

Development of a 3D microfluidic Lab on a Chip for light spectrometry analysis of enzyme kinetics

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Abstract— This paper presents the development of a low cost three dimensional light spectrometer microfluidic device manufactured with 3D printing technologies for the study of enzyme kinetics.

I. INTRODUCTION

The rationale set out by Michaelis & Menten in 1913 determines the steady-state kinetics of enzyme-catalyzed biochemical reactions. These kinetic analyses can inform us on the efficiency of enzyme catalysis, substrate preference and inhibition characteristics [1]. Michaelis-Menten kinetics relies on the study of product formation (or substrate depletion) over time, which is often determined by recording the characteristic UV or visible light during the biochemical reaction. We use simple electronics components and advanced manufacturing for 3D microfluidic devices to develop a low cost light spectrometer suitable for monitoring enzyme kinetics. We tested the device by performing kinetic analysis of the hydrolysis of p-nitrophenol-phosphate (pNPP) using the phosphatase enzyme.

II. MICROFLUIDIC DEVICE DESIGN

The experiment studies the light absorption (at 405nm) of a pNPP solution at differing concentrations. We prepared a 10 mM stock solution and made subsequent solutions down to 0.019 mM using the doubling dilution method. The light spectrometer consists of an Ultra Violet Light-Emitting Diode (LED) (Bivar, UV3TZ-405-15) emitting at 405 nm and a visible light phototransistor (PT) (Vishay, TEPT4400 with 10 k Ω resistor). To protect from unwanted exposure coming from the surroundings and light leakage we applied heat-shrink tubing around the LED and phototransistor, leaving a 2 mm window at the top of the lens. We glued the LED and PT

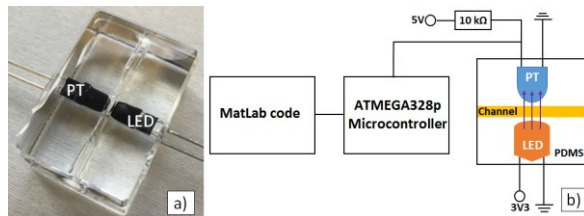


Figure 1. a) Microfluidic device used for experiments, with LED and PT embedded in PDMS & b) Diagram of experimental setup.

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facing each other, across a 1.5 mm channel on a 3D printed ABS plastic negative mold. We manufactured the polydimethylsiloxane (PDMS) microfluidic device following a 3D soft-lithography method we developed previously [2, 3]. Fig. 1 shows the final device. The voltage reading transmitted by the PT, which correlates to the amount of light received, is measured with an ATMEGA328p (Atmel Corp., USA) at a 100 ms interval for 30 s. The measurement was repeated 5 times for each concentration.

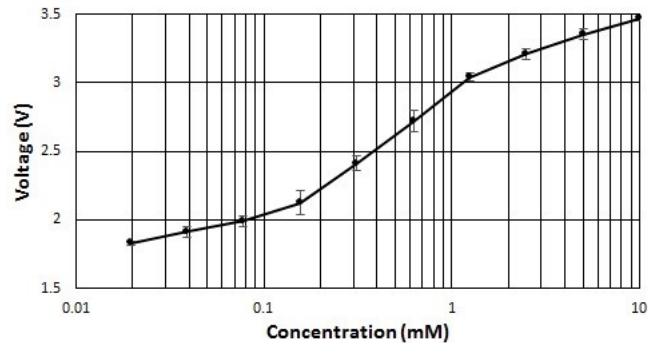


Figure 2. Voltage reading vs. concentration of pNPP. The PT exhibits a log output voltage vs. light intensity input hence the logarithmic scale.

III. RESULTS

This experiment was able to show that voltage correlates to concentration of pNPP. The 3D microfluidic device was capable of detecting a wide range of concentrations, from 10 mM to 0.019 mM, as shown by Fig. 2. Rapid prototyping of 3D microfluidics is an important approach for new low cost integrated systems which use light absorption based analysis.

IV. FUTURE WORK

This experiment is a crucial step in developing the underlying technology for a more complex microfluidic device that will be able to mix and analyze different concentrations of enzyme-catalyzed biochemical reactions over time. This proposed system will create an integrated, and low cost, solution for the study of enzyme kinetics.

REFERENCES

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