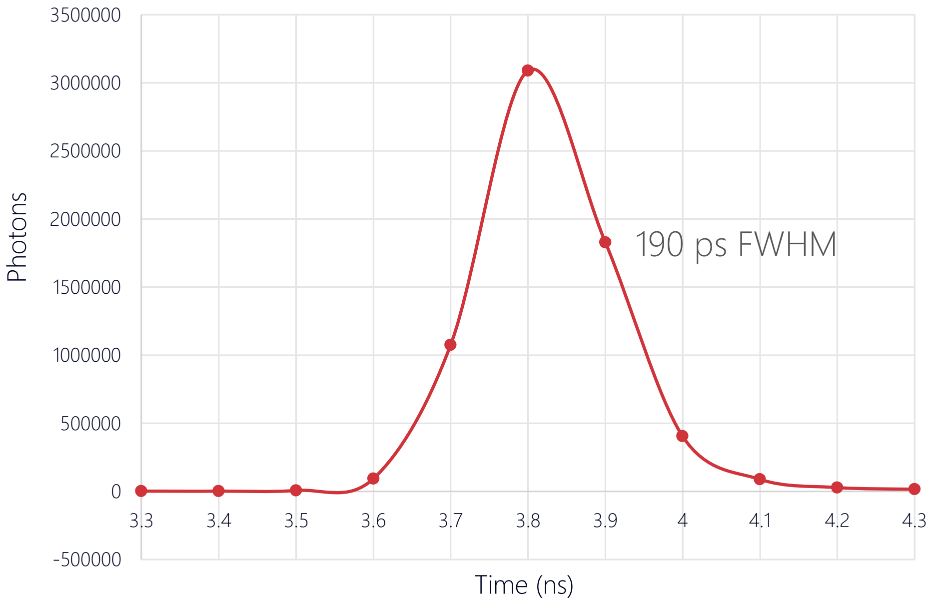
IRF

EXPERIMENTAL DETERMINATION

GOLD NANOPARTICLES



SCATTERING MILK SOLIDS



IRF – INSTRUMENT RESPONSE FUNCTION

MATHEMATICAL APPROXIMATION

The Overal IRF, quantifies the timing uncertainty in a system.

The overall IRF in a setup that includes laser, passive optical components comprising the central part of the setup, detector, and recording electronics, can be approximated by the square root of the sum of squares of the IRF of the individual components

$$Overall\ IRF \cong \sqrt[2]{IRF_{laser}{}^{2} + IRF_{optics}{}^{2} + IRF_{detector}{}^{2} + IRF_{electronics}{}^{2}}$$

The overall IRF is an important parameter to evaluate fluorescence measurement accuracy, and the ability to discriminate between samples, treatments or conditions.

IRF

MATHEMATICAL APPROXIMATION – example 1

- Laser = 6 ps
- Optics = 4 ps
- Si Detector = 50 ps
- Electronics = 20 ps

$$Overall\ IRF \cong \sqrt[2]{IRF_{laser}{}^{2} + IRF_{optics}{}^{2} + IRF_{detector}{}^{2} + IRF_{electronics}{}^{2}}$$

⇨ $\text{Overall IRF} \cong 54\ \text{ps}$ (FWHM)

IRF

MATHEMATICAL APPROXIMATION – example 1

- Laser = 6 ps
- Optics = 4 ps
- Si Detector = 50 ps
- Electronics = 4 ps

$$Overall\ IRF \cong \sqrt[2]{IRF_{laser}{}^{2} + IRF_{optics}{}^{2} + IRF_{detector}{}^{2} + IRF_{electronics}{}^{2}}$$

⇨ $\text{Overall IRF} \cong 51\ \text{ps}$ (FWHM)

IRF

MATHEMATICAL APPROXIMATION – example 2

- Laser = 6 ps
- Optics = 4 ps
- InGaAs Detector = 150 ps
- Electronics = 20 ps

$$Overall\ IRF \cong \sqrt[2]{IRF_{laser}{}^{2} + IRF_{optics}{}^{2} + IRF_{detector}{}^{2} + IRF_{electronics}{}^{2}}$$

⇒ $\text{Overall IRF} \cong 152\ \text{ps}$ (FWHM)

IRF

MATHEMATICAL APPROXIMATION – example 2

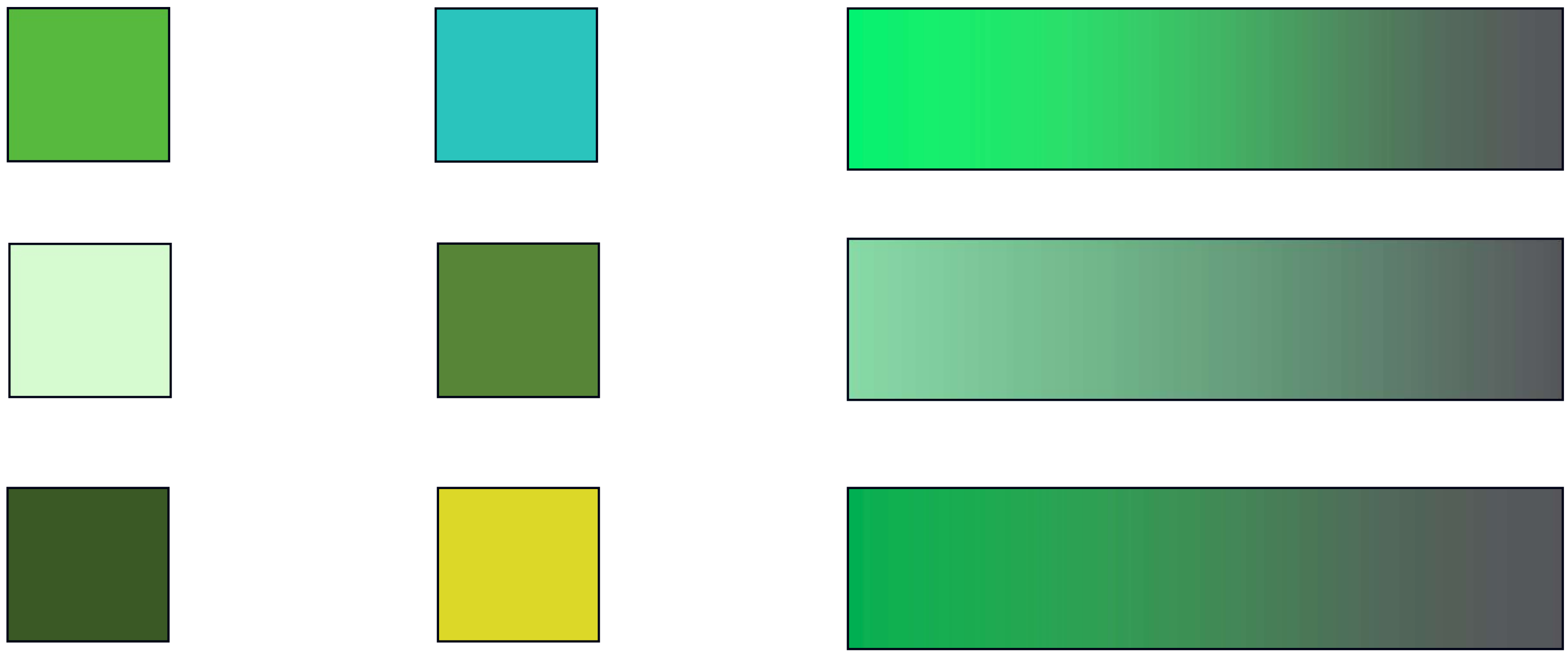
- Laser = 6 ps
- Optics = 4 ps
- InGaAs Detector = 150 ps
- Electronics = 4 ps

$$Overall\ IRF \cong \sqrt[2]{IRF_{laser}{}^{2} + IRF_{optics}{}^{2} + IRF_{detector}{}^{2} + IRF_{electronics}{}^{2}}$$

⇨ $\text{Overall IRF} \cong 150\ \text{ps}$ (FWHM)

INTENSITY – COLOR SPECTRUM - LIFETIME

Intensity = ∑concentration of kinds   Spectrum = type of molecule   Lifetime = environment and interaction

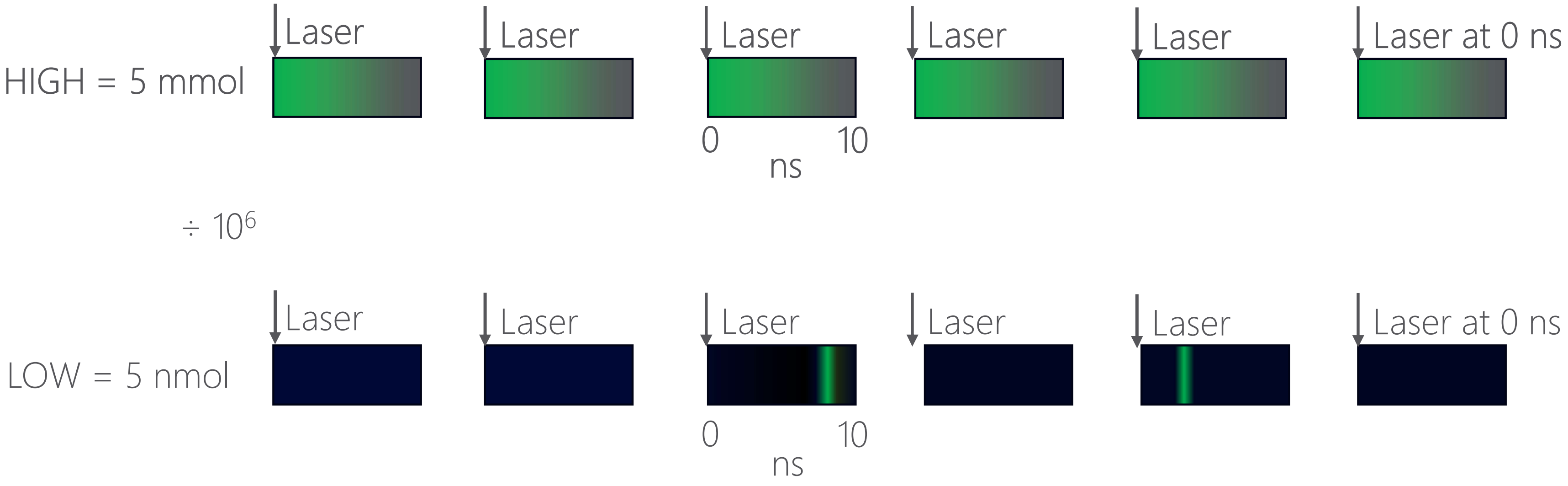


sample 1

sample 2

sample 3

Fluorescence decay at high and very low concentrations



HIGH = 5 mmol

Laser

Laser

Laser

Laser

Laser

Laser at 0 ns

0 10

ns

÷ 10^6

LOW = 5 nmol

Laser

Laser

Laser

Laser

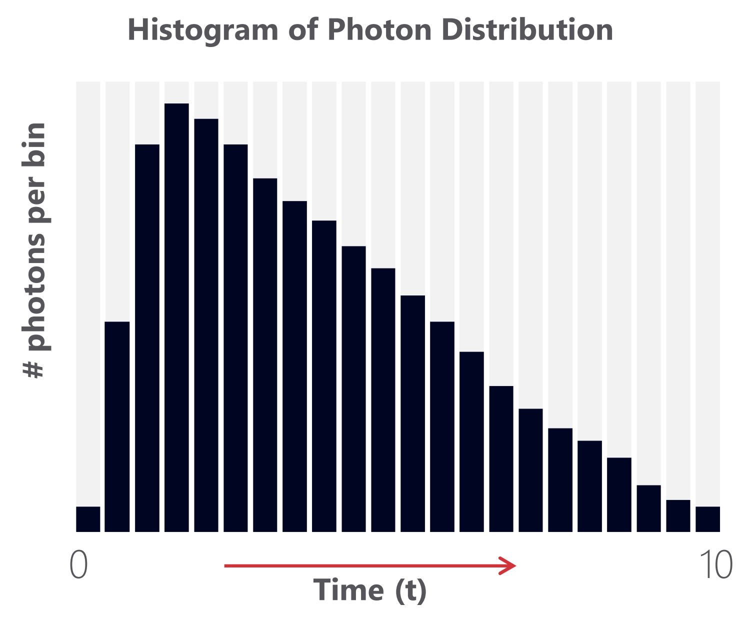
Laser

Laser at 0 ns

0 10

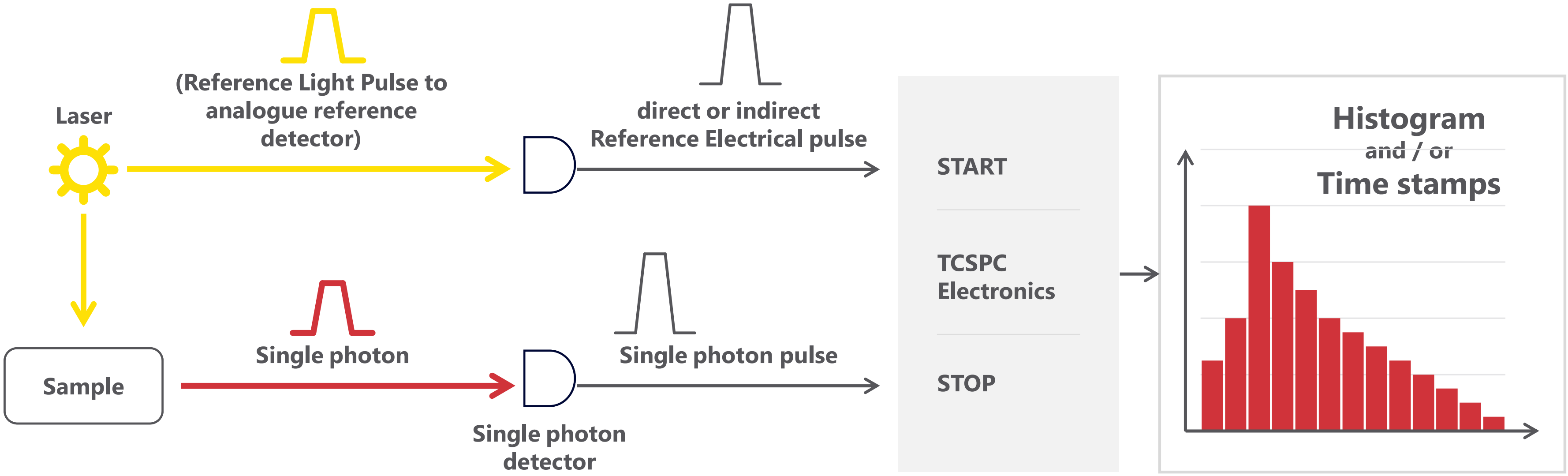
ns

Principle of Time-Correlated Single Photon Counting (TCSPC)



TCSPC – implementation

TCSPC – schematic with laser, detector and electronics



Laser

(Reference Light Pulse to analogue reference detector)

direct or indirect Reference Electrical pulse

Sample

Single photon

Single photon pulse

Single photon detector

START

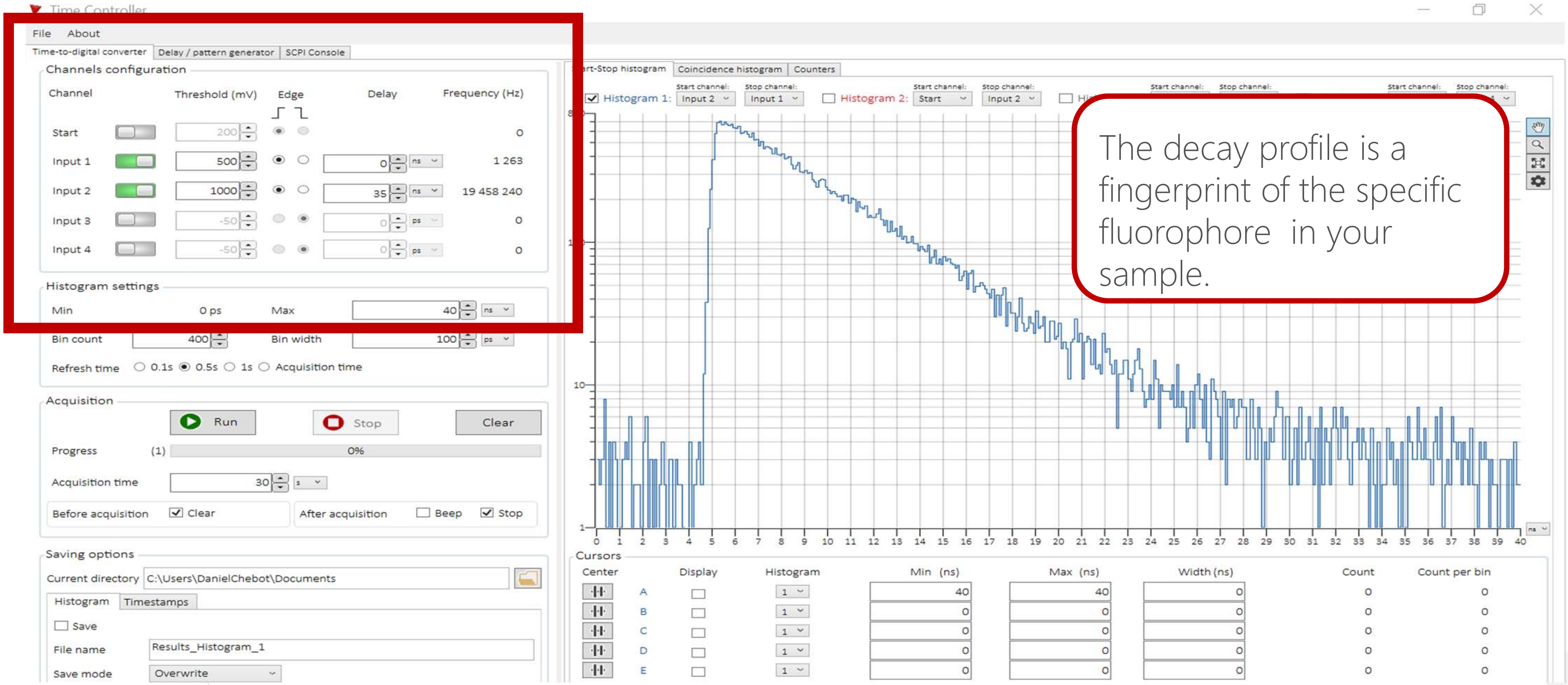
TCSPC Electronics

STOP

Histogram
and / or
Time stamps

ID900 Time Controller

TCSPC – implementation in the id900 Time Controller



Channels configuration

Channel	Threshold (mV)	Edge	Delay	Frequency (Hz)
Start	200	● ○		0
Input 1	500	● ○	0 ns	1 263
Input 2	1000	● ○	35 ns	19 458 240
Input 3	-50	○ ●	0 ps	0
Input 4	-50	○ ●	0 ps	0

Histogram settings

Min 0 ps
Max 40 ns
Bin count 400
Bin width 100 ps
Refresh time ○ 0.1s ● 0.5s ○ 1s ○ Acquisition time

Acquisition

Progress (1) 0%
Acquisition time 30 s
Before acquisition ☑ Clear
After acquisition ☐ Beep ☑ Stop

Saving options

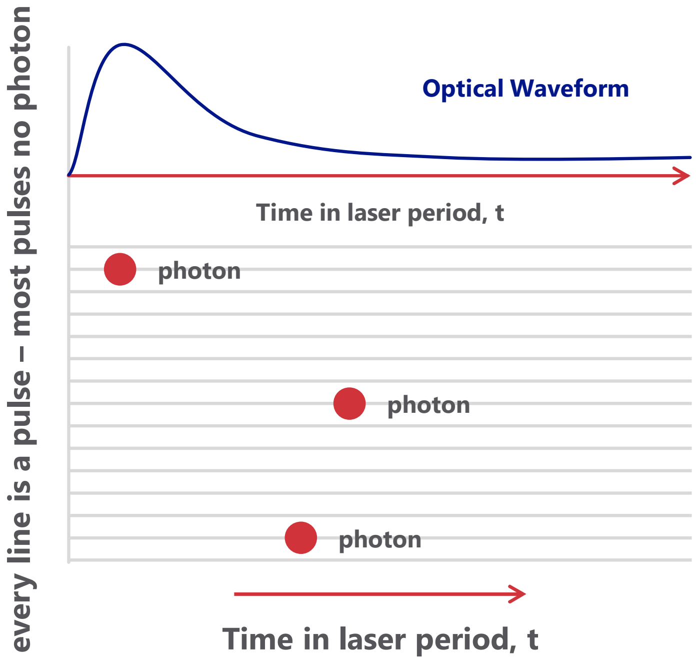
Current directory C:\Users\DanielChebot\Documents
Histogram | Timestamps
☐ Save
File name Results_Histogram_1
Save mode Overwrite

☑ Histogram 1: Start channel: Input 2 Stop channel: Input 1
☐ Histogram 2: Start channel: Start Stop channel: Input 2

Center		Display	Histogram	Min (ns)	Max (ns)	Width (ns)	Count	Count per bin
	A	☐	1	40	40	0	0	0
	B	☐	1	0	0	0	0	0
	C	☐	1	0	0	0	0	0
	D	☐	1	0	0	0	0	0
	E	☐	1	0	0	0	0	0

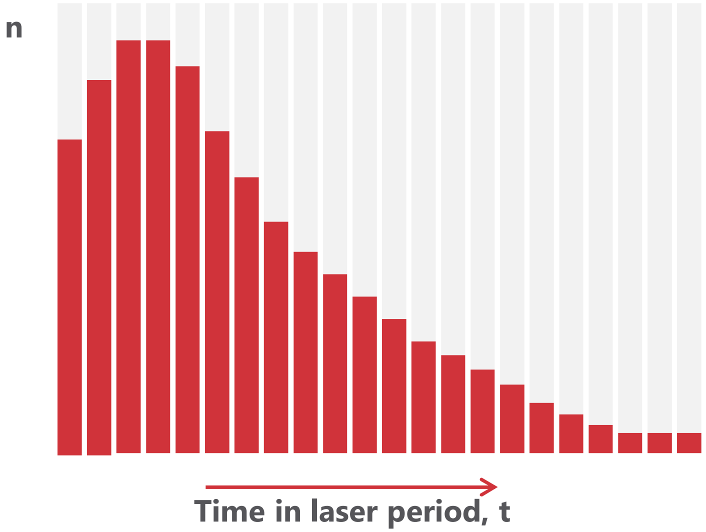
The decay profile is a fingerprint of the specific fluorophore in your sample.

Principle of Time-Correlated Single-Photon Counting – recap

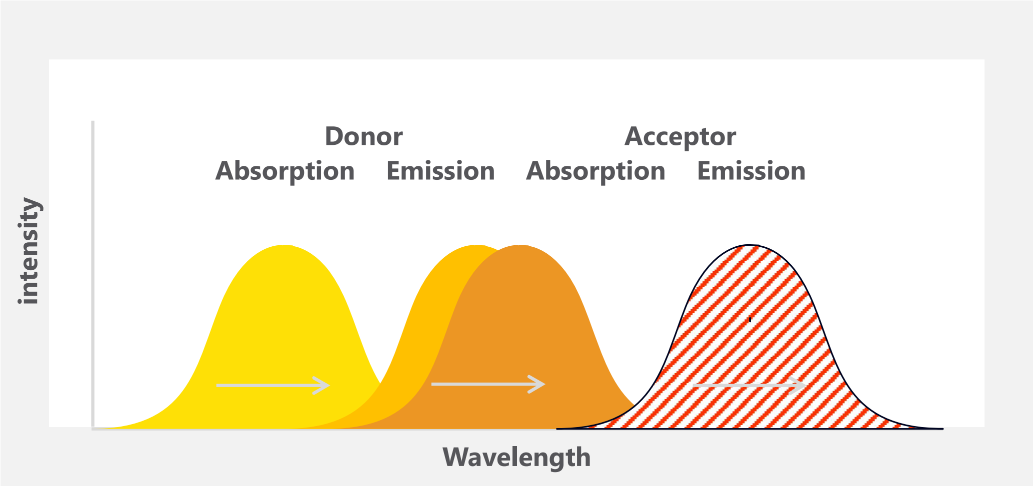


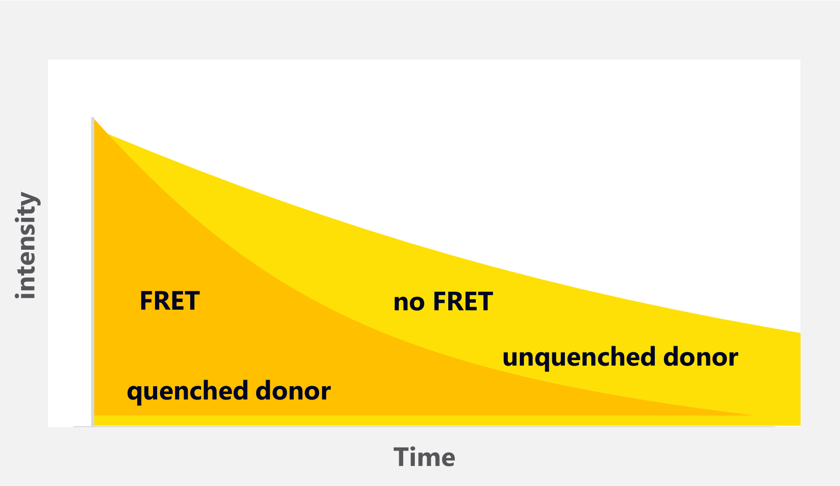
Repeated measurement of photon time within pulse period

HISTOGRAM of photon distribution



One application of TCSPC: Förster Resonance Energy Transfer - FRET





FRET: Donor and Acceptor 'Pair'

spectral overlap

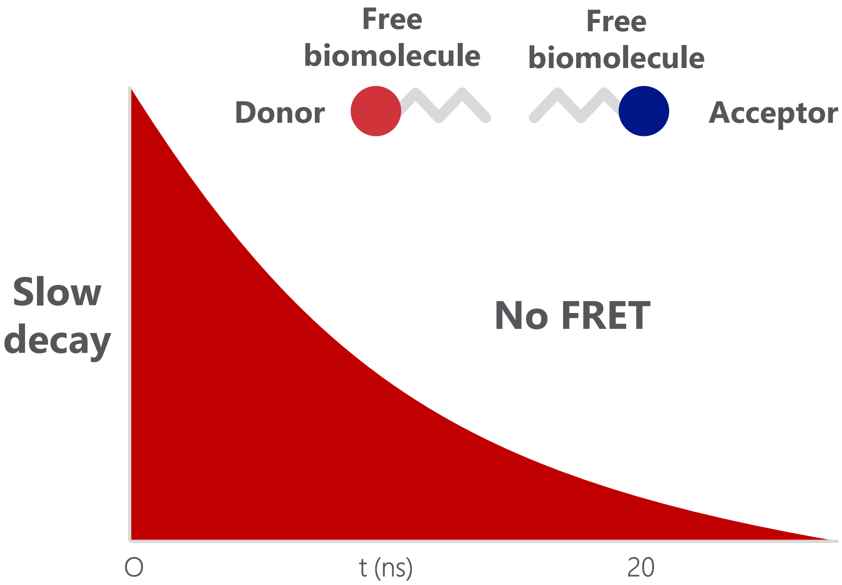
FRET fraction – double-exponential decay

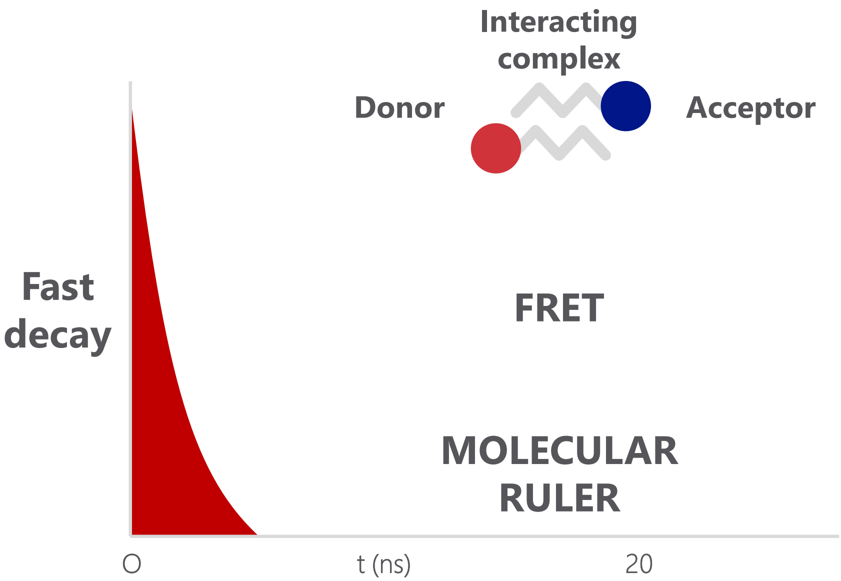
$$\tau_{\text{observed}} = \%\text{interacting} * \tau_{\text{snort}} + \%\text{non-interacting} * \tau_{\text{long}}$$

FRET distance – a very sensitive Molecular Ruler

$$E = 1 / [\ 1 + (r/R_o)^{6}:\ ]$$

FRET – *dynamic* Molecular Interactions





Determination of FRET distance and FRET fraction requires only the acquisition of the donor fluorescence

02

NIR Fluorescence Lifetime

Singlet Oxygen

New inorganic (synthetic) dyes

Quantum Dots (e.g. lead sulfide-based)

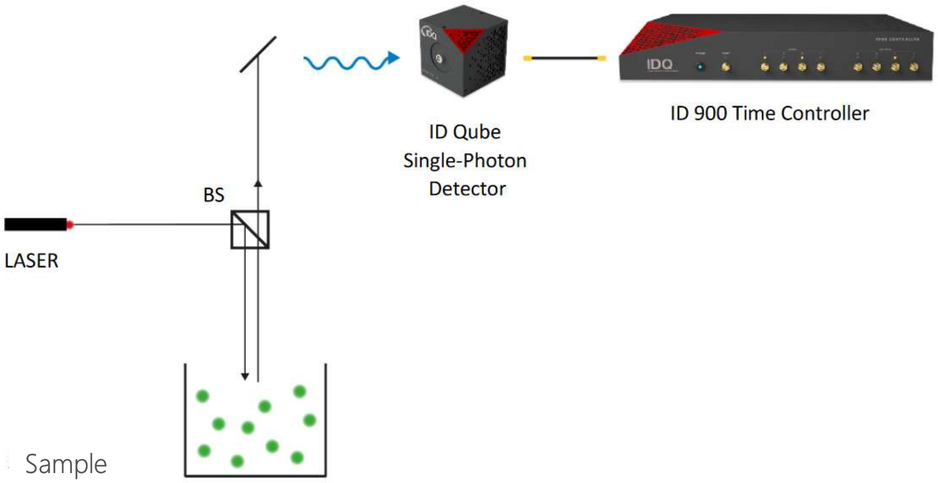
Single-Walled Carbon nanotubes (SWCNTs)

2D Material Surfaces

NIR Fluorescence Lifetime

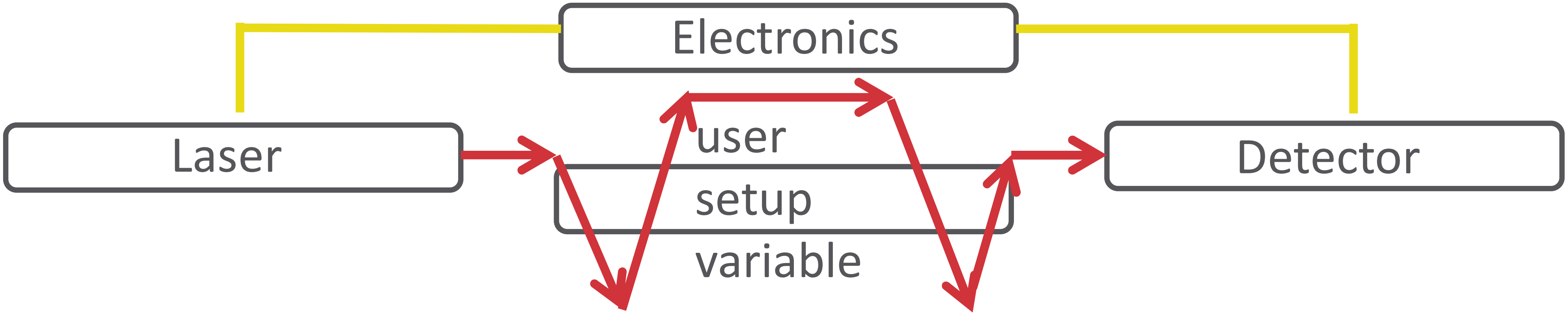
SCHEMATIC OF BASIC MODULAR SETUP

- 3 IDQ parts: * light source * single photon detector(s) * electronics
- 1 (BIG) part: customer setup



SCHEMATIC OF BASIC MODULAR SETUP

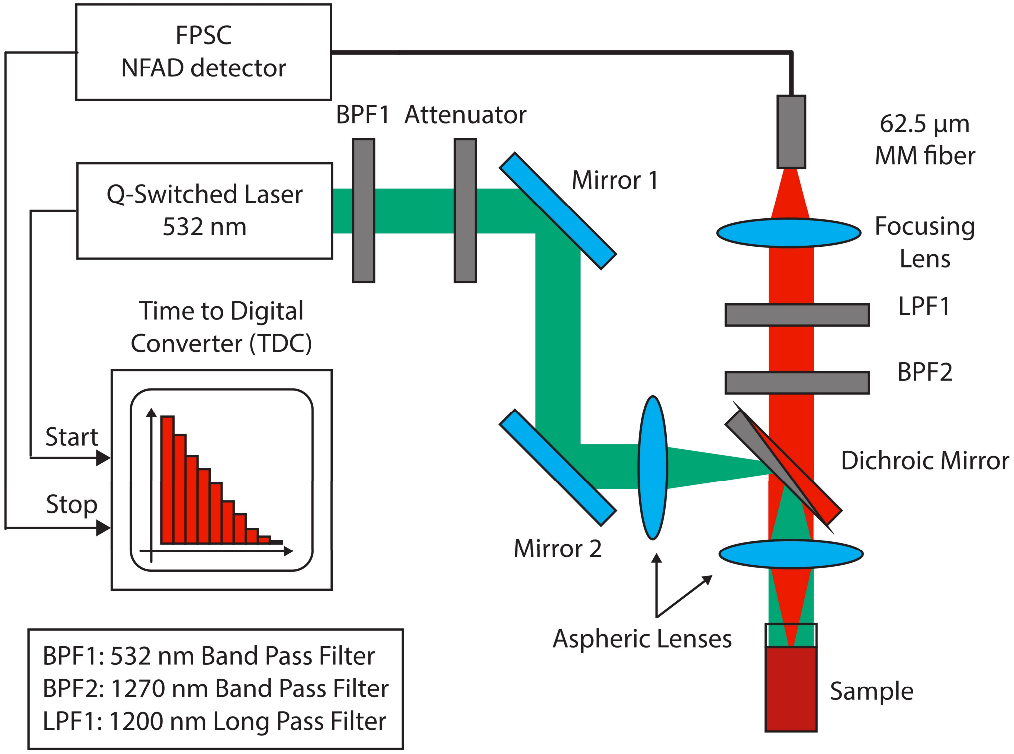
- 3 IDQ parts: * light source * single photon detector(s) * electronics
- 1 (BIG) part: customer setup



Example of the basic modular setup for NIR Fluorescence Lifetime

Example 1. Singlet oxygen: Actual setup for luminescence

- A sample, with a photosensitizer like Rose Bengal creates singlet oxygen upon excitation with a pulsed laser.
- The singlet oxygen luminescence is detected with a NIR-sensitive InGaAs SPAD (single photon avalanche diode).

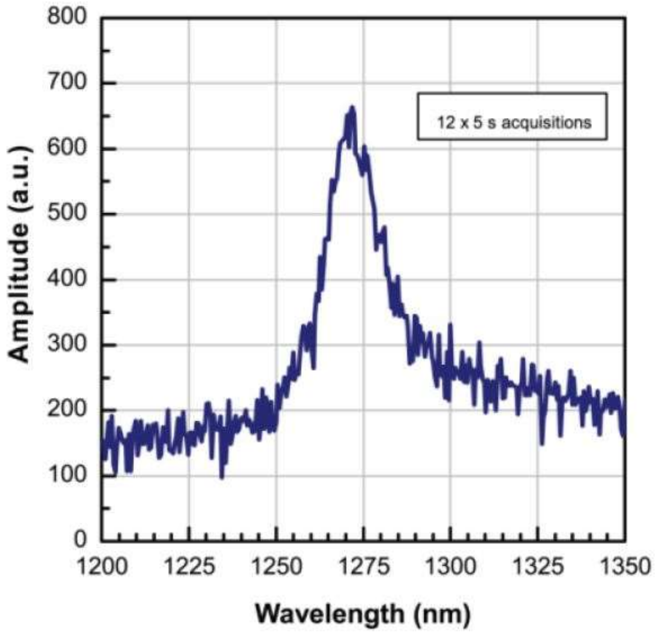


BPF1: 532 nm Band Pass Filter
BPF2: 1270 nm Band Pass Filter
LPF1: 1200 nm Long Pass Filter

Boso, G et al (2015)

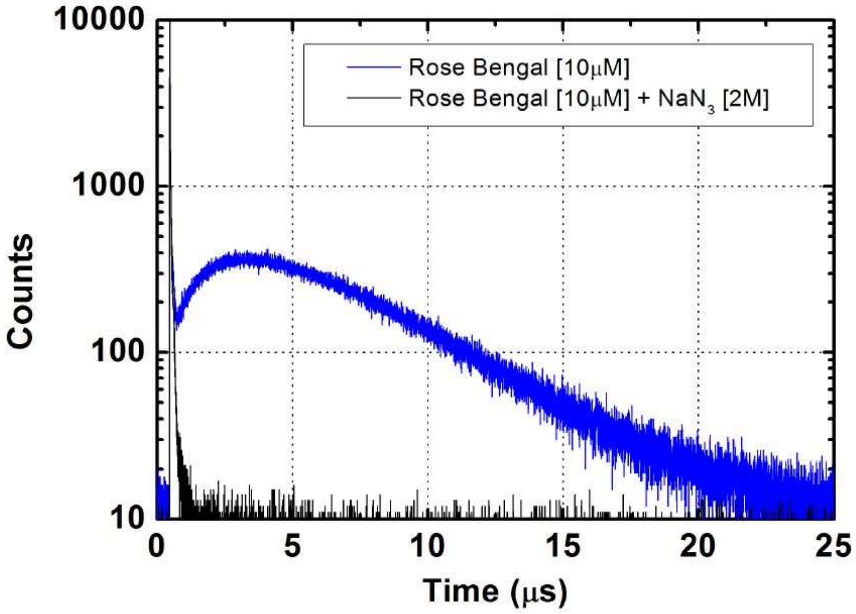
Singlet-Oxygen luminescence spectrum

- Detection of the faint singlet oxygen luminescence signal is difficult as illustrated by the long acquisition time (60 seconds) needed to acquire the characteristic luminescence spectrum, even under optimized conditions.

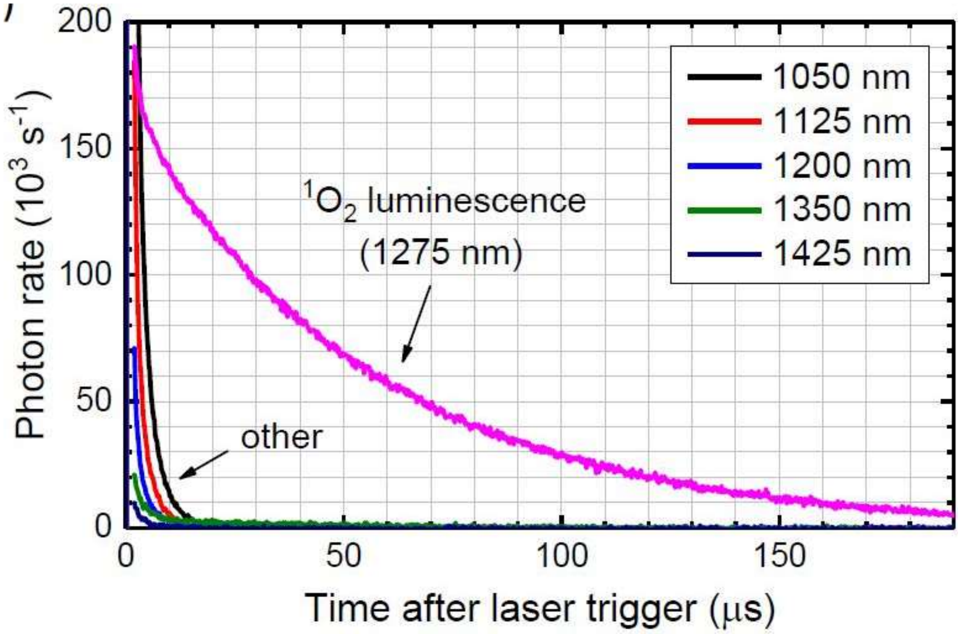


Boso, G et al (2015)

Improving $^1O_2$ luminescence lifetime detection



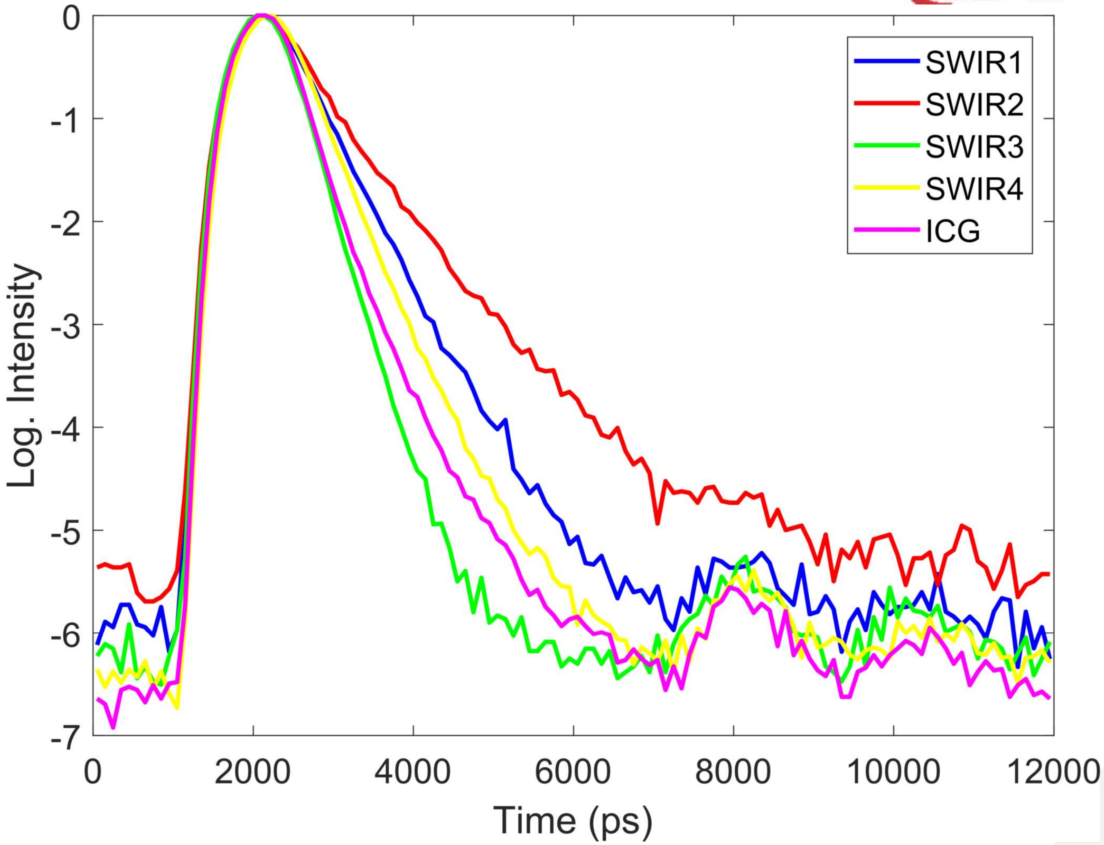
Boso, G et al (2015)



Lin, CW et al (2019)

Example 2. Development of New Short Wave Infrared (NIR) Inorganic Dyes

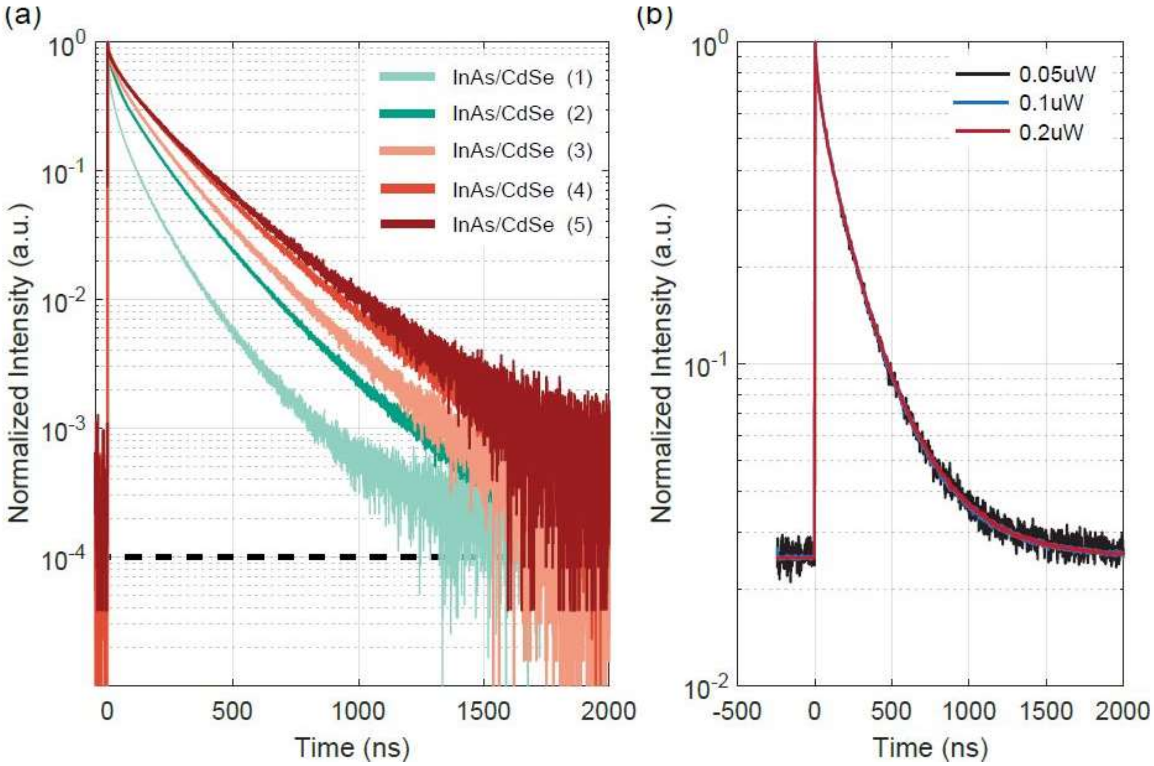
- SWIR Dyes have a short lifetime
- The overall IRF is an issue – means careful deconvolution
- The NIR-SPAD id230 and TCSPC id900 are not limiting in the overall IRF



(courtesy of Steven Hou, MGH)

Example 3a. QUANTUM DOTS: long lifetimes and environment sensitivity

- In contrast to organic fluorophores, quantum dots can have very long lifetimes, in the range from tens to hundreds of nanoseconds. However, the lifetime of quantum dots often is multi-exponential, as seen in the graph to the right of ensemble PL lifetimes for quantum dots with InAs core and CdSe shell
- The luminescence lifetime of quantum dots can vary with their environment and can be taken advantage of, e.g. as in the use of quantum dots linked to porphoryns and used as oxygen sensor

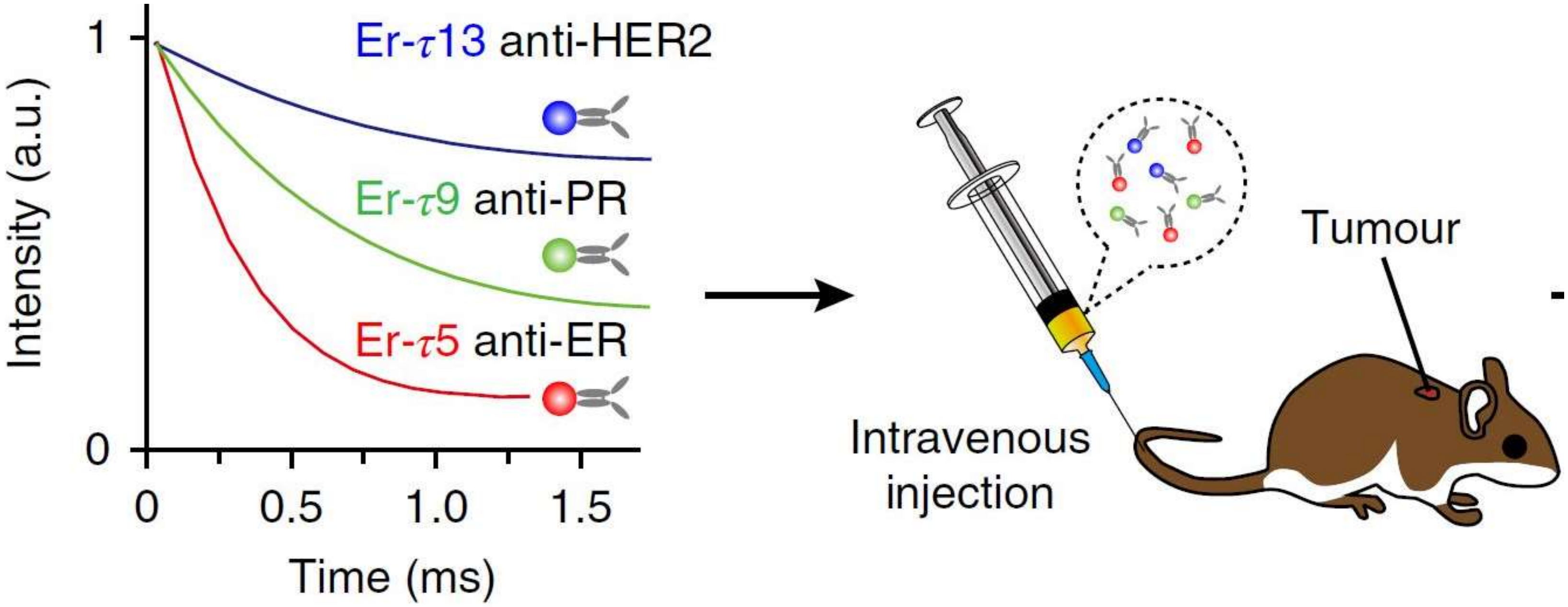


Bertram SN, Spokoyny B *et al* (2019) ACS Nano

Example 3b. NIR-II Erbium-doped nanoparticles

Conjugated with antibodies for tumor detection in mice

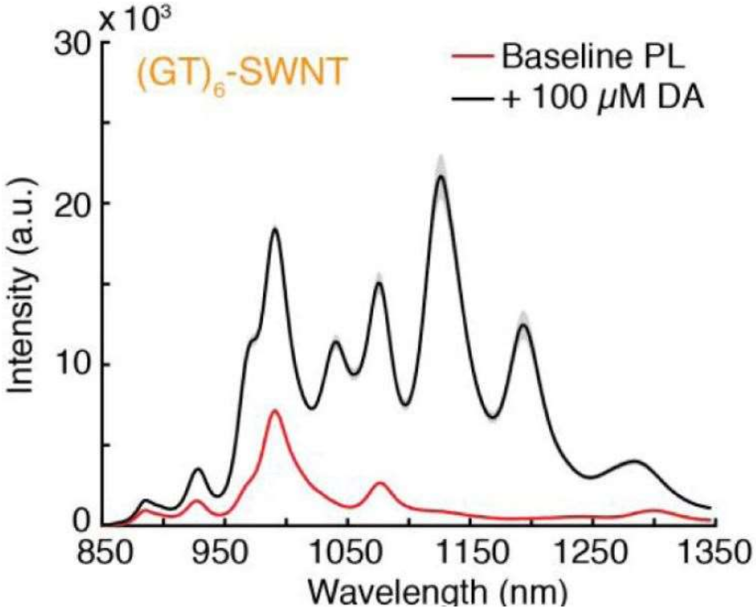
Lifetimes of 100s of ms

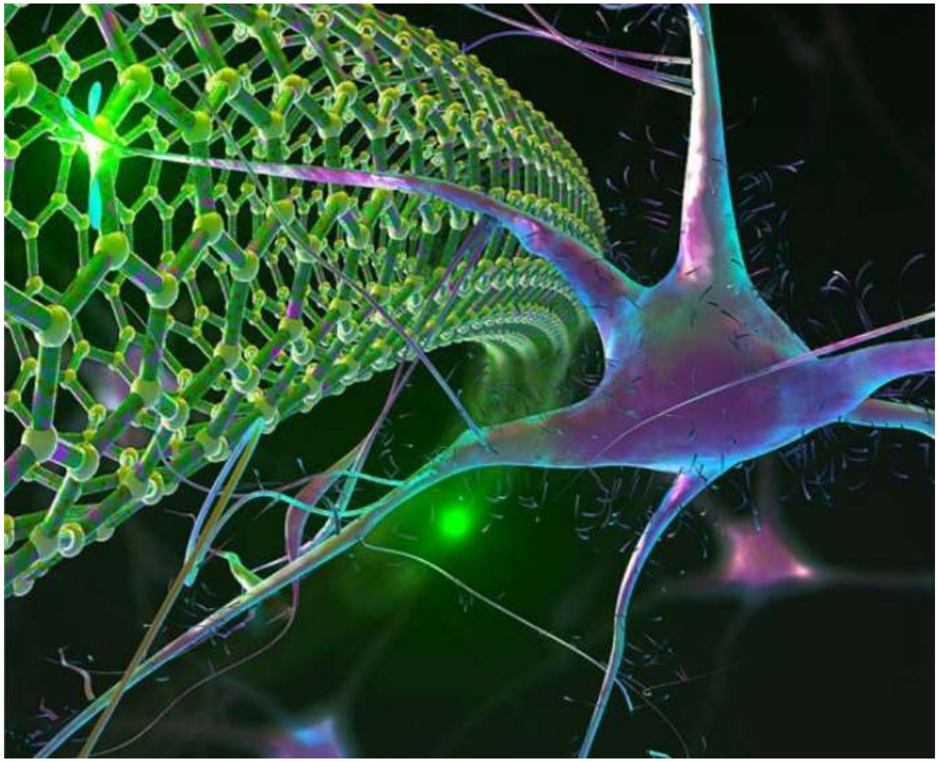


Fan, Y *et al* (2018) Nature Nanotechnology

Example 4. Single-Walled Carbon NanoTubes (SWCNTs)

- ‘pure’: CW Lin and RB Weisman
- modified: CW Lin et al.
- functionalized: Beyene et al. (2018): dopamine sensor

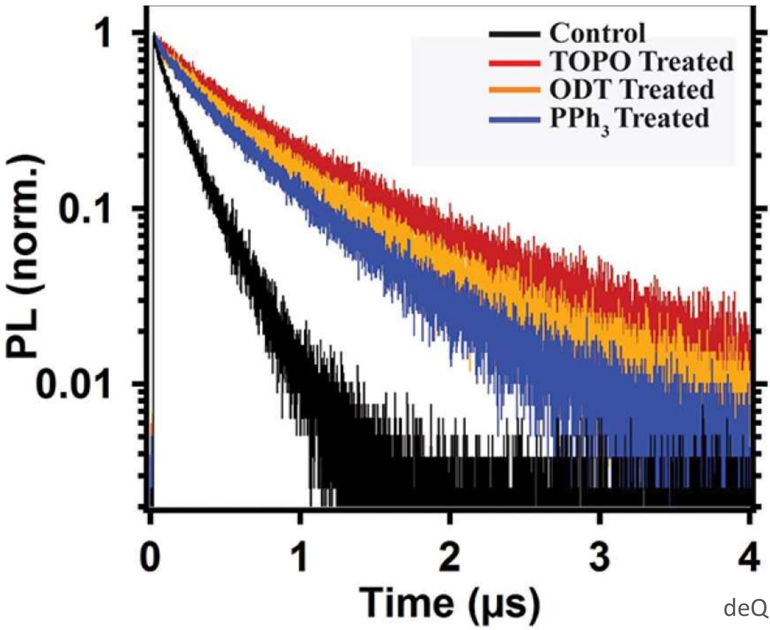




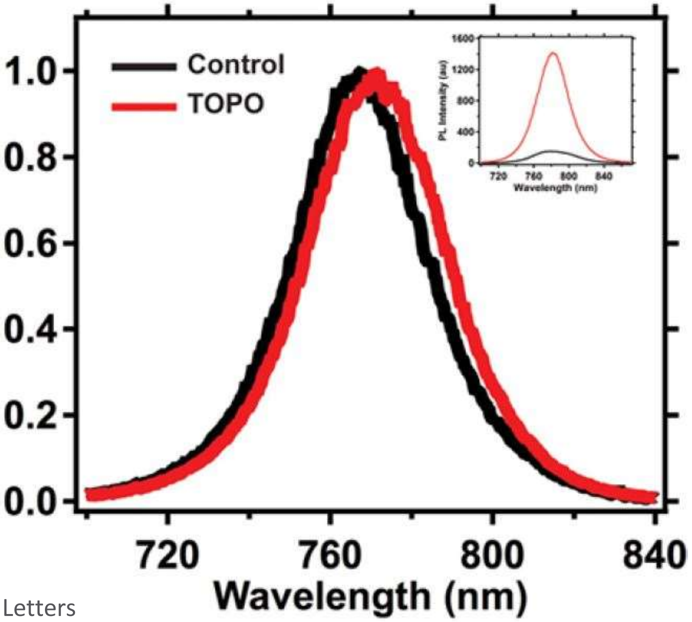
Courtesy of Markita Landry

Example 5. Perovskite Thin Films for solar cells

Photoluminescence Lifetimes: 100 ns to > 8 µs



Luminescence wavelength 720 – 820 nm



deQuilettes *et al* (2018) Energy Letters

Recap: Fluorescence at the single-photon level

Why

- Can do things that one can not do using analogue techniques
- Highest sensitivity
- High time resolution

How

Single Photon Detectors and Electronics
High performance for Best science

CONCLUSIONS

- IRF relevance to Single Photon Counting
- Fluorescence lifetime, one of the characteristics of fluorescence
- Time-domain FRET, one of the methods using TCSPC

- Some examples of the use of NIR single photon fluorescence, including:
  - Singlet oxygen fluorescence
  - Novel NIR fluorophores
  - Quantum Dots
  - Carbon nanotubes and other low-dimensional nanomaterials

- Technological advances in NIR single photon detectors drive science and commercial applications.

- Tendency for detection further in the red, NIR, and SWIR

- Contact us to discuss whether our instrumentation can help with your investigation or application.

A few words about IDQ’s Quantum Sensing technologies

Low-light level sensing

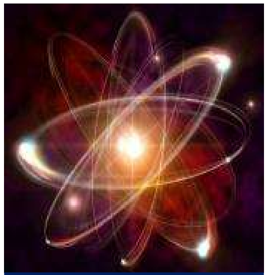
SPADs

SNSPDs

Quantum sensing solutions

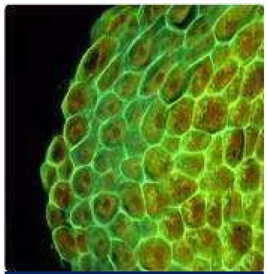
Precision timing & control

Time Controller



Quantum physics

Communication



Life science

Material science

OTDR & LIDAR

Security & Surveillance

Oil & gas

ID Quantique

Quantum.
Trust enabled for the future

Q & A

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ID Quantique

Founded in 2001

3 Product lines:

1. Quantum Random Number Generation
2. Quantum-Safe Security
3. Quantum Sensing

High-quality engineering

Best-in-class performance

Trust

Operational simplicity

THANK YOU.

Rik.vanGorsel@idquantique.com