DYE-FLUORESCENCE LED-SPEC: A BATTERY-OPERATED, ON-CHIP, WAVELENGTH-TUNABLE OPTICAL SOURCE FOR DETECTION OF BIOCHEMICALS

Long Que, P. Selvaganapathy, Bhaskar Mitra, Chester G. Wilson,
and Yogesh B. Gianchandani
Department of Electrical Engineering and Computer Science
University of Michigan, Ann Arbor

ABSTRACT

This paper reports a wavelength-tunable microfluidic optical source for the fluorescence of dye-labeled biochemicals. A pulsed micro-arc is struck between a metal anode and a microfluidic cathode filled with an aqueous solution of $BaCl_2$. Atomic transitions from the Ba atoms, which are sputtered into the glow region, provide strong emissions at 493 nm and 454 nm wavelengths. Undesirable wavelengths are blocked out by an integrated optical filter. The emission is used to fluoresce calf thymus DNA tagged with SYBR™ green dye, achieving brightness levels comparable to commercially-available macro-scale apparatus. This method can be easily adapted for other fluorophores.

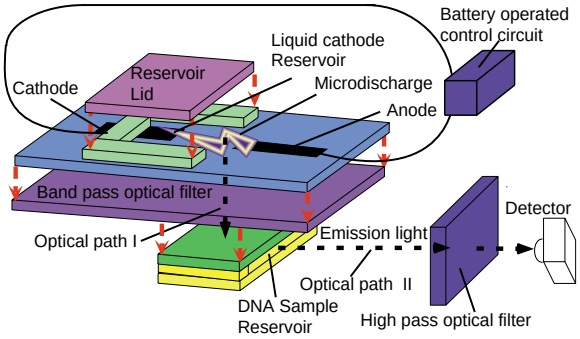
Keywords: DNA, fluorescence, microfluidic, microdischarge

I. INTRODUCTION

Fluorescence detection is a widely used technique in medical and biochemical diagnostics for studying the structure and dynamics of biomolecules [1,2]. In particular, the fluorescence of tagged dyes is used to detect DNA. A typical fluorescence imaging system consists of an excitation source, an analysis chamber/channel, and a detection setup. Recent efforts at miniaturization have focused on solid-state diodes and lasers as optical sources [3-6], which have sophisticated manufacturing processes, and use non-standard compound semiconductors. In contrast, this paper reports a micromachined liquid-based optical source which is battery-operated, wavelength-tunable, and can be integrated with microfluidic diagnostic systems. It uses a pulsed arc between a metal anode and a doped liquid cathode. Atomic and molecular transitions of impurities sputtered from the liquid cathode (at room temperature and atmospheric pressure) provide the desired spectral output which stimulates fluorescent dyes. This is named the *dye-fluorescence LEd-SpEC (liquid-electrode spectral-emission chip)*, and is based on an approach for spectral measurement of inorganic impurities in water [7-9].

II. MICROSYSTEM DESIGN

The dye-fluorescence LEd-SpEC device (Fig.1a) consists of six glass layers and an integrated optical filter. The upper three layers form the microdischarge source chip, including a metal anode and the liquid cathode reservoir, while the bottom three layers form the DNA sample reservoir. The DNA sample reservoir, which is aligned to the optical source, can be part of a larger fluidic system or a disposable plastic chip. In the figure, the downward-pointing arrows indicate how the system is assembled.



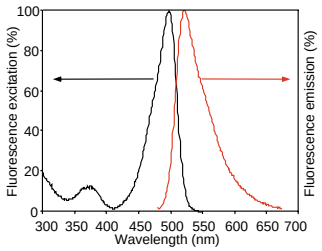
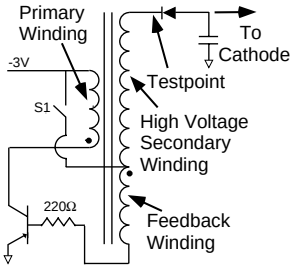


Fig. 1: (a-left): Exploded view of the miniaturized device. (b-right): The dsDNA-bound SYBR green dye is most efficiently excited near 498 nm. The emission peak is at 522 nm [1].

The present manifestation of the device is intended for fluorescing SYBR green dye (Molecular Probes, Inc.), tagged to calf thymus dsDNA. The excitation of this dye peaks over 491-503 nm while emission peaks over 510-600 nm (Fig. 1b). The liquid cathode used is 20% W/V $BaCl_2$. The emission spectrum of Ba provides peaks at 454 and 493 nm, corresponding to 25% and 97% efficiency for this dye, respectively [1]. The proximity of the absorption and emission peaks and the miniature size of the instrument pose a challenge in the optical design of the device. A dichroic band-pass filter (350-500 nm), chosen for its low sensitivity to incident angle and its superior reliability over other types of filters (e.g., interference filter), separates the optical source from the sample reservoir to block unwanted wavelengths [10]. The filtered emission from the discharge pulse follows optical path I (Fig. 1a), and stimulates fluorescence in the biochemical sample reservoir, which is detected along optical path II. Path I and II are orthogonal to minimize the possibility of stray light feeding-through from the arc to the detector. In addition, a high pass (>500 nm) optical filter placed along path II blocks stray light scattered by the sample from entering the detector. All the glass layers are #7740 Pyrex™ glass (506 μm thick) which has transmittance >80% for 300-700 nm wavelengths.



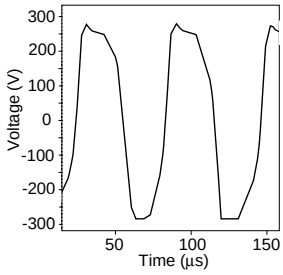


Fig. 2: (a-left): Schematic of the ringing choke converter high voltage circuit.
(b-right): Waveform at test point obtained by SPICE simulation of the circuit driven by a 1.5 V battery.

The microdischarge is powered by a pulsed high voltage generated by a 3 V battery-operated circuit (Fig. 2a). The design is based on the ringing choke converter [11], which consists of a transistor-transformer oscillator in which secondary windings on the transformer boost the voltage. When the switch S1 is closed, a small base current starts to flow into the transistor. The rising collector current leads to increasing base current due to positive

feedback from the transformer. This drives the transistor into saturation. The rate of change of current then decreases, and the voltage induced in the feedback winding decreases. The base current decreases and transistor turns off. Due to the sudden decrease of collector current, a large voltage drop occurs across the primary winding, and consequently on the secondary winding. Figure 2b shows the SPICE simulated waveform at this test point (for which the circuit is driven by a 1.5 V battery). This high voltage AC waveform is rectified and used to charge up the capacitor, which powers the cathode for the pulsed discharge.

III. EXPERIMENTAL RESULTS AND DISCUSSION

A fabricated dye-fluorescence LEd-SpEC device and its circuit are shown in Fig. 3. The microdischarge source chip and the DNA sample reservoir were processed separately before the stack is bonded together. The final device footprint measures 10 mm × 20 mm.

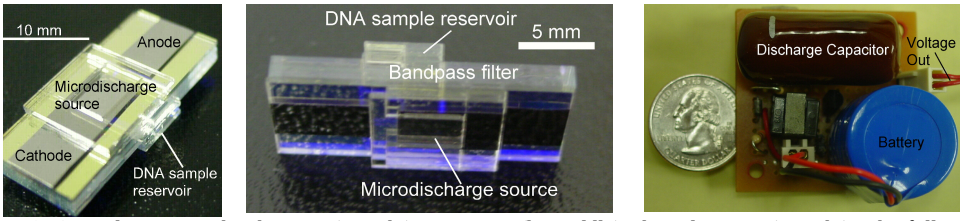


Fig. 3: Optical micrographs showing (a: right): top view; (b: middle): the side view. (c: right): The fully self-contained battery-operated circuit shown against a U.S. quarter dollar.

Figure 4a shows the measured electrical output of the circuit as it drives the microdischarge. The capacitor charges up to the breakdown voltage and discharges very rapidly through the ionized media. As the output voltage drops, the discharge extinguishes, allowing the cycle to be repeated. Figure 4b shows that the measured high voltage signal at the test point identified in Fig. 2a is similar to the simulated results in Fig. 2b.

The spectra produced by the microdischarge before and after progressing through the on-chip optical band-pass filter are shown in Fig. 5. The 454 and 493 nm peaks characteristic to Ba are evident in the unfiltered spectrum, along with a number of lines characteristic to nitrogen and other atmospheric gases. The two primary Ba lines pass through the filter. The fluorescent image of the DNA with SYBR green dye is shown in Fig. 6c. This image has almost the same quality as that by a commercial lamp source (Fig. 6b). A reference image of a water sample without dye (Fig. 6a) remains dark. Note that all three images were obtained on the same device, microscope, and camera. Even better performance could be achieved by optimizing the optical coupling between the microdischarge and the sample, such as by integrating focusing optics.

IV. CONCLUSION

This effort successfully demonstrates that a microfluidic chip with an integrated microdischarge source can be used to locally fluoresce biochemicals. The microfluidic nature of the source makes it low cost and amenable for integration with diagnostic systems. Fluorescence of SYBR dye-tagged calf thymus DNA is demonstrated using $BaCl_2$ solution as the cathode. This device can be tuned to a variety of wavelengths by the appropriate selection of chemicals.

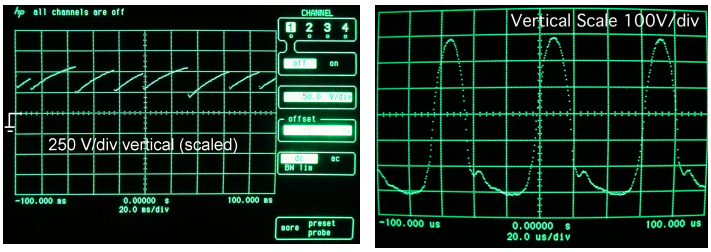


Fig. 4: (a: left): Oscilloscope trace of circuit output while creating pulsed arcs with a 3 V battery; (b: right): Measured waveform at the test point in the absence of a discharge, with a 1.5 V battery.

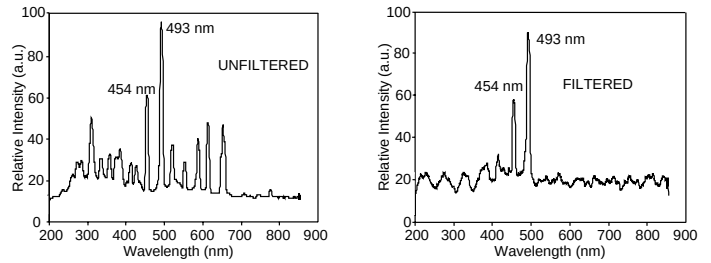


Fig.5: Measured spectrum of the pulse-driven $BaCl_2$-based microdischarge source without the filter (a-left); and with a (350 nm-500 nm) band pass dichroic color filter (b-right).

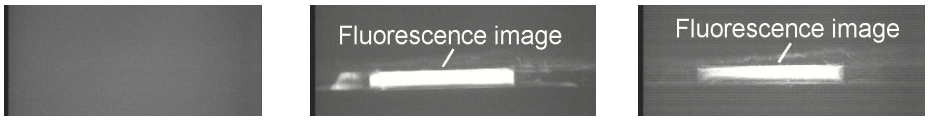


Fig. 6: The measured image of water (a-left), the glowing DNA image using the filtered lamp source (b-middle) and pulse-driven microdischarge source (c-right).

ACKNOWLEDGEMENTS

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REFERENCES

[1] R.P. Haugland, *Handbook of fluorescence probes and research chemicals*, Molecular Probes, Inc. Eugene, OR, 1996

[2] A. Pease, D. Solas, E. Sullivan, M. Cronin, C.P. Holmes, P. Fodor, "Light-generated oligonucleotide arrays for rapid DNA sequence analysis," *Proc. Natl. Acad. Sci.*, 91, pp. 5022-6, 1994

[3] H. Chou, C. Spence, A. Scherer, S. Quake, "A microfabricated device for sizing and sorting DNA molecules," *Proc. Natl. Acad. Sci.*, 96, pp. 11-13, 1999

[4] J.Webster, M.A. Burns, D.T. Burke, C.H. Mastrangelo, "Monolithic capillary electrophoresis device with integrated fluorescence detector," *Anal. Chem*, 73, pp. 1622-6, 2001

[5] M. Warren, W.C. Sweatt, J.R. Wendt, C.G. Bailey, C.M. Matzke, D.M. Arnold, S.A. Kemme, A.A. Allerman, T.R. Carter, R.E. Asbill, S. Samora, "Integrated micro-optical fluorescence detection system for microfluidic electrochromatography," *SPIE*, v. 3878, pp. 185-192, 1999

[6] E. Thrush, O. Levi, K. Wang, M. Wistey, J. Harris Jr., S. Smith, "Integrated semiconductor fluorescent detection system for biochip and biomedical applications," *SPIE*, 4626, pp. 289-96, 2002

[7] G. Jenkins, A. Manz, "Optical emission detection of liquid analytes using a micro-machined DC glow discharge device at atmospheric pressure," *μTAS*, pp. 349-50, 2001

[8] C. Wilson, Y. Gianchandani, "LEd-SpEC: Spectroscopic detection of water contaminants using glow discharges from liquid electrodes," *Proc. IEEE Intl. MEMS Conf.*, pp. 248-51, Jan 2002

[9] L. Que, C. Wilson, J.de La Rode, Y. Gianchandani, "A water spectroscopy microsystem with integrated discharge source, dispersion optics, and sample delivery," *Transducers'03*, pp.32-35, Boston, 2003

[10] Model number K30-635 and K43-456, Edmund Industrial Optics, Inc. Barrington, NJ

[11] L.H. Light, P.M. Hooker, "Transistor D.C. Convertors," *Proc. of IEE, B*, 102, pp. 775-786, 1955