High Performance
Photon Counting

User Manual

NUV and Blue ps Diode Lasers

Designed and manufactured
in cooperation with

LASOS Lasertechnik GmbH

Becker & Hickl GmbH

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High Performance
Photon Counting

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BDL-375-SMC
BDL-405-SMC
BDL-440-SMC
BDL-473-SMC
NUV and Blue Picosecond Diode Lasers

 

Picosecond pulsed operation or CW operation
Free-beam operation or coupling into optical fibre
Correction of beam-profile and astigmatism
High power density in focused spot
60% of power delivered into single-mode fibre
Compatible with all commonly used fibre couplers
Repetition rate from 20 MHz to 80 MHz
Wavelengths of 375 nm, 405 nm, 440 nm, and 473 nm
Fast on/off control, multiplexing capability
Excellent timing stability
All driving and control electronics integrated
Simple +12V power supply
Compatible with the bh TCSPC systems

Designed an manufactured in cooperation with LASOS Lasertechnik GmbH

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1st Edition, May 2006

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Overview

The BDL-375-SMC, BDL-405-SMC, BDL-440-SMC and BDL-473-SMC lasers emit at typical wavelengths of 375 nm, 405 nm, 440 nm, and 473 nm, respectively. The lasers can be switched between picosecond pulse operation and CW operation.

In the picosecond mode, pulse repetition rates of 20 MHz, 50 MHz, and 80 MHz can be selected. The pulse width is in the range of 40 ps to 90 ps, the CW-equivalent power between 0.2 and 1.5 mW. The high repetition rate and the short pulse width make the BDL-SMC lasers ideally suited for time-correlated single photon counting (TCSPC) applications [8, 9].

In the CW mode an output power of up to 40 mW is available. The lasers can thus be used for a number of applications that require both continuous excitation at high power and excitation with ps pulses. Typical examples are single-molecule experiments by fluorescence correlation, anti-bunching and burst-integrated fluorescence lifetime or multi-parameter-spectroscopy [11, 13, 21]. By switching between CW and ps operation, these techniques can be applied to the same sample or, in some cases, even to the same molecules.

All BDL-SMC lasers come with a beam corrector that corrects both for beam shape and astigmatism. The lasers are compatible with all commonly used single-mode fibre couplers. Due to the excellent correction of the beam up to 70% of the laser power can be coupled into a single-mode fibre. Thus, high laser power can be focused into a diffraction limited spot. The lasers are thus excellent excitation sources for single-molecule spectroscopy and time-resolved laser scanning microscopy.

The BDL laser modules have a TTL controlled shutdown input that can be used to switch the laser off and on within a time of 1 us. Thus, lasers of different wavelength can be multiplexed at microsecond periods. In laser scanning microscopes the shutdown function is used to switch off the laser during the line and frame flyback.

The BDL-SMC lasers are operated from a simple wall-mounted +12 V power supply. The complete control and driving electronics is contained in the laser module. The design of the BDL-SMC lasers results in exceptionally low RF noise radiation and low timing drift between the electrical trigger output and the light pulses.

General Description

System Components

The BDL-SMC laser is shown in Fig. 1. The power supply module is shown left. It is a simple wall-mounted +12V stabiliser. Fig. 1, middle shows the laser switch box. The box contains the key switch mandatory for class 3B lasers, and a switch to select between three pulse frequencies and CW operation. Input connectors for control signals are located at the back of the switch box. Via the control connectors, the laser can switched on and off at µs rate (see Fig. 15, page 15). Moreover, the laser power can be controlled by an analog signal of 0 to 10V, and the frequency can be switched by external TTL signals (see ‘Control Inputs’, page 13).

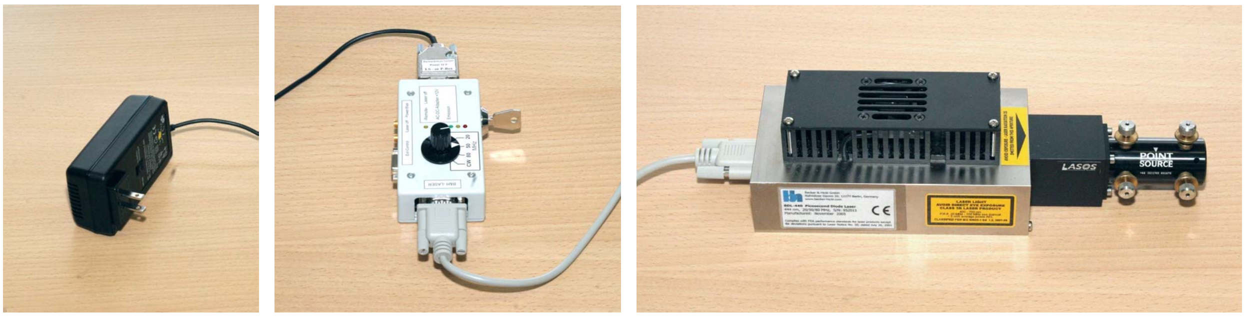


Fig. 1: BDL-SMC laser. Left: Wall mounted power supply. Middle: Switch box with safety key, switch for frequency and CW / pulsed operation and control signal inputs. Right: Laser module containing the complete driving and control electronics.

Fig. 1, right, shows the laser module. It contains the complete pulse generator and driver electronics, the control electronics and an the active temperature stabilisation of the laser diode. The diode itself is mounted on a peltier cooler inside the laser module. The beam profile corrector is attached to the outside of the laser housing. The front end of the corrector has threaded holes that fit to the standard 1” pitch of the commonly used fibre couplers or manipulators. Fig. 1 shows the BDL-SMC laser with a fibre manipulator from Point Source, UK.

Status Indicators, Connectors and Controls

The back panel of the laser head is shown in Fig. 2. The left LED indicates that the laser is active. The LED flashes when the power of the laser is on and the ‘/Laser Off’ signal is ‘high’ or unconnected. The other two LEDs show the status of the cooler of the laser diode. The right LED is on when the cooling of the laser diode is active. It may turn off after some time of operation when the diode has been cooled down and almost no cooling power is required to hold it at constant temperature. The red LED in the middle turns on when the cooling power is high. It normally turns off after some minutes of operation.

The 15-pin sub-D connector connects the power supply and control signals from the laser switch box to the laser. The lasers are delivered with appropriate connecting cables, so that user access to the 15 pin connector is not normally needed. For pin assignment please see page 37.

Fig. 2: Back panel of the BDL-SMC laser

A trigger output signal is available at an SMA connector. The shape of the signal is shown in Fig. 10, page 11. Depending on the laser power, he amplitude of the synchronisation signal may vary between about 100 and 300 mV.

There are two potentiometers at the back of the laser module. The 'Power' adjust changes the operating voltage of the driving generator. The 'Bias' adjust changes the bias voltage of the laser diode. Higher voltage and higher (positive) bias give higher output power. The best pulse shape is obtained with minimum (negative) bias and high driving power. Please see also 'Picosecond Operation of Laser Diodes', page 20.

Please note that the power of the BDL-SMC lasers can also be changed by an analog input signal. By using the DCC-100 detector controller card of the bh TCSPC systems [3, 9] the laser power can be controlled per software, see Fig. 14, page 14. Controlling the power per software is not only more convenient than turning a potentiometer but also makes it easier to restore previously used settings.

Laser Switch Box

The laser switch box is shown in Fig. 3. The box contains the mandatory laser safety elements of class 3B lasers: a key switch and emission indicators. Laser action is indicated by four LEDs of different coulour, at least one of them being visible through any laser safety eyewear. The 'Laser Off' LED shows the status of the laser on/off signal.

Fig. 3: Laser switch and connection box

The connectors for the control signals are shown in Fig. 3, right. There are two SMA connectors, one for the on/off signal and one for the analog power control signal. The same signals can be connected to a 15 pin sub-D connector. This connector has also inputs for switching between 20, 50, and 80 MHz, and CW operation. Please note that the frequency switch must be in the 'CW' position when electronic frequency control is used. For pin assignment and signal specification, please see 'Control Inputs', page 13.

The 15 pin connector at the laser side can be used as a 'remote interlock connector'. The connector can be pulled off or plugged in at any time without causing damage to the laser.

Operating the BDL-SMC Lasers

Free-Beam Operation

The BDL-SMC lasers can be used both in free-beam or fibre coupled systems. For free-beam operation the lasers are used without a fibre coupler attached, see Fig. 4.

Fig. 4: Free-beam operation of the BDL-SMC laser

The beam diameter is about 0.7 mm. The beam profile at 1 m distance from a BDL-405-SM laser is shown in Fig. 5. The definition of the profile improves with increasing power. Because of the high peak power in the pulsed mode the beam profile is slightly better than in the CW mode.

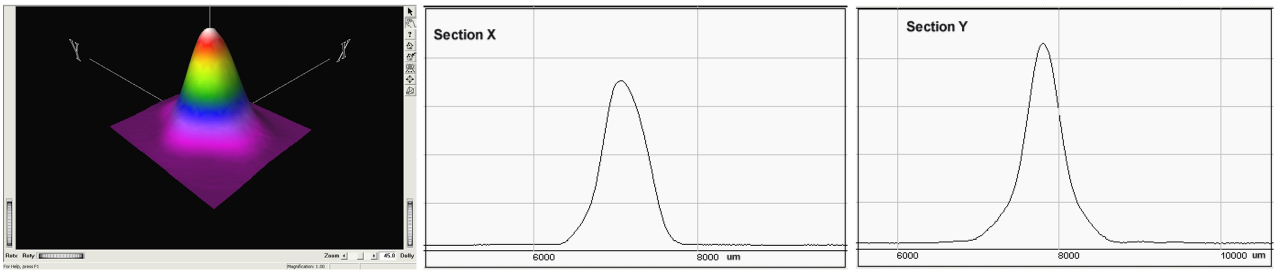


Fig. 5: Beam profile in 1m distance from the laser. BDL-405-SMC, CW mode, 30 mW

The collimator and beam correction optics of the BDL-SMC lasers is aligned during manufacturing. Once the optics are aligned the elements are fixed in place permanently. Therefore, please do not attempt to change anything in the optics of the laser. If you need focusing, other beam diameters, or other beam shape, please use external optics. The intensity distribution in the focus of a 200-mm lens is shown in Fig. 6.

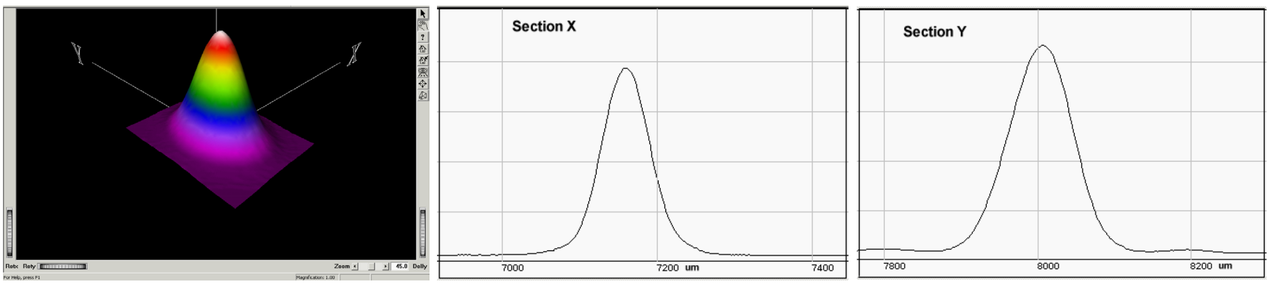


Fig. 6: Intensity distribution in the focus of a 200-mm lens. BDL-405-SMC, CW mode, 30 mW

Fibre Coupling

Optical fibres consist of a core with high index of refraction and a cladding with lower index of refraction. The light is kept inside the core of the fibre by total internal reflection. Optical fibres come in two different versions - multi-mode fibres and single-mode fibres.

Multi-mode fibres have diameters from typically 50 µm to 1 mm. Any light within a given input cone is transferred to the output. Coupling light into a multi-mode fibre is therefore relatively easy. However, rays of different angles to the optical axis have different optical path lengths and, consequently, different transit times. Therefore a light pulse coupled into a multi-mode fibre spreads out in time. For a fibre of 1m length illuminated at maximum numerical aperture the transit time spread is on the order of 100 ps. The transit time spread is independent of the diameter of the fibre [14]. The second drawback of multi-mode fibres is that the light leaves the end of the fibre from the whole cross section of the core, and within a cone of large angle. Focusing the light from the fibre into a small spot, at best, yields a de-magnified image of the core cross section. A diffraction-limited spot cannot be obtained.

Single-mode fibres have core diameters on the order of 3 µm. Because of the small diameter only a single wave mode is transmitted through the fibre. Coupling into a single-mode fibre is difficult not only because of the small diameter but also because of the relatively small angle of the input light cone. However, the light leaves the fibre output as a single wave mode. That means, the light virtually comes from an infinitely small spot. Light transmitted by a single-mode fibre can therefore focused into a diffraction-limited spot. Moreover, because only a single wave mode is transmitted, there is virtually no transit time spread.

Because of the superior features of single-mode fibres the BDL-SMC lasers are available with single-mode fibre coupling. The lasers are compatible with almost any of the commonly used single-mode fibre couplers.

Alignment of Point-Source Coupler

The fibre-coupling system of Point Source Ltd., UK, uses special fibres that have a focusing lens permanently attached to the fibre input. Both the lens and the fibre are assembled in a cylindrical adapter that is inserted into a fibre manipulator, see Fig. 7. The Point-Source system thus avoids any alignment at the sub-µm scale [20]. The result is high efficiency and extraordinarily good long-term stability. Due to the good long-term stability commercial laser scanning microscope almost exclusively use the Point Source system.

The fibre manipulator has four adjustment screws, A1, A2, B1 and B2. Inside the manipulator, the fibre input adapter is pressed against the alignment screws by a spring-loaded counter-bearing. Thus, the fibre adapter can both be shifted and tilted by turning the adjustment screws. Under normal use, e.g. after removing and re-inserting the fibre, only fine adjustments are required. It is then sufficient to adjust the front screws, A2 and B2, for maximum image intensity. Do not turn the screws by more than 1/2 turn. Once the manipulator is totally misaligned you have to go through the complete alignment procedure.

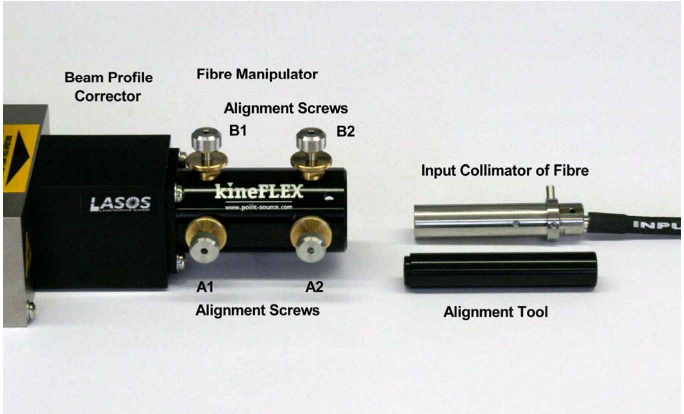


Fig. 7: Front end of the BDL-405SM laser. Beam profile corrector, fibre manipulator with alignment screws, input adapter of the single-mode fibre, and alignment tool.

The complete alignment procedure is illustrated in Fig. 8. For the first steps an alignment tool is required, see Fig. 7. The tool is a tube which has a pinhole in the optical axis.

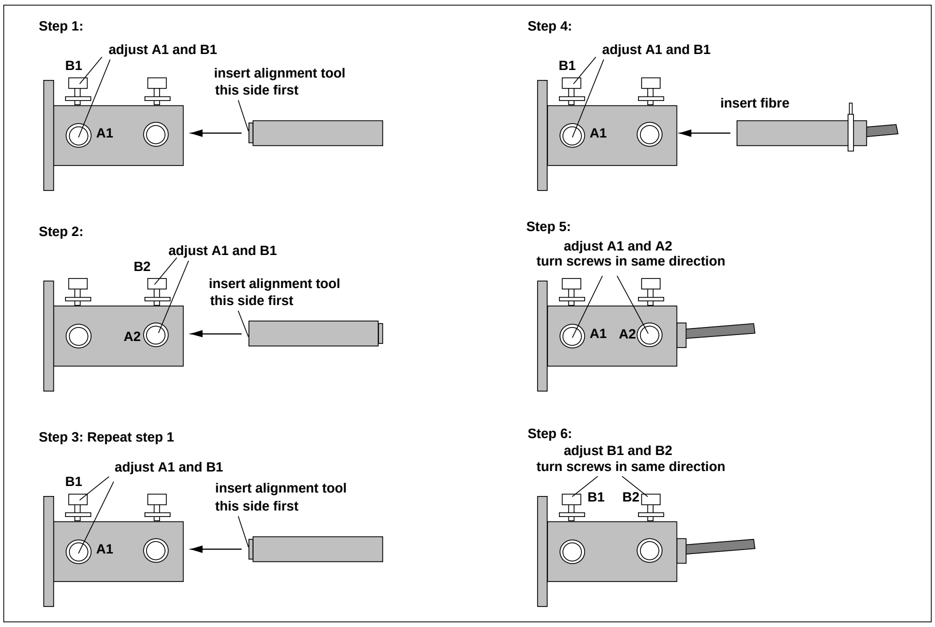


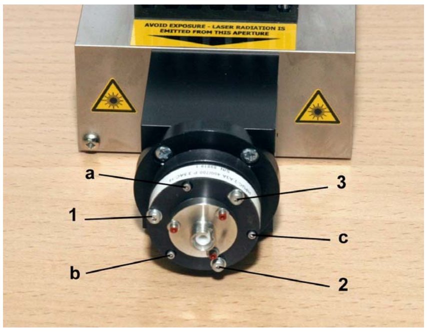
Fig. 8: Steps of the alignment procedure

To align the fibre coupler, proceed as follows [20]:

1) Insert the alignment tool as indicated in Fig. 8 and adjust A1 and B1 for maximum throughput.
2) Reverse the alignment tool and adjust A2 and B2 for maximum throughput.
3) Repeat step 1. After step 3 the optical axis of the fibre manipulator is aligned with the axis of the laser beam.
4) Insert the fibre. Adjust A1 and B1 for maximum output intensity.
5) Adjust A1 and A2 for maximum intensity. This step is a lateral shift of the optical axis. Therefore turn both screws in the same direction until you find the setting that yields maximum intensity.
6) Adjust B1 and B2 for maximum intensity. This step is a lateral shift of the optical axis. Therefore turn both screws in the same direction until you find the setting that yields maximum intensity.

Alignment of OZ Optics Coupler

The OZ Optics coupler consists of two plates, separated by a resilient O ring. The plate holding the fibre receptacle is tilted against the laser head adapter by three screws, 1,2,3, see Fig. 9, left. The laser head adapter contains a lens that focuses the laser beam into the core of the fibre. By adjusting the three screws one can move the core of the fibre laterally with respect to the focus [19]. To adjust the focus longitudinally the connector of the fibre has a differential thread, see Fig. 9, right. When the adjustment is complete the front plate is locked against the base plate by tightening the lock screws, a, b, c. The longitudinal focus adjustment is locked by tightening the focus lock nut, see Fig. 9, right.



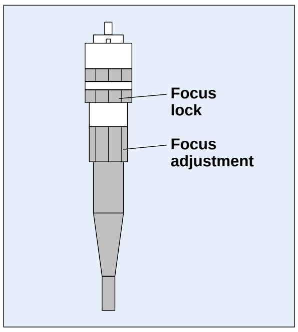


Fig. 9. Left: OZ Optics coupler, with adjustment screws, 1,2,3, and lock screws, a, b, c. Right: Fibre connector with focus adjustment

For fine adjustment it is normally sufficient to loosen the lock screw a, b, c, by a quarter turn and turn the adjust screws, 1,2,3, until maximum intensity is transmitted through the fibre. Then tighten the lock screws one after another in small steps, see below, alignment step 14.

If the coupler is totally misaligned so that no light is transmitted through the fibre, proceed as described below [19].

7) Without a fibre, examine the output on a sheet of paper. If the image on the screen is not centred, adjust the lateral position of the focusing lens with respect to the laser beam. To do this, loosen the tilt adjustment screws, 1,2,3, by about half a turn, and apply lateral pressure to the side of the coupler flange. This will shift the lateral position of the coupler flange with respect to the laser head adapter. Once the image has been centred, tighten the tilt adjustment screws to their original position.
8) Insert a multi-mode fibre of a core diameter of 50 to 100 µm. Observe the transmitted light on a sheet of paper. Make sure that the lock screws, a, b, c, are not pressing against the base plate. Adjust the tilt screws, 1,2,3, until you obtain maximum intensity and a symmetrical intensity pattern on the paper.
9) Tighten the tilt screws evenly, while maintaining maximum intensity and a symmetrical intensity pattern. No more than 1.27 mm (0.05”) space should be left between the base adapter and the coupler flange.
10) Remove the multi-mode fibre and put in the single-mode fibre. At least a small amount of light should be transmitted through the fibre. Adjust the tilt screws, 1,2,3, until you obtain maximum throughput. Important: The Airy disk pattern in the focus of the lens may have secondary maxima. If the throughput is unacceptably low, make sure that you are in the central peak of the Airy disk.
11) Adjust the focus by rotating the focus adjustment of the fibre connector. It may be necessary to readjust the tilt screws.

12) Once the best focus is achieved, tighten the nut that locks the focus adjustment.
13) Turn in the three lock screws, a, b, c until the just make contact with the laser head adapter.
14) Tighten the lock screws by an additional quarter turn. If the throughput drops, adjust the lock screws slightly until optimum coupling efficiency is restored.

Trigger Output

TCSPC measurements require a timing reference signal from the laser [8, 9]. To minimise timing drift the BDL-SMC lasers derive a trigger signal directly from the laser diode. The electrical pulse shape is shown in Fig. 10.

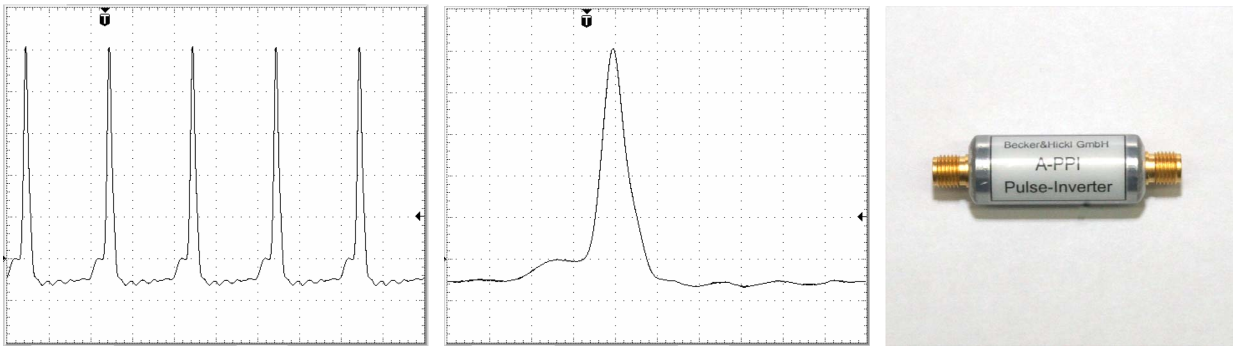


Fig. 10. Left and middle: Electrical pulse from the trigger output, 50 mV/div., left 10 ns / div., middle 2 ns/div. Recorded with Tektronix TDS 3052 Oscilloscope. Right: A-PPI pulse inverter

The polarity of the trigger pulse is positive. To connect the pulse into the SYNC input of a bh TCSPC module, please use an A-PPI pulse inverter, see Fig. 10, right. The adapter is delivered with all bh TCSPC modules.

The time difference between the trigger pulse and the light pulse is less than 1 ns and does not change appreciably for different output power and for different repetition rate. The shift of the light pulse with the power referred to the trigger pulse is shown in Fig. 11. The repetition rate was 50 MHz, the power was varied between 0.3 mW and 1.3 mW. The total shift with the power is about 200 ps.

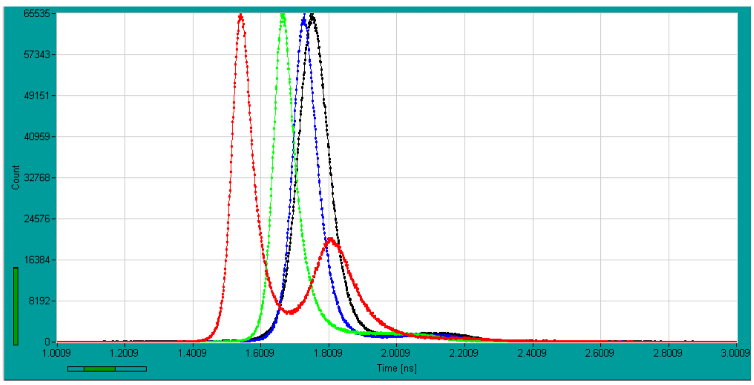


Fig. 11: Shift of the light pulse with the output power referred to the trigger pulse. Left to right: Power 0.3 mW, 0.5 mW, 0.8 mW and 1.3 mW. Recorded with bh SPC-730 TCSPC module [9] and Hamamatsu R3809U MCP-PMT [12].

When using the BDL-SM lasers in conjunction with the bh TCSPC modules please keep in mind that the TCSPC modules use reversed start stop. Reversed start-stop operation of TCSPC requires a reference pulse at the end of the signal period or, better, at the end of the recorded time interval

[8, 9]. At the high repetition rate of the BDL-SMC lasers the next laser pulse is no more than 50 ns away so that a reasonable recording is achieved without problems. However, it is not always clear which laser pulse actually stops the time measurement. It can happen that the stop pulse is not the same laser pulse that excited the detected photon but a pulse from a period before or after. Stopping with a pulse from a different period is no problem if the laser pulses have a constant period and no pulse-to-pulse jitter. The BDL-SMC lasers have, however, selectable pulse periods. Moreover, the clock oscillator of a diode laser may have a pulse-to-pulse jitter of some 10 ps. If the reference pulses come from the wrong signal period the position of the recorded signal in the TAC range changes when the laser period is changed. Moreover the pulse-to pulse jitter adds to the transit time spread of the TCSPC system.

To stop the TAC with the correct laser pulse, the reference signal should be delayed so that the reference pulse arrives after a photon pulse from the same period [8, 9]. The correct delay in the reference channel is the detector transit time, plus the width of the recorded time interval, plus a few ns for the TAC start delay. The relation of the detector and reference delay is shown in Fig. 12.

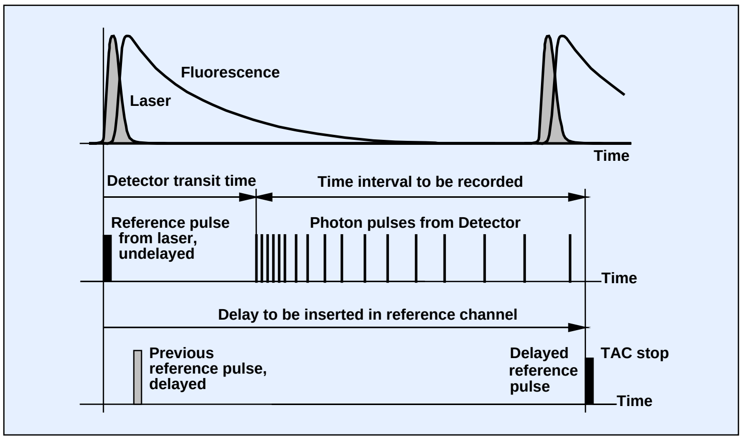


Fig. 12: Reversed start-stop should be used with a delay in the reference channel to stop the TAC with the correct laser pulse [9]

Typical signal transit times of detectors are

MCP PMTs	1 ns
Hamamatsu R7400 TO-8 PMTs	5 - 6 ns
Hamamatsu H5783 photosensor modules	5 - 6 ns
bh PMC-100 detector module	5 - 6 ns
30 mm side-window PMTs	25 ns
XP 2020 linear focused PMTs	25 ns

A good stop delay to start with is 15 ns or 3 m cable for an MCP PMT and 25 ns or 5 m cable for TO-8 PMTs. Please see [9] for details.

Control Inputs

The control input connectors at the switch box are shown in Fig. 13.

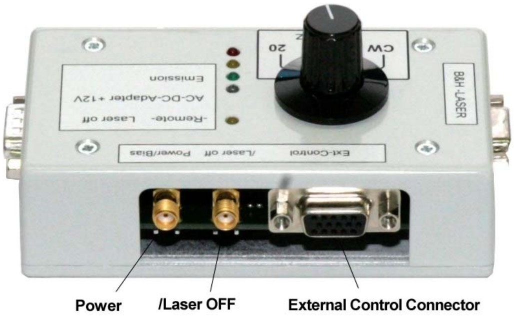


Fig. 13: Control inputs

The pin assignment of the external control connector is

1	not connected	9	not connected
2	Frequency 20 MHz	10	not connected
3	Frequency 50 MHz	11	not connected
4	Frequency 80 MHz	12	Power / Bias, 0 to +10 V
5	GND	13	not connected
6	not connected	14	not connected
7	/Laser Off	15	GND
8	not connected		

Pin 2,3,4, Frequency select pins

Frequency select pins, CMOS compatible. The laser works at the selected frequency when the corresponding pin is at 'high' or open and the other pins are at 'low' or connected to ground.

Important: The frequency select pins are connected in parallel to the frequency select switch. They can only be used when the frequency select switch is in the 'CW' position. In all other positions of the switch the pin corresponding to the frequency selected is connected to ground. Please make sure that the source of the control signals connected to pin 2, 3 and 4 is short-circuit proof.

Pin 5, Ground

Reference pin for all signals and power supply '-' pin.

Pin 7, /Laser Off

Connecting this pin to TTL/CMOS 'Low or' GND switches the laser off. The laser beam is shut down and the trigger output becomes inactive. After disconnecting the pin from GND or switching to TTL/CMOS 'high' the laser resumes normal operation (see Fig. 15). Leave the pin open if you want the laser to run continuously. Please notice that the laser does not deliver trigger pulses when it is switched off by /Laser Off = 'low'. For bh TCSPC modules this is no problem. However, if the /Laser Off signal is pulsed at high rate the SPC module will display an *average* SYNC rate, i.e. a rate lower than the frequency selected by the frequency selection switch.

The /Laser OFF signal can also be connected to an SMA connector, see Fig. 13. The SMA input is connected in parallel with pin 7 of the sub-D connector.

Pin 12, Power / Bias

An input voltage applied to pin 12 changes the bias of the laser diode. The voltage can be in the range of 0 V to +10 V. The output power increases with the voltage. The Power / Bias signal can also be connected to an SMA connector, see Fig. 13. The SMA input is connected in parallel with pin 12 of the sub-D connector.

Pin 15, Ground

Reference pin for all signals and power supply '-' pin.

Software Control of the Laser Power

The 'Power / Bias' input can be used for electronic power control of the BDL-SMC lasers. The power control signal is connected either to an SMA connector or to pin 12 of a 15-pin connector, both located at the back of the laser switch box. Controlling the laser power electronically is particularly convenient in TCSPC systems that use the bh DCC-100 detector controller [3], see Fig. 14, left.

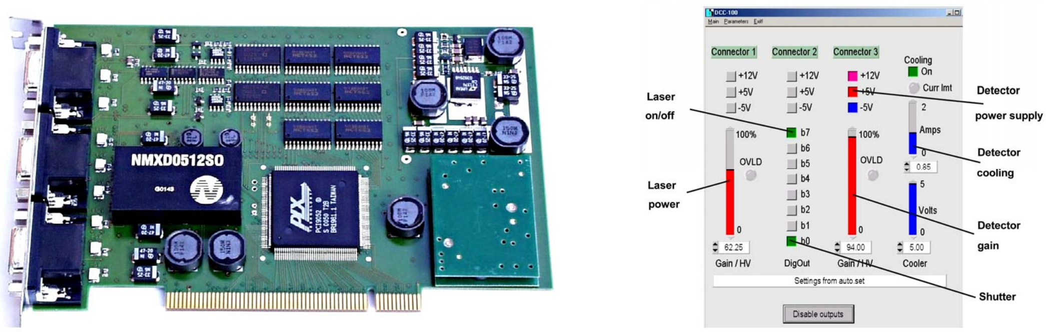


Fig. 14. Left: DCC-100 detector controller card. Right: DCC-100 software panel

Fig. 14, right shows the software control panel of the DCC-100. The 'Connector 1' channel is used to control the power of the laser, while 'Connector 3' controls the detector. The 'b7' bit of the digital outputs ('Connector 2') is used to switch the laser on and off.

Laser OFF Signal

The BDL-SMC lasers can be switched on and off by applying a TTL/CMOS signal to pin 7 of the sub-D connector. TTL Low or connecting the pin to GND switches the laser off. The '/Laser OFF' signal works both in the picosecond mode and in the CW mode. The reaction times are typically

	ps mode	CW mode
/Laser OFF low to emission on	< 1 µs	3 µs
/Laser OFF high to emission off	< 100 ns	3 µs

The on-off behaviour is shown in Fig. 15. The curves were recorded by a pin photodiode connected to a Tektronix TDS 3052 oscilloscope. *Important*: If you want to test the switching behaviour, apply a reverse bias to the photodiode and switch the oscilloscope to an input impedance of 50 Ω. To obtain sufficient signal amplitude at 50 Ω it may be necessary to use an avalanche photodiode.

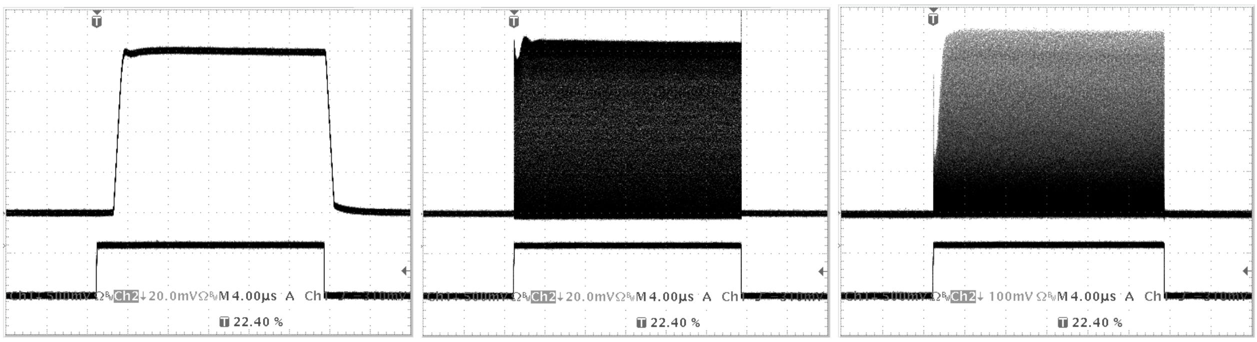


Fig. 15: On/Off behaviour of the BDL-SMC laser. Left to right: CW mode, 20 MHz, 80 MHz. Upper trace laser intensity, lower trace /Laser OFF signal. Recorded with avalanche photodiode connected to Tektronix TDS 3052. Time scale 4 µs per division.

The variation of the shape of the laser pulses after the off/on transition is shown in Fig. 16. 40 µs TTL high pulses were applied to the /Laser OFF input, and the sequence was accumulated in the Scan Sync Out mode of an SPC-830 TCSPC module for 10^5 on/off transitions [9]. The photons were detected by an R3809U-52 MCP-PMT [12]. Each curve of the sequence represents an interval of 500 ns. As can be seen from Fig. 16, right, the pulse shape is stable after 2 µs. The shift of the pulses within the first 2 µs is less than 30 ps.

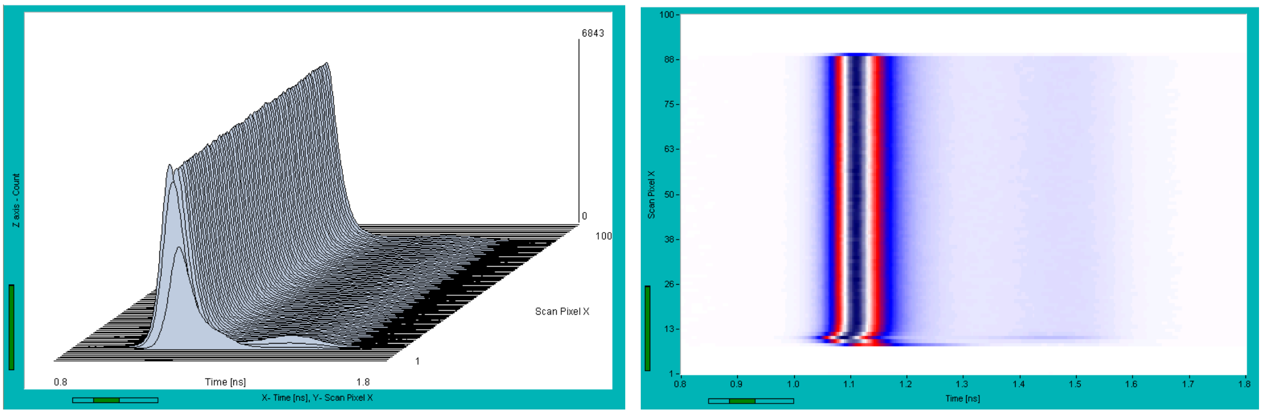


Fig. 16: Transient pulse shape variation of the BDL-SMC lasers after transition from /Laser OFF = low to /Laser OFF = high. 40 µs TTL high pulses were applied to the '/Laser OFF' input. Repetition rate 50 MHz. SPC-830 TCSPC module with R3809U MCP-PMT, triggered sequential recording in Scan Sync In mode. Each curve of the sequence represents an interval of 500 ns. Left: Curve plot. Right: Contour plot.

TCSPC Systems with the BDL-SMC Lasers

A wiring diagram of a TCSPC system with a BDL-SMC laser, a PMC-100 detector, a DCC-100 detector controller, and an SPC TCSPC module is shown in Fig. 17.

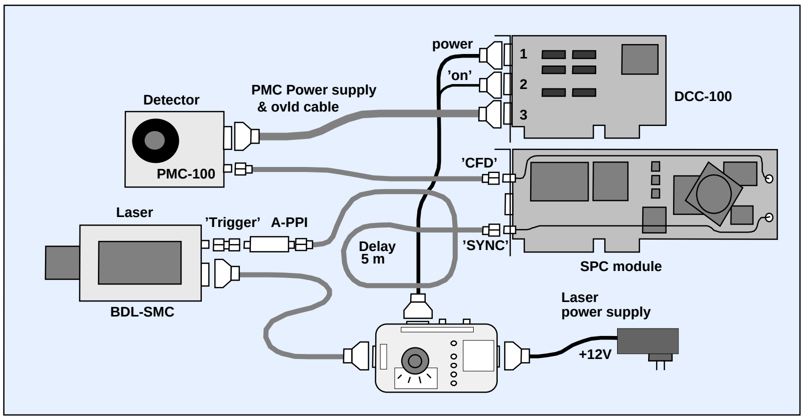


Fig. 17: Connection diagram of a TCSPC system with a BDL-SMC laser, a PMC-100 detector, a DCC-100 detector controller and an SPC module

The DCC-100 module controls both the laser and the detector. The laser power can be changed via a control signal from connector 1 of the DCC-100. The emission is switched on and off via connector 1 (see also Fig. 14).

The trigger output pulses of the lasers are inverted by an A-PPI pulse inverter and fed into the SYNC (stop) input of the SPC module. A cable of 5 m length is used to place the stop pulse after the photon pulses originating from photons detected in the signal period of a particular trigger pulse (see Fig. 12).

A PMC-100 detector is used to detect the light from a sample. The PMC-100 is controlled via connector 3 of the DCC-100. The DCC-100 controls the gain of the detector, provides the power supply for the internal preamplifier, high-voltage generator, and cooler of the PMC-100. In case of overload, the DCC-100 shuts down the high voltage of the PMT of the PMC-100.

The single-photon pulses of the PMC-100 are fed into the CFD (start) input of the SPC module.

A large number of modifications of the TCSPC setup are possible. The detector may be replaced with an ultra-fast R3809U MCP PMT, with a single-photon avalanche photodiode, or with a multi-spectral detector assembly. For recording fluorescence lifetime images, the TCSPC system may also be connected to a laser scanning microscope, see 'Laser Scanning Microscopy', page 29. For details please see [9].

Multiplexing Lasers

By controlling several lasers via their /Laser OFF inputs the lasers can be multiplexed at periods down to the microsecond range. Normally laser multiplexing is used in conjunction with multiplexed TCSPC operation [8, 9]. That means, the routing signal inputs of the TCSPC module are used to direct the photons of the individual lasers into separate photon distributions.

To simplify the generation of the multiplexing and routing signals the DDG-200 digital delay generator card is available, see Fig. 18. The card is controlled via the software panel shown in Fig. 18, right.

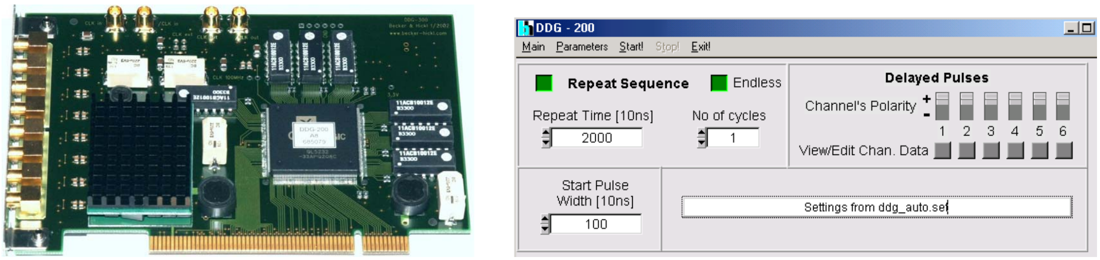


Fig. 18. Left: DDG-200 card for multiplexing control. Right: Software panel of the DDG-200

The DDG-200 can be programmed to multiplex up to four lasers. Multiplexing periods can be programmed at any time scale from a few 100 ns to 10 milliseconds. The control signals can be defined non-overlapping, i.e. with gaps of some 100ns duration between the individual lasers. This guarantees that any crosstalk is avoided, even if the routing and multiplexing signals get delayed in the connecting cables.

A TCSPC system with two multiplexed lasers is shown schematically in Fig. 19. A bh DDG-200 card is used to generate the 'ON' signals for the lasers and routing signals for the SPC module. The control sequence is shown in the lower right corner. The lasers are switched on alternatingly. Simultaneously with the laser switching, a bit at the routing input of the SPC card is toggled between 0 and 1. Consequently, the SPC module records the signals of the two lasers into different memory blocks.

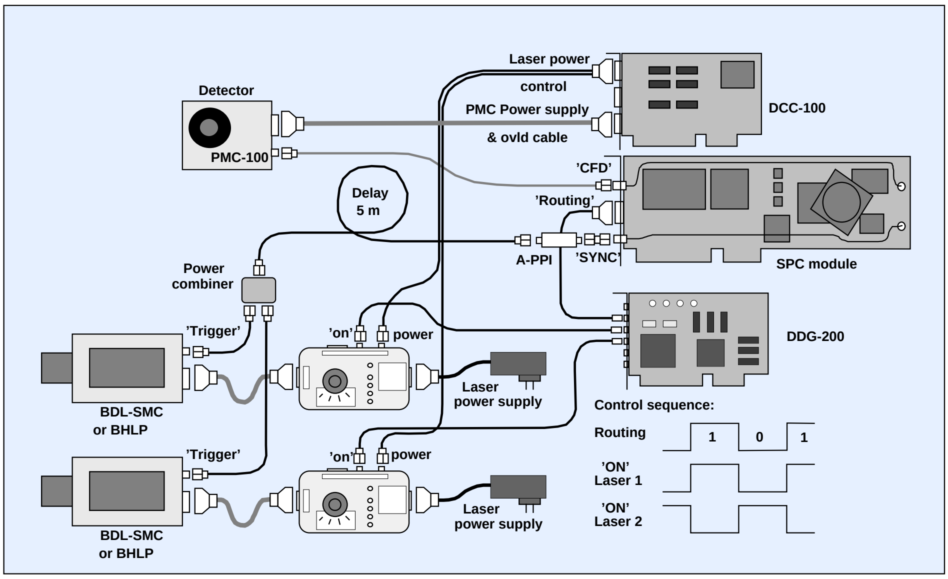


Fig. 19: TCSPC system with two multiplexed lasers

Please note that multiplexing can be used even in combination with multidetector operation, sequential recording, and imaging by TCSPC scanning techniques [9]. Moreover, the bh blue BDL-SMC lasers can be multiplexed with the BHLP-700 red and NIR lasers [5]. An application to chlorophyll measurements is described in [8]. A BDL-405 (405 nm) and a BHLP-700 (650 nm) laser are multiplexed, and the signals at 540 nm and 700 nm are recorded simultaneously by two detectors. The setup is able to record both the fluorescence of flavins and of chlorophyll independently of photochemical and non-photochemical quenching transients, see also 'Excitation Wavelength Multiplexing', page 27.

Laser Safety

The BDL-SMC lasers are class 3B laser products. The laser safety regulations dictate that the lasers be labelled with the stickers shown in Fig. 20, and that the labels and the location of the labels on the lasers be described in the manual. The laser class is indicated on the laser by an ‘explanatory label’, Fig. 20, left. The laser aperture is marked with the aperture labels, Fig. 20, middle and right.

Fig. 20. Left to right: Explanatory label, aperture labels.

Moreover, each laser has a manufacturer identification, as shown in Fig. 21.

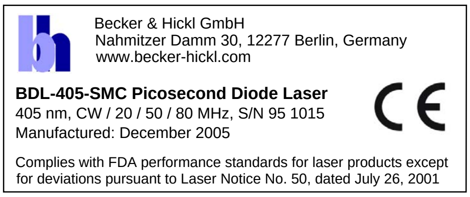


Fig. 21: Manufacturer identification label

The position of the labels on the laser modules is shown Fig. 22.

Fig. 22: Location of the labels on the lasers

Laser safety regulations forbid the user to open the housing of the laser, or to do any maintenance or service operations at or inside the laser. Use of controls or adjustments or performance of procedures other than specified herein may result in hazardous radiation exposure or damage to the laser module.

Moreover, do not look into the laser beam through lenses, binoculars, microscopes, camera finders, telescopes, or other optical elements that may collimate the light into your eye. When using the lasers in combination with a microscope make sure that the beam path to the eyepieces is blocked for the laser wavelength when the laser is on. If an optical fibre connected to a 3B laser has to be replaced, the laser has to be turned off.

It is required to have a ‘remote interlock connector’ that can be pulled to turn off the laser reliably. In that case, use the 15 pin connector at the laser side of the laser switch box. The connector can be pulled off or plugged in at any time without causing damage to the laser.

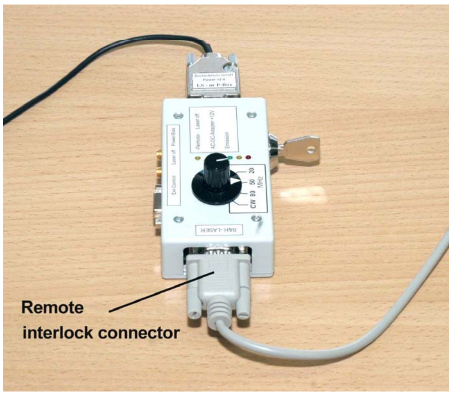


Fig. 23: Remote interlock connector: Pull the 15 pin connector at the laser side of the switch box to turn off the laser

Understanding Picosecond Diode Lasers

Picosecond Operation of Laser Diodes

The BDL-SMC lasers are based on commercially available blue laser diodes [17]. Picosecond pulsing of laser diodes requires to drive extremely short current pulses trough the pn junction of the diode. Unfortunately commercial laser diodes are not optimised for this kind of operation. In particular, the junction capacitance C_j and the lead inductance L_l form an LC low pass filter that impedes a fast voltage rise across the diode junction. The situation is shown in Fig. 24.

For low driving power the generator pulse initiates a damped sine-wave voltage across the diode junction. When the first positive peak reaches the forward conducting voltage of the diode, current starts to flow through the junction. As long as the laser threshold is not reached the light pulse is weak and broader than the current pulse.

If the driving power is increased the first positive peak drives a substantial forward current through the diode junction. The dynamic impedance of the junction drops dramatically, preventing the voltage at the junction to increase much above the forward voltage. The current through the junction exceeds the laser threshold for a short fraction of the sine wave period, and a short light pulse is emitted.

If the driving power is increased further the forward current pulse and consequently the light pulse becomes stronger. The decrease in the dynamic resistance of the pn junction and the nonlinearity of the laser emission cause the optical pulse width to decrease. Eventually, the subsequent peaks of the sine wave start to drive a forward current through the diode junction resulting in a tail or afterpulses of the light pulse.

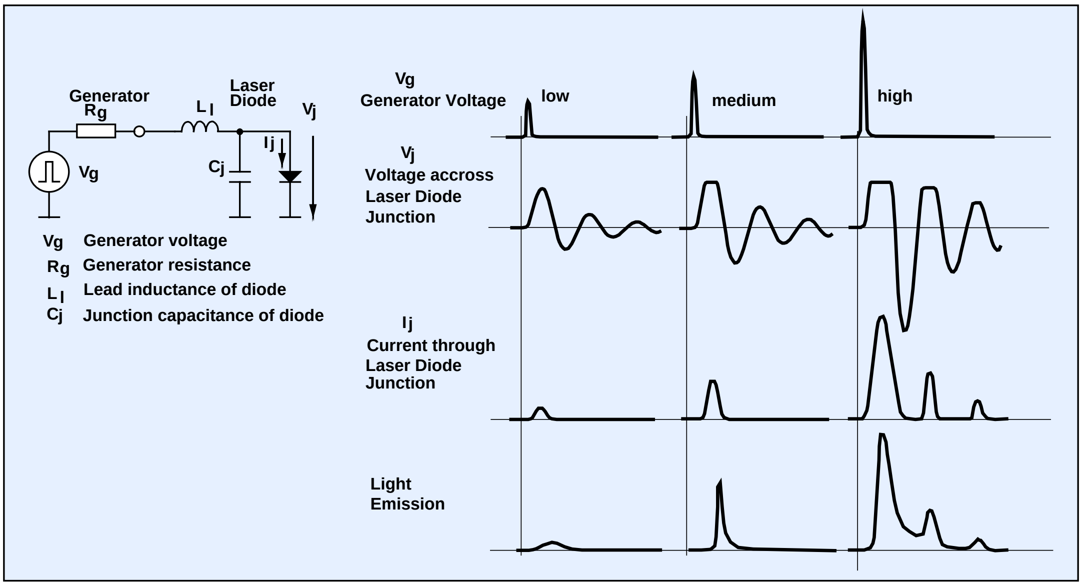


Fig. 24: Junction voltage Vj and junction current Ij in a picosecond laser diode for different driving pulse amplitude Vg

The behaviour of the junction current explains why there is a relation between the pulse shape and the pulse power. Good pulse shapes can be obtained only at moderate optical power. Using a stronger laser diode does not generally help. It can actually make the situation worse because the junction capacitance of the larger laser diode is higher.

An additional control parameter is obtained by adding a bias voltage to the driving pulse. For blue laser diodes, which have a forward conducting voltage of 4 to 5 V, the bias can be positive (in forward direction) or negative (in reverse direction). The influence of the diode bias is shown in Fig. 25.

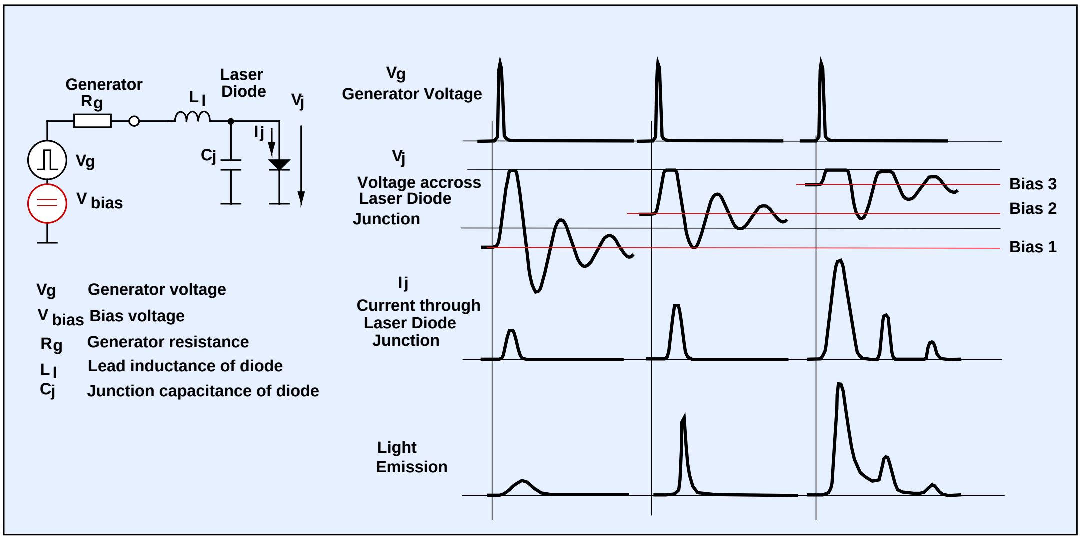


Fig. 25: Junction voltage Vj and junction current Ij in a picosecond laser diode for different diode bias voltage

Positive bias results in higher output power, but makes afterpulsing more likely. Reverse bias helps to suppress afterpulses but reduces the power. In general the best optical pulse shape is obtained by using high driving amplitudes and a bias as low (negative) as possible.

It should be noted that the operating conditions of picosecond pulsed laser diodes are different from those of modulated laser diodes used in communication equipment. A modulated laser diode is always forward biased, and there is a continuous forward current through the laser diode. Consequently, the diode junction has a low dynamic impedance that shorts the junction capacitance. The speed of the diode is then determined mainly by the lead inductance and the generator impedance.

Average Power and Peak Power

The typical pulse width for a picosecond laser diode is in the range of 40 to 100 ps. For a repetition rate in the 20 to 80 MHz range the duty factor is on the order of 300. As shown in Fig. 26, the result is a relatively high peak power even for low average (CW equivalent) power.

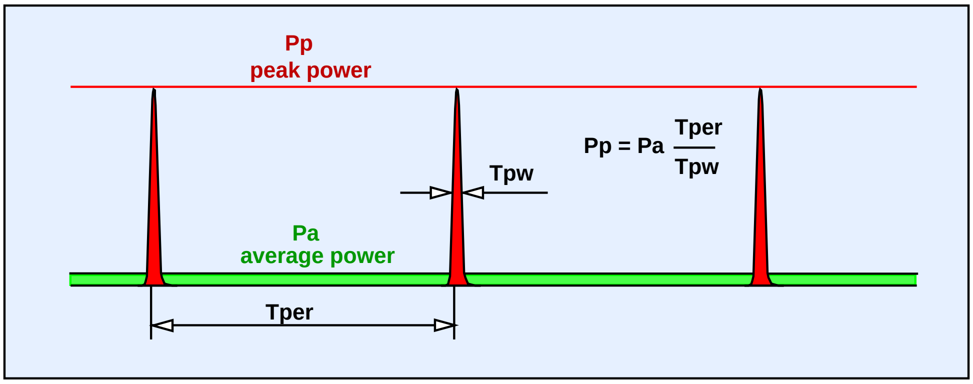


Fig. 26: Relation between peak power, average power, pulse width and pulse period

For ps diode lasers, the optical peak power is far beyond the permissible steady state power for the laser diodes used. Due to the short pulse width the high peak power does not cause any thermal damage. However, damage may also occur by extremely fast nonlinear optical effects. It is therefore recommended to avoid unnecessarily high peak power, and not to exceed the power at which substantial afterpulses develop.

Pulse Shape

Fig. 27 through Fig. 30 show pulse shapes, pulse width and peak power for different BDL-SMC lasers. It should be noted here that the Nichia laser diodes used in the BDL lasers are continuously improved. For example, the optical output power of the 405 nm diodes has been increased by a factor of 10 within two years. At the same time, there was a substantial improvement in efficiency, i.e. in the output power for a given forward current. Fig. 27 through Fig. 30 should therefore considered to demonstrate the general relation between power and pulse parameters, not the quantitative values.

Pulse shapes for a BDL-405-SMC laser for different average optical power at 50 MHz are shown in Fig. 27.

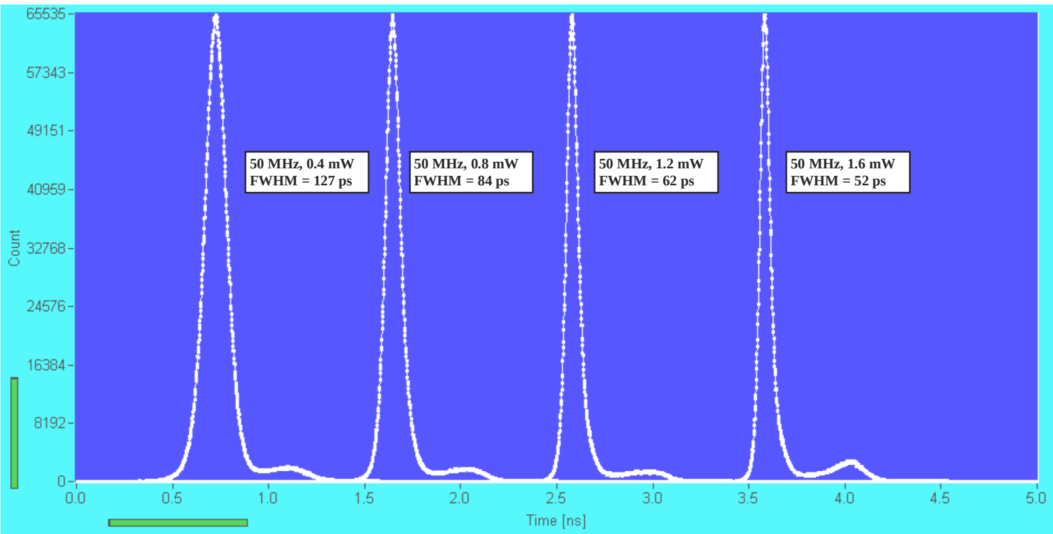


Fig. 27: Pulse shapes for a BDL-405-SMC laser at 50 MHz. Recorded with Hamamatsu R3809U-50 MCP [12] and BH SPC-730 TCSPC module [9].

The curves were recorded with a Hamamatsu R3809U-52 MCP PMT and a bh SPC-730 TCSPC module. The R3809U-52 was operated at 3 kV, yielding an instrument response function (IRF) of 30 ps fwhm. *Important*: The instrument response function of the R3809U-52 has a shoulder of about 300 ps duration [8, 9, 12]. The afterpulse visible in the recorded pulse shape, in a large part, comes from that shoulder, not from the laser pulse itself.

The pulse width decreases continuously with increased output power. The best pulse shape for 80 MHz, 50 MHz, and 20 MHz is obtained at 1.6 mW, 1 mW, and 0.4 mW, respectively. Typical curves of the peak power and the pulse width are shown in Fig. 28.

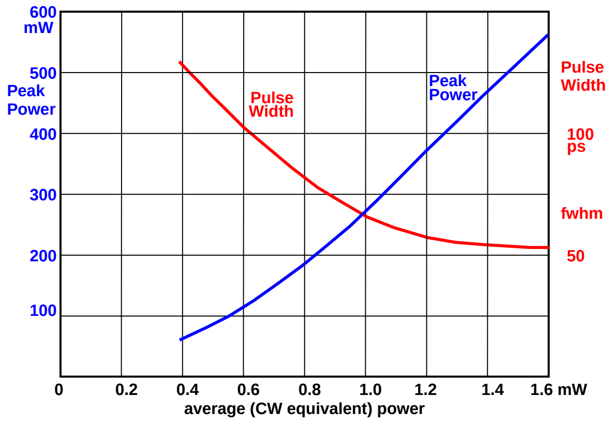


Fig. 28: Pulse width and peak power for a BDL-405 versus average power at 50 MHz repetition rate. Pulse width corrected for 30ps IRF width of detection system.

Typical pulse shapes of the BDL-440-SMC and the BDL-473-SMC are shown in Fig. 29 and Fig. 30.

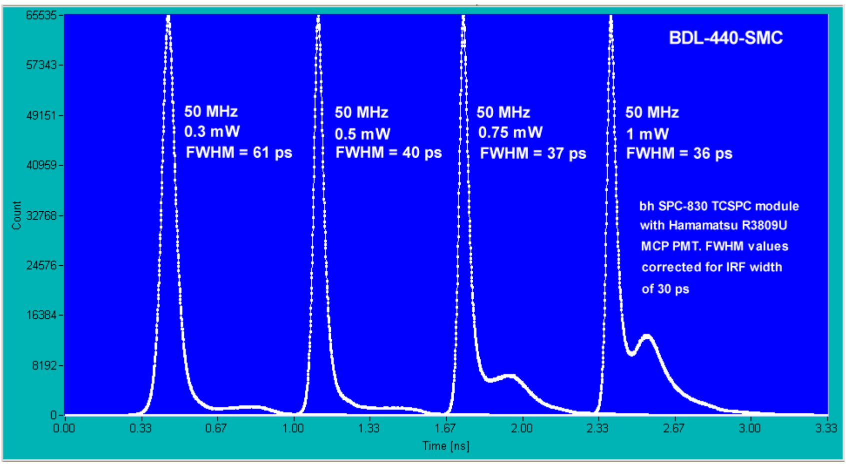


Fig. 29: Pulse shapes for a BDL-440-SMC laser at 50 MHz. Recorded with Hamamatsu R3809U-50 MCP and bh SPC-830 TCSPC module.

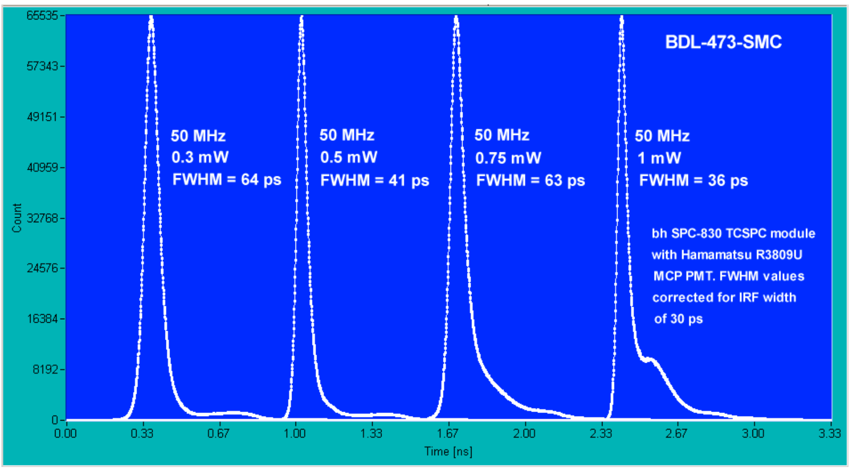


Fig. 30: Pulse shapes for a BDL-473-SMC laser at 50 MHz. Recorded with Hamamatsu R3809U-50 MCP and bh SPC-830 TCSPC module.

Application to Fluorescence Lifetime Spectroscopy

Fluorescence Lifetime Experiments

The BDL-SMC lasers in conjunction with the bh TCSPC modules make fluorescence lifetime measurements an easy task. A simple fluorescence lifetime system is shown schematically in Fig. 31.

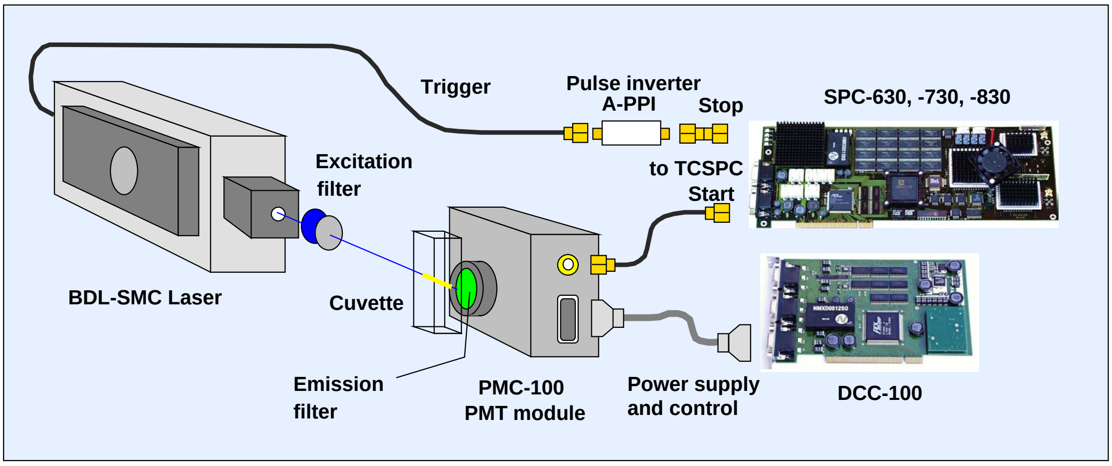


Fig. 31: Fluorescence lifetime measurement setup

For samples with strong scattering possible background emission of the laser diode may be removed by a bandpass filter in the excitation path. Moreover, it may be convenient to have variable ND filter placed in the excitation path.

After passing the filters the laser beam is sent into the sample cell. The fluorescence light is detected at an angle of 90° from the excitation beam. The detector is a bh PMC-100 module. It is controlled via a DCC-100 detector controller card. The PMC-100 is located close to the sample cell. The fluorescence light is collected directly, i.e. without an additional transfer lens. The detection wavelength interval is selected by a bandpass filter at the input of the detector.

Important: In the BDL-SMC lasers the polarisation of the laser beam is horizontal. Sending a horizontally polarised laser beam into the sample and detecting the fluorescence under an angle of 90° from the excitation would result in detecting only I_s components of the fluorescence, i.e. projections of the electrical field vectors perpendicular to the polarisation of the laser. This would result in large distortion of the measured decay functions by rotational depolarisation [8, 9, 18]. In the setup shown in Fig. 31 the laser is therefore turned by 90°. Polarisation effects can further be reduced by tilting the laser or placing a polariser in the beam path. Unfortunately the exact angles depend on the numerical aperture of the detection light path which is not exactly predictable.

The PMC-100 detector is controlled by a DCC-100 detector controller. The DCC-100 provides the power supply for the PMT module, the preamplifier, and the cooler of the PMC-100. Moreover, it provides software controlled detector gain and overload shutdown.

The photon pulses of the PMC-100 are connected directly to the TCSPC module. Any bh SPC module can be used. The stop (timing reference) signal comes from the trigger output of the laser.

An example of a fluorescence decay measurement is shown in Fig. 32. Stilben (blue curve) and Rhodamin 110 (red curve) were excited by a BDL-405-SMC laser. The black curve is the IRF obtained from a scattering solution.

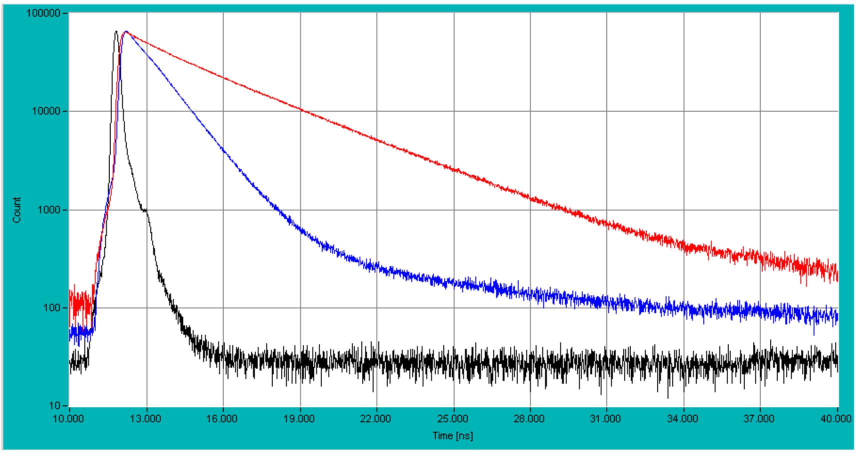


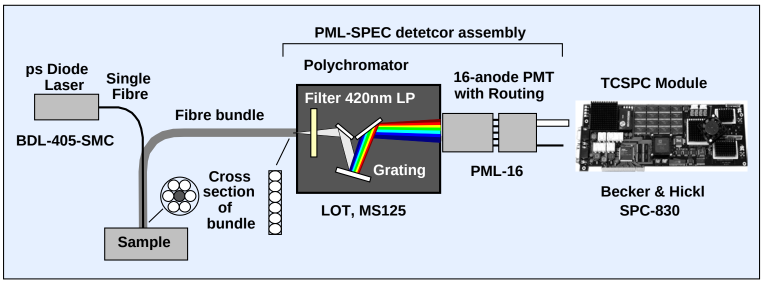
Fig. 32: Fluorescence decay curves of stilben (blue) and rhodamin 110 (red), excited at 405 nm (black). Time scale 3 ns per division, time channel width 12 ps.

Despite of its simplicity the setup features high sensitivity and good time resolution. Another advantage is that light reflected at the emission filter and the photocathode of the detector is not focused back into the sample. Therefore the setup has less problems with optical reflections then more complex optical systems.

In all fluorescence measurement that use deconvolution of the fluorescence data from the instrument response function (IRF) the laser must be operated at the same power for the fluorescence measurement and the IRF measurement. As shown in Fig. 27, page 22, to Fig. 30, the shape of the laser pulse changes with the power. Changing the laser power between the recordings may therefore lead to a wrong shape of the IRF and, consequently, to a poor fit of the data and large lifetime errors for fast lifetime components.

Autofluorescence of Tissue

A simple optical setup for single-point multi-spectral measurements of tissue autofluorescence is shown in Fig. 33, left. A BDL-405-SMC or a BDL-375-SMC laser is used for excitation. A fibre probe is used to excite the sample and to collect the fluorescence light. The probe contains 7 multi-mode fibres of 0.5 mm diameter. The central fibre delivers the laser, the surrounding fibres collect the fluorescence. The detection system consists of a bh 'PML-Spec' multi-wavelength assembly and an SPC-830 TCSPC module. The detection system records decay curves in 16 wavelength intervals simultaneously. Multi-spectral fluorescence decay data of human skin obtained this way are shown in Fig. 33, right.



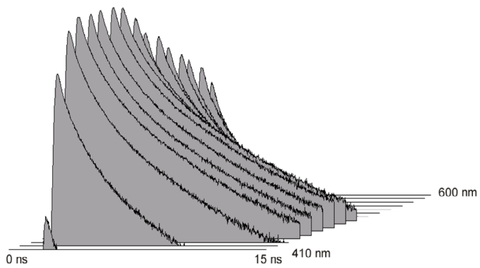


Fig. 33: Left: Optical setup for single-point autofluorescence measurement. Right: Multi-spectral fluorescence decay data of human skin. Time scale 0 to 15 ns, wavelength scale 410 to 600 nm, intensity scale logarithmic from 500 to 30,000 counts per channel.

The count rates obtained from biological tissue are surprisingly high. At an excitation wavelength of 405 nm a count rate of $2 \cdot 10^6$ s^{-1} could be obtained at an excitation power of only 60 µW.

Recording Chlorophyll Transients

The fast on/off switching capability of the BDL-SMC lasers can be used to record excitation-driven transient fluorescence phenomena.

Typical examples of transient fluorescence effects are the ‘Kautski effect’ or the ‘fluorescence transients’ of chlorophyll in living plants [16]. When a dark-adapted leaf is exposed to light the intensity of the chlorophyll fluorescence starts to increase. After a steep rise the intensity falls again and finally reaches the steady-state level. The rise time is of the order of a few milliseconds to a second, the fall time can be from several seconds to minutes. The initial rise of the fluorescence intensity is attributed to the progressive closing of reaction centres in the photosynthesis pathway. Therefore the quenching of the fluorescence by the photosynthesis decreases with the time of illumination, with a corresponding increase of the fluorescence intensity. The fluorescence quenching by the photosynthesis pathway is termed ‘photochemical quenching’. The slow decrease of the fluorescence intensity at later times is termed ‘non-photochemical quenching’.

Results of a non-photochemical quenching measurement are shown in Fig. 34. The fluorescence in a leaf was excited by a bh BDL-405-SMC picosecond diode laser. The fluorescence was detected by a bh PML-SPEC multi-wavelength detection assembly [2], see Fig. 33. Decay curves in the 16 wavelength intervals of the PML-SPEC were recorded by a bh SPC-803 TCSPC module. Simultaneously with the switch-on of the laser a recording sequence was started in the TCSPC module. 30 recordings were taken in intervals of 2 seconds; Fig. 34 shows four selected steps of this sequence. The decrease of the fluorescence lifetime with the time of exposure is clearly visible.

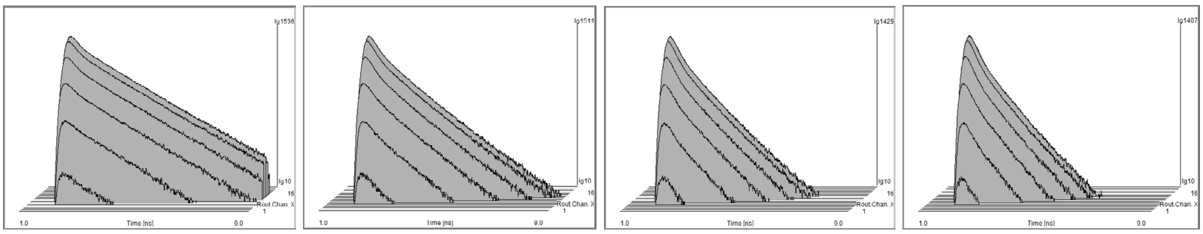


Fig. 34: Non-photochemical quenching of chlorophyll in a leaf, excited at 405 nm. Recorded wavelength range from 620 to 820 nm, time axis 0 to 8 ns, logarithmic display, normalised on peak intensity. Left to right: 0 s, 20 s, 40 s, and 60 s after start of exposure.

Fig. 35 shows fluorescence decay curves at selected wavelengths versus the time of exposure, extracted from the same measurement data set as Fig. 34. The sequence starts at the back and extends over 60 seconds. Also here, the decrease of the fluorescence lifetime with the time of exposure is clearly visible.

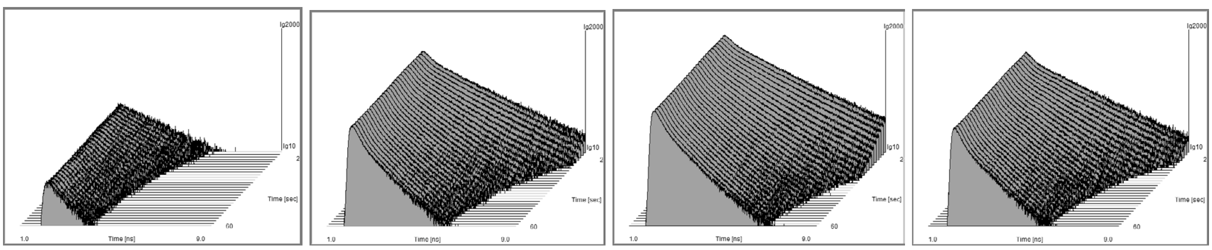


Fig. 35: Non-photochemical quenching of chlorophyll in a leaf, excited at 405 nm. Fluorescence decay curves in different wavelength channels versus time of exposure. 2 s per curve, sequence starts from the back. Extracted from same measurement data as Fig. 34.

The non-photochemical transients shown above occur on a time scale of several 10 seconds. Good results are therefore obtained by recording a single sequence of decay curves at an acquisition time of a few seconds per curve.

The photochemical quenching transients are much faster. Recording these transients requires a resolution of less than 100 µs per step of the sequence. Of course, the number of photons detected in a time this short is too small to build up a reasonable decay curve. Photochemical quenching transients must therefore be recorded by triggered sequential recording [8, 9]. The principle is shown in Fig. 36. The excitation laser is periodically switched on an off via the '/laser off' signal. Each 'on' phase initiates a photochemical quenching transient in the leaf; each 'off' phase lets the leaf recover. Within each 'on' phase a fast sequence of decay curves is recorded in the TCSPC module. The measurement is continued for a large number of such on-off cycles, and the results are accumulated.

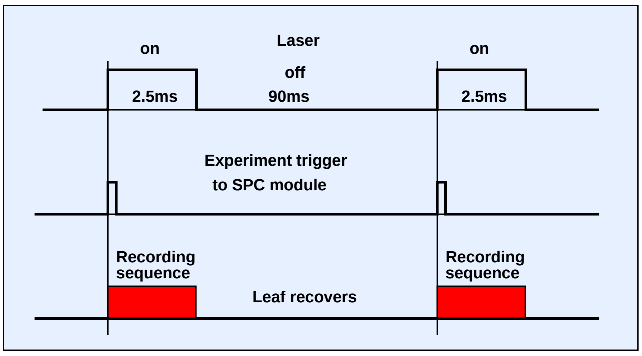


Fig. 36: Triggered sequential recording of photochemical quenching transients. The laser is cycled on and off. Each 'on' phase starts a photochemical quenching transient in the leaf. A sequence of waveform recordings is taken within each 'on' phase. A large number of such on/off cycles is accumulated to obtain enough photons with the individual steps of the accumulated sequence.

A typical result is shown in Fig. 37. The 'on' time was 2.5 ms. Within this time a sequence of 50 decay curves of 50 µs collection time each was recorded. The 'off' time was 90 ms. 20,000 of such on-off cycles were accumulated.

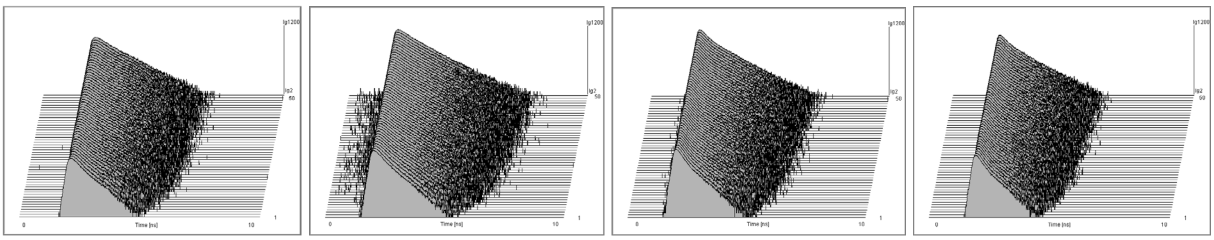


Fig. 37: Photochemical quenching of chlorophyll in a leaf. Fluorescence decay curves in different wavelength channels versus time. Triggered sequential recording, 50 µs per curve, 20,000 measurement cycles accumulated

Excitation Wavelength Multiplexing

Excitation wavelength multiplexing is used to excite different fluorophores during the same measurement. Compared with sequential measurement at different excitation wavelengths fast multiplexing has the advantage that changes in the fluorescence behaviour of the sample have the same effect on all fluorescence signals recorded.

A typical application of multiplexed excitation are measurements at living plants. Green leaves show the typical chlorophyll fluorescence around 700 nm, and a blue-green fluorescence from flavinoids. Due to the Kautski effect, the fluorescence intensity and lifetimes vary with the time of exposure and are thus different for different excitation wavelength and different excitation intensity. It is therefore difficult to obtain comparable results for different excitation conditions. An experiment that avoids this problem is shown in Fig. 38.

The sample is excited by two lasers. A BDL-405-SMC and a BHLP-700 (650 nm) are multiplexed. The light from the sample is split into a 515-nm and a 700-nm component by a dichroic mirror and two bandpass filters. The fluorescence components are detected by two PMT modules. The PMTs are connected to the TCSPC module via a HRT-41 router [1]. Thus, both fluorescence components are recorded simultaneously. One routing bit is required to separate the photons of both detectors. A second routing bit is used to separate the photons excited by the two lasers. The stop signal for the TCSPC module comes from the synchronisation outputs of the lasers. Because only one laser is active at a time, the pulses can be combined by a simple power combiner.

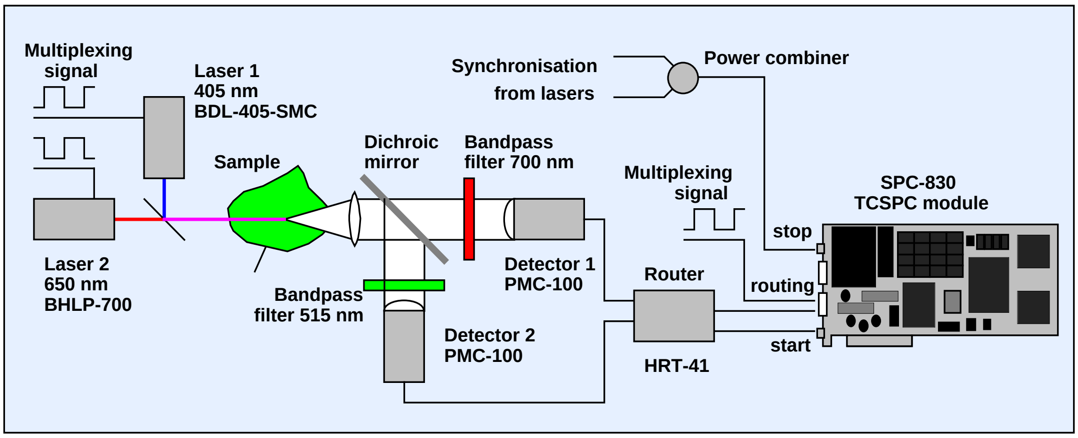


Fig. 38: Simultaneous measurement at two excitation and two emission wavelengths

A typical result is shown in Fig. 39. Fluorescence decay curves for a fresh leaf are shown left, results for a dry leaf right. The synchronisation signal of the 650 nm laser was delayed by 3 ns to make the curves better distinguishable. The multiplexing period was 50 ms. At this rate the lifetime is modulated by photochemical quenching, but not by non-photochemical quenching. Therefore, different lifetimes of the 695 nm emission are obtained for both wavelengths. No such effect is seen in the 695 nm emission of the dry leaf. The green emission at 515 nm from the fresh leaf has a considerably lower intensity, a shorter lifetime, and a multi-exponential decay profile. This indicates that a strong, non-uniform quenching process is at work. Both the intensity and the lifetime of the green emission increase in the dry leaf.

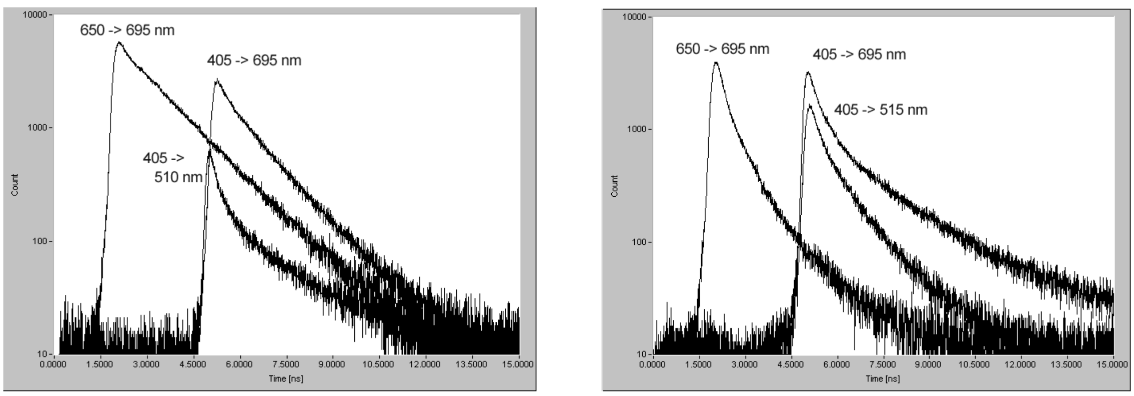


Fig. 39: Dual-wavelength excitation and dual-wavelength detection of the fluorescence of a fresh leaf (left) and a dry leaf (right). Multiplexed excitation at 405 nm and 650 nm, dual-detector recording at 515 nm and 695nm.

Laser Scanning Microscopy

Laser scanning microscopes can relatively easily be upgraded for fluorescence lifetime imaging by multi-dimensional TCSPC [8, 9]. FLIM is especially easy with multiphoton microscopes [7, 10]. The Ti:Sapphire laser of these microscopes is an almost ideal excitation source for FLIM. Standard confocal microscopes, however, use only continuous lasers. Upgrading a standard confocal microscope with FLIM therefore requires a suitable pulse excitation source to be added [23].

All confocal microscopes couple the visible lasers into the optical beam path via single-mode fibres. Most of the microscopes use the coupler and fibre manipulator systems of Point Source Ltd., UK. Attaching a BDL-SMC laser to a confocal microscope is therefore relatively easy. The only requirement is that a free input fibre be available. Many microscopes have a 405 nm continuous diode laser integrated. The excitation problem of FLIM is then easily solved by attaching the fibre of this laser to a BDL-405-SMC. Experiments requiring high continuous power can, in most cases, by performed in the CW mode of the BDL-405-SMC. It is therefore not necessary to switch the fibre between the BDL-405-SMC and the diode laser of the microscope.

Lasers of 440 or 473 nm wavelength can, in principle used in the same way. However, coupling the laser light into the sample requires a dichroic beamsplitter of the correct wavelength in the scanner optics. The 473 nm laser can often be coupled via the 488 nm beam path. Unfortunately, replacing the 488 nm Argon laser with the 473 nm diode laser is often not acceptable. Therefore, please contact bh or the manufacturer of your microscope before you consider attaching a 473 nm laser to it.

Wiring diagrams for a single-detector FLIM system and a multi-spectral FLIM system are shown in Fig. 40, left and right. For details, please see [6] and [9].

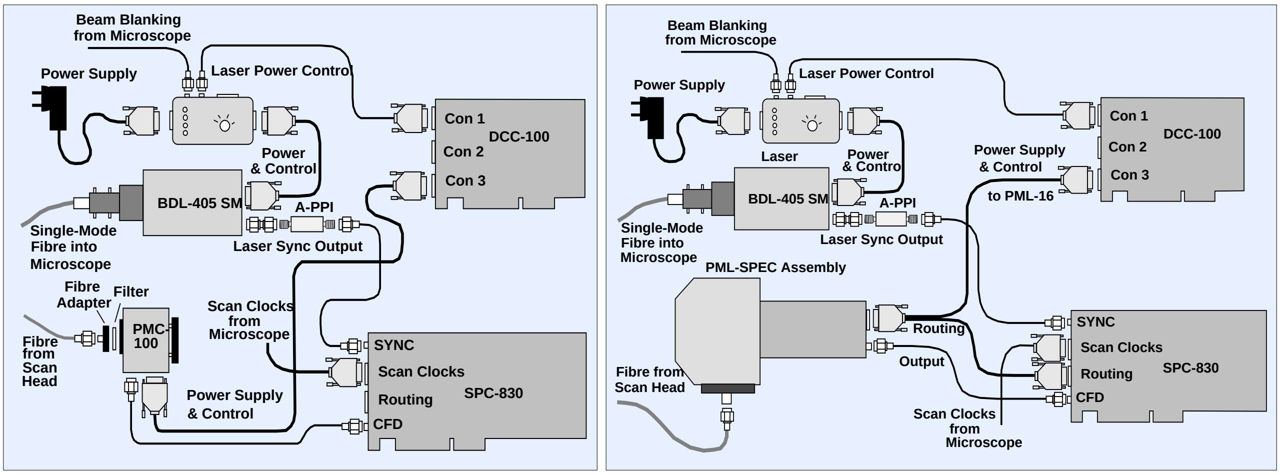


Fig. 40: Wiring diagrams of a single-detector FLIM system (left) and a multi-spectral FLIM system (right)

For FLIM, the excitation power required in the focus of the microscope objective lens is not particularly high. Many samples can be exited with less than 50 µW. However, in scanning microscopes the back aperture of the objective is often over-illuminated to obtain a uniform intensity distribution over the whole aperture. Moreover, a substantial fraction of the excitation light gets lost in the scanner and the microscope optics. The high coupling efficiency of the BDL-SMC lasers is therefore a considerable benefit. There is sufficient power margin, and the laser can be operated at a power that yields optimum pulse shape.

Fig. 41 shows fluorescence lifetime images of plant tissue recorded in a Zeiss LSM 510 upgraded with a bh BDL-405-SMC laser and SPC-830 TCSPC module. Plant tissue makes good test samples because it contains a wide variety of different fluorophores. The fluorescence decay functions are therefore multi-exponential. Left to right, Fig. 41 shows images of the lifetime of the fast component, the lifetime of the slow component, and the ratio of the amplitudes of the fast and the slow component of the fluorescence.

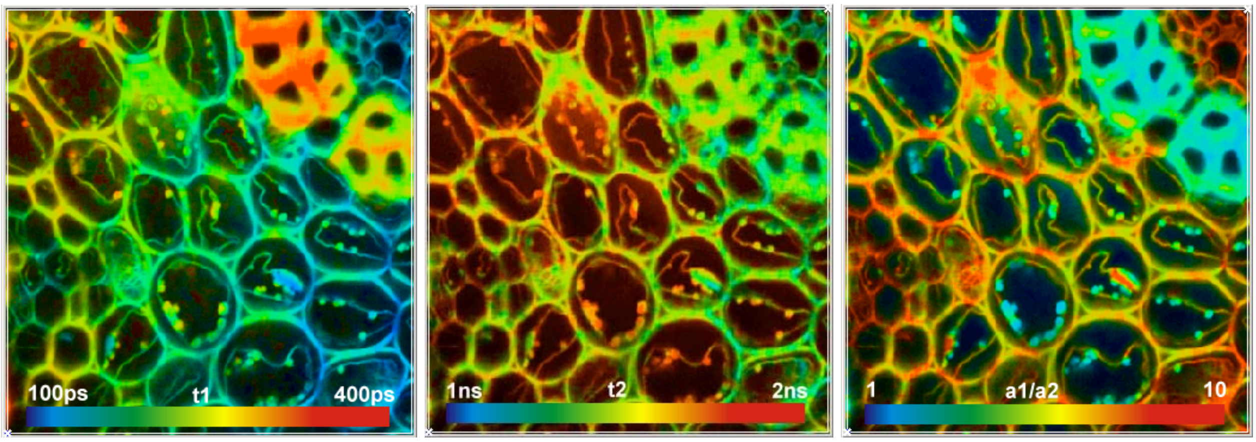


Fig. 41: Fluorescence lifetime images of plant tissue. Double-exponential decay analysis, left to right: Lifetime of fast component, lifetime of slow component, ratio of amplitudes of fast and slow component. bh BDL-405-SMC laser, Zeiss LSM 510, bh SPC-830 TCSPC module.

Fluorescence Correlation Spectroscopy (FCS)

Fluorescence correlation spectroscopy (FCS) is based on exciting a small number of molecules in a femtoliter volume and correlating the fluctuations of the fluorescence intensity. The fluctuations are caused by diffusion, rotation, intersystem crossing, conformational changes, or other random effects. The technique dates back to a work of Magde, Elson and Webb published in 1972 [15]. Theory and applications of FCS are described in [22].

The required femtoliter volume can be obtained by one-photon excitation and confocal detection or by two-photon excitation, see Fig. 42. The principle is the same as in a laser scanning microscope. A continuous or high-repetition rate laser beam is focused into the sample through the microscope objective lens. The fluorescence light from the sample is collected by the same lens, separated from the laser by a dichroic mirror, and fed through a pinhole in the upper image plane of the microscope lens. In a confocal microscope the fluorescence light from above or below the focal plane is not focused into the pinhole and therefore substantially suppressed. With a high-aperture objective lens the effective sample volume is of the order of a femtoliter, with a depth of about 1.5 µm and a width of about 400 nm.

Due to its good beam quality the BDL-SMC lasers are excellently suitable for FCS experiments. The laser can be either free-beam coupled into the microscope, or fibre coupling may be used. FCS experiments and other single-molecule techniques especially benefit from the capability of the BDL-SMC lasers to be operated both in the ps and in the CW mode. Thus, combined FCS / lifetime experiments [10] or burst-integrated fluorescence lifetime (BIFL) experiments [21] can be performed in the ps mode , whereas pure correlation experiments can take advantage of the high power available in the CW mode [11].

FCS and BIFL experiments can be performed efficiently in the ‘FIFO’ or ‘Time Tag’ mode of the bh TCSPC modules. Please see [8, 9] for details. An example of a combined FCS/lifetime measurement is shown in Fig. 43.

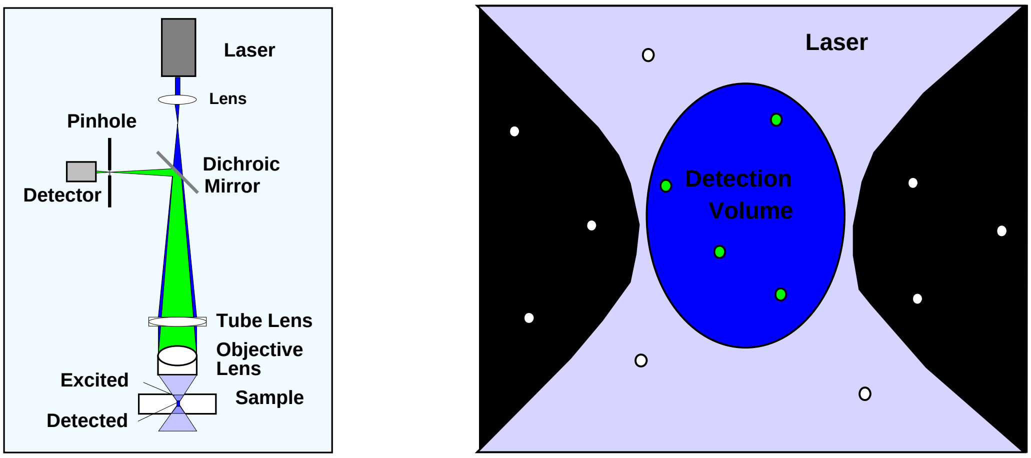


Fig. 42: Fluorescence correlation spectroscopy. Left: Basic optical setup. Right: Beam waist of laser and confocal detection volume

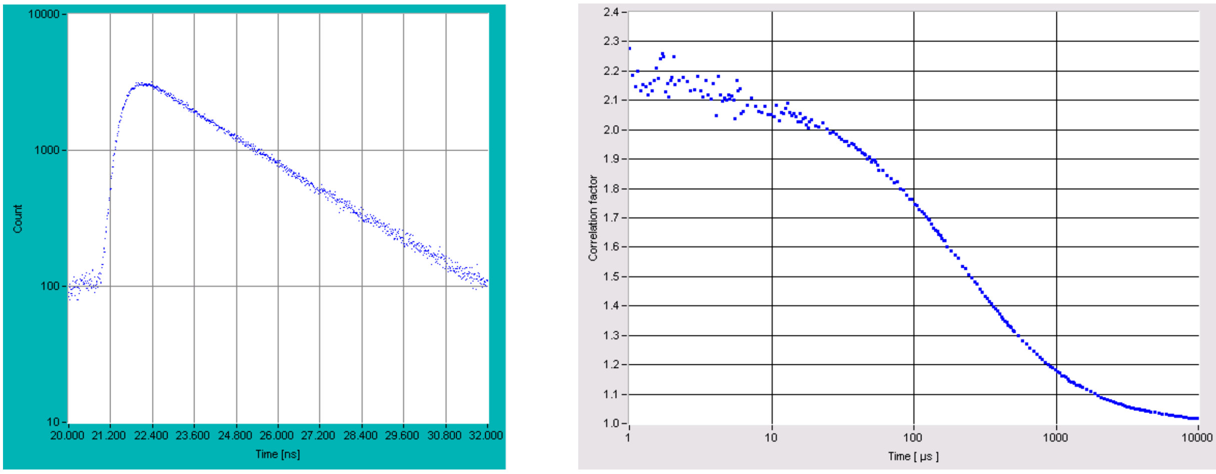


Fig. 43: Combined FCS / Lifetime measurement of a dye solution. Left: Fluorescence decay curve. Right: FCS curve.

Once the optical system is setup correctly, FCS measurements on highly diluted dye solutions (on the order of 10^{-9} mol/l) are relatively easy. This is not necessarily the case for FCS measurement in living cells. Especially in transfected cells the fluorophore concentration cannot be accurately controlled. It is usually much higher than required for FCS. The number of molecules in the focus can easily be on the order of 100 or even 1000, resulting in an extremely small amplitude of the correlation function [10]. Reasonable FCS results from such specimens can, of course, only be obtained if the fluctuation of the laser power are smaller than the fluctuation of the number of molecules in the focus.

Fig. 44 shows autocorrelation curves of the laser intensity for a BDL-405-SMC laser. The curves resemble FCS results obtained from a samples that does not show any intrinsic intensity fluctuations. Fluctuations at times shorter than 1 ms would show up as bumps in the curves, fluctuations at longer time scales as an offset from C = 1. No such effects are visible in Fig. 44. That means the BDL-SMC lasers can be used for FCS down to correlation coefficients smaller than 1.001.

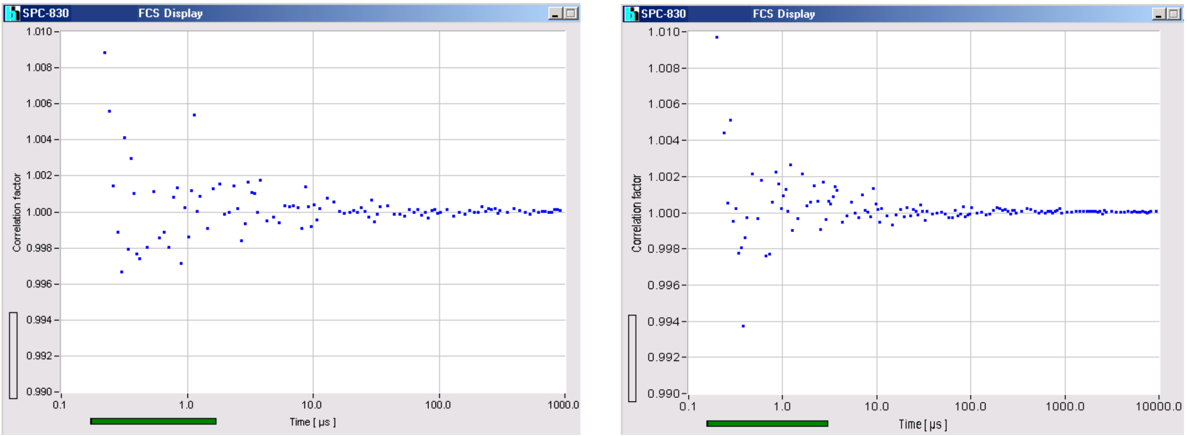


Fig. 44: Autocorrelation (FCS) curves of the laser intensity. BDL-405-SMC laser, recorded over 5 minutes with R3809U MCP PMT and SPC-830 TCSPC module. Left: pulsed operation. Right: CW operation. Scale of correlation coefficient from 0.99 to 1.01.

Specification

BDL-375-SMC

Optical

Repetition Rate	20-50-80 MHz, or CW operation	
Wavelength	370 nm to 380 nm, typ. 375 nm	
Pulse Width (FWHM, at 1 mW power, 50 MHz)	50 to 90 ps	
Peak Power	10 to 100 mW [1]	
Average Power	20 MHz:	0.05 mW to 0.16 mW [2]
(Average CW equivalent power,	50 MHz:	0.1 mW to 0.4 mW [2]
user adjustable)	80 MHz:	0.15 mW to 0.6 mW [2]
	CW mode:	0.5 mW to 5 mW [2]
Beam diameter before coupler	0.7 mm, TEM_{00} mode	
Polarisation	horizontal	
Coupling efficiency into fibre, typically	20%	
Stability of Repetition Rate	± 100 ppm	
Pulse-to Pulse Jitter	< 20 ps	
Reaction time to 'Laser on' signal (pulsed mode)	1 µs	
Reaction time to 'Laser on' signal (CW mode)	3 µs	
Power and pulse shape stabilisation after switch-on	3 min	
Fibre coupler	all 1" footprint couplers: Point Source, Schäfter&Kirchhoff, OZ Optics, Linus	

Trigger Output

Pulse Amplitude	+100 to +300 mV (peak) into 50 Ω
Pulse Width	1 ns
Output Impedance	50 Ω
Connector	SMA
Delay from Trigger to Optical Pulse	< 500 ps
Jitter between Trigger and Optical Pulse	< 10 ps

Control Inputs

Frequency 20 MHz	TTL / CMOS high [3]
Frequency 50 MHz	TTL / CMOS high [3]
Frequency 80 MHz	TTL / CMOS high [3]
CW operation	TTL / CMOS high [3]
Laser ON / Off	TTL / CMOS low [3]
External Power Control	analog input, 0 to + 10V

Power Supply

Power Supply Voltage	+ 9 V to +12 V
Power Supply Current	300 mA to 1 A [4]
Power Adapter	AC-DC power adapter, with key switch and control box in cable

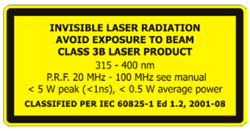
Mechanical Data

Dimensions	160 mm x 90 mm x 60 mm
Mounting Thread	two M6 holes

Maximum Values

Power Supply Voltage	0 V to +15 V
Voltage at Digital Control Inputs	-2 V to +7 V
Voltage at Ext. Bias Input	-12 V to + 12 V
Ambient Temperature	0 °C to 40 °C [5]

1) Typical values, sample tested. Depends on pulse width and selected power.
2) Recommended power adjust range. Lower power gives broader pulses, higher power gives ringing in pulse shape. Power levels above the given range can be selected, but may impair the lifetime of the laser diode.
3) All inputs have 10 kΩ pull-up resistors. Open input is equivalent to logic 'high'.
4) Dependent on ambient temperature. Cooling current changes due to temperature regulation of laser diode
5) Operation below 13 °C may result in extended warm-up time.



Caution: Class 3B laser product. Avoid direct eye exposure. Light emitted by the device may be harmful to the human eye. Please obey laser safety rules when operating the devices. Complies with US federal laser product performance standards.

BDL-405-SMC

Optical

Repetition Rate	20-50-80 MHz, or CW operation
Wavelength	401 nm to 410 nm, typ. 405 nm
Pulse Width (FWHM, at 1 mW power, 50 MHz)	50 to 90 ps
Peak Power	80 to 500 mW [1]
Average Power	20 MHz: 0.12 mW to 0.6 mW [2]
(Average CW equivalent power,	50 MHz: 0.3 mW to 1.6 mW [2]
user adjustable)	80 MHz: 0.4 mW to 2.4 mW [2]
	CW mode: 5 mW to 40 mW [2]
Beam diameter before coupler	0.7 mm, TEM_{00} mode
Polarisation	horizontal
Coupling efficiency into fibre, typically	60%
Stability of Repetition Rate	± 100 ppm
Pulse-to Pulse Jitter	< 20 ps
Reaction time to ‘Laser on’ signal (pulsed mode)	1 µs
Reaction time to ‘Laser on’ signal (CW mode)	3 µs
Power and pulse shape stabilisation after switch-on	3 min
Fibre coupler	all 1” footprint couplers: Point Source, Schäfter&Kirchhoff, OZ Optics, Linus

Trigger Output

Pulse Amplitude	+100 to +300 mV (peak) into 50 Ω
Pulse Width	1 ns
Output Impedance	50 Ω
Connector	SMA
Delay from Trigger to Optical Pulse	< 500 ps
Jitter between Trigger and Optical Pulse	< 10 ps

Control Inputs

Frequency 20 MHz	TTL / CMOS high [3]
Frequency 50 MHz	TTL / CMOS high [3]
Frequency 80 MHz	TTL / CMOS high [3]
CW operation	TTL / CMOS high [3]
Laser ON / Off	TTL / CMOS low [3]
External Power Control	analog input, 0 to + 10V

Power Supply

Power Supply Voltage	+ 9 V to +12 V
Power Supply Current	300 mA to 1 A [4]
Power Adapter	AC-DC power adapter, with key switch and control box in cable

Mechanical Data

Dimensions	160 mm x 90 mm x 60 mm
Mounting Thread	two M6 holes

Maximum Values

Power Supply Voltage	0 V to +15 V
Voltage at Digital Control Inputs	-2 V to +7 V
Voltage at Ext. Bias Input	-12 V to + 12 V
Ambient Temperature	0 °C to 40 °C [5]

1) Typical values, sample tested. Depends on pulse width and selected power.
2) Recommended power adjust range. Lower power gives broader pulses, higher power gives ringing in pulse shape. Power levels above the given range can be selected, but may impair the lifetime of the laser diode.
3) All inputs have 10 kΩ pull-up resistors. Open input is equivalent to logic ‘high’.
4) Dependent on ambient temperature. Cooling current changes due to temperature regulation of laser diode
5) Operation below 13 °C may result in extended warm-up time.

Caution: Class 3B laser product. Avoid direct eye exposure. Light emitted by the device may be harmful to the human eye. Please obey laser safety rules when operating the devices.
Complies with US federal laser product performance standards.

BDL-440-SMC

Optical

Repetition Rate	20-50-80 MHz, or CW operation	
Wavelength	436 nm to 448 nm, typ. 440 nm	
Pulse Width (FWHM, at 1 mW power, 50 MHz)	40 to 90 ps	
Peak Power	40 to 250 mW [1)]	
Average Power	20 MHz:	0.07 mW to 0.2 mW [2)]
(Average CW equivalent power,	50 MHz:	0.3 mW to 1 mW [2)]
user adjustable)	80 MHz:	0.4 mW to 1.2 mW [2)]
	CW mode:	1 mW to 20 mW [2)]
Beam diameter before coupler	0.7 mm, TEM_{00} mode	
Polarisation	horizontal	
Coupling efficiency into single-mode fibre, typically	60%	
Stability of Repetition Rate	± 100 ppm	
Pulse-to Pulse Jitter	< 20 ps	
Reaction time to 'Laser on' signal (pulsed mode)	1 µs	
Reaction time to 'Laser on' signal (CW mode)	3 µs	
Power and pulse shape stabilisation after switch-on	3 min [5)]	
Fibre coupler	all 1" footprint couplers: Point Source, Schäfter&Kirchhoff, OZ Optics, Linus	

Trigger Output

Pulse Amplitude	+100 to +300 mV (peak) into 50 Ω
Pulse Width	1 ns
Output Impedance	50 Ω
Connector	SMA
Delay from Trigger to Optical Pulse	< 500 ps
Jitter between Trigger and Optical Pulse	< 10 ps

Control Inputs

Frequency 20 MHz	TTL / CMOS high [3)]
Frequency 50 MHz	TTL / CMOS high [3)]
Frequency 80 MHz	TTL / CMOS high [3)]
CW operation	TTL / CMOS high [3)]
Laser ON / Off	TTL / CMOS low [3)]
External Power Control	analog input, 0 to + 10V

Power Supply

Power Supply Voltage	+ 9 V to +12 V
Power Supply Current	300 mA to 1 A [4)]
Power Adapter	AC-DC power adapter, with key switch and control box in cable

Mechanical Data

Dimensions	160 mm x 90 mm x 60 mm
Mounting Thread	two M6 holes

Maximum Values

Power Supply Voltage	0 V to +15 V
Voltage at Digital Control Inputs	-2 V to +7 V
Voltage at Ext. Bias Input	-12 V to + 12 V
Ambient Temperature	0 °C to 40 °C [5)]

1) Typical values, sample tested. Depends on pulse width and selected power.
2) Recommended power adjust range. Lower power gives broader pulses, higher power gives ringing in pulse shape. Power levels above the given range can be selected, but may impair the lifetime of the laser diode.
3) All inputs have 10 kΩ pull-up resistors. Open input is equivalent to logic 'high'.
4) Dependent on ambient temperature. Cooling current changes due to temperature regulation of laser diode
5) Operation below 13 °C may result in extended warm-up time.

Caution: Class 3B laser product. Avoid direct eye exposure. Light emitted by the device may be harmful to the human eye. Please obey laser safety rules when operating the devices. Complies with US federal laser product performance standards.

BDL-473-SMC

Optical

Repetition Rate	20-50-80 MHz, or CW operation
Wavelength	467 nm to 476 nm, typ. 473 nm
Pulse Width (FWHM, at 1 mW power, 50 MHz)	40 to 90 ps
Peak Power	40 to 250 mW [1]
Average Power (Average CW equivalent power, user adjustable)	20 MHz: 0.07 mW to 0.2 mW [2] 50 MHz: 0.3 mW to 1 mW [2] 80 MHz: 0.4 mW to 1.2 mW [2] CW mode: 0.5 mW to 10 mW [2]
Beam diameter before coupler	0.7 mm, TEM_{00} mode
Polarisation	horizontal
Coupling efficiency into single-mode fibre, typically	60%
Stability of Repetition Rate	± 100 ppm
Pulse-to Pulse Jitter	< 20 ps
Reaction time to ‘Laser on’ signal (pulsed mode)	1 µs
Reaction time to ‘Laser on’ signal (CW mode)	3 µs
Power and pulse shape stabilisation after switch-on	3 min [5]
Fibre coupler	all 1” footprint couplers: Point Source, Schäfter&Kirchhoff, OZ Optics, Linus

Trigger Output

Pulse Amplitude	+100 to +300 mV (peak) into 50 Ω
Pulse Width	1 ns
Output Impedance	50 Ω
Connector	SMA
Delay from Trigger to Optical Pulse	< 500 ps
Jitter between Trigger and Optical Pulse	< 10 ps

Control Inputs

Frequency 20 MHz	TTL / CMOS high [3]
Frequency 50 MHz	TTL / CMOS high [3]
Frequency 80 MHz	TTL / CMOS high [3]
CW operation	TTL / CMOS high [3]
Laser ON / Off	TTL / CMOS low [3]
External Power Control	analog input, 0 to + 10V

Power Supply

Power Supply Voltage	+ 9 V to +12 V
Power Supply Current	300 mA to 1 A [4]
Power Adapter	AC-DC power adapter, with key switch and control box in cable

Mechanical Data

Dimensions	160 mm x 90 mm x 60 mm
Mounting Thread	two M6 holes

Maximum Values

Power Supply Voltage	0 V to +15 V
Voltage at Digital Control Inputs	-2 V to +7 V
Voltage at Ext. Bias Input	-12 V to + 12 V
Ambient Temperature	0 °C to 40 °C [5]

1) Typical values, sample tested. Depends on pulse width and selected power.
2) Recommended power adjust range. Lower power gives broader pulses, higher power gives ringing in pulse shape. Power levels above the given range can be selected, but may impair the lifetime of the laser diode.
3) All inputs have 10 kΩ pull-up resistors. Open input is equivalent to logic ‘high’.
4) Dependent on ambient temperature. Cooling current changes due to temperature regulation of laser diode
5) Operation below 13 °C may result in extended warm-up time.

Caution: Class 3B laser product. Avoid direct eye exposure. Light emitted by the device may be harmful to the human eye. Please obey laser safety rules when operating the devices. Complies with US federal laser product performance standards.

Connector Pin Assignment, all BDL Lasers

External control connector at the laser switch box

1	not connected	9	not connected
2	Frequency 20 MHz	10	not connected
3	Frequency 50 MHz	11	not connected
4	Frequency 80 MHz	12	Power / Bias, 0 to +10 V
5	GND	13	not connected
6	not connected	14	not connected
7	/Laser Off	15	GND
8	not connected		

15 pin connectors at the laser and at the left side of the laser switch box

1	not connected	9	Test output, internal bias
2	Frequency 20 MHz	10	+12 V from power supply
3	Frequency 50 MHz	11	not connected
4	Frequency 80 MHz	12	Power / Bias, 0 to +10 V
5	GND	13	not connected
6	not connected	14	not connected
7	/Laser Off	15	GND
8	Test output, internal +7V		

9 pin connector at the right side of the laser switch box

1	+12 V from power supply	6	+12 V from power supply
2	+12 V from power supply	7	not connected
3	not connected	8	GND
4	GND	9	GND
5	GND		

Dimensions

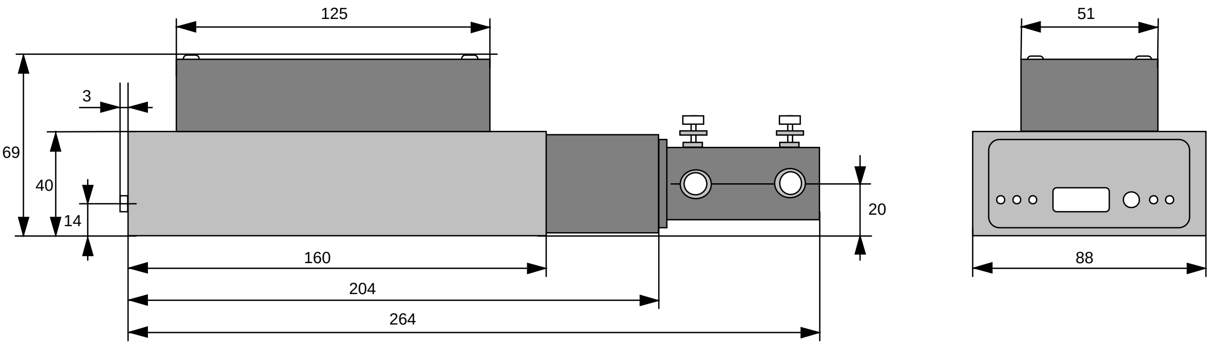


Fig. 45: BDL-SMC lasers, dimensions in mm. Laser shown with Point Source Coupler.

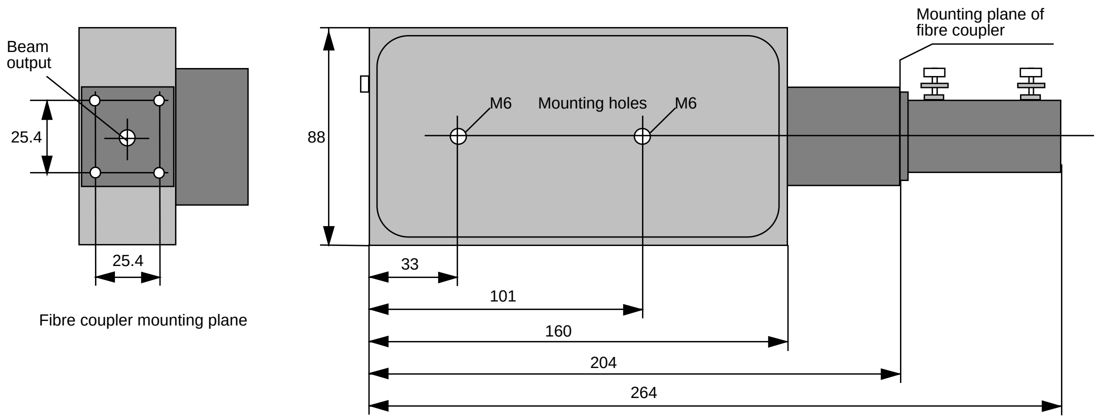


Fig. 46. Left: Mounting plane of fibre coupler. Right: Bottom view, with M6 mounting holes

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