MODULAR AND PORTABLE TIME-RESOLVED FLUORESCENCE MEASUREMENT SYSTEM

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INTRODUCTION

With the increasing importance of monitoring-based preventive medicine and advances in the development of fluorometric assays, small and more affordable time-resolved fluorescence measurement techniques are gaining acceptance in biomedical applications [1], [2]. Often these devices are only designed to detect basic properties of a marker and do not have the essential features that would enable the detection of more complex processes in a fluorometric assay.

DEVICE

The measuring device developed consists of two components, the excitation module shown in Fig. 1 and the detection module shown in Fig. 2. Both modules are powered and controlled by a computer via a standard USB Type C port.

Excitation
- Monolithic LD driver with pulse generation or signal pass-through operating modes
- Configurable energy and current limits for pulse energy control and LD protection
- Sample excitation modes: CW mode, square wave or pulse train modulation
- Generation of a reference signal that is transmitted to the detector electronics

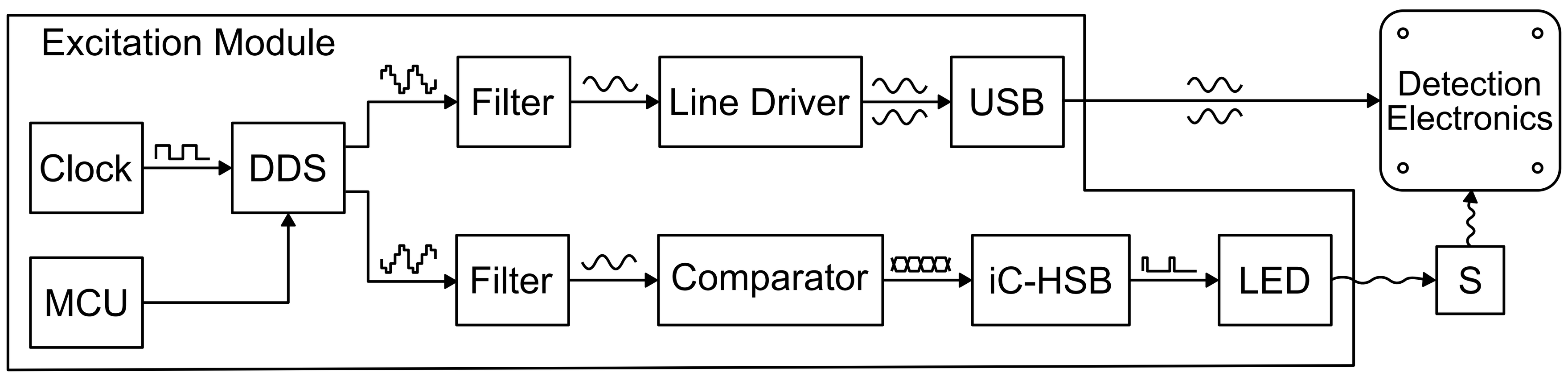

Fig. 1. Block diagram of the signal paths in the excitation module.

Detection
- Temperature-stabilized power supply that supports different types of SiPM detectors
- Simple front-end electronics with voltage-controlled variable gain amplifier
- Frequency down-conversion using heterodyning signal processing technique
- Single microcontroller for digitizing the signal and calculating the fluorescence lifetime

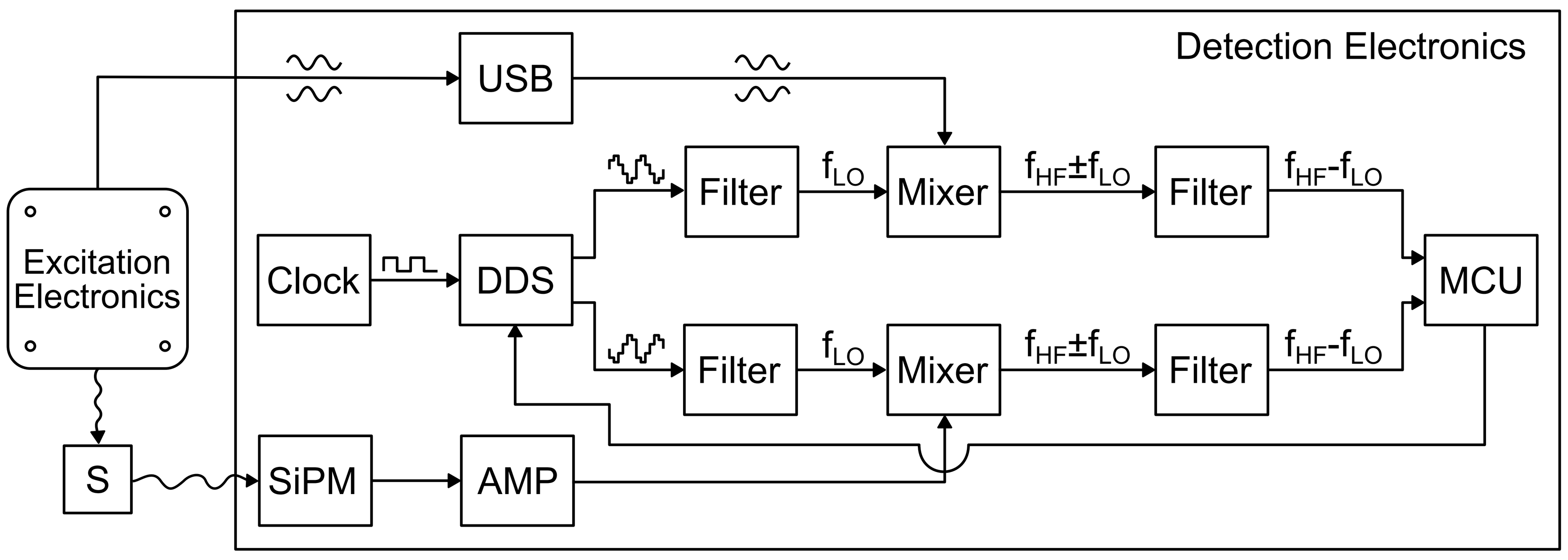

Fig. 2. Block diagram of the signal paths in the detection module.

The optical configuration can be freely designed depending on the measurement task. Fig. 4 shows a possible design for the characterization of liquid samples in a semi-microcuvette. This setup is printed entirely from polylactide (PLA) using a standard 3D printer for fused deposition modeling.

RESULTS

The apparent lifetime measured with the developed device shows a decrease depending on the pH value, see Fig. 5. At a pH value of 6, the emission is dominated by Ac_{H+}, as the pH value increases, the ratio between [Ac^-] and [Ac_{H+}] changes, causing the apparent lifetime of the sample to decrease. Based on the measured standard deviation of each point, an accuracy of pH ± 0.032 (95% probability interval of ± 2σ) could be predicted.

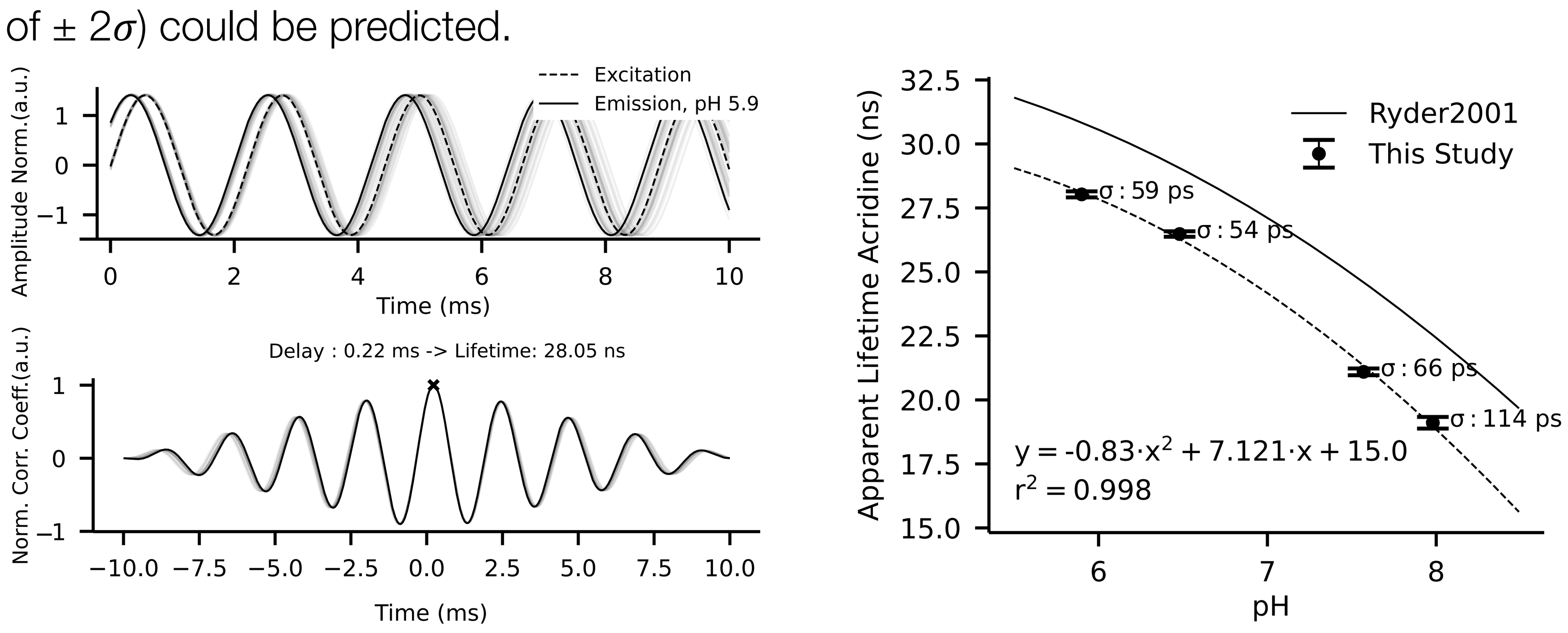

Fig. 5. Left: Cross-correlation of reference and sample signal to calculate the lifetime of the marker. Right: Measurements of the apparent lifetime with the developed device in comparison to [6].

A comparison of the measurements carried out in this study with [6] shows a parallel curve with an offset. This offset in the apparent lifetime is due to the early stage of development of the device, where some characterization parameters related to the heterodyning performance and the delays caused by the electronics are still missing.

EVALUATION

First device evaluation: Investigation of the time-resolved fluorescence of the pH-sensitive marker acridine. Acridine is one of the indicators with the longest pH lifetime response (10 ns) [3] and has the potential to achieve similar accuracy to electromechanical sensors. The fluorescence emission spectra for neutral acridine (Ac^-) and protonated acridine (Ac_{H+}) as well as the kinetic scheme are shown in Fig. 3. Each measured sample consists of 1 ml of 0.1 M aqueous K-phosphate buffer at different pH values with an acridine concentration of 0.001 M.

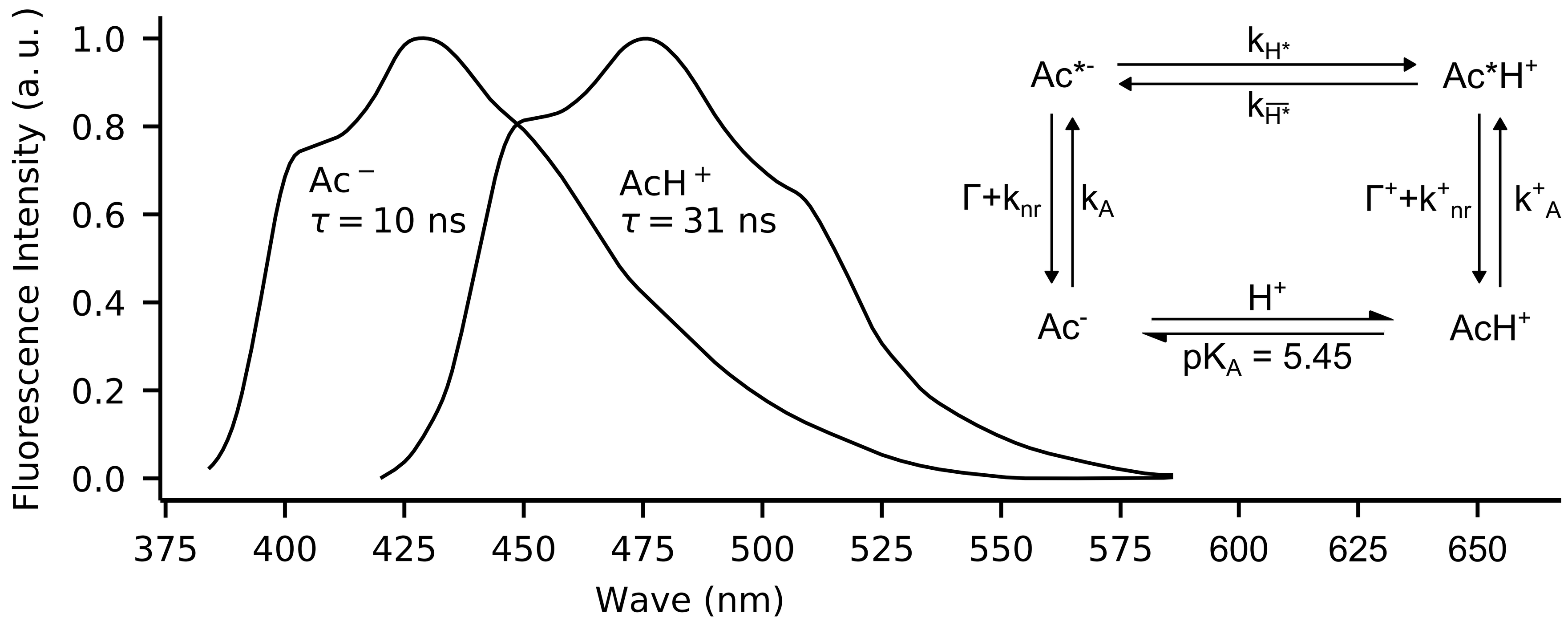

Fig. 3. Fluorescence emission spectra of Ac^- and Ac_{H+}. Kinetic scheme for proton transfer reactions of acridine in the ground state and in the excited state. The figure is an adaptation from [4, Fig. 2], [5, Fig. 7.49].

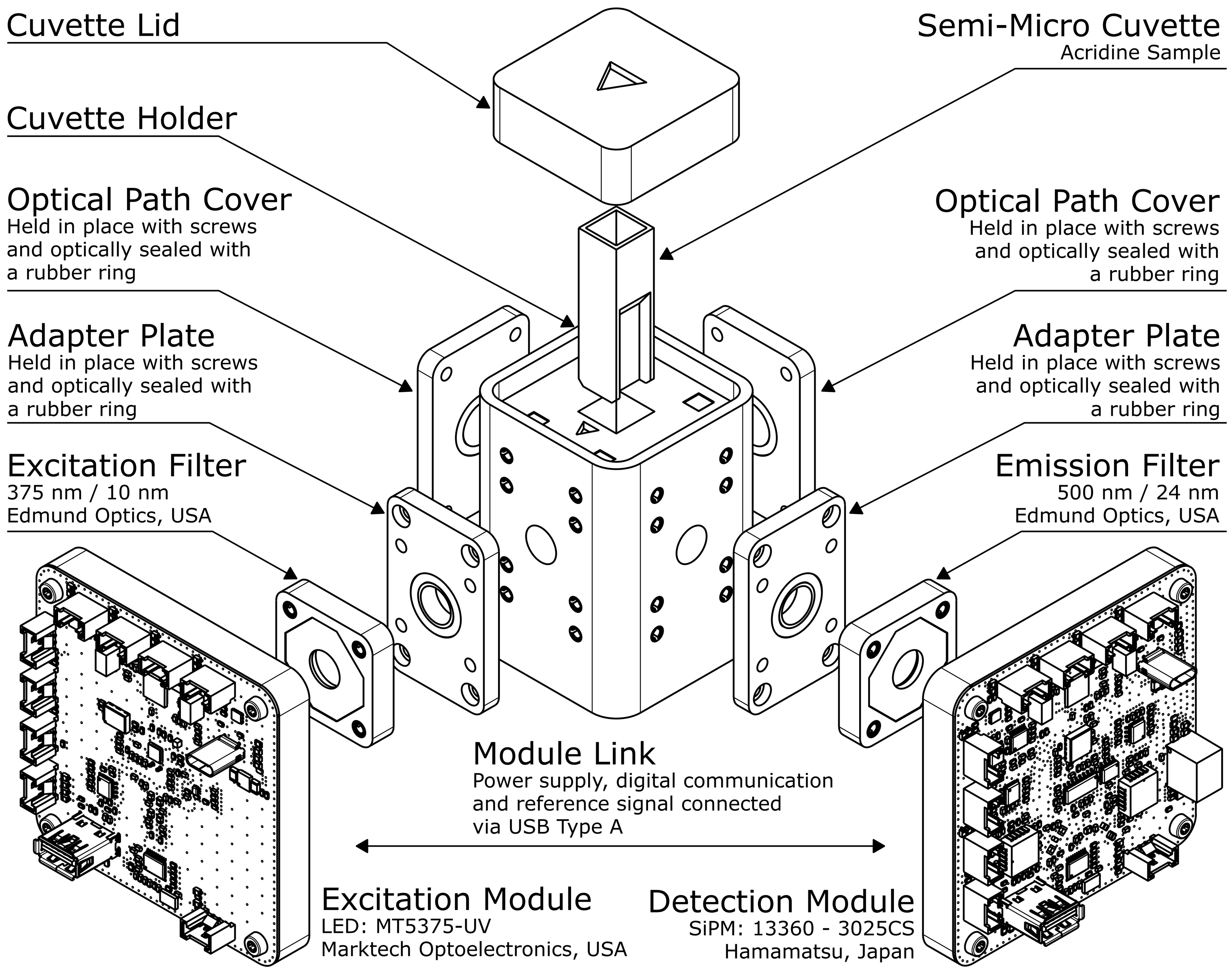

Fig. 4. Possible device architecture that can be used to characterize liquid samples in a semi-microcuvette. Measurement setup for the first evaluation with acridine fluorescent marker.

CONCLUSION

The developed device attempts to combine the advanced measurement methods of desktop systems for time-resolved fluorescence measurements with the cost and size advantages of point-of-care devices. Further testing is required to fully evaluate the performance of the device. In the future, it could provide researchers with an affordable, portable and versatile system for time-resolved fluorescence measurements.

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