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High Speed Low Noise Multiplexed Three Color Absorbance Photometry

Khaled M. Dadesh, G.K. Kurup, and Amar S. Basu, *Member, IEEE*

Abstract*— Multispectral photometry is often required to distinguish samples in flow injection analysis and flow cytometry; however, the cost of multiple light detectors, filters, and optical paths contribute to the high cost of multicolor and spectral detection systems. This paper describes frequency division multiplexing (FDM), a simple approach for performing multi-wavelength absorbance photometry with a single light detector and a single interrogation window. In previous efforts, modulation frequencies were <10 KHz, resulting in a detector bandwidth of <20 Hz. This paper presents a high frequency FDM circuit which can increase the oscillation frequencies to several 100 KHz, improving the detection bandwidth by a factor of 10 while still maintaining low cost. Light from 3 different LED sources are encoded into unique frequency channels, passed through the detection cell, and later demodulated using phase-sensitive electronics. Electronic multiplexing couples all light sources into a single optical train without spectral filters. Theory and high frequency considerations are demonstrated. Simultaneous three color absorbance detection is demonstrated in solutions and in flowing droplet microreactors. This technique can potentially reduce the cost of multicolor photometry by replacing expensive optical components with low-cost electronics.

I. Introduction

Optical detection methods have been widely used in analytical chemistry in applications ranging from health sciences, environmental monitoring, and homeland security. The need for in-situ, low cost, and miniature devices has motivated the use of light emitting diode (LED) and photodetectors (PD) in portable detection systems. LEDs have a low cost (<$10), low power consumption (<50mW), longer lifetime (>50,000 hrs), a robust structure, reasonable chromaticity (<100nm bandwidth), and are commercially available over a wide spectral range (247-1550 nm) [1]. PDs have proved to be low cost and versatile detectors in photometry applications after Anfält *et al*. published the first use of a photodiode with LED in 1976 [2].

Hauser et al. 1993 [3] were the first who published on the uses of a blue LED with PD for photometry measurements. Since then, many contributions have been made using single wavelength photometry [1]. Although the single wavelength photometry is a useful technique in many basic chemical analysis measurements, it lacks the ability to differentiate between multiple analytes. Multi-wavelength photometry can offer the required specificity; however, it requires a more complex optical design, including gratings and arrayed light detectors (CCDs, photodiode arrays). In addition to the added cost and complexity, multi-wavelength systems can have lower sensitivity due to losses in the grating and the poor low-light performance of CCD based detectors compared to photomultiplier tubes (PMTs). In microfluidic systems, the reduced sensitivity is particularly problematic due to the small path lengths. The integration of multiple optical paths is also challenging [4].

Chance et al. 1971[5] proposed his first commercial dual wavelength spectrophotometer (DWS). A time-sharing technique was accomplished using a rotating wheel with

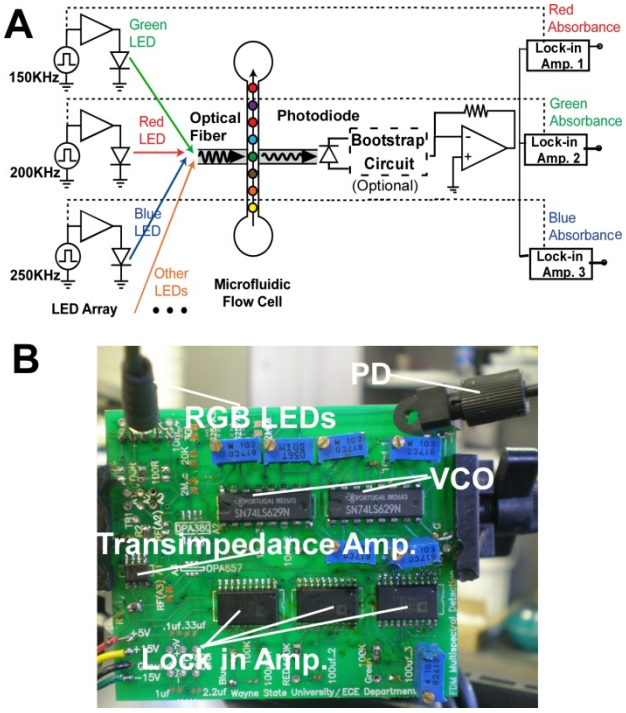

***Figure 1**: A) A schematic diagram of multi-spectral absorbance detection using FDM. Absorbance at red (636 nm), green (574 nm), and blue (470 nm) wavelengths are simultaneously monitored using a simple modulation/demodulation scheme. B) A photo of the implemented circuit to the low cost of electronic components, this approach can be economically scaled to include many LEDs spanning a wide spectral range. Note: Bootstrap circuit is optional.*

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K. M. Dadesh is with Electrical and Computer Engineering Department at Wayne State University, Detroit, MI 48202, USA,Tel: 313-384-8262; fax: 313-577-1101; e-mail: k_dadesh@wayne.edu.

G.K.Kurup is with Electrical and Computer Engineering Department at Wayne State University, Detroit, MI 48202mailto:.

Amar S. Basu is with Electrical and Computer Engineering Department and Biomedical Engineering Department at Wayne State University, Detroit, MI 48202, USA. Tel: 734-096-6688; fax: 313-577-1101; e-mail: abasu@eng.wayne.edu.

properly selected interference filters. In 1980. Werner [6] published an advanced DWS with two selecting interference filters and a small vibrating mirror as time sharing unit. In 2004, Werner [7] reported a micro-dual-wavelength spectrophotometer. He used a time division multiplexing technique to chop the light of two LEDs alternatively with 2 KHz rectangular pulses. The produced florescence signals were detected using a miniature photomultiplier, lock-in amplifier, and a low pass filter with .03 s time constant.

Oh et al, 1998 [8] reported a technique to perform a simultaneous detection of multiple gases (i.e., CO and CO_2) by use of the wavelength modulation spectroscopy (WMS) with diode lasers; they used two different frequencies 40 and 50 kHz for their experiments and two lock-in amplifiers to extract each WMS signal. Suzuki et al, 2003 [9] used the same previous technique to measure absorbance with flow-injection analysis system; they used four different LEDs with four different frequencies at which the highest frequency did not exceed 10 KHz. We previously reported the use of multispectral absorbance photometry of organic dyes [4]; the maximum frequency used was 10 KHz. Although these frequencies are commonly used in lock-in detection, the resulting measurement bandwidth is not suitable for high speed detection in flow cytometry or high throughput microfluidic systems.

In this paper, we present a multispectral, high-speed, and low-noise detection system, which improves bandwidths of the previous efforts by >1 order of magnitude. We measured the absorbance of three food dyes using a red, green, and blue LED and also demonstrate high speed detection (1 ms time constant) on an array of droplets being serially passed through the detector. The setup uses a frequency division multiplexing (FDM) technique to transmit three different modulated light signals with three different frequencies on a single optical path. It uses a single light detector and a single detection window, which simplifies the optical design. A phase sensitive detection technique using economical lock-in demodulators and low-pass filters were employed to separate the absorbance measurements of each light signal. A schematic diagram and photo for the implemented circuit are shown in Figure 1.

II. THEORETICAL BACKGROUND

A. FDM Principle

In radio broadcasting, frequency-division multiplexing (FDM) is defined as the assigning of signal spectra in frequency channel such that each signal spectrum does not overlap all the others [10]. Each signal spectrum can be extracted using a bandpass filter. For example, N signals each is bandlimited to f_m Hz, and each is modulated with a carrier frequency f_{C1} , f_{C2} , ..., f_{CN}; if the modulation method is double side band (DSB), the spectral density of every modulated signal has a bandwidth of $2f_m$ and each is centered at different carrier frequencies $f_{C1}, f_{C2}, ..., f_{CN}$. These carrier frequencies have to be chosen far enough apart such that each signal spectral density is separated from all the others. Figure 2 illustrates the concept for three signals that have arbitrary shapes.

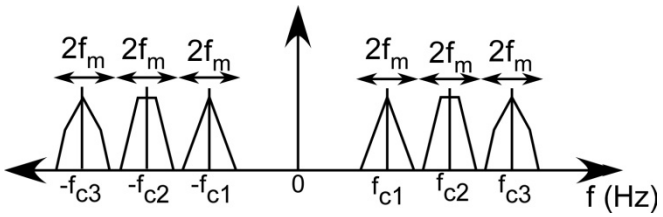

Figure2. *Frequency domain of FDM signal*

B. Principle of Lock-In amplifier

This section reviews the basic principle of the lock-in amplifier with some emphasis on the AD630 lock-in amplifier that was used in our measurement setup. Lock-in amplifiers, also known as phase sensitive detector, have the advantage, over other amplifiers, of extracting a signal of known frequency and phase from a highly noisy environment. The orthogonality of the sine waves is the core principle behind the operation of the lock-in amplifier. If we have a signal $V_s(t)$, with frequency f_s, and peak V_s, this signal can be expanded by Fourier series to a summation of infinite number of sine and cosine wave at which the fundamental component will have the same frequency f_s. The lock-in amplifier multiplies the input signal $V_s(t)$ by a reference signal, usually square wave with frequency f_o and peak V_o. The output of the lock-in amplifier will be a signal that has a DC component and many other harmonics. If $f_o = f_s$, the amplitude of the DC component will be proportional to $(V_r V_O/2)$ [10]. A low pass filter can be used to pass the DC component and reject all the harmonics. The time constant or band width of the low pass filter determines the maximum detection speed of the circuit.

C. Signal detection and amplification

Photodiodes are quantum detectors, which we used in our setup. They exist in different types, sizes, and characteristics; they exhibit excellent performance at low costs. Silicon photodiodes when operated under zero or reverse bias have low intrinsic noise and leakage currents due to their relatively wide bandgap. In addition to these advantages, their extreme linearity, due to the quantized nature of light to electricity conversion, made them a suitable candidate to be used in our FDM setup for the proposed absorbance measurements [12].

The main objective of this research is to increase the bandwidth of the FDM system by a factor of ten. The inherent capacitance of the photodiodes, C_{PD}, (about 10 pf) slows down the response of the front end of the detection circuitry. The transimpedance amplifier usually employs a large feedback resistance, R_f , to convert the low current produced by the PD to an appreciable voltage signal. Both this resistance and the capacitance of the PD that determine the time constant, i.e., $\tau = R_f * C_{PD}$ or the cutoff frequency, i.e., $f_c = 1/(2\pi * R_f * C_{PD})$ of the front end circuitry [11]. To minimize this time constant, our group previously [4] used a moderate value of R_f, and a second non-inverting amplifying stage to provide additional gain. In practice, the two-stage approach was found to reduce the overall bandwidth and signal linearity. In this work, we revert back to a single, high gain transimpedance stage. To minimize

the time constant, we attempted to add a bootstrapped cascade circuit reported by M. Boukadoum and A. Obaid 2007 [13]. This circuit reduces the effective resistance observed by C_{PD}. This greatly reduces circuit time constant, allowing us to detect modulation at > 100 KHz. However, it added nonlinearity and complexity to the circuit without any performance improvements. Instead, the best performance was achieved using a single high speed, low noise transimpedance amplifier (OPA380, Texas Instruments).

III. Experimental Setup

Figure 1A shows a block diagram of a multispectral absorbance detection setup using FDM. Each of the three, red, blue, and green, LEDs, was modulated using three voltage controlled oscillators (VCO, SN74LS629N; Texas Instruments), via three rapid-response and high-precision comparators (LM311; Texas Instruments). The modulation frequencies of the green (570 nm), red (636 nm), and blue (470 nm) LEDs were 150, 200, and 250 KHz, respectively. The frequencies were chosen so that their Fourier spectra do not overlap. A potentiometer in series with the LED was used to adjust the relative intensity of each color.

A bundle of 32 multimode optical fiber waveguides (Industrial Fiber Optics) was used to combine the light from the three LEDs and guide it to the flow cell, a 1.5 mm cross junction (Value Plastics). Fiber bundles were epoxied into holes drilled into the LED lens to increase light coupling. The light transmitted through the flow cell was guided using a single core, multimode, 1500 µm diameter optical fiber to a high-speed PD detector housed in a "connector-less" style plastic fiber optic package (IF-D91; Industrial Fiber Optics). The two optical fiber were affixed to the opposite ends of the cross junction.

Light absorption by the sample modulates the amplitude of each frequency channel depending on the sample's absorption coefficient at the corresponding wavelength. The generated photo-current in the PD was converted to a voltage signal using the high-speed low-resistance front end described in the previous section. Consequently, the photodetector signal's Fourier spectrum contains frequency channels representing the light intensities of the respective LEDs.

The photodiode signal was supplied to the three lock-in amplifiers. Each lock-in amplifier was tuned to one of the three operating frequencies to extract the intensity from each channel. The output of each lock-in amplifier was passed through a low-pass filter that has 1 ms time constant (~133KHz bandwidth). This time constant is 25 times smaller than that used by Werner [6].

IV. Results and Discussion

A. Multispectral Absorbance photometry of FD&C dyes:

To demonstrate simultaneous, three color measurements, we flowed a mixture of three FD&C dyes (i.e., red, blue, and green) through the detection system. We started with ~0.13 ml dye in 4ml of de-ionized (DI) water as a stock solution. This is denoted as 100% concentration in the subsequent graphs. Concentrations in Figures 3A-C are normalized to this value. The solution was titrated with DI water to obtain a set of serial dilutions with concentrations ranging from 0-100%. For each concentration, three simultaneous absorbance measurements for each channel were recorded. The absorbance was calculated as $AU = -log_{10}(V/V_o)$ where V is the measured DC voltage at the output of each low pass filter and V_o is the measured DC voltage at the output of each low pass filter when DI water is in the flow cell.

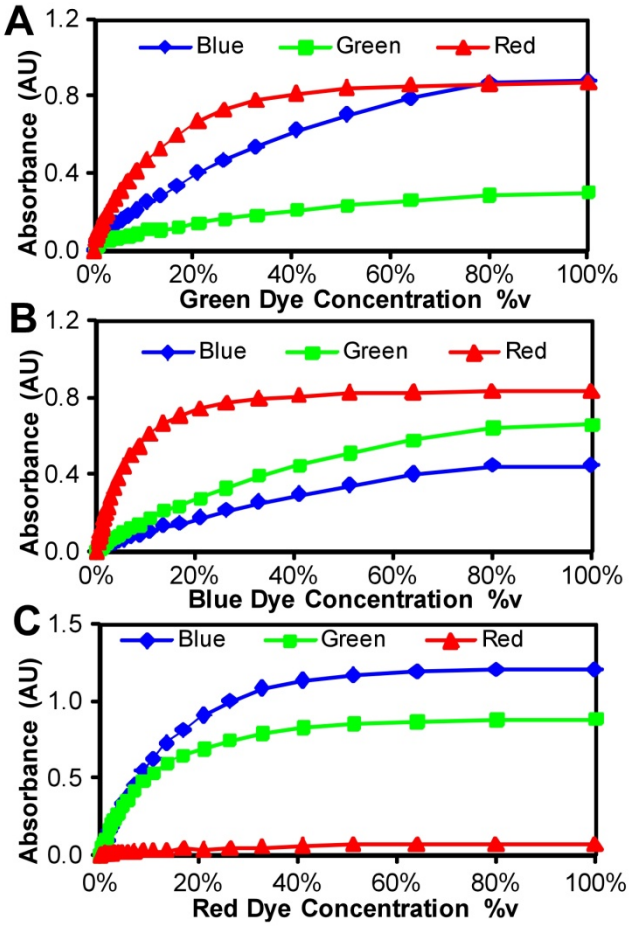

***Figure 3**: Multiplexed absorbance measurements of food dyes at 3 wavelengths. (A & B) FD&C Blue and green absorb all three wavelengths. (C) FD&C Red absorbs blue and green light, but passes red light.. The results are in agreement with the known absorbance spectra of the 3 food dyes.*

Figures 3A-B show the ability to simultaneously perform three wavelength absorbance measurements. The figures show that FD&C red strongly absorbs blue and green light, but not red light. In contrast, both FD&C blue and green strongly absorbs all colors. These results agree with the known absorbance spectra of the dyes. This experiment validates the system in performing independent measurements at a high modulation frequency. Absorbance increases logarithmically with concentration, consistent with Beer's law. At high absorbance, the signal saturates, as is common with typical absorbance meters. The logarithmic region defines the concentration range of the detector.

B. *Analysis of droplet microreactors*

Figures 4A-C show an inline absorption measurement of droplet microreactors containing red blue, and green FD&C dyes. The figures reveal the ability to perform high speed multispectral absorption measurement of three different colors using the FDM photometer. The rate of the droplet flow was 12 drops/sec, and the concentration of the dye was about 12.5% (v/v). Figures 4A-C present the change in the output voltage of each of the lock-in amplifiers. This result agrees with the expected absorption of each dye. The time constant of 1 ms of the low pass filter allows for high speed measurement. Although the fluidic setup limited the throughput to 12 drop/sec, we expect the circuit to be able to handle 1-2 orders of magnitude higher. The effect of the flow cell on the shape of the drop manifests itself as a distortion of the peaks in the red light signals.

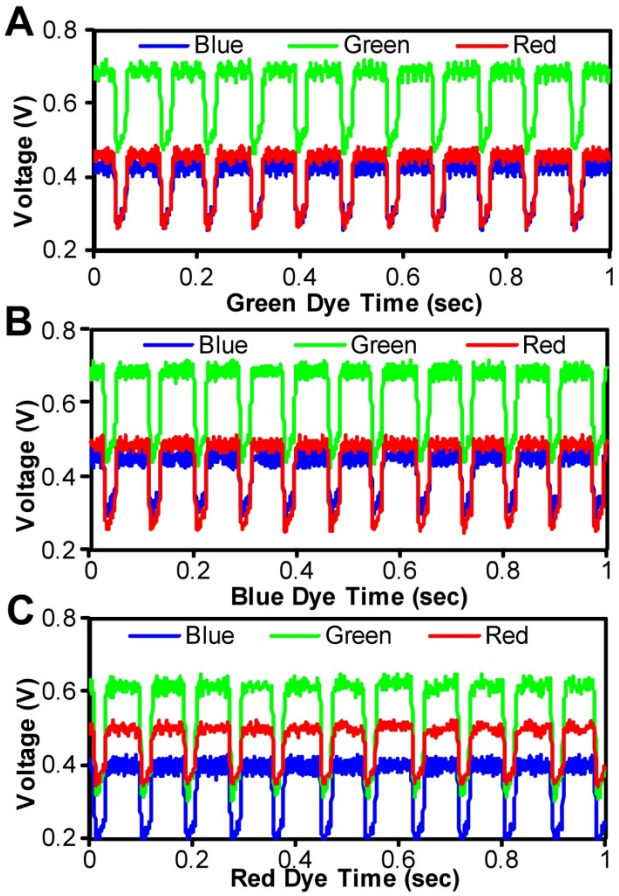

Figure 4. *Inline, multispectural analysis of droplet microreactors using the FDM photometer for; A) Green Dye, and B) Blue Dye, C) Red Dye.*

V. CONCLUSIONS

Using the FDM technique at high frequency enables us to reduce the time constant of a lock in detection system to about 1 ms, which enables multispectral high speed absorption measurements of liquid samples as well as droplet microreactors. FDM allows the spectral filtering using electronics instead of optical filters. This system can potentially provide several benefits: 1) *Scalability*: additional channels can be added economically. LEDs with a wide spectral range (200-2000nm) are commercially available. 2) *Low Cost:* achieved by using a single light detector and interrogation window, low-cost optoelectronics, and fewer optical components. In addition to the cost, it is simple, compact and consumes low power (<3W). 3) *Sensitivity*: Lock-in detection inherently reduces measurement noise up to 100dB, enabling nanomolar sensitivity [14]. We are presently working on improving the coupling of the optics to the flow cell to achieve high speed measurements using smaller tubing. This technique can potentially be expanded to multiplexed fluorescence detection.

REFERENCES

[1] M. O'Toole and D. Diamond, "Absorbance Based Light Emitting Diode Optical Sensors and Sensing Devices". *Sensors,* vol. 8, pp. 2453-2479, April 2008.

[2] T. Anfalt,.; A. Graneli,.; M. Strandberg, " Probe photometer based on optoelectronic components for the determination of total alkalinity in seawater," *Analytical Chemistry,* vol. 48, pp. 357-360, 1976.

[3] P. C. Hauser, D.W.L Chiang, " A photometric detector based on a blue light-emitting diode", *Talanta*, vol. 40, pp.1193-1200, 1993.

[4] G. Kurup and A.S. Basu, "Multispectral Absorbance Photometry with a Single Light Detector Using Frequency Division Multiplexing," *Proc. Micro Total Analysis Systems*, Groningen, The Netherlands: 2010.

[5] B. Chance, D Mayer, and V. Legallis, *Anal Biochem,* vol. 42, pp. 494–504, 1971.

[6] S. Werner, "A high performance dual-wavelength spectrophotometer and fluorometer," *J. Biochem Biophys. Methods* Vol.2,pp. 171– 81, 1980.

[7] S. Werner, "A high performance micro-dual- wavelength spectrophotometer (MDWS)," *J.. Biochem. Biophys. Methods,* vol. 58, pp. 15–24, 2004.

[8] D.B. Oh, M.E. Paige, and D.S. Bomse, "Frequency modulation multiplexing for simultaneous detection of multiple gases by use of wavelength modulation spectroscopy with diode lasers," *Applied Optics*, vol. 37, pp. 2499-2501, 1998.

[9] Y. Suzuki, H. Hori, M. Iwatsuki, and T. Yamane, "A four-wavelength channel absorbance detector with a light emitting diode-fiber optics assembly for simplifying the flow-injection analysis system," *Analytical Sciences*, vol. 19, pp. 1025-1028, 2003.

[10] R. Ziemer, and W. Tranter, "Principles of Communications; Systems, Modulation, and Noise,"6th edition, John Wiley & Sons, 1976, ch. 3.

[11] H. John, J. H. Scofield,. "Frequency-domain description of a lock-in amplifier," *American Journal of Physics* (AAPT), *vol. 62, pp. 129–133, Feb. 1994.*

[12] P. C. D. Hobbs, "Building Electro-Optical Systems-Making It All work," by John Wiley & Sons, 2000, ch. 3 and 18.

[13] M. Boukadoum and A. Obaid, "high-speed, low input current transimpedance amplifier for led-photodiode pair," in Conf. Rec. 2007 *IEEE Int. Conf. on Signal Processing and Communications*, Dubai, United Arab Emirates.

[14] L. Novak, P. Neuzil, J. Pipper, Y. Zhang, and S. Lee, *Lab on a Chip,* vol. 7, , pp. 27-29, 2007.