

DEVELOPMENT OF SOLID STATE LASER-INDUCED-FLOURESENCE DETECTION SYSTEM

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ABSTRACT

This paper reports benchmark test results from an all-solid-state Laser-Induced-Flouresence (LIF) system that is being tested side-by-side with a conventional PMT/Argon gas laser detector in the application of microdevice electrophoresis. The goals of this development are reductions in (1) cost, (2) size, (3) parts-number, (4) overall failure-modes and, (5) required power consumptions without losing the sensitivity of the system. We demonstrate the proof of the concept of using the first generation of laser-diode/avalanche photodiode (LD/APD) prototype.

KEYWORDS: Laser-Induced-Flouresence (LIF), Low Cost DNA Sequencer, Electrophoresis, Laser Modulation

INTRODUCTION

Laser-induced-fluorescence detection is an essential component of many μ TAS instruments. Although revolutionary improvements are being made in fluidics, parallelism and system integration, the cost, physical size and power requirements of conventional LIF detectors are still a bottleneck, in particular for point-of-care instruments.

We are developing a compact fiber-coupled module of LIF that will integrate an avalanche photodiode and blue-laser-diode. In principle this small integrated system can be packaged to near ten cubic centimeters in size, can be produced at low cost, and can replace the bulky and expensive discrete-laser/PMT optics system in a large number of μ TAS instruments. Although discrete solid-state devices have been previously demonstrated in MEMS detectors, the benefits have not been fully realized due to insufficient practical packaging, high fluorescence noise floor, scattered light, and other compromises in detector performance. We are developing an alternative system by integrating solid-state components.

EXPERIMENTAL

Figure 1 shows the configuration of the laser-diode/avalanche photodiode (LD/APD) prototype. We tested the prototype on a microdevice, developed for a single-lane DNA sequencer at Whitehead Institute for Biomedical Research (WIBR), with a channel dimension $20\text{cm}^L \times 130\mu\text{m}^W \times 60\mu\text{m}^D$; and fluorescein standards ($0.002\mu\text{mol/L}$ - $1\mu\text{mol/L}$) to estimate the sensitivity and detection limit of the discrete LD and APD. Since an advantage using LD is a capability of modulation, we compared and analyzed the signals between continuous LD mode and modulation LD

mode + data synchronization. The LD is modulated at the frequency 24kHz and a lock-in-amp automatically synchronizes output signal with the low pass filter 100Hz. We also performed ssDNA electrophoresis (4% LPA gel and 170V/cm) on the single-lane DNA chip with 25-times-diluted commercial ladder samples (Promega, CXR), in order to verify the system under realistic time response.

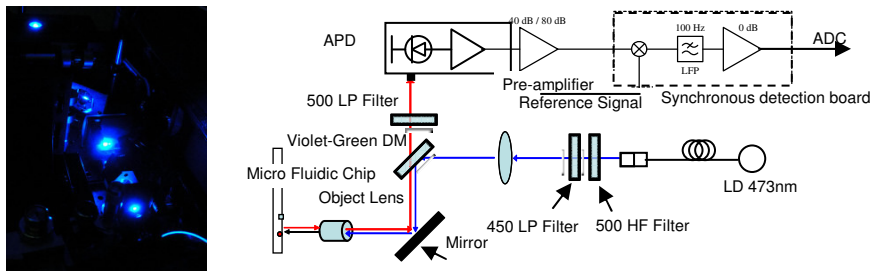


Figure 1. Prototype all-solid-state LIF detector (top half: Block diagram illustrating op-amp and lock-in amp, bottom half: Optics, left: LD light source and optics picture in operation) LD: Nichia Aquamarine Laser Diode. APD: Hamamatsu APD C5460.

RESULTS AND DISCUSSION

First tests revealed that the LD's spontaneous emission was limiting SNR, therefore band-pass (450nm-500nm) and low-pass (>500nm) filters were added. We found that the LD output driven at 100mA was more than sufficient to excite fluorescein standards when detecting the fluorescent light by the PMT and the APD (threshold is about 60mA).

Figure 2 illustrates the effects of modulation LD mode and data synchronization by the lock-in-amp. The red line with triangle markers in Figure 2 is the signal by modulation/synchronization whereas the blue line with circle markers is the signal by continuous laser and direct detection (without lock-in-amp). The test conditions of the two operation modes are exactly the same, i.e., 0.05 μ mol/L dye concentration, standard APD, and 40dB pre op-amp. The noise with the continuous LD mode is about 0.5V_{pp} and it is reduced to less than 0.1V_{pp} by the modulation LD/synchronization. The gain of the sensitivity is about 5X if the modulation/synchronization is used in this particular case. The calibration results, thus far, indicate a detection limit about 0.003 μ mol/L

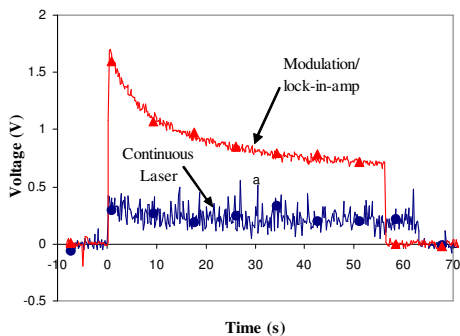


Figure 2: Effects of modulation LD and lock-in-amp mode. The fluorescent signal decays with time due to relaxation.

(not shown in this paper) using the modulation LD mode/high sensitive APD/lock-in-amp, which is good many applications but may be insufficient for some applications.

The digitized data of an electrophoresis run were processed by Fourier transformation in order to reduce noise and the processed results are shown in Figure 3. The figure clearly shows the DNA ladder peaks and achieves a SNR within about 1 decade of the highly optimized PMT system at WIBR.

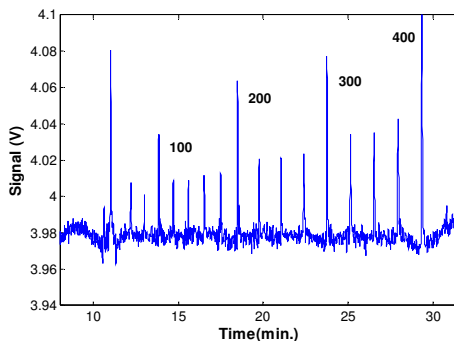


Figure 3: Electrophoresis results, Promega ssDNA ladder (CXR), 25- times-diluted, has peaks at 20-bp intervals up to 200bp and 25-bp intervals up to 400bp.

CONCLUSIONS

We have demonstrated the concept of the all-solid-state LIF prototype. The results of the modulation/synchronization mode are encouraging and we think that this method will lead to Frequency Division Multiplexing (FDW) for future parallelism. Although we did not fully optimize the first prototype, we could achieve a factor of 5 size reduction (see figure 4) and a significant components cost reduction compares to the PMT system. The apparent power for new system is about 10VA whereas the conventional LIF system at WIBR requires about 45kVA. The lower power consumption will significantly benefit in massively paralleled systems and portable point of care instruments.

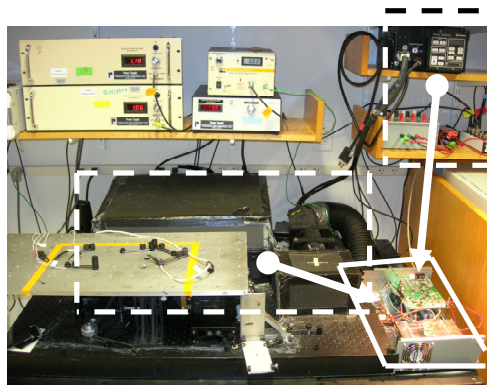


Figure 4: Size comparison: Prototype (Solid line Box) and WI single lane DNA sequencer (Dot line Box)

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