Supplementary

1. Hardware supplementary information.

As is mentioned in the manuscript, an extra excitation light is included in the design for maximum flexibility. The interchangeable LED is attached to the developed system by using hidden magnets which allow for placement in the correct position, avoiding the usage of mechanical parts.

The light source can be chosen in almost any wavelength using commercial LEDs for optical applications with a power of 1W. The use of a heatsink in this unit is mandatory to avoid overheating. The ON/OFF control of the extra LED unit is performed by using a digital output of the microcontroller (Pin D9), which is connected to a NPN transistor that activates the corresponding relay (Figure S1b). Evidently, an external DC power supply is necessary for this purpose, a cellular USB charger type being a cheap option.

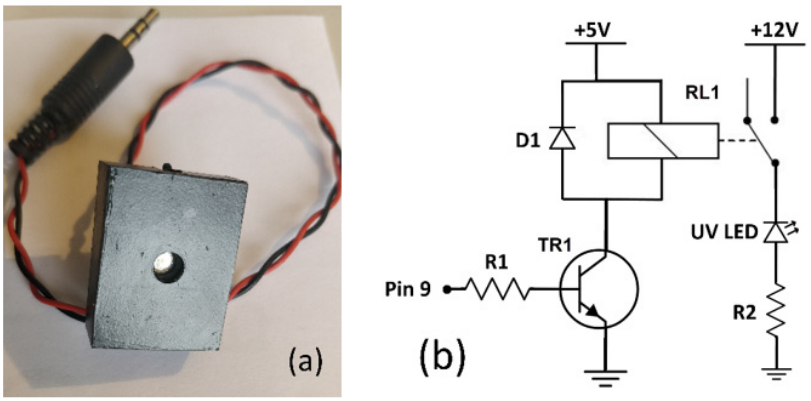


Figure S1. a) External detachable UV-LED 365 nm unit employed in this work. Two internal small disk magnets allow the proper alignment of the light source in the right place. b) Schematic electronic components used for the extra source light control. R1= 10 kΩ, D1= 1N4007, TR1=2N2222, R2= 110 Ω, RL1= SRD 12VDC SL C, UV LED 365 nm 1W.

Figure S2 shows the extra LED unit working. The C12880MA spectrometer (showed on the top of the picture) directly receives the fluorescent light at 90º with respect to the light source. In this arrangement, no mirrors nor filters are necessary.

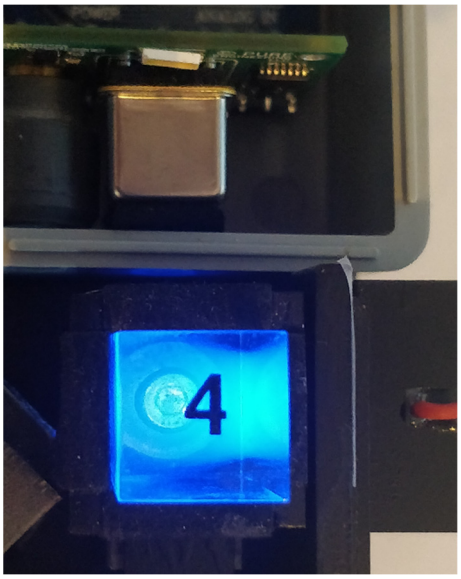


Figure S2. External UV-LED unit (365 nm) illuminating a fluorescent quinine sample.

On the other hand, the excitation light can be provided by the laser diode included in the GroupGets PCB. In this case, a flat mirror is mounted at 45º in the cuvette compartment in front of the spectrometer, increasing the amount of light reaching the spectrometer, as shown in Figure S3. The C12880MA (shown at the bottom of the figure) receives the fluorescent light from the sample. With this cell arrangement, filters are not necessary either, avoiding the usage of mechanical parts in the system.

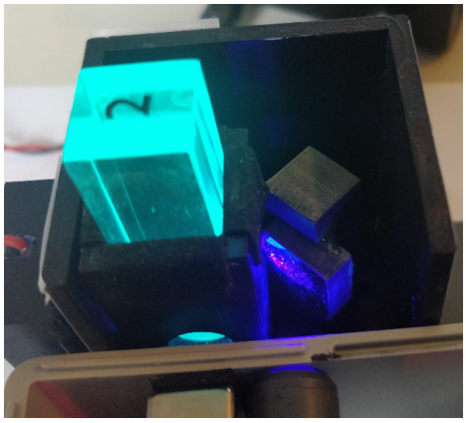
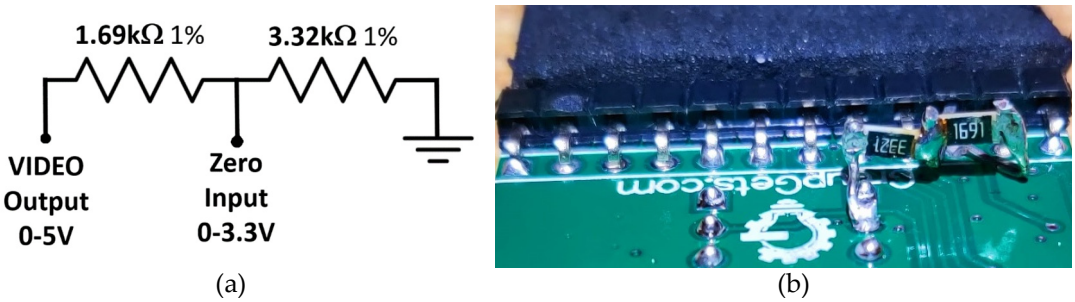


Figure S3. Internal design of the measurement cell when the 405nm laser unit is employed. The incident light reaches the flat mirror at 45º which allows the illumination of the fluorescent sample.

The C12880MA spectrometer has a typical 5V output, as indicated in the datasheet [1]. A reversible adaptation for the GroupGets breakout board has been implemented changing from 5 V to 3.3 V, which is the maximal value for the Zero-Arduino-compatible microcontrollers or for the microcontrollers working at 3.3 V exclusively. For this purpose, a simple voltage divider is the simplest and easiest solution. Two resistors with ±1% tolerance (250 mW SMD size 1206) were employed with an appropriate ratio (1.69kΩ/3.32kΩ) for the voltage divider, as shown in Figure S4a). The resistors are soldered reversibly at the bottom layer of the GroupGets breakout board ver.1 (see Figure S4 for picture and schematics).



(a) (b)

Figure S4. a) Electrical scheme of the voltage divider employed on the GroupGets breakout board. b) The output VIDEO pin pass through the voltage divider before being redirected into the microcontroller board.

2. Software supplementary information.

The main goal of the software development has been to acquire a single averaged spectrum in the spreadsheet for each measurement process, both for the blank and for the analyzed samples. The code size was minimized, so that it could be uploaded on microcontrollers with low memory. Part of the microcontrollers' flash memory is used to store

fixed information through using the add-on for the Excel spreadsheet. For the UNO-compatible microcontroller, the memory associated with the code program takes up to 95% of the total space.

The resultant spectrum stored in the Excel spreadsheet comes from averaging 1-1000 spectra, minimizing random noise and improving the S/N ratio. The code program allows the accumulation of the measured spectra, performing the averaging before sending the data to the spreadsheet.

Finally, the integration time control can be varied from 10 μs to 2 seconds, providing a wide range of sensitivity. An integration time of more than 1 hour, with an accuracy of a few microseconds, is available if it is necessary. By using the microcontroller internal timer, the program is able to measure the exact number of microseconds employed during a single measurement, as described in the datasheet. Once this value is perfectly established, it can be used to normalize the measured spectra with different integration times.

2.1. System subroutines

The developed source code program, with comments for easy understanding, can be provided by the authors upon request. In the following, a brief summary of the different routines employed during the measurement process is provided.

- *Setup routine:* set the controls pins mode as output or input. Only the START/STOP and VIDEO pins are set as input. Next, initialize the variable to the by defect values, clearing the arrays which store both the spectrum and averaging values. It is only possible for the ZERO microcontrollers to set the A/D converter to 12 bits of resolution, the default value being 10 bits. The serial communication port is set to 9600 bauds speed. This low speed minimizes issues during the writing of data into the Excel spreadsheet. It is highly recommended not to interact with Excel during the measurement process or the spectrum data writing.
- *Clock routine:* this toggles the CLK pin a selected number of times, generating a sequence of pulses by using a classical loop. The CLK line, in combination with other spectrometer control lines, is employed for managing different operations of the C12880MA. Toggling the CLK pin can be performed by using high-level programming sentences (codenamed "*digitalWrite*" in the following box) or by directly managing the microcontroller ports. If high level language is employed (slow mode), the routine can generate a maximum clock frequency signal around 100 kHz for the three microcontrollers tested.

```
/* Pulse clock function
void pulse_clk (unsigned long  cycles){
for(int i = 0; i < cycles; i++){
digitalWrite(SPEC_CLK, HIGH);
delayMicroseconds(delayTime);
digitalWrite(SPEC_CLK, LOW);
delayMicroseconds(delayTime);
}
} //end pulse_clk
```

- Managing directly the microcontroller ports, it is possible to reach around 700 kHz with UNO and MEGA microcontrollers but, for ZERO, it is possible to go further, reaching up to 2.67 MHz. In this case, it is possible to control the signal frequency performing a fine tuning of the timing control signal to obtain precisely a 1 MHz CLK frequency. In these conditions, a nearly direct relationship with the integration time given in microseconds can be obtained. The following piece of code for the ZERO microcontroller has been employed (shown in the box below) for the fine tuning of the CLK signal to 1 MHz, using a digital oscilloscope. The number of "nop" assembler sentences allows for the precise controlling of the square wave symmetry (shown in the box below).

```
F_pulse_clk1MHz (unsigned long cycles){
for(unsigned long i = 0; i < cycles; i++){
__asm__("nop\n\t""nop\n\t""nop\n\t""nop\n\t""nop\n\t""nop\n\t");  // 6 cycles A_ZERO
REG_PORT_OUTTGL1 = PORT_PB09;     // Toggle the output HIGH and LOW
__asm__("nop\n\t""nop\n\t""nop\n\t""nop\n\t""nop\n\t");  // 5 cycles A_ZERO
__asm__("nop\n\t""nop\n\t""nop\n\t""nop\n\t""nop\n\t");  // 5 cycles A_ZERO
__asm__("nop\n\t""nop\n\t""nop\n\t""nop\n\t""nop\n\t");  // 5 cycles A_ZERO
__asm__("nop\n\t""nop\n\t""nop\n\t""nop\n\t");  // 4 cycles A_ZERO
REG_PORT_OUTTGL1 = PORT_PB09;     // Toggle the output HIGH and LOW
__asm__("nop\n\t""nop\n\t""nop\n\t""nop\n\t""nop\n\t");  // 5 cycles A_ZERO
// freq 1.04MHz +W480ns -W480ns nop 6,H,5,5,5,4,L,4 BEST symmetry
// freq 1.02MHz +W480ns -W500ns nop 6,H,5,5,5,4,L,5 best fit with external t_int
// freq 1.00MHz +W520ns -W480ns nop 6,H,5,5,5,5,L,5 extra time, worst t_int
}
}
```

Figure S5, shows a snapshot of the three control signals (ST, CLK and LIGHT) during the acquisition of a spectrum with the ZER0 microcontroller. The signals have 3.3V of amplitude. CLK signal (cyan line) has a frequency of 926 kHz with a low width of 490 ns and high width of 590 ns. ST signal is in a low state and the excitation light control pin is HIGH.

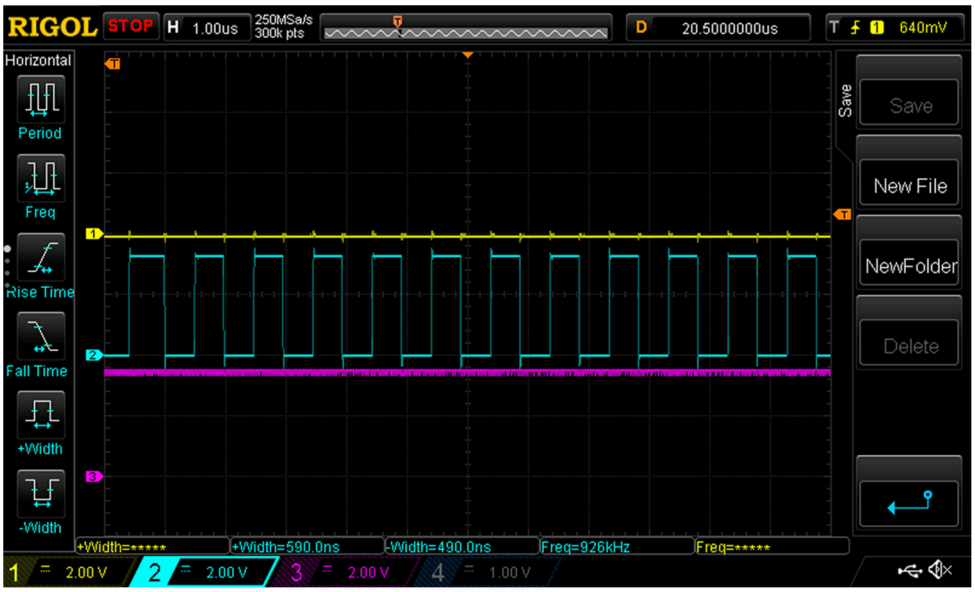


Figure S5. Oscilloscope snapshot for the generation of a 1MHz CLK square wave signal (cyan). ST signal is in high state (yellow) and LIGHT signal is in low state (pink) during the measurement process. The signals are generated with the Zero microcontroller by using a 3.3 V voltage level.

- *Spectrometer read routine:* two different routines (fast and slow mode) have been developed, as stated previously, the main difference being the CLK signal generation mode and the pin controls. This routine identifies as input parameters the type of excitation light (LASER/UVLED) to be activated. The excitation light is activated before the measurement process starts for source auto-stabilization, typically within 10 ms, but it can be modified depending on the light source properties. The whole integration time includes the period where the ST line is set to HIGH and 48 CLK pulses after ST is set to LOW. A total of 40 CLK extra pulses are necessary to shift the output data corresponding to each photodiode to match with the spectrometer calibration data at each wavelength. A lower or higher number of these CLK pulse values shifts the spectrum forward or reverses it at the output string variable. Figure S6 show the timing diagram of the three control signals for a single measurement timing process. Lastly, the resulting spectrum is stored in an array sequentially with one CLK pulse for each data, finishing the routine.

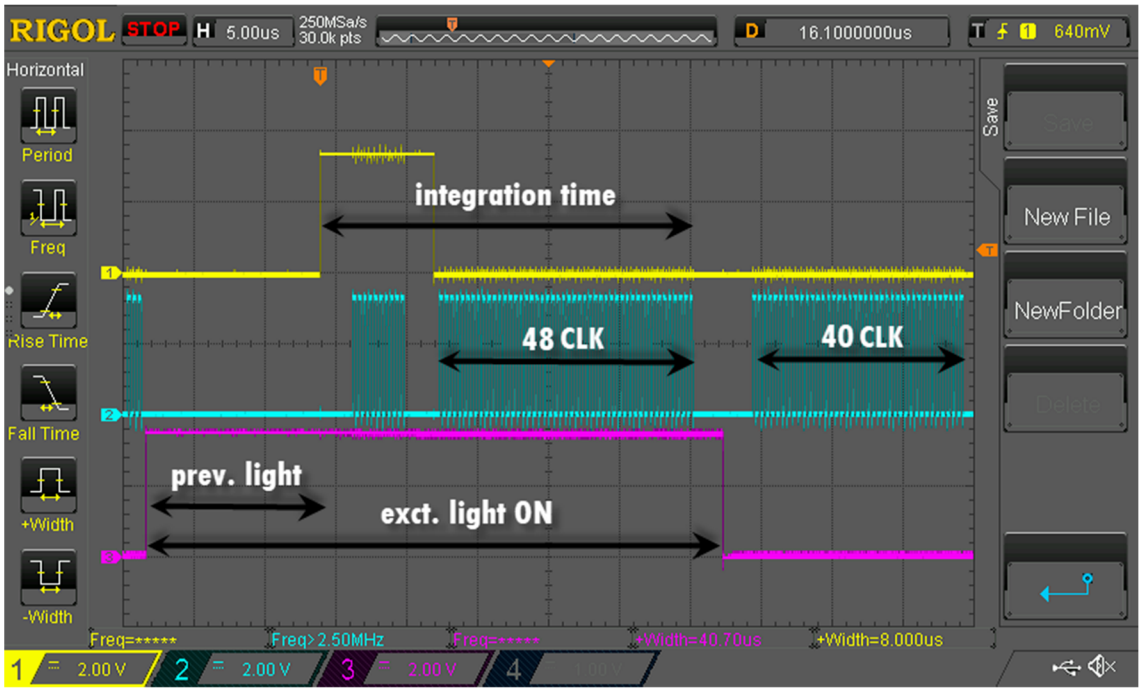


Figure S6. Oscilloscope snapshot for the timing of a single spectrometer measurement. CLK signal (cyan), ST signal (yellow) and excitation light (purple). The integration time is approximately 26 µs, which contains 48 CLK pulses. The following 40 CLK pulses are necessary to match the microprocessor data output string to the classical spectrum format.

- *Wavelength calibration routine:* this calculates the corresponding wavelength values for each element in the array by using the six coefficients of the polynomial calibration function supplied by the manufacturer based on the spectrometer serial number.
- *Wait button routine:* this checks for a change on a digital input (D12) status, which is actuated by the external button pressing. When the button status changes during a selected time interval, this means that the button has been pressed intentionally, and the routine is terminated.
- *Export data to datasheet routine:* this prepares and formats the data resulting from the measurement to be stored in an external spreadsheet, by using the typical spectrum structure in two columns: one for the wavelength of the light (in nanometers) and one for the corresponding digitally averaged light intensity value.

- *Loop routine:* this is repeated endlessly. When the actual measurement is finished, it waits to restart the procedure again. In this way, the blank sample measurement and the sample itself can be easily stored without modifying the initial parameters. The routine starts cleaning the memory for the averaged array and it waits until the initial parameter values are set: integration time, number of spectra for averaging and the chosen excitation light. If the typed values in the spreadsheet are out of the allowed range, then a set of the default values are established for these variables (10 μs, 10 averaging spectra and 405 nm, respectively). The wide range of accessible values for the integration time (10 - 2000000 μs) make the use of an "unsigned long" as variable type necessary, which is read as a "float type" variable from the Excel add-on. Following this, the routine waits until the user sets the cuvette with the solution in place. Once the button is pressed, the measurement process is performed and the data are written in the data sheet. Then, the program waits for a change in the status of the digital line (D12) associated to the pushbutton to restart the measurement process.
- *Head routine:* this handles information about the measurement parameter ranges and their corresponding values between the Excel datasheet and the control software developed for the microcontroller. It is important to point out that all the data variables are stored in the microcontroller internal memory (see Table 2 in the manuscript for each microcontroller's memory capacities). For this reason, and trying to save as much memory as possible, some definitions, constant variables and string messages are stored in the flash memory of the microcontroller by using the command Serial.print(F("Message")). With this strategy, it is possible to employ the microcontroller SRAM internal memory up to 98%,for storing run-time variables.

3. Blank and sample subtraction procedure.

In the raw spectra of the sample and the blank, there is a strong peak corresponding to the scattering of the light from the excitation source. This peak has an intensity much higher than the emission peaks of the sample and this can interfere in its quantification. In the case of using a laser light source, such as the one provided by the Sony SLD3132VF diode, the half-width of this peak is small, a few nanometers around the wavelength specified in its data sheet, 405 nm. In the case of the UV LED used, this half-width is significantly larger, a few tens of nanometers. For a given fluorescent substance, if the peak corresponding to the fluorescent emission light is located at wavelengths sufficiently far from the emission wavelength, the influence of the light scattering is small, and the use of light absorption filters is not strictly necessary. Otherwise, the scattered light can overlap with the fluorescence signal, causing a distortion of the experimental results.

As mentioned in the manuscript, the Hamamatsu C12880MA spectrometer is very reliable and produces very reproducible spectra with low background noise, which is further minimized by using averaging. In this regard, the spectra of the fluorophores shown in Figure 6 have been averaged, subtracting the blank contribution.

A more exhaustive analysis can be made of the blank spectrum corrected for the dark current (the reading in the absence of the excitation light), and its influence on the sample spectrum, corrected from the blank. Figure S7 shows both magnified fluorescence spectra (% signal values of the A/D converter scale) with a 405 nm excitation light for a 1 μM fluorescein solution (blue line), and the spectrum of the blank under the same experimental conditions (black line), corrected from dark current. Within the violet box, the strong influence on the wavelength's interval around 405 nm (laser emission light) can be noted. In this case, a distortion with an approximate width of 20-30 nm is obtained.

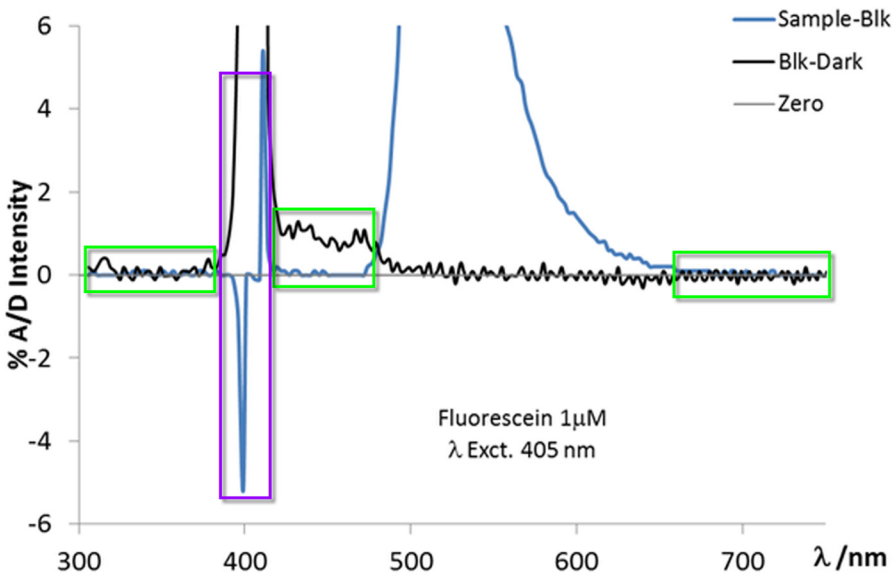


Figure S7. Fluorescent spectrum magnified, for 1 μM Fluorescein (blue line) compared with its associated blank spectrum (black line) corrected from the dark current. Excitation light: 405 nm.

In Figure S7, three green boxes have been marked where the effects of the scattered light in the corrected sample spectrum are practically negligible (both at shorter wavelengths and above the 405 nm zone of influence). That is, the subtraction of the sample data from the blank data produces a corrected spectrum, which virtually eliminates the light scattering effects not associated with the sample. It can be pointed out in Figure S7 that the blank spectrum corrected from the dark current contains a scattered light signal which can be attributed to the effects of the solution, cuvette, etc. These effects are very similar to those undergone by the sample under the same experimental conditions. Therefore, by subtracting the spectrum of the sample from the blank (blue line), these effects are completely corrected. The detection and quantification limits are reached when the signal due to the sample is very low and comparable with the blank signal.

Figure S8 shows the same results for the Fluorescein solution under the same conditions, but using the 365 nm UV LED as an excitation source. The same colored boxes with the same meanings have also been marked. The spectrum of the sample is now a red line, maintaining the same coding as in the manuscript.

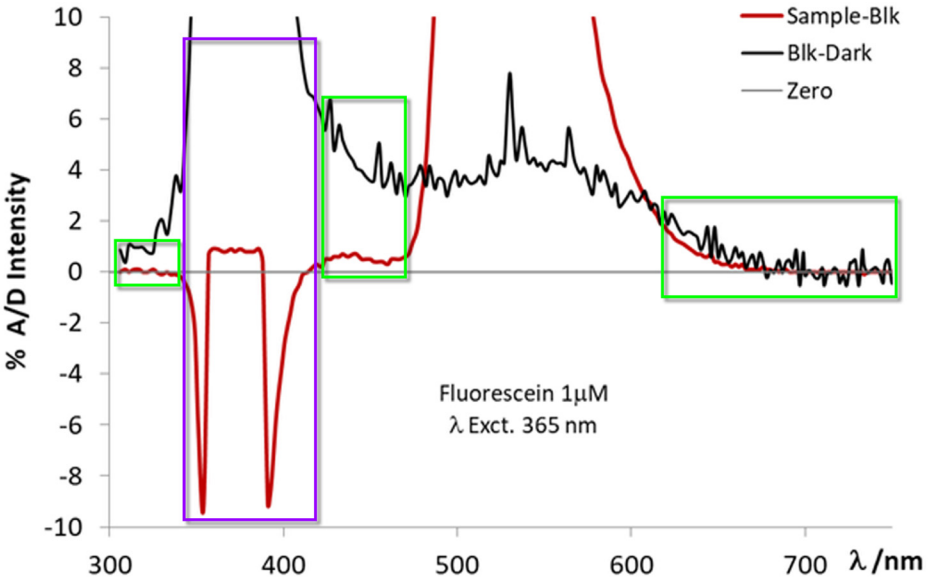


Figure S8. Fluorescent spectrum magnified, for 1 µM Fluorescein (red line) compared with its associated blank spectrum (black line), corrected from the dark current. Excitation light: 365 nm.

In this case, it can be noted that the influence of the excitation light is broader, about 80 nm. However, outside this interval of influence of the excitation light, similar considerations to the aforementioned can be made in relation to the correction of the scattered light not attributable to the sample.

For the case of Quinine, under the same experimental conditions, the use of the 405 nm laser is not operative for its detection and quantification, as shown in Figure 6 of the manuscript. Thus, only the analysis of the spectrum using 365 nm excitation light is performed (shown in Figure S9).

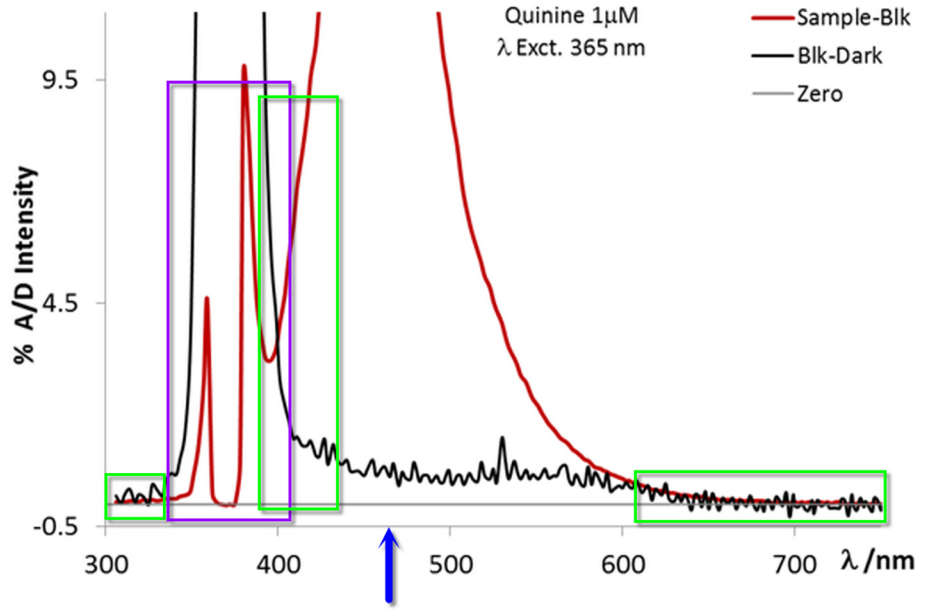


Figure S9. Fluorescent spectrum magnified for 1 µM Quinine (red line) compared with its associated blank spectrum (black line) corrected from the dark current. Blue arrow indicates the maximum wavelength for the fluorescence. Excitation light: 365 nm.

Under these conditions, due to the wider bandwidth of the UV LED, there is a wavelengths interval (approx. 390 - 430 nm) where two contributions to the spectrum can be clearly noted: one from the excitation light and another one from the sample. The traditional optical designs employ bandpass filters which, in the UV range, are relatively inexpensive. However, these kind of bandpass filters usually present a low transmission value as a drawback (about one-fifth and having not very sharp spectral edges). For this reason, the use of an absorption bandpass filter for narrowing the wavelength range for our excitation light (365 nm UV LED, 20 mW max) would not be appropriate. Consequently, the use of an absorption bandpass filter has not been considered for this low-power design.

If the quantification of the sample is performed by measuring the area under the emission peak, a deconvolution process has to be implemented. For this purpose, an additional free software, such as Fittyk (https://fityk.nieto.pl/), could be employed. However, if the peak intensity value at the maximum of the emission wavelength (blue arrow in Figure S9) is used for the quinine quantification process, the influence of excitation light scattering not due to the sample is negligible. Outstanding calibration results have been obtained, which are comparable to those obtained by commercial equipment with higher cost and complexity.

4. Raw experimental spectra

The following figures show the experimental spectra obtained for each fluorophore investigated. Worked solution concentrations are indicated in the corresponding legend. The inset in the figures shows the dilute concentration range.

a) Fluorescein

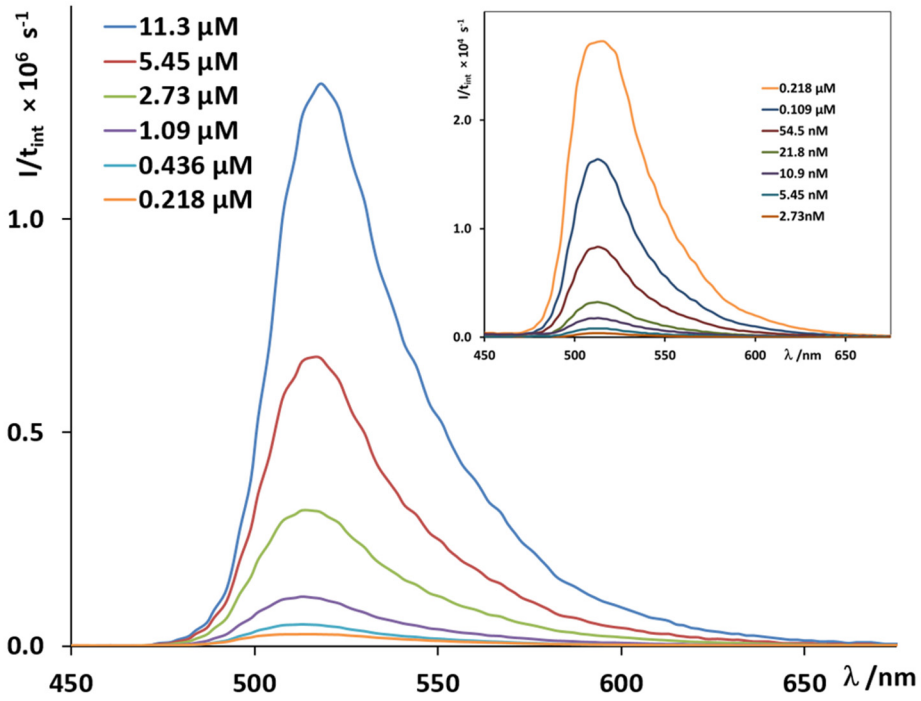


Figure S10. a) Fluorescence spectra at different concentration levels for Fluorescein at 405 nm.

b) Rhodamine B

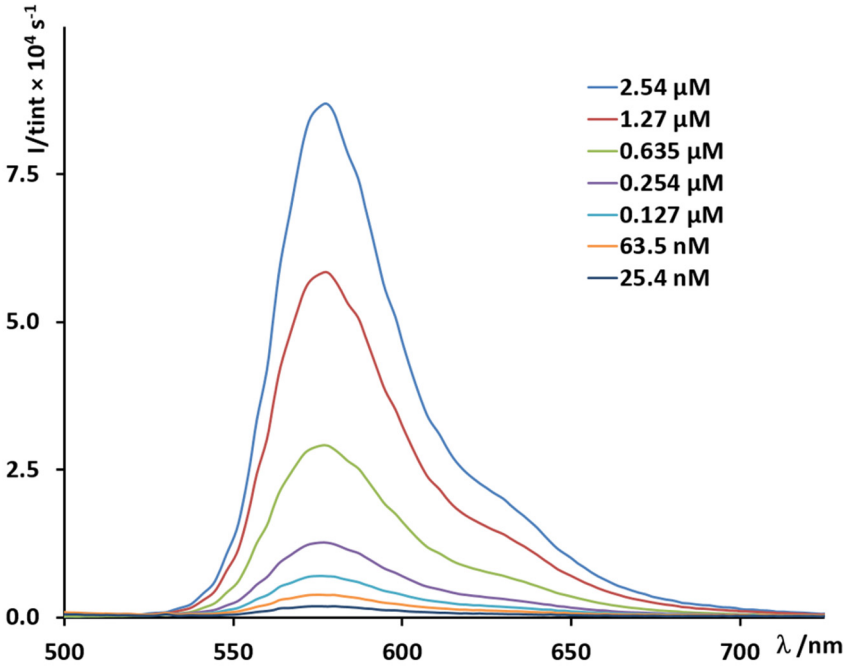


Figure S10. b) Fluorescence spectra at different concentration levels for Rhodamine B at 405 nm.

c) Rivoflavin:

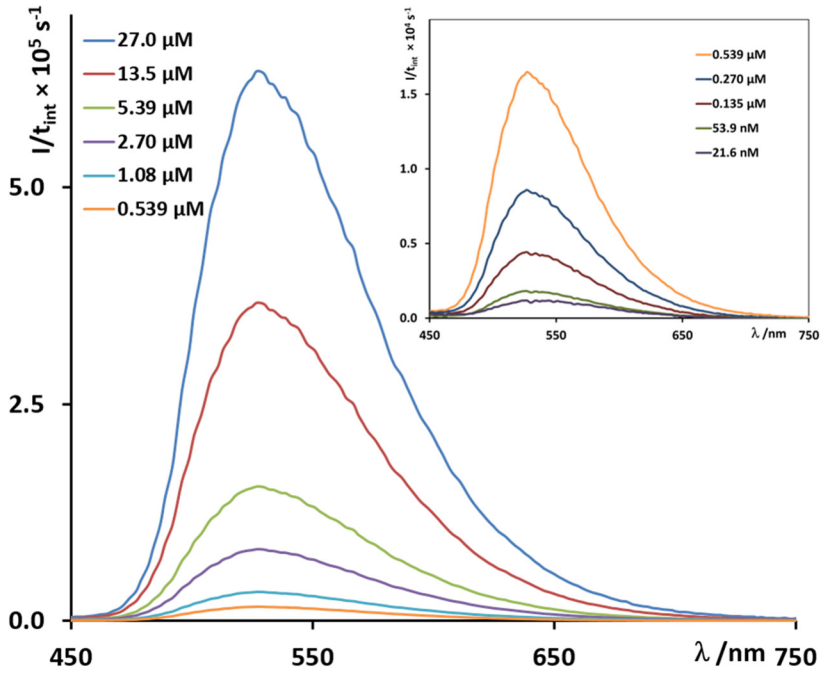


Figure S10. c) Fluorescence spectra at different concentration levels for Rivoflavin at 405 nm.

d) Ru(bpy)₃

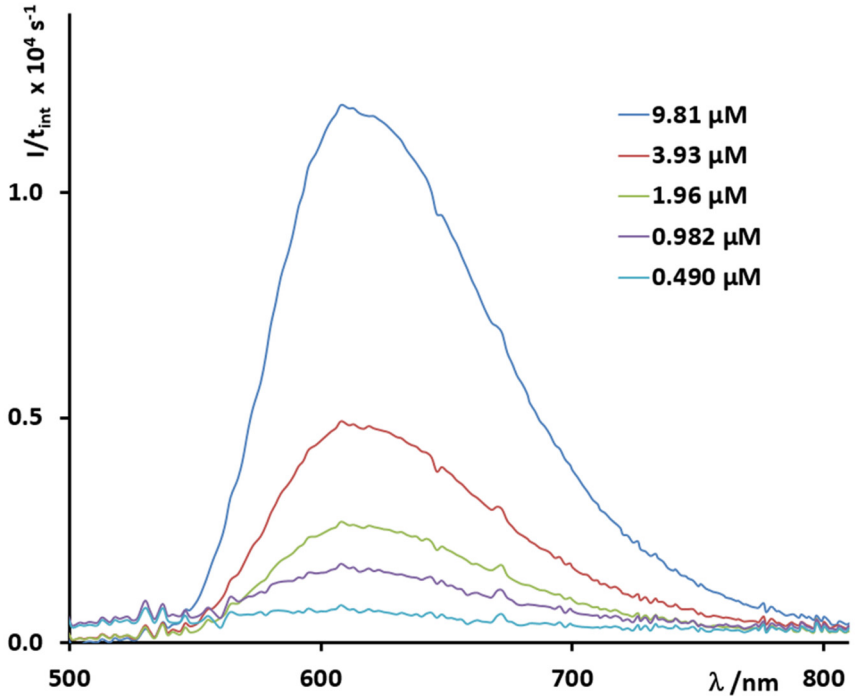


Figure S10. d) Fluorescence spectra at different concentration levels for $Ru(bpy)_3$ at 365 nm.

5. Logarithmic calibration plots

The following figures show the calibration plots (log I/t_i vs log C) for each fluorophore investigated, obtained from peak intensity values at the maximum emission wavelength.

a) Fluorescein

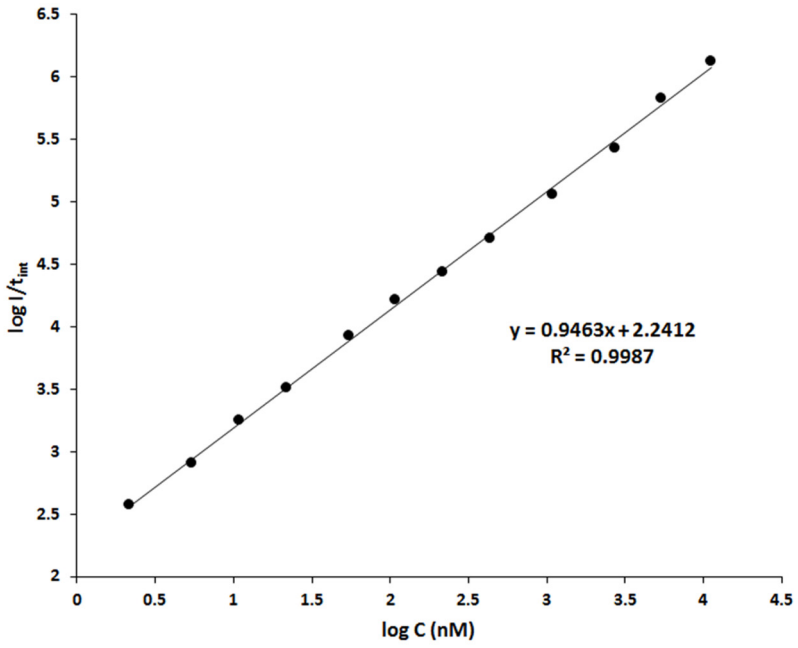


Figure S11. a) Logarithmic calibration plot for Fluorescein at 405 nm.

b) Rhodamine B

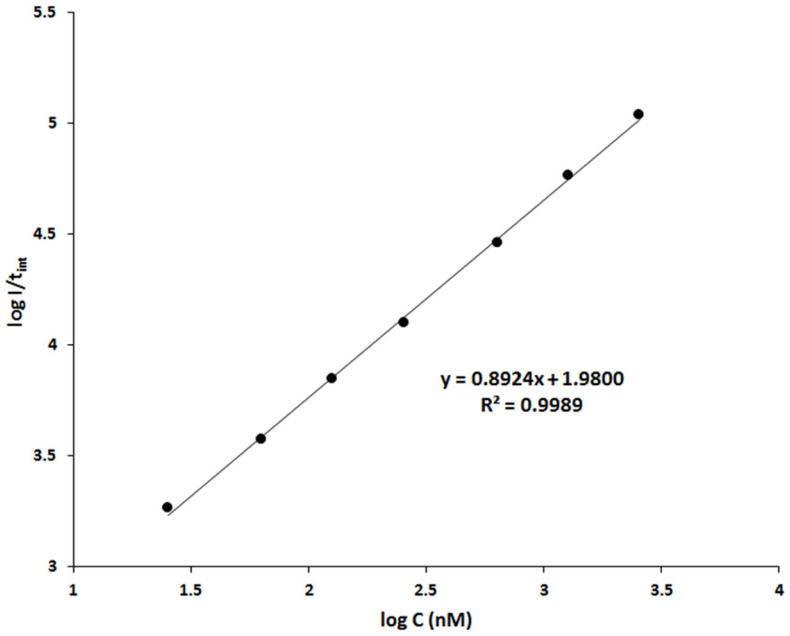


Figure S11. b) Logarithmic calibration plot for Rhodamine B at 405 nm.

c) Rivoflavin

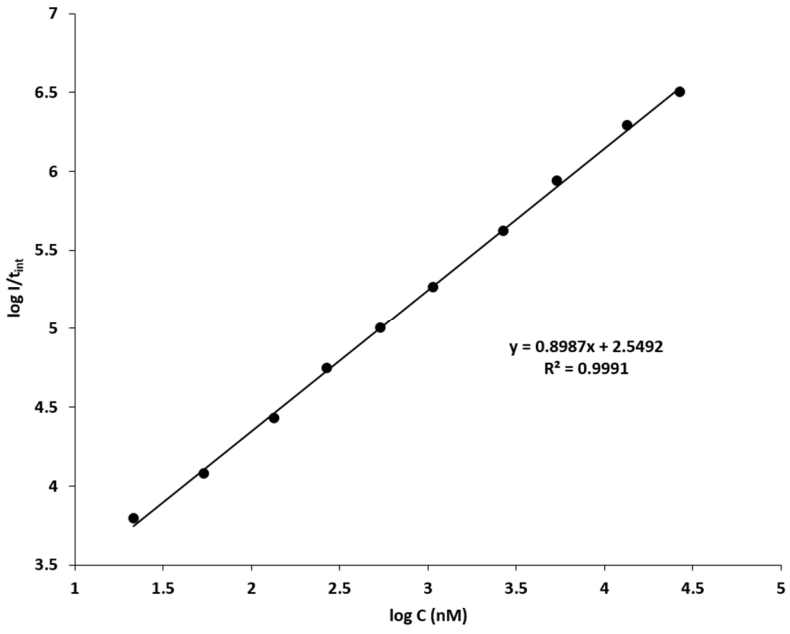


Figure S11. c) Logarithmic calibration plot for Rivoflavin at 405 nm.

d) Ru(bpy)$_3$

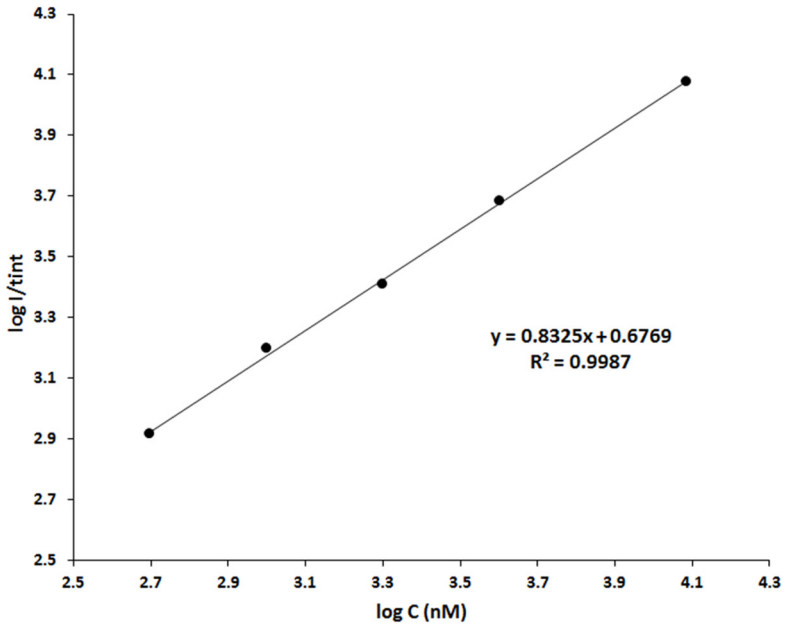


Figure S11. d) Logarithmic calibration plot for $Ru(bpy)_3$ at 365 nm.

References

1. Hammatsu Mini-spectrometer C12880MA DataSheet. Available online: https://www.hamamatsu.com/content/dam/hamamatsu-photonics/sites/documents/99_SALES_LIBRARY/ssd/c12880ma_kacc1226e.pdf (accessed on 10 April 2023).