IEC TS 62607-3-3

Edition 1.0 2020-09

TECHNICAL SPECIFICATION

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Nanomanufacturing – Key control characteristics – Part 3-3: Luminescent nanomaterials – Determination of fluorescence lifetime of semiconductor quantum dots using time correlated single photon counting (TCSPC)

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IEC TS 62607-3-3:2020-09(en)

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NANOMANUFACTURING – KEY CONTROL CHARACTERISTICS –

Part 3-3: Luminescent nanomaterials – Determination of fluorescence lifetime of semiconductor quantum dots using time correlated single photon counting (TCSPC)

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Technical Specifications are subject to review within three years of publication to decide whether they can be transformed into International Standards.

IEC TS 62607-3-3, which is a Technical Specification, has been prepared by IEC technical committee 113: Nanotechnology standardization for electrotechnical products and systems.

The text of this Technical Specification is based on the following documents:

Enquiry draft	Report on voting
113/490/DTS	113/529/RVDTS

Full information on the voting for the approval of this Technical Specification can be found in the report on voting indicated in the above table.

This document has been drafted in accordance with the ISO/IEC Directives, Part 2.

A list of all parts in the IEC 62607 series, published under the general title *Nanomanufacturing – Key control characteristics*, can be found on the IEC website.

The committee has decided that the contents of this document will remain unchanged until the stability date indicated on the IEC website under "http://webstore.iec.ch" in the data related to the specific document. At this date, the document will be

- reconfirmed,
- withdrawn,
- replaced by a revised edition, or
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INTRODUCTION

Fluorescence lifetime is considered as the average time that luminescent materials spend in the excited state before emitting a photon and returning to the ground state. Fluorescence lifetime can vary widely from picoseconds to hundreds of nanoseconds, even to microseconds or milliseconds, depending on the type of luminescent nanomaterials.

Fluorescence lifetime is an important property of luminescent materials. Fluorescence lifetime does not depend on fluorophore concentration, absorption by the sample, thickness of the sample, method of measurement, fluorescence intensity, photo-bleaching, and/or excitation intensity. It is affected by external factors, such as temperature, polarity of solvent, and the presence of fluorescence quenchers. Fluorescence lifetime is sensitive to internal factors that are dependent on fluorophore structure.

The possible applications of measuring fluorescence lifetime include the following:

a) determine the environment that the sample molecules inhabit, e.g. viscosity, pH value, temperature, polarity, and solvation, etc.;
b) uncover the size and shape of the sample molecules, and the distances between different parts of the molecules;
c) learn about the contributions of each component in a mixture of sample molecules, through time-resolved spectra of overlapping emissions;
d) reveal the molecular interactions;
e) obtain the kinetic and dynamic rates.

Time-correlated single photon counting (TCSPC) is a widely used, sensitive, reproducible and precise technique to measure the photon arrival time in applications characterized by a strong demand in terms of temporal resolution such as fluorescence lifetime spectroscopy and imaging, photon migration and time of flight measurements.

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NANOMANUFACTURING – KEY CONTROL CHARACTERISTICS –

Part 3-3: Luminescent nanomaterials – Determination of fluorescence lifetime of semiconductor quantum dots using time correlated single photon counting (TCSPC)

1 Scope

This part of IEC 62607, which is a Technical Specification, provides a method for determining the fluorescence lifetime of semiconductor quantum dots (QDs) using the time correlated single photon counting (TCSPC) technique. TCSPC is suitable for testing fluorescence lifetime in the range from picoseconds to nanoseconds. This document is only applicable to liquid samples that are stable dispersions of QDs. It is not applicable to solid samples.

This document includes:

- outlines of the experimental procedures,
- data processing, and
- case study.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use – Specification and test methods*

ISO 385, *Laboratory glassware – Burettes*

ISO 648, *Laboratory glassware – Single-volume pipettes*

ISO 1042, *Laboratory glassware – One-mark volumetric flasks*

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3 Terms, definitions, and abbreviated terms

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

3.1.1
time correlated single photon counting
TCSPC
technique based on detecting single photons of periodical light signal, measuring the detection times, and building up the distribution of the photon numbers with respect to the detection time

3.1.2
fluorescence lifetime

parameter describing the time evolution of the decay of the fluorescent radiant intensity

Note 1 to entry: For a single exponential decay, the decay means that fluorescent radiant intensity decreases along the time axis to 1/e of the initial maximum value.

[SOURCE: "Glossary of terms used in photochemistry, 3rd edition (IUPAC Recommendations 2006)" *Pure Appl. Chem.* 79, 293-465 (2007)]

3.2 Abbreviated terms

TCSPC	time correlated single photon counting
IRF	instrumental response function
OPO	optical parametric oscillator
QDs	quantum dots
SHG	second harmonic generation
THG	third harmonic generation
FHG	fourth harmonic generation
YAG	yttrium aluminium garnet
EHT	extra-high tension

4 Test principle

TCSPC is an established technique for the measurement of fluorescence lifetimes in the picosecond and nanosecond time scale. Detection of single photon delay times following multiple pulsed excitations yields a distribution that is equivalent to a fluorescence intensity decay that would be observed following a single pulse excitation of an ensemble of fluorophores in the same sample. The TCSPC technique requires a high repetition rate light source to accumulate a sufficient number of photon events for precise statistics in the probability histogram of arrival times.

Figure 1a is an illustration of how the histogram is formed over multiple cycles. In the example, fluorescence is excited repetitively by short laser pulses. The time difference between excitation and emission is measured by electronics that act like stopwatches. In Figure 1b, the typical result in time-resolved fluorescence experiments is a histogram with an exponential drop of counts towards later times.

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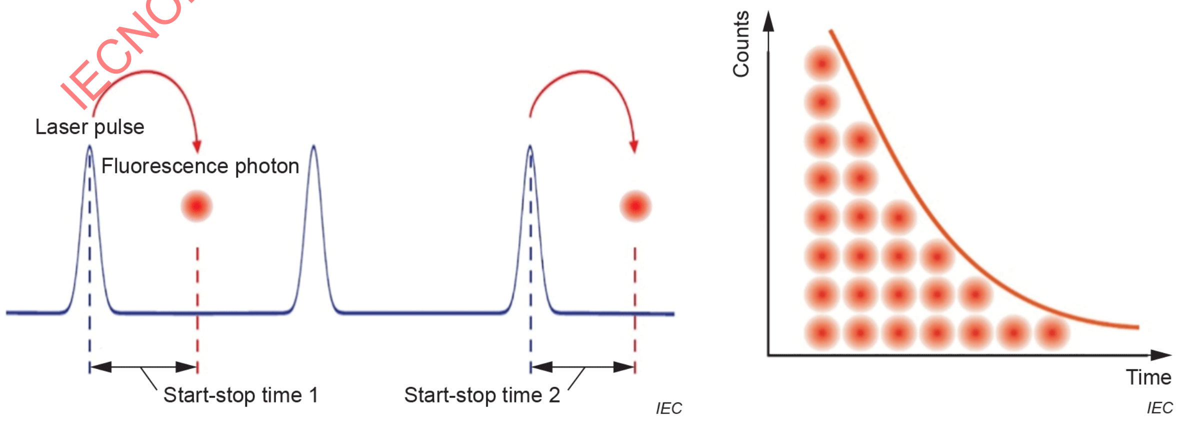

a) b)

Figure 1 – The schematic of start-stop times in time-resolved fluorescence measurement with TCSPC

5 Sample preparation

For the measurement of fluorescence lifetimes of QDs with TCSPC, a homogeneous and stable dispersion of QDs sample is needed. The solvent should be compatible with the surface chemistry of QDs sample to produce a clear dispersion, while not interfering with the fluorescence lifetime of QDs sample.

When performing the dilution, care should be taken to avoid QDs aggregation due to desorption of ligand molecules from QDs. Compatible solvent with 0,1 % to 0,5 % mass fraction of ligand molecules dissolved can be employed to dilute the QDs samples, which ensures the diluted QDs are stable and well-dispersing.

Measure the lifetime of QDs samples with different concentrations and assure that it does not change significantly with concentration. If the lifetime varies significantly, QDs aggregation may take place. To avoid aggregation, increase the concentration of ligand molecules or use a more compatible solvent.

During the analysis, reagents shall be recognized analytical grade, water shall be at least Grade 2, in accordance with ISO 3696 as appropriate.

All volumetric glassware shall be class A, in accordance with ISO 385, ISO 648 or ISO 1042 as appropriate.

A case study for determining fluorescence lifetime of semiconductor QDs is shown in Annex A.

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6 Measurement

6.1 TCSPC fluorescence spectrometer

TCSPC is based on the detection of the arrival times of individual photons after optical excitation of a sample. A TCSPC fluorescence spectrometer usually contains light source, detector, constant fraction discriminator (CFD), time-to-amplitude converter (TAC), analog-to-digital converter (ADC) and memorizer (MEM), as shown in Figure 2.

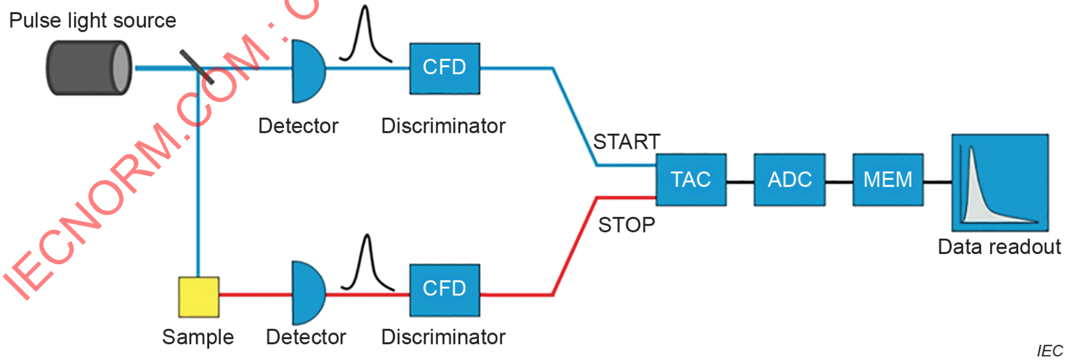


Figure 2 – The working schematic of TCSPC fluorescence spectrometer

The wavelength of the light source is selected to match excitation wavelength of the QDs sample. Table B.1 shows typical laser input excitation sources used in TCSPC.

6.2 Measurement procedure

6.2.1 Instrument preparation

Prepare the instrument following the user manual and operation instructions.

6.2.2 Fluorescence lifetime decay curve measurement

a) The absorption spectrum of QDs sample is recorded and the absorption peak wavelength is measured.

b) The emission spectrum is recorded with QDs sample excited at the absorption peak or a shorter wavelength.

NOTE Take CdTe QDs as an example. The absorption peak of CdTe QDs is located below 500 nm; so, set the excitation wavelength below 500 nm (e.g. 474 nm), with an emission wavelength of 620 nm.

c) According to the absorption peak wavelength, choose the appropriate light source and set the appropriate instrument parameters.

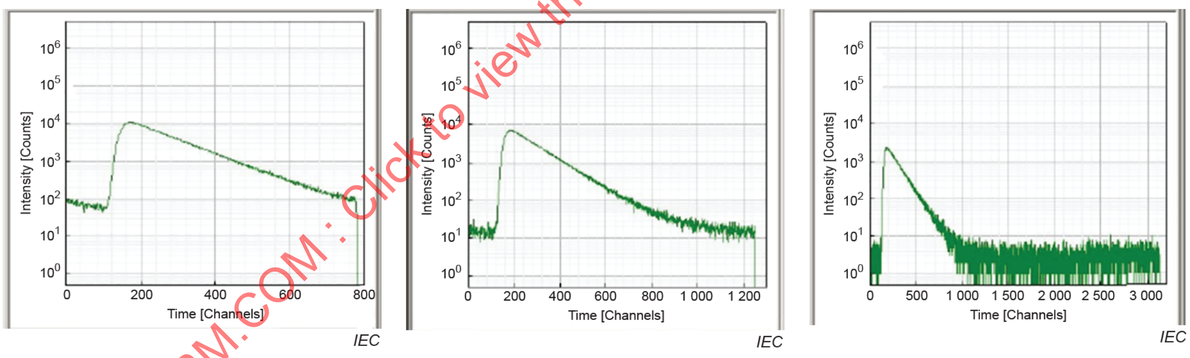
NOTE Take CdTe QDs as example. Choose the picosecond pulsed diode lasers light source in the range 200 nm to 400 nm, pulse width of < 1 ns, pulse repetition rate of 0 MHz to 80 MHz, and power of 0,2 mW to 2 mW.

d) An appropriate volume of sample (50 μL to 2 000 μL) is added into a quartz cuvette. Then the cuvette is put into the instrument sample holder. Make sure the position of the sample is in the excitation beam.

e) Estimate the expected lifetime and review the pulse repetition rate. The pulse repetition period of the light source should not be shorter than 10 times the longest lifetime to be measured. The pulse width should be set to 50 ps to 300 ps; and the light source (CW) power should be set to 0,2 mW to 2 mW.

f) Check the detector signal (in counts per second). Ensure that the signal is below saturation and pulse pile-up level. With TCSPC, the signal count rate from the detector should not exceed 5 % of the rate of the exciting light source.

g) Set up the measurement parameters and ensure to gain a proper spectrum with a short trailing stretch, as shown in Figure 3b. To obtain a proper fitting result, the far end of the tail that only contains detector background, noise and no valuable information on the decay kinetics should be excluded.



a) narrow measurement range b) suitable measurement range c) broad measurement range

Figure 3 – Examples for typical single exponential decay curves obtained in different measurement ranges

NOTE For single exponential decay, the number of collected photons is set as 2 000 to 10 000. It is enough for most luminescent materials to set the number of collected photons as 2 000 to 10 000. A higher number of collected photons needs more unnecessary testing time.

h) Start the measurement, and record the fluorescence decay curve. The measurement will stop manually or automatically when the number of collected photons reaches the set value.

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6.2.3 IRF measurement

In a large number of cases, IRF measurement is not required for the analysis of phosphorescence decay measurements. The IRF width is negligibly small compared to the intrinsic lifetimes of the decay, and the analysis with the tail-fit routine is sufficient. However, for the correct determination of short lifetimes (much shorter than the width of the excitation pulse), reconvolution is necessary, and thus the IRF needs to be measured.

a) Take 30 % silica aqueous solution as a scatterer instead of the sample, and set the value of emission wavelength to be the same as the excitation wavelength.
b) The IRF should be measured under similar conditions compared to the previous measurement of the fluorescence decay.
c) The IRF is typically measured to the same peak height as that of the decay measurement. But this is not absolutely necessary for the subsequent data analysis.

6.2.4 Data analysis

Use exponential tail-fit analysis to fit a sample decay to a model function with one or more exponential terms to estimate lifetimes.

Use the exponential tail-fit analysis only to analyse sample decay measurements that are not (or not significantly) affected by the finite width of the IRF.

For a precise measurement of short lifetimes, use exponential reconvolution fit to analyse sample decay, taking into account the IRF.

7 Test reports

The test report shall include but not be limited to the following information:

a) report number;
b) test date;
c) tester, the reviewers;
d) ambient temperature and humidity of the measurement;
e) sample description, including name, solvent, concentration, manufacturer, sample preparation method, etc.
f) instrument description, including manufacturer, model, instrument warm-up procedure, etc.;
g) analysis method description, including fitting method of the lifetime.

8 Uncertainty source

Currently, the uncertainties associated with measuring the lifetime of semiconductor QDs should be estimated from various origins as listed below:

- instrument calibration;
- measurement environment;
- data analysis method.

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Annex A
(informative)

Case study for determining fluorescence lifetime of semiconductor quantum dots

A.1 General

Annex A provides an example of the determination of the fluorescence lifetime of semiconductor QDs colloidal dispersion using TCSPC.

A.2 QDs sample

Make water-soluble transfer treatment for oil-soluble semiconductor QDs, and disperse the QDs in a PBS solution whose pH is 7,4.

A.3 Instruments

The fluorescence lifetime decay curve of the sample was recorded on a fluorescence spectrophotometer, equipped with nanosecond flashlamp (nF920), microsecond flashlamp (µF920 xenon flashlamp), and laser input (474,8 nm). A cuvette of 500 µL was used for fluorescence lifetime measurement.

A.4 Measurement conditions for TCSPC

Measurement conditions were set as follows.

a) The measurement range of fluorescence lifetime was 0 ns to 500 ns.

b) The number of collected photons was set as 10 000.

c) The excitation wavelength was chosen as 475 nm.

d) The emission wavelength was set as 585 nm.

A.5 Procedures for measurement

A.5.1 Instrument preparation

Heat up the thyratron trigger within the lamp head at least 5 min before the experiment. Check the gas pressure, cables, gas tubes and optical fibre. The nF920 nanosecond flashlamp was chosen as excitation source. For standard operating conditions, EHT is 6,8 kV and frequency is 40 kHz. For normal start-up, the auto-start option is selected. Ensure the gas pressure is as required (0,45 bar ± 0,05 bar for standard operating conditions). When the warm-up period of approximately 5 min has expired, switch the lamp and EHT on and stepwise ramp up the frequency (auto-start).

The lamp is in proper operating conditions when the measured frequency is stable within the range of ±50 Hz. Initially this tolerance interval may be exceeded, but after 2 min to 3 min of operation the lamp should be stable. If it is not, refilling with gas, or even re-servicing the lamp is required.

A.5.2 Fluorescence lifetime decay curve measurement

a) 300 µL of CdTe QDs with 0,01 mg/mL in pH 7,4 PBS was added into a 500 µL quartz cuvette. Then the cuvette was put into the instrument sample holder. Make sure the position of the sample is in the excitation beam.

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b) The absorption spectrum and emission spectrum of the sample were tested respectively. The excitation wavelength was set at the peak value 475 nm of the absorption spectrum. The parameters of the flashlamp were modulated on the basis of excitation wavelength. The emission wavelength was set at the peak value 585 nm of the emission spectrum.

c) Have an understanding of the expected lifetime (based on the chemical components of QDs, fluorescence lifetime can be predicted through experience or by reviewing literature). For reference, Table B.2 shows the range of fluorescence lifetimes of commonly used semiconductor QDs. Then review the pulse repetition rate of the light source. The pulse repetition period of the light source should not be shorter than 10 times the longest lifetime to be measured.

NOTE For sample excitation with the standard nF920 flashlamp (operating at 40 kHz) the repetition rate is, for the vast majority of lifetime measurements, low enough and there will be no need to further reduce the repetition rate. However, for alternative TCSPC light sources with much higher pulse repetition rates, the issue of selecting an appropriate pulse rate is of significant relevance.

d) Check the detector signal (in counts per second). Ensure that the signal is below saturation and pulse pile-up level. With TCSPC the signal count rate from the detector should not exceed by more than 5 % the rate of the exciting light source. For operation with the standard nanosecond flashlamp (40 kHz), this means the detector signal shall not exceed 2 000 counts per second. Change the amount of signal by adjusting the iris setting and/or the band width in excitation and emission.

e) Set up the measurement parameters.

f) Start the measurement, and record the fluorescence lifetime decay curve. The measurement stops automatically when the number of collected photons reaches the set value.

A.5.3 IRF measurement

The fluorescence lifetime of the QDs sample is relatively short, so the IRF was measured.

Take 30 % silicone aqueous solution as a scatterer, and set the value of emission wavelength to be the same as the excitation wavelength.

A.6 Results of fluorescence lifetime decay curve

Figure A.1 is a typical fluorescence lifetime decay curve of the QDs sample.

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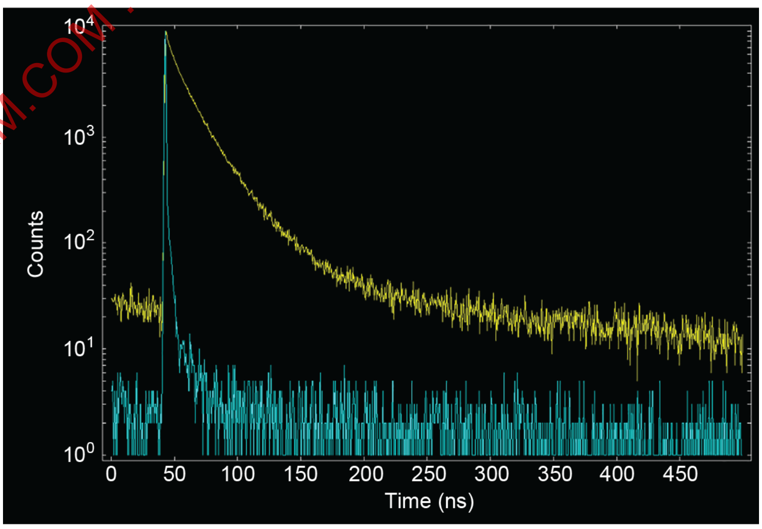

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Figure A.1 – Typical fluorescence lifetime decay curve

In Figure A.1, the yellow line is the fluorescence lifetime decay curve of the QDs sample, and the blue line is IRF.

A.7 Data analysis

a) When the measurement is finished, choose the fitting range, and fit the data. The result is shown in Figure A.2.

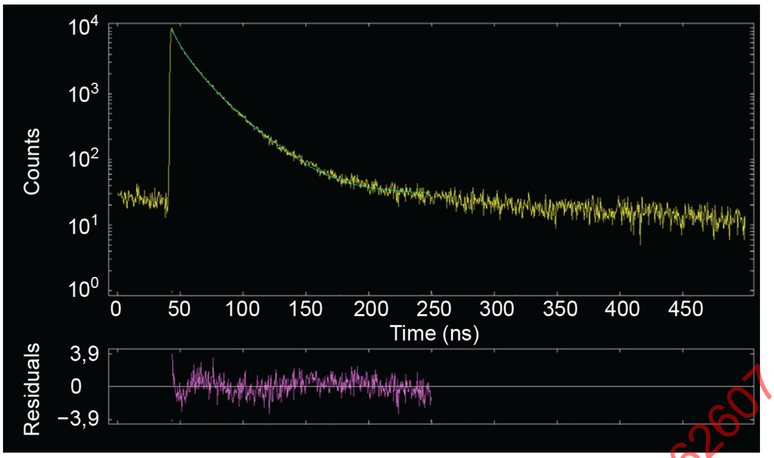

Figure A.2 – Fitting result curve and IRF curve

In Figure A.2, the yellow line is the fluorescence lifetime decay curve of the QDs sample after deducting the IRF, the blue line is the fitting curve, and the purple line is the error curve.

b) A double exponential fitting is used for the QDs sample. The lifetimes are 8,5 ns (30,43 %) and 24,6 ns (69,57 %); χ^2 = 1,282.

c) When fitting is finished, the fitting files are saved.

d) The measurements were repeated three times for each sample, and the average values were calculated.

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Annex B
(informative)

Typical laser input excitation sources used in TCSPC

Laser input are excitation source options besides flashlamp. Table B.1 shows typical excitation sources used in TCSPC. The excitation source, including but not limited to Table B.1, is chosen according to the tested materials. For example, a 475 nm laser is used for CdTe QDs, whereas a 350 nm laser is chosen for ZnSe QDs.

Table B.1 – Typical laser input excitation sources used in TCSPC

Light source	Wavelength range nm	Pulse width ps	Repetition rate (typ.) MHz	Power (CW) mW
Diode laser	375, 405, 440, 475	50 to 300	0 to 80	0,2 to 2
Diode laser	635, 650, …, 1 300	30 to 300	0 to 80	0,2 to 10
Titanium sapphire laser (Ti:Sa)	700 to 980	0,2 to 2	78 to 90	200 to 1 300
Ti:Sa, Pulse picker	700 to 980	0,1 to 1	0 to 9	< 100
Ti:Sa, SHG	350 to 490	0,1 to 1	78 to 90	100
Ti:Sa, THG	250 to 320	0,1 to 1	78 to 90	20
Ti:Sa + OPO	1 050 to 1 600	0,1 to 1	78 to 90	40 to 240
Ti:Sa, OPO + SHG	525 to 660	0,1 to 1	78 to 90	60 to 200
Dye laser	400 to 900	10	80 to 125	50
Nd-YAG laser	1 064	15	50 to 80	10 000
Nd-YAG lasers + SHG, THG FHG	532, 354, 266	15	50 to 80	100 to 4 000
Chip laser	1 064, 532, 354, 266	1 500	< 0,01	20 to 1
Fibre laser	800, 1 600	0,2	80	20
Synchrotron	X-ray to IR	> 1 000	5	< 1

Table B.2 – Fluorescence lifetime of commonly used semiconductor QDs

Type of QDs	Fluorescence lifetime	Reference
CdSe	11 ns to18 ns 20 ns 48 ns	Mol. Cryst. Liq. Cryst., 2012, 566, 120-125. Small, 2012, 8, 2652-2658. AIP Conf. Proc., 2014, 1591, 420-422.
CdSe/ZnS	> 10 ns	Nat. Methods, 2008, 5, 763-775.
CdSe/CdS	33 ns to 36 ns	AIP Conf. Proc., 2014, 1591, 420-422.
CdSe-CdS/ZnS	14 ns	J. Phys. Chem. C, 2014, 118, 10424−10433.
CdTe	> 10 ns 32 ns to 38 ns	Small, 2012, 8, 2652-2658. ISRN Spectrosc., 2012, 894385(1-8).
CdTe/CdS	41 ns to 45 ns	ISRN Spectrosc., 2012, 894385(1-8).
$\mathrm{CdTe}_{1-x}\mathrm{Se}_{x}$ ($0 \le x < 1$)	> 80 ns	J. Alloys Compd., 2017, 699, 216-221.
Mn-doped ZnSe	20 µs to 1 000 µs	Anal. Chem., 2011, 83, 9076-9081.
InAs	700 ps	Acta Phys. Sin., 2014, 63, 027801.
InAs/GaAs	> 1 ns	Phys. Rev., B 1997, 56, 10435.
AgInZnS	0,44 to 52,14 ns	Catal. Lett., 2019, 149, 1800.
PbS	2,27 ± 1,15 µs	J. Lumin., 2017, 188, 252-257.

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