Phase Sensitive Detection of Lateral Flow Test Strip Signal – Proof of Concept

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***Abstract*—Lateral flow test strips are paper strips that determine the presence of a disease-causing biomarker as a purple-colored test spot. Quantification of the amount of biomarker present requires a reader that estimates the intensity of the purple-colored spot. The aim of this project is to detect different intensities of purple using the concept of reflection and phase-sensitive detection (PSD) and present a proof-of-concept for a Lateral flow test strip reader based on basic electronic circuits without any added image processing.**

***Index Terms*—lateral flow test strip, phase-sensitive detection, LASER**

I. Introduction

Gold Nanoparticles based Lateral flow immunoassays are paper-based assays at the point-of-care for the diagnosis of a large number of diseases. These paper test strips determine the presence of certain disease-specific biomarkers in bodily fluids qualitatively as well as quantitatively. While the presence of a purple-coloured test spot on the test strip gives a qualitative result (can be seen by the naked eye), quantification of the results requires a reader that can estimate the intensity of the purple spot. The intensity determines the concentration of the biomarker present [1]. This project focuses on reading intensities of different purple scale paper strips using basic electronic circuits without any added image processing. Our main aim is to check the feasibility/ proof-of-concept that a better version of our setup can be used as a Lateral flow test strip reader.

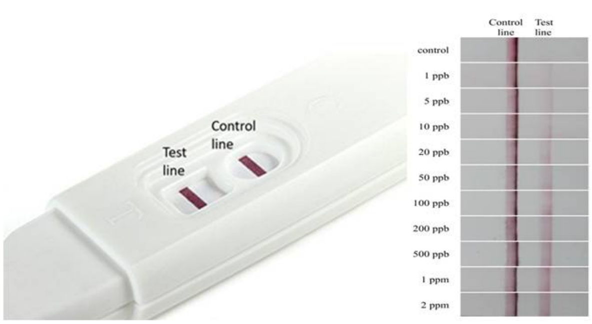

Fig. 1. Lateral Flow test Strip showing various intensities corresponding to various concentrations of Biomarker [1]

Our setup uses a green laser, a Photodiode as a sensor and the concept of reflection to estimate different purple intensities. Purple color is a mixture of blue and red; hence, a green laser is chosen as a light source. Green laser light when shined on the purple test strip, will get absorbed and reflect less light and vice versa in case of the white test strip (no spot). The reflected light is then directed onto the photodiode. Plastic Optical Fibers (POF) are used to direct the light from the laser to the test strip and finally to the photodiode. The concept of phase-sensitive detection is also used to make system detection insensitive to ambient light (noise).

II. CIRCUIT USED

A. LASER and BPW21 Photo-diode

The intensity of the purple test strips are measured by lighting them with a TL532D1250A model of green LASER, being a coherent and collimated source. The wavelength of the green Laser is 532nm with operating power less than 10mW. The LASER is excited by a 5V, dc pulsed square wave of 80Hz. The LASER requires a minimum current of 200mA to drive which is given by a driving circuit, as shown in Fig. 2. The driving circuit is made from a hex NOT gate IC-7406. Since a NOT gate in that IC can support a max 50mA current flow, hence four NOT gates input and output terminals were shorted and the LASER is connected at the V_{cc} pin and shorted output pin.

The BPW21 photo-diode is a silicon photodiode suitable for applications from 350 nm to 820 nm. It operates in reverse biased condition and is used to detect the reflected rays in terms of photodiode current. The photo-diode has maximum sensitivity at 550 nm and has a response of more than 90% for green wavelength, which is the wavelength of operation.

B. I-V Converter

The response of the photodiode to the reflected rays is in the current. Therefore, the photodiode current is converted to voltage using an operational amplifier based I to V converter circuit as shown in the Fig. 2. The signal is further amplified using an amplifier before feeding it into the phase-sensitive detector (PSD) circuit.

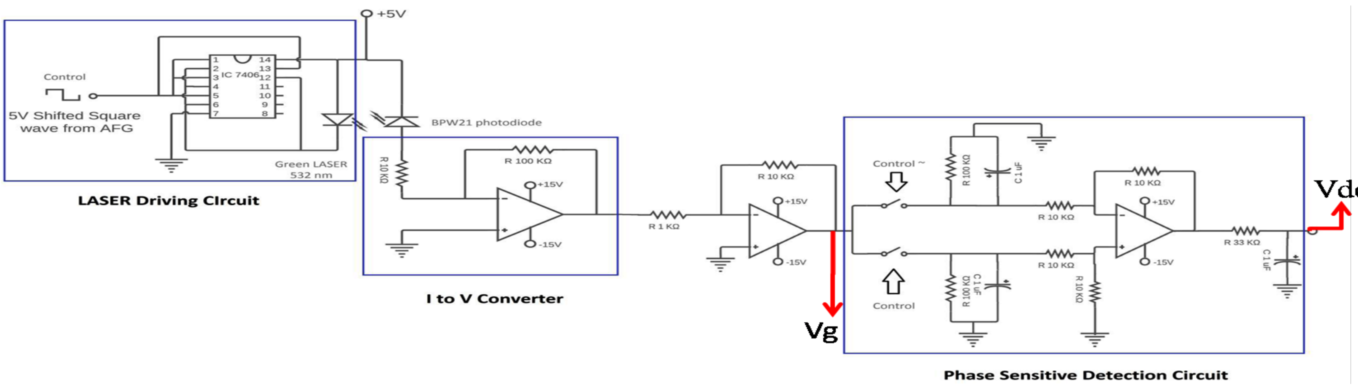

Fig. 2. Circuit diagram for Experimental Setup

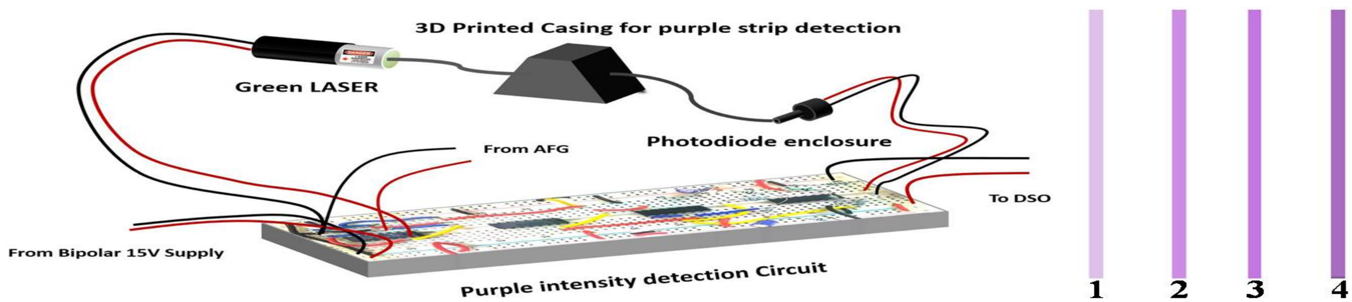

Fig. 3. Schematic of Experimental Assembly with different purple intensity test strips

C. Phase Sensitive Detection

The PSD circuit is used to detect very small signal by rejecting the ambient noise. The LASER is excited by a 5V, dc pulsed square wave of 80Hz. When LASER is ON, both the output signal as well as noise due to external sources is obtained at V_g, while, when LASER is OFF, only ambient noise is obtained at V_g. The output signal, V_g (Fig. 2) has been given to two switches. CD 4066 is used for switching operations. The control signals, control and ~control(as shown in Fig. 2) are of equal amplitude and 180 degrees out of phase. When control is high, the signal along with noise is passed through the switch and stored in the corresponding envelope. When control is low, ~control is high, and only the ambient noise signal is passed through the switch and stored in the corresponding envelope. Both capacitors hold a charge during OFF cycles of corresponding control signals and are subtracted to give output voltage, independent of ambient noise.

D. Subtractor

The role of the subtractor circuit is to eliminate the common noise signal in both the upward and downward envelope detectors. The lower envelope detector circuit (as shown in Fig. 2) has a capacitor which gets charged according to the detected signal plus ambient noise amplitude. Whereas the upper envelope detector circuit gets charged only with the ambient noise in the next cycle. The subtractor circuit finally eliminates noise by subtracting the two signals from envelope detectors and offer the sensed purple concentration at the output along with some ac component, which has been taken care of by the low pass filter.

III. EXPERIMENTAL ASSEMBLY

The entire functional purple intensity detector circuit has seven components:

1) Green LASER source (532 nm).
2) 3D printed casing containing the measurand strip.
3) Photo-diode (BPW21).
4) Signal conditioning circuit.
5) Arbitrary Function Generator (AFG).
6) Bipolar DC power supply.
7) DC voltmeter.

The LASER is shined on the purple strip using input optical fiber (for directionality and diffraction free channelling). The reflected light is gathered using output optical fibre and allowed to fall on the photodiode. These two fibers are placed orthogonally and are embedded in the 3D printed casing, as shown in Fig. 3. The photodiode produces a corresponding current output, which is then conditioned to remove unwanted noise components by the signal conditioning circuit, which is then displayed on a DSO and voltmeter. AFG is used for providing a control clock signal. A LP filter with a cut-off frequency of 5Hz is designed to get dc output for 80Hz input signal.

IV. EXPERIMENTS PERFORMED AND RESULTS

A. LASER Calibration & Characterization

The experiments are performed to check the optimum operating voltage and frequency of the Laser. The Laser is calibrated for operating voltage by checking the response of sensor at V_g with different operating voltages of the laser at a fixed frequency of 600Hz and is plotted in Fig. 4. The Laser does not excite at a voltage below 2V. The operating voltage of 5V is chosen, seeing maximum, V_g response of the sensor and highest operating voltage suggested by the manufacturer. The frequency response of the laser is checked by directly shining the photodiode with the laser at an operating voltage(fixed) of 5V and is plotted in Fig. 5. The response is maximum for lower frequency, and so 80Hz is chosen.

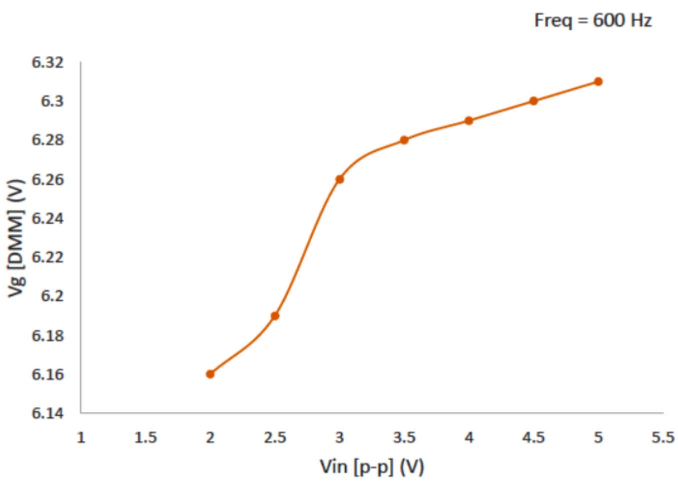

Fig. 4. Operating Voltage Response of the Laser

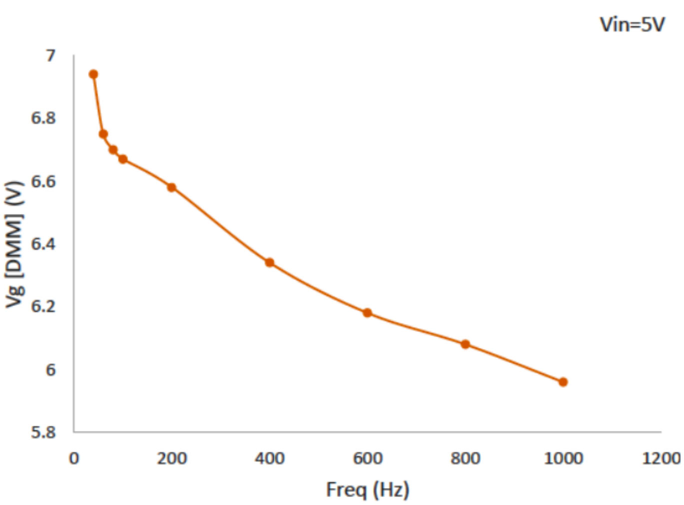

Fig. 5. Frequency Response of the Laser

B. Repeatability

Repeatability measures the variation in measurements taken by a single instrument or person under the same conditions over a different period of time. The measurements are taken for eight days and are plotted in Fig. 7. The output voltage, V_{dc}, of the darkest shade of purple(test strip 4) is subtracted from every shade of test strips, including white to normalise. The voltage(V_{dc}) decreases with the decrease in the intensity of purple test strip. The pattern remains the same for all eight days with a significant difference between two test strips as shown in Fig. 7.

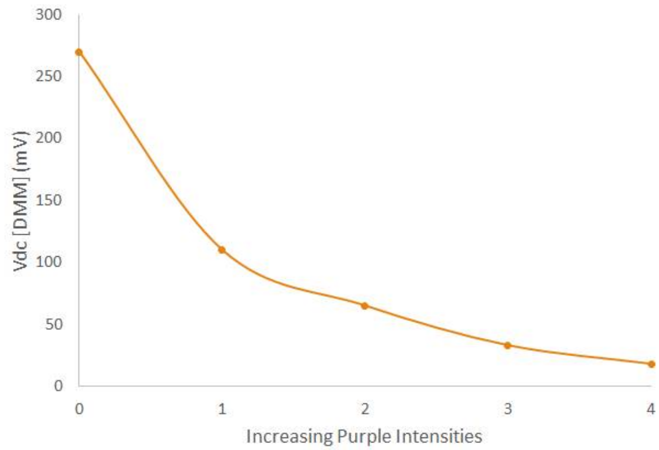

Fig. 6. Response with varying pink intensity

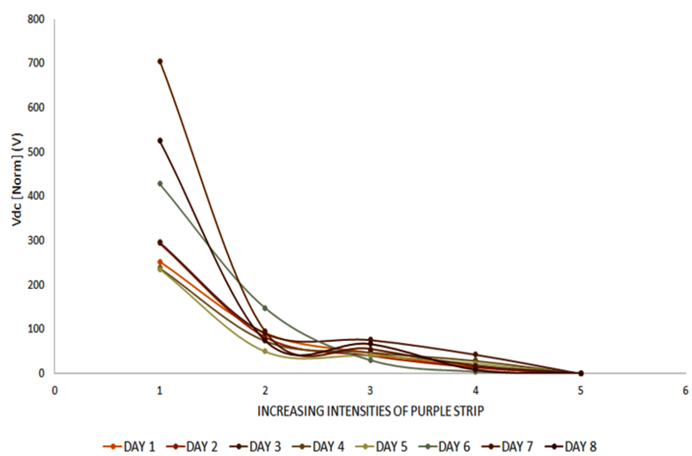

Fig. 7. Repeatability

C. Reproducibility

Reproducibility is the variation in measurement when taken using different test strips samples by different observers at different point of time. Our setup does not use hardboard test strips, and so the orientation of the plane of different test strips is different for the incident light. Though a similar decreasing trend can be observed for different samples, the corresponding dc voltage difference in intensities of test strips is not the same in our setup.

V. INFERENCES AND CONCLUSIONS

The different intensities of purple test strips are identified suppressing the ambient noise. Repeatibility is tested and verified as a scientific method for measurements. Reproducibility is difficult in our setup as it is highly sensitive to the orientation of the plane of the test strip. Hence, a more optimum setup with very well defined dimensions is required. The reflection is both diffused and regular, but our setup concentrates more on the regular reflection by placing both the optical fibre at positions obeying the law of reflection. Our setup should collect all the reflected rays for better result and reproducibility.

VI. FUTURE WORK

In later stages, as an extension of this project for the scope of improvements, we can utilize lens assembly instead of output fibre to maximise the gathering of reflected light (both regular and diffused reflections). Also, we can ensure the plane of incidence normal to the incident and reflected lights by using a glass slide over the test strip.

CONTRIBUTION AND ACKNOWLEDGEMENT

Contribution of Jasmeen, Shaurya and Ashish in the following:

- Laser and Sensor calibration & characterization: All
- Circuit design & Experimental Setup: All
- Logistics: All
- Analysis: All
- Report: All

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[1] Guo, Zebin, et al. "Lateral flow test for visual detection of silver (I) based on cytosine-Ag (I)-cytosine interaction in C-rich oligonucleotides." Microchimica Acta 184.11 (2017): 4243-4250.