

NADPH/AO

Emitter-Detector Modules

Manual

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APPENDIX

Schreiber U, Klughammer C (2009) New NADPH/9-AA module for the DUAL-PAM-100: Description, operation and examples of application. PAN 2: 1-13

Contents

1 Safety Instructions

1.1 General Safety Instructions

- Read safety instructions and the operating instructions prior to operation of the device and its accessories.
- Pay attention to all safety warnings.
- Keep device away from water or high moisture areas.
- Keep the device away from dust, sand, and dirt.
- Do not put the device near sources of heat.
- Ensure that neither liquids nor foreign bodies get inside the device.
- Ensure sufficient ventilation.
- Connect the device to the control unit as indicated in the operating instructions.
- The device should only be repaired by qualified personnel.

1.2 Special Safety Instructions

The NADPH/9-AA emitter (DUAL-ENADPH) of the NADPH/9-AA module produces ultraviolet radiation in the UV-A range which can be hazardous to the eye or skin. Operate the NADPH/9-AA emitter only when it is securely attached to the optical unit ED-101US/MD. Do not look directly at the optical port of the NADPH/9-AA emitter when the emitter is connected to the DUAL-PAM-100 Power-and-Control-Unit.

2 NADPH/9-AA Emitter-Detector Module

The module is designed for measuring NADPH/NADH and 9-AA fluorescence with suspensions of isolated chloroplasts microalgae and cyanobacteria.

Excitation and detection wavelengths of the NADPH/9-AA Emitter-Detector Module are optimized for fluorometric NADPH determinations. The module's excitation and detection wavelengths are also suited to measure 9-amino acridine fluorescence (see Fig. 9, page 19). This section considers only NADPH fluorescence.

2.1 NADPH/9-AA Emitter Unit (DUAL-ENADPH)

NADPH measuring light: 365 nm

Chlorophyll fluorescence measuring light: 620 nm

Actinic light: Far red LED lamp: 740 nm. Chip-on-board (COB) LED array: 635 nm for continuous actinic illumination, maximum 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Saturating single turnover flashes, maximal 200 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, adjustable between 5 and 50 μs . Multiple turnover flashes, maximal 20 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, adjustable between 1 and 1000 ms.

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 400 g (incl. cables, 1 m long)

2.2 NADPH/9-AA Photomultiplier Detector Unit (DUAL-DNADPH)

Signal detection: Blue-sensitive photomultiplier with filter compartment and cover for filter compartment.

Dimensions: 10 cm x 6.5 cm x 10.5 cm (L x W x H).

Weight: 460 g (incl. cable, 1 m long).

Detector filter: Filter sandwich (BG 39/KV 418/DT Cyan) transmitting from 420-550 nm.

Connection to ED-101US/MD Optical Unit: Perspex rod 10 x 10 x 100 mm (L x H x W) with black metal tube and Allen wrench.

Fluorescence standard: Fluorescence standard solution (Lumogen® F Violet 570) and 2 mm pinhole to reduce limit fluorescence reaching the (2 mm diameter)

PM-101/N: Amplifier box: To be connected to the NADPH/9-AA Photomultiplier Detector Unit. **Design:** Aluminum chassis with texture paint. Line input 115/230 V AC, 50-60 Hz, 0.04/0.02 A. Two rotary buttons to select of 6 coarse amplification factors with 11 subdivisions. **Dimensions:** 11 cm x 11 cm x 7 cm (L x W x H). **Weight:** 700 g

2.3 Setup of the NADPH Module

The NADPH/9-AA Emitter Unit (DUAL-ENADPH) has two modulated light sources for fluorescence excitation: the UV-A source (365 nm) is used to excite NADPH fluorescence, and the red source (620 nm) is suited to excite chlorophyll fluorescence. The principal arrangement of the module components is outlined in Fig. 1.

The detector unit is situated perpendicularly to fluorescence excitation by the DUAL-ENADPH emitter. The right-angled setup reduces the amount of measuring light reaching the photomultiplier and, thus, the level of artifactual background signals. It is important that ED-101US/MD ports which are not used are closed to prevent external light from reaching the interior of the optical unit. Generally, do not operate the DUAL-DNADPH at high light-exposed sites, cover the ED-101US/MD top, and also the filter slot.

The NADPH/9-AA Photomultiplier Detector Unit (DUAL-DNADPH) only measures NADPH fluorescence. To record chlorophyll and NADPH fluorescence in parallel, the setup in Fig. 1 must be extended by a detector for chlorophyll fluorescence.

In Fig. 2, the detector for chlorophyll fluorescence is a DUAL-DB or a DUAL-DR detector unit. Its signal cable is plugged into socket "DETECTOR 1". The LED array of the detector can provide actinic light when it is connected to the upper 15-pin socket of the control unit. Fluorescence can also be measured by a photodiode detector DUAL-DPD shielded by an RG9 glass filter against measuring light. In this case, photodiode the signal cable is plugged into socket "DETECTOR 1".

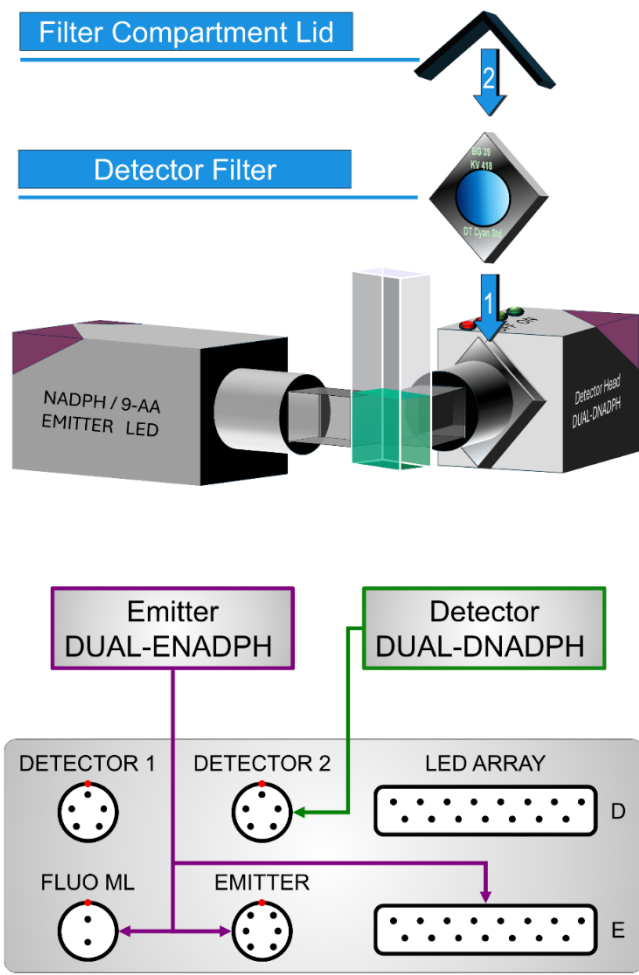


Fig. 1: Setup for NADPH Fluorescence Measurements

Outline of the arrangement of emitter and detector. The NADPH filter sandwich must be placed in the filter compartment of the detector unit. The filter compartment must be closed by the filter compartment lid. For measurements, both units must be attached to the ED-101US/MD Optical Unit. See the DUAL-PAM-100 manual for details.

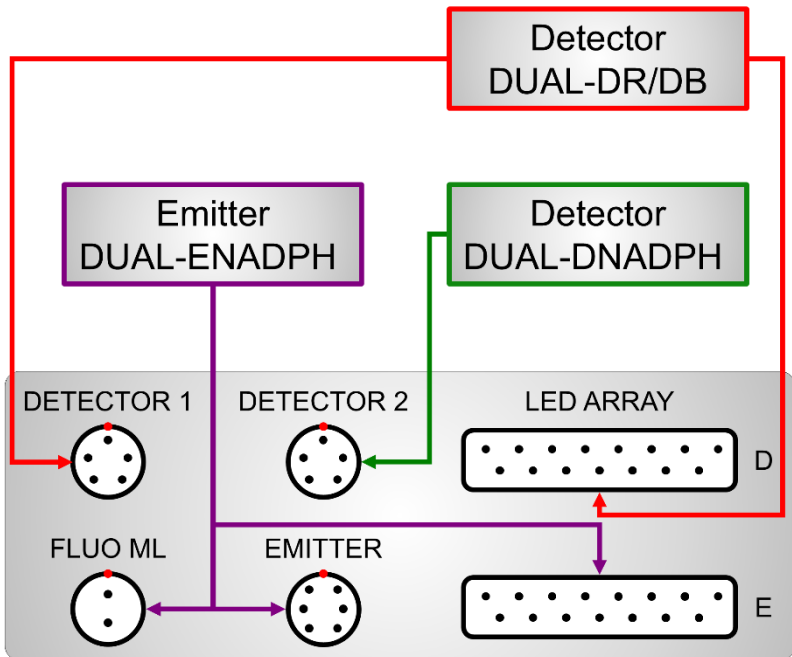
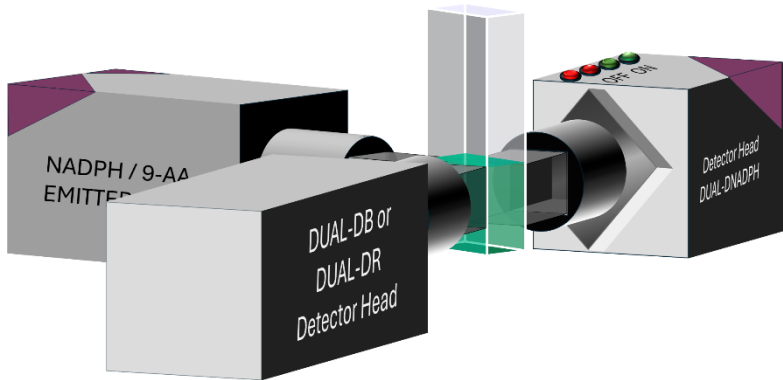


Fig. 2: Setup for NADPH and Chlorophyll Fluorescence Measurements

Outline of the arrangement of emitter and detector of the NADPH module plus a measuring head for chlorophyll fluorescence. The setup requires the ED-101US/MD Optical Unit which is not shown. See the DUAL-PAM-100 manual for details.

2.3.1 Installing the NADPH/9-AA Photomultiplier Detector Unit (DUAL-DNADPH)

Connect the DUAL-DNADPH to the optical unit ED-101US/MD using the special metal tube (Fig. 3). Place the Perspex rod inside the tube. The tube acts as a light guide and reduces losses of fluorescence radiation between the sample cuvette and detector. Fig. 3 provides instructions on how to mount the detector unit.

Connect the DUAL-DNADPH to the DETECTOR 2 input of the control unit (Fig. 1). The housing possesses a socket for the power cable of the Power Supply PM-101/N unit. The power supply provides voltage to the photomultiplier of the DUAL-DNADPH. The voltage and, thus, the photomultiplier sensitivity can be varied by the COARSE and FINE switches in front of the power supply. The total span of sensitivity is about 1:230 (Table 1). Note that sensitivity and settings are not strictly linearly related.

Table 1: DUAL-DNADPH Power Supply: Settings and Signal

The table shows typical data. All data are relative to the signal amplitude at settings COARSE=1 and FINE=0.

SWITCH		SIGNAL	SWITCH		SIGNAL
COARSE	FINE		COARSE	FINE	
1	0	1	3	0	57
2	0	12	3	1	63
3	0	57	3	2	69
4	0	123	3	3	75
5	0	168	3	4	80
6	0	202	3	5	87
6	11	227	3	6	93
			3	7	99
			3	8	105
			3	9	111
			3	10	117
			3	11	124

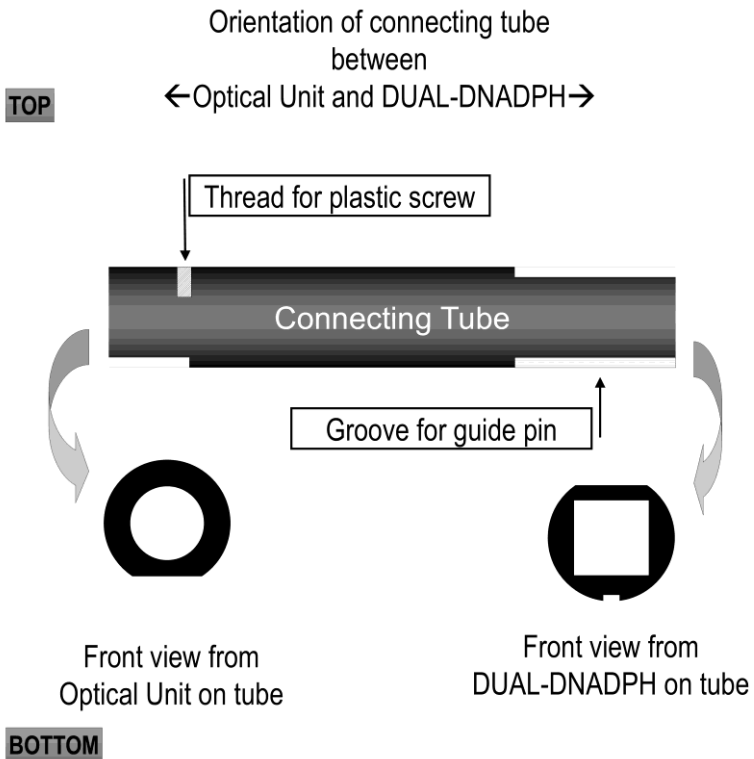


Fig. 3: Connecting Tube for DUAL-DNADPH

Schematic illustration of connecting tube between Optical Unit and DUAL-DNADPH. The connection tube is made from anodized aluminum. For proper function it must contain a Perspex tube (10 x 10 x 100 mm). The ends of the Perspex rod differ, and it is not radially symmetrical.

At the end directed towards the Optical unit (left in the drawing), a plastic screw can be inserted to fix the Perspex rod. Rotate the rod so that the thread for the plastic screw is on top. Below the thread for the plastic screw, the tube shows a flat section. When the tube is inserted in the Optical Unit, a metal screw of the Optical Unit can be turned in until it presses against the flat section and thereby fixes the tube.

On the opposite end, the tube has a groove at the lower side. At the bottom of the DUAL-DNADPH entrance port, a guide pin is located. The guide pin fits onto the groove and prevents any rotary movement. When the tube is inserted, the flat portion on the tube side above the groove is positioned below a thread for a plastic screw of the DUAL-DNADPH entrance port. Turning in a plastic screw fixes the DUAL-DNADPH to the tube.

At the Power Supply PM-101/N unit, select appropriate voltage using the AC VOLTAGE switch on the back of the housing. Connect power supply to line power. If the red LED lamp lights up right after the power supply is turned on, the photomultiplier must be switched on by pressing the green push button. During use, the photomultiplier is switched off automatically when receiving too much light or when the voltage applied is too high. The photomultiplier can also be manually turned off by the red push-button.

With sample in place, start with lowest setting of the Power Supply PM-101/N unit. Increase voltage in small increments until the signal level is around 2000. in

Care must be taken that the photomultiplier is not exposed to ambient light because repeated exposure to high light intensities can result in a significant decrease in the signal/noise ratio of the DUAL-DNADPH unit.

2.3.2 Blue Fluorescence Standard

Delivery of the NADPH/9-AA module includes a fluorescence standard solution (Blue F Standard) in a 2 ml glass vial. The standard is a highly dilute ethanolic solution of Lumogen F Violet. The absorption and emission properties of this dye (Fig. 4) are stable. When excited at 365 nm excitation, Lumogen F Violet emits blue fluorescence.

The vial must stay closed! Keep the glass vial clean. Remove dust particles before use, as their fluorescence may contribute to the signal.

For measurements, place the glass vial into the Optical Unit ED-101US/MD instead of a 10x10 mm cuvette. Make sure that the

bottom port and all side ports of the Optical Unit are closed, either by the miniature stirrer or by a mirrored rod.

The fluorescence intensity of the Blue F Standard is higher than NADPH fluorescence of biological samples. To match the fluorescence signal of the standard to that of biological samples, place a 2 mm pinhole plate in front of the detector filter (compare Fig. 1).

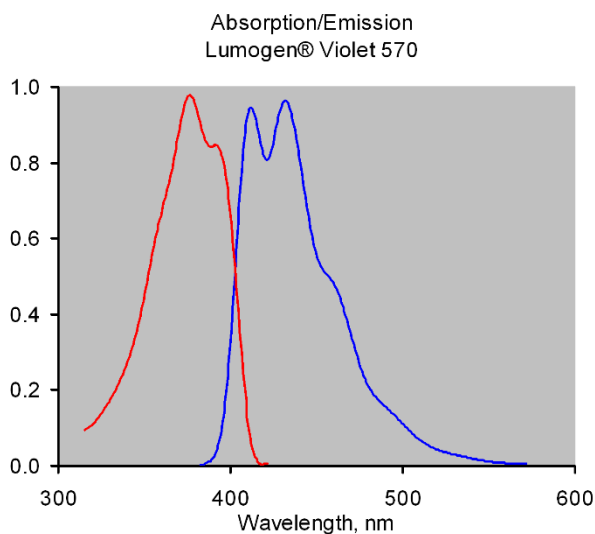


Fig. 4: Fluorescence Standard Spectra

Absorption (red) and emission (blue) spectrum of Lumogen F Violet 570.

2.3.3 Applications of the Standard

System performance

Checking system performance, like signal/noise ratio and signal stability at defined photomultiplier gain.

NADPH signal comparison

NADPH signal amplitudes of different experiments can be compared by normalizing the NADPH signal to the standard signal. This requires recording the standard signal within each experiment under identical optical conditions and instrument settings.

As outlined in PAN (2009) 2: 1-13 (see Appendix), measurements of *in vivo* NADPH fluorescence changes are impeded by a number of complicating factors (see section on: *Important points for optimal NADPH measurements* in Appendix). The *in vivo* NADPH fluorescence signal is very small and sometimes in the same order of magnitude as instrument related noise and disturbances. Therefore, it is important for the user to know what part of the overall signal changes is instrument related. On the other hand, if a particular light-induced change has been observed with a biological sample, it is reassuring that under identical conditions no corresponding change can be observed with the Blue F Standard (see example of application below).

It is recommended to obtain a record of the signal/noise performance of the NADPH/9-AA module with the Blue F Standard briefly after delivery by running the Slow Kinetics trigger file test-NADPH.STM at defined PM Gain setting (e.g. 6/6, i.e. setting 6 coarse/setting 6 fine). Beforehand, all software-controlled instrument settings should be standardized by selecting Walz_NADPH.def under "User Settings" (see the Amin Manual for information on user settings). Typical testNADPH.STM records comparing the responses of the Blue F Standard with that of

a biological sample (suspension of *Chlorella vulgaris*) are shown below. When the measurement with the Blue F Standard is repeated under identical conditions later, possible changes in signal/noise characteristics can be assessed. A gradual decline in signal amplitude is normal, due to declines in PM sensitivity and LED intensity.

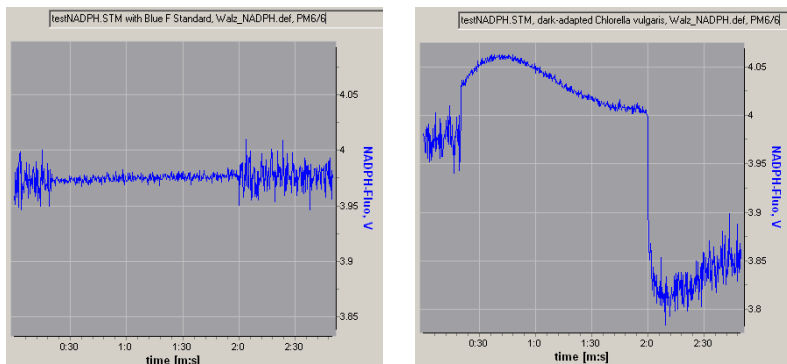


Fig. 5: Test Measurements

Experiments were automatically performed by the Slow Kinetics Triggered Run “test-NAPPH.STM”. See main manual on the use of triggered runs. Left: Blue F Standard (left). Right: a suspension of *Chlorella vulgaris*. Both experiments used identical experimental conditions. 20 s after start of the Triggered Run the Actinic Light (AL intensity setting 8) was turned on and Measuring Light (ML) frequency switched from 200 Hz to 5000 Hz. After 2 min AL was turned off and ML frequency switched back from 5000 Hz to 200 Hz. Display at 256 Average Points.

2.3.4 Key Publication

Kauny J, Sétif P (2014): NADPH fluorescence in the cyanobacterium *Synechocystis* sp. PCC 6803: A versatile probe for in vivo measurements of rates, yields and pools. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* Volume 1837: 792–801
<https://doi.org/10.1016/j.bbabi.2014.01.009>

3 **Acridine Orange/Yellow Fluorescence Emitter-Detector Module**

Excitation and detection wavelengths of the Acridine Orange/Yellow fluorescence emitter-detector module are designed for fluorescence measurements of the dyes acridine orange and acridine yellow (see Fig. 9).

3.1 **DUAL-EAO Emitter Head**

Measuring light: Blue excitation LED lamp with emission peak at 455 nm.

Dimensions: 10.5 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 400 g (incl. cables, 1 m long)

3.2 **DUAL-DAO Detector Head**

Signal detection: Photodiode detector with filter set for green fluorescence (500-570 nm) and preamplifier of pulse modulated signal.

Measuring light: LED emitter (620 nm) for excitation of Chl fluorescence (measured *via* standard DUAL-DR or DUAL-DB or DUAL-DPD at an angle of 90°).

Actinic light: Chip-On-Board LED array emitting maximally at 635 nm. Maximum PAR intensities for continuous red actinic illumination = 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, for single turnover flashes = 200 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, adjustable between 5 and 50 μs , and for multiple turnover flashes 20 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, adjustable between 1

and 1000 ms. Blue (emission maximum at 460 nm) LED-array with maximum PAR of 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Dimensions: 15 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 460 g (incl. cable, 1 m long)

3.3 Setup of the AO Module

The DUAL-EAO Emitter Head excited the acridine dyes “acridine orange” or “acridine yellow” with blue modulated measuring light (see Fig. 9). The green fluorescence from these acridine dyes is detected at right angles by the DUAL-DAO Detector Head (Fig. 6).

The DUAL-DAO Detector Head emits red modulated light for excitation of chlorophyll which can be measured simultaneously to acridine fluorescence by a standard DUAL DR or DUAL DB unit at an angle of 90° (Fig. 7). The DUAL-DAO Detector Head is additionally equipped with LEDs for blue and red actinic light.

The proper connection of measuring heads is illustrated in Fig. 6 and Fig. 8.

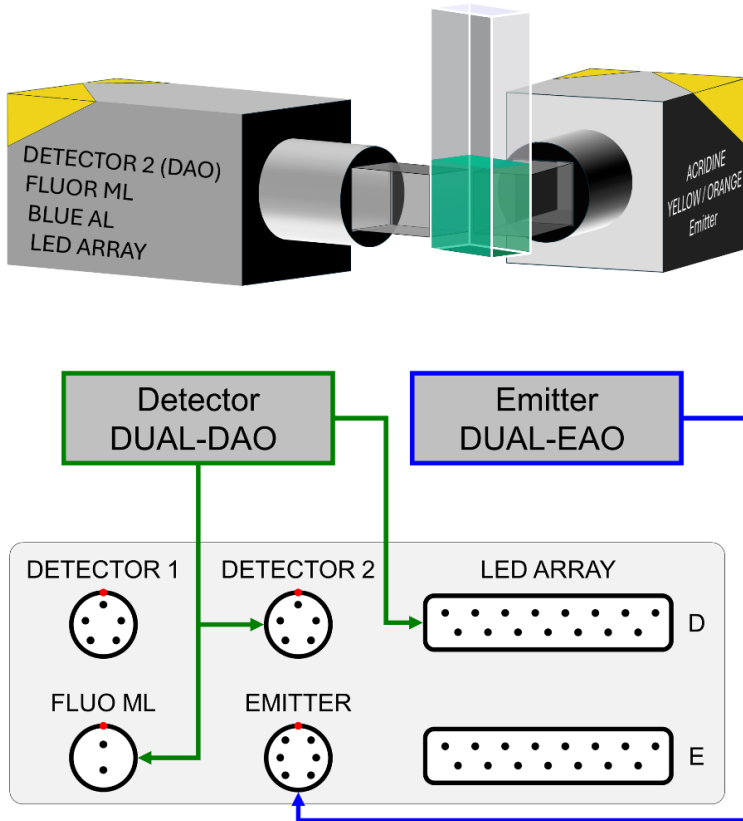


Fig. 6: Setup for Measuring Acridine Derivatives AO or AY

Top: The measuring heads of the AO module are oriented at right angles to minimize the offset signal arising from measuring light reaching the photodetector. For measurements, both units must be attached to the ED-101US/MD Optical Unit. See the DUAL-PAM-100 manual for details.

Bottom: The unit DUAL-EAO is connected to the EMITTER socket: it emits blue modulated measuring light to excite acridine orange (AO) or acridine yellow (AY).

The green acridine fluorescence is detected by the DUAL-DAO Detector Head which is plugged in the DETECTOR 2 socket of the DUAL-PAM-100 Power-and-Control-Unit. When connected to the socket FLUO ML, the DUAL-DAO emits red modulated measuring light to excite chlorophyll fluorescence. Measuring chlorophyll fluorescence requires an additional detector which is not present in this figure. Connecting the DUAL-DAO Detector Head to socket LED ARRAY (D) is required for actinic light emission.

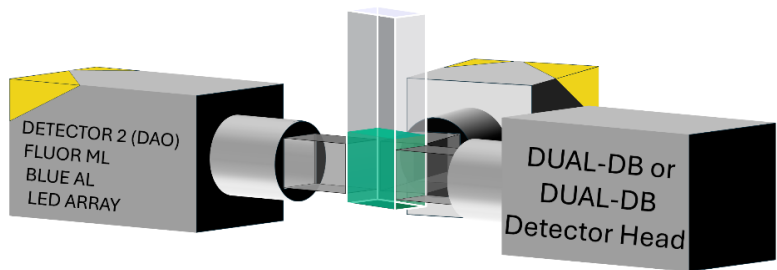


Fig. 7: Setup for Measuring Acridine Derivatives AO and AY Plus Chlorophyll Fluorescence

The figure corresponds to Fig. 6 except a detector for chlorophyll fluorescence is added (DUAL-DB or DUAL-DR). Both the AO and the chlorophyll detector are oriented at right angles to the emitter head. The right-angled arrangement reduces the offset signal caused by measuring light reaching the photodetector.

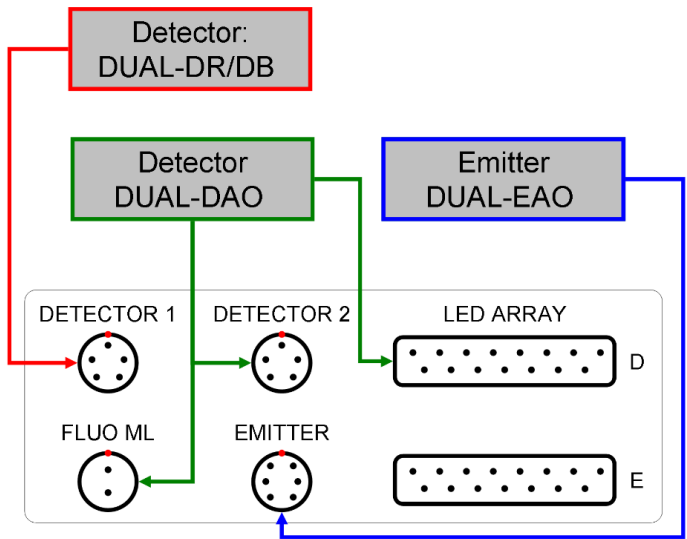


Fig. 8: Connecting the AO Module plus a Chl Fluorescence Detector

The AO module is connected as shown in Fig. 6. Additionally, a detector for chlorophyll fluorescence (DUAL-DR or DUAL-DB) is connected to socket DETECTOR 1.

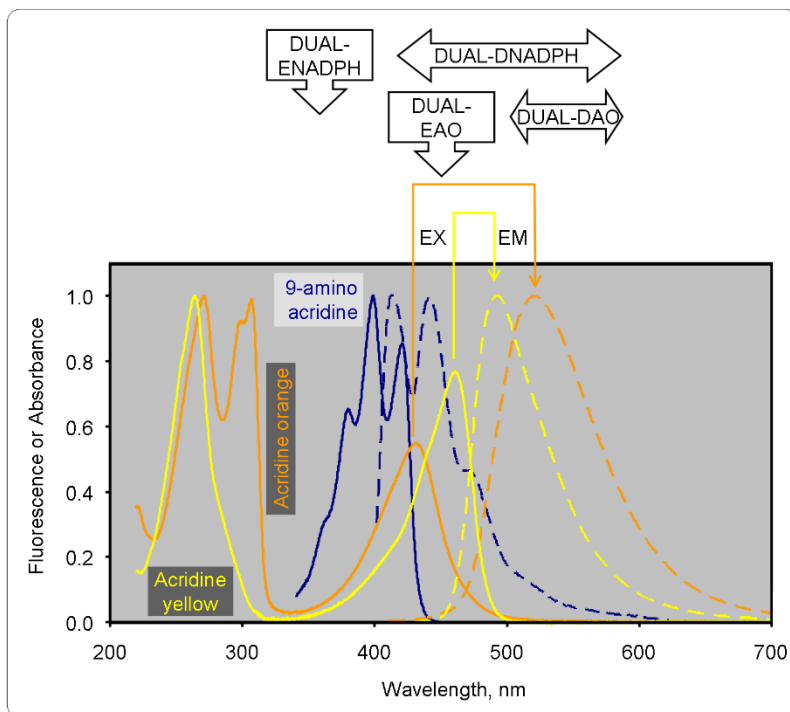


Fig. 9: Spectra of Acridine Derivatives

Normalized absorbance spectra (solid orange and solid yellow lines), fluorescence excitation spectra (solid blue line), and fluorescence emission (dashed lines) of 9-amino acridine (blue), acridine orange (orange) and acridine yellow (yellow). Wavelength positions of excitation light emitted by the DUAL-ENADPH and the DUAL-EAO are indicated in boxes with downward facing arrows; double arrows define the wavelength range of the detection windows of the DUAL-DNADPH and the DUAL-DAO units. Data source for acridine orange and acridine yellow: Du H, Fuh RA, Li J, Corkan A, Lindsey J S, (1998) *Photochem Photobiol* 68: 141-142. Data source for 9-amino acridine: Grzesiek S, Otto H, Dencher NA (1989) Δ pH-induced fluorescence quenching of 9-aminoacridine in lipid vesicles is due to excimer formation at the membrane. *Biophys. J.* 55: 1101-1109

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