

Design of a Portable and Reliable Fluorimeter With High Sensitivity for Molecule Trace Analysis

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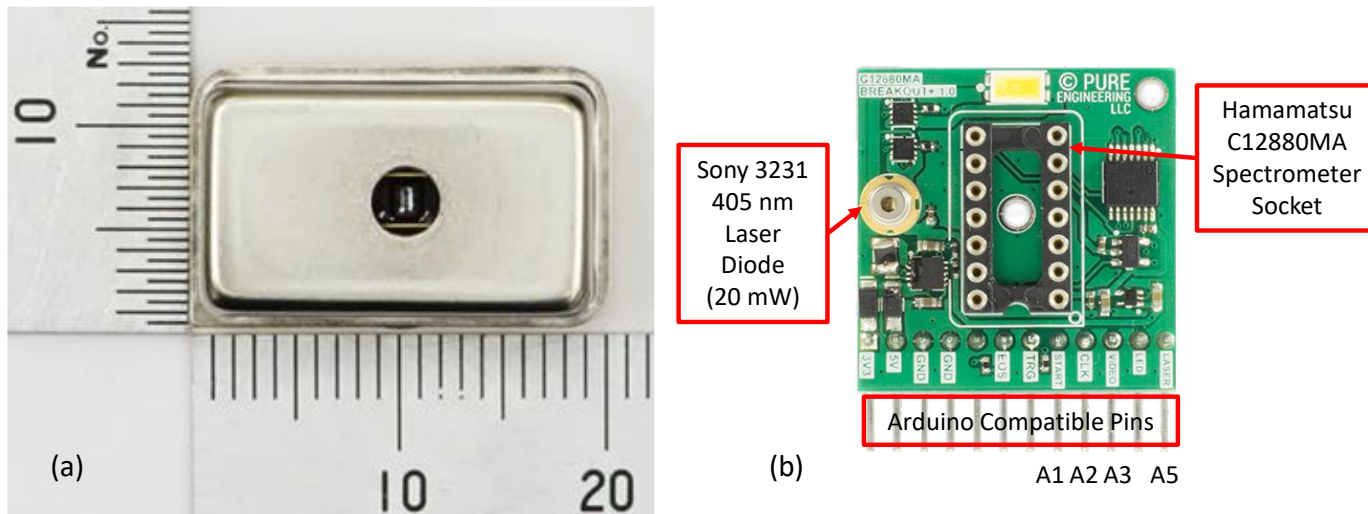
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Objetives

- Design an equipment for fluorescence measurements
- Portable for in-situ assays
- High sensitivity for molecule trace analysis (ppb limit)
- Microprocessor controlled (Arduino compatible)
- Variable excitation sources for flexibility
- USB interfaced through PC computer
- Data acquisition through Microsoft Excel Worksheet
- Low cost (less than 500€)

Hardware

- C12880MA Mini-Spectrometer from Hamamatsu Photonics (a)
- GroupGets Breakout v.1 for Hamamatsu C12880MA Spectrometer Integration with Arduino Microcontroller (b)



Selectable excitation sources: LED (365 nm) and Laser (405 nm)

Hamamatsu Mini-Spectrometer C12880MA DataSheet

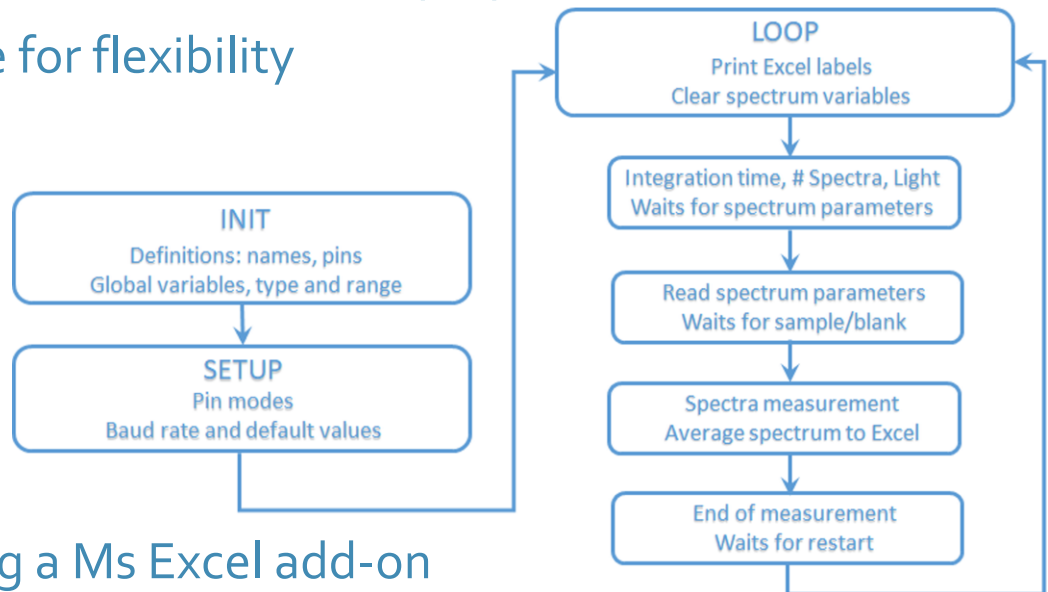
https://www.hamamatsu.com/content/dam/hamamatsu-photonics/sites/documents/99_SALES_LIBRARY/ssd/c12880ma_kacc1226e.pdf

C12880MA Breakout Board v2 by GetLab

<https://groupgets-files.s3.amazonaws.com/hamamatsu/uspectrometer/C12880MA%20Breakout%20Board%20v2%20-%20Datasheet%20-%20201.2.pdf>

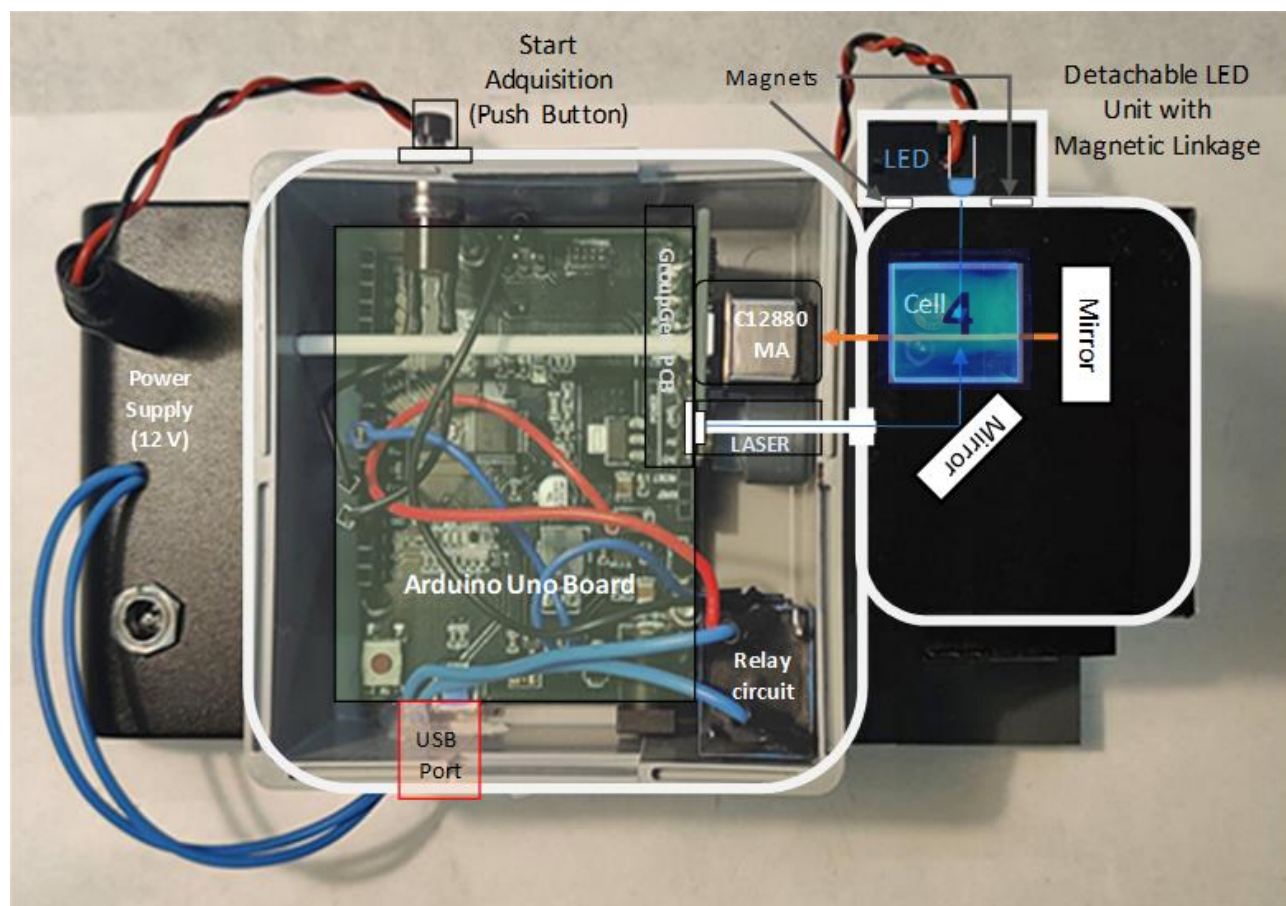
Software

- Code adapted for different Arduino compatible microcontrollers: UNO, Mega2560 and WeMos
- Measurement procedure controlled with only a pushbutton
- Variable Integration Time for flexibility
- General Flow Diagram



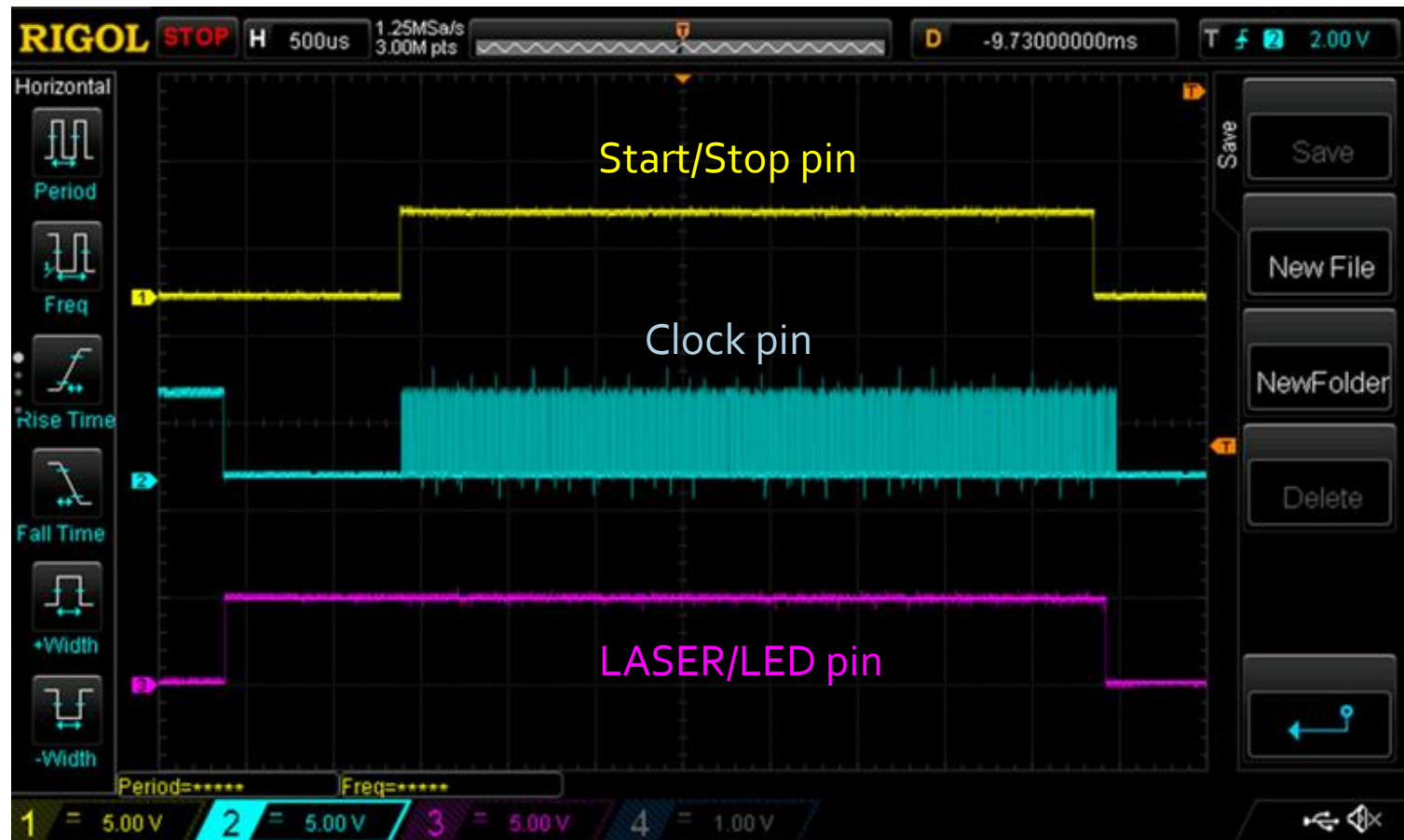
- Parameters input by using a Ms Excel add-on

Prototype Fluorimeter Design



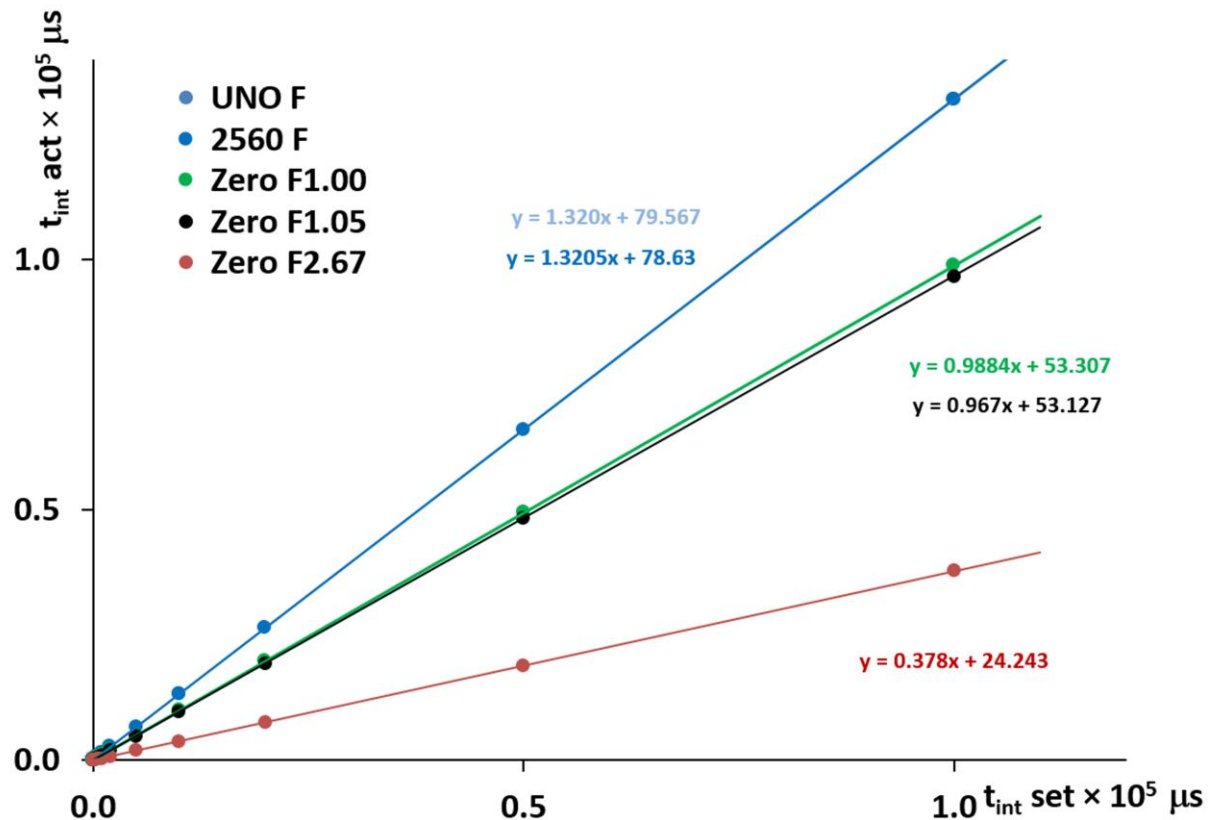
González-Arjona, D.; Roldán González, E.; López-Pérez, G.; Domínguez Pérez, M.M.; Calero-Castillo, M. Coulometer from a Digitally Controlled Galvanostat with Photometric Endpoint Detection. *Sensors* **2022**, *22*, 7541

Measuring Process Timing Diagram

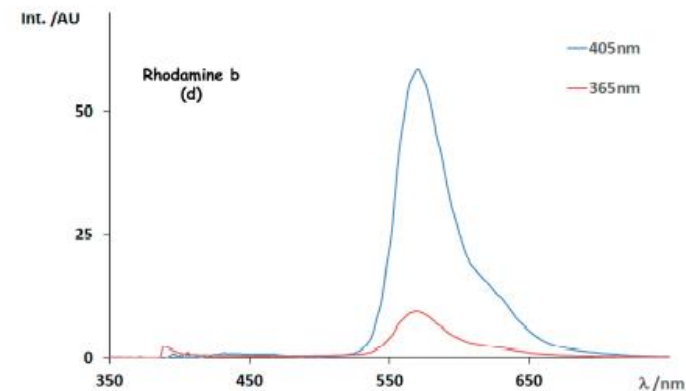
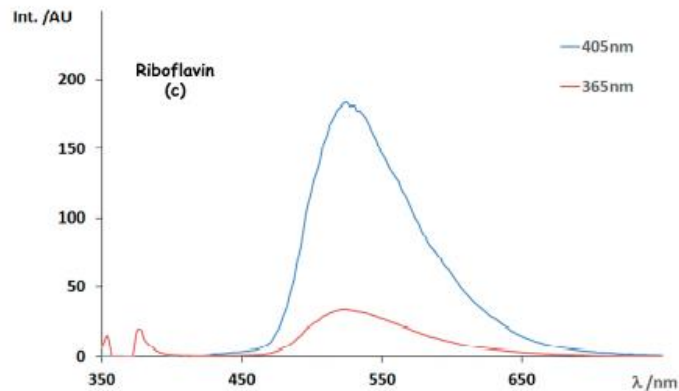
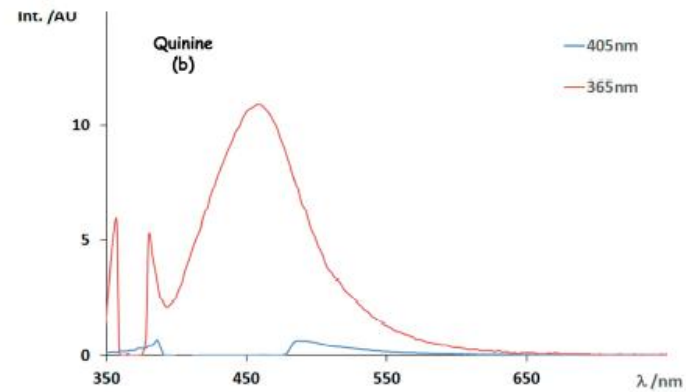
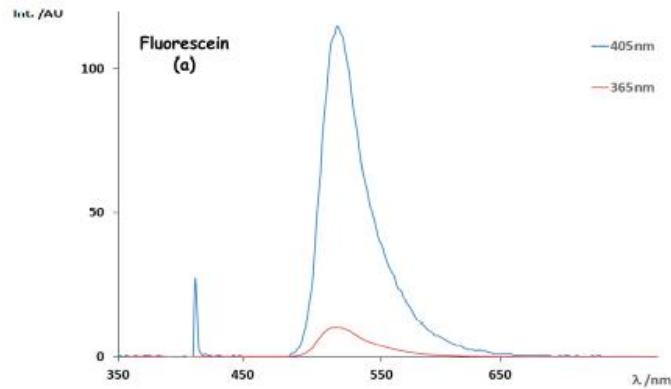


Variable Integration Time

Linear relationship between the integration time and the input parameter



Experimental Emission Spectra



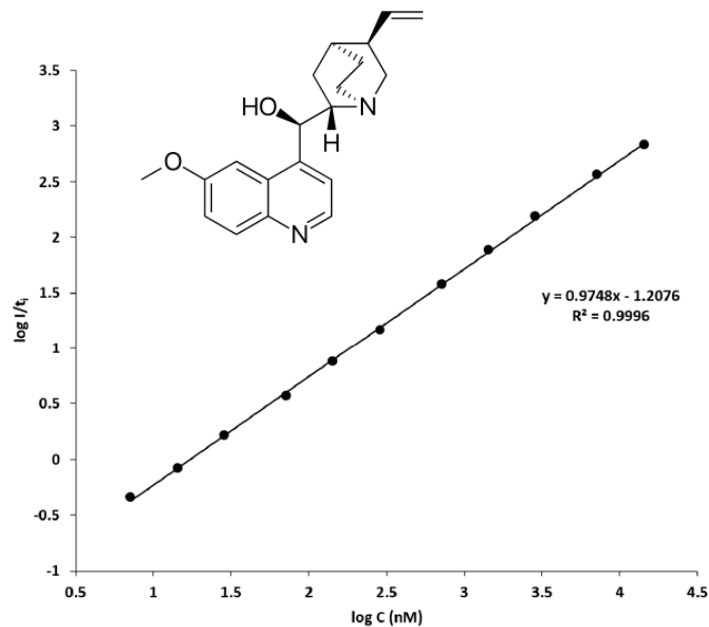
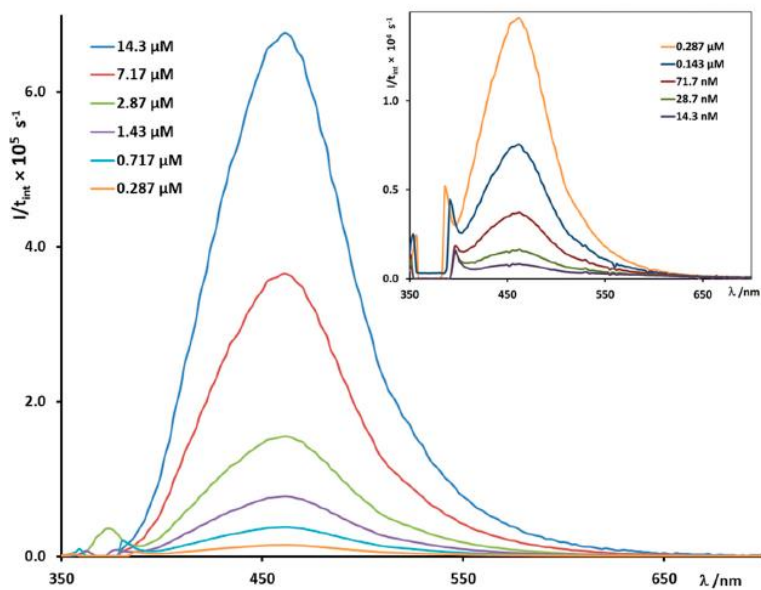
405 nm (LASER) 365 nm (UVLED)

All samples contain a 1,0 M solution of the indicated fluorophores

Analytical Validation

Analyte	Linear Range (nM)	LOD (nM)	LOQ (nM)	Linearity (%)	R ²
Fluorescein	1.9–11,000	1.2	1.9	98.9	0.9987
Quinine	1.5–14,000	1.1	1.5	99.4	0.9996
Rhodamine B	2.4–2500	1.3	2.4	98.5	0.9989
Riboflavin	2.1–27,000	1.3	2.1	98.9	0.9991
Ru(bpy) ₃	12.8–9800	2.1	12.8	97.9	0.9987

Quinine



Anion Chloride Determination

Quenching Method

Stern-Volmer Kinetics

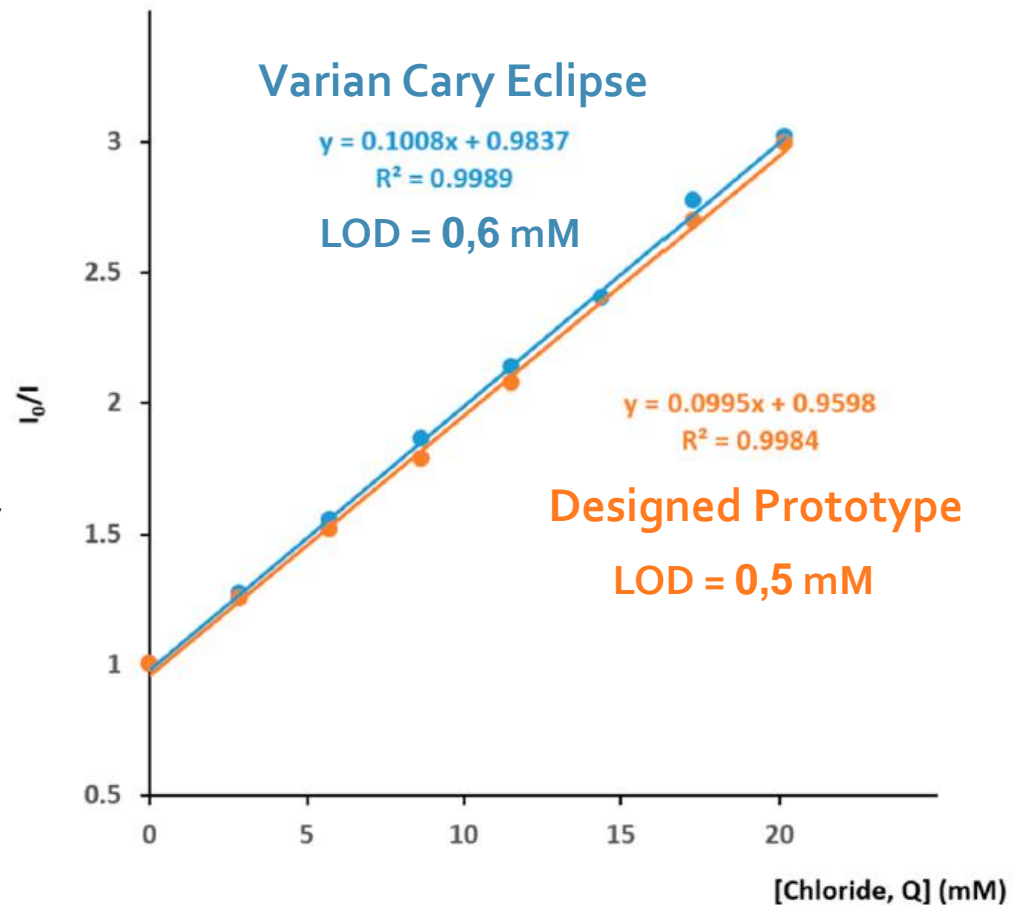
$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$

I_0 : Fluorescent Emission Intensity
in the absence of Quencher

I : Fluorescent Emission Intensity
in the presence of Quencher

K_{SV} : Stern–Volmer Constant

$[Q]$: Quencher Concentration



Fluorescence Equipments Comparative

Features	AVANTES AvaSpec-ULS2048CL-EVO	Ocean QE-Pro Fluoresce Bundle	This Work
Wavelength (nm)	200–1160	Configuration-dependent	340–850
Spectral Resolution (nm)	1–20	Configuration-dependent	15 nm
Integration time	9 μ s–59 s	8 ms–60 min	25 μ s–2 min
Excitation Source	Avalight—HPLED modules *	365 nm *	365 nm (LED)
Fiber Optic	FCR-FLTIP-IND *	Optional *	405 nm (Laser diode)
Software	AvaSoft-Basic	Proprietary	Optional *
Power supply	For each module	External	External (Excel®)
Detector Type	CMOS	Not available	Included
Sample	Optical Fiber	Optical Fiber	MEMS
Dimensions (mm)	177 × 127 × 44.5	Cuvette (sample holder) *	Cuvette
Weight (Kg)	1.135 + other modules *	182 × 110 × 47	Optical Fiber *
Platform	Proprietary	1.150 + power supply 0.45 *	120 × 80 × 60
Communication	USB 3.0	Proprietary	0.40
Cost (EUR)	Not available	USB 2.0	Open Source
		Accessories: 1200	USB 2.0
		Spectrometer not included	Complete: 500

FINAL REMARKS

- 1) Versatile, Portable and Low-cost Fluorimeter
- 2) Low Levels for Trace Analysis (ppb ranges)
- 3) Suitable for in situ Sample Analysis
- 4) Possibility of Extending the System Capabilities with Minimal Changes