# Detection of Urinary Tract Infections on lab-on-chip device by measuring photons emitted from ATP bioluminescence

Shilun Feng, Tao Dong*- *IEEE Member*, and Zhaochu Yang

***Abstract*— A microfluidic Lab-on-chip (LOC) platform for in vitro detecting Urinary Tract Infections (UTI) for clinical diagnostic applications has been built. Based on one commercial adenosine 5'-triphosphate (ATP) assay kit, one chip designed before was applied to detect UTI with the help of photomultiplier tube (PMT) and quantitative determination was made by measuring the photons of light emitted in the bioluminescent reaction of ATP with the enzyme luciferase. The chip had been tested and materials had been well prepared before testing the PMT detecting system. The data from PMT were visualized by the Labview™, appearing good linearity between voltage values and the concentration of the ATP ranging from $2\times10^{-12}$ M to $2\times10^{-8}$ M. Fresh urine sample with different amounts of *Escherichia coli* had been measured by the system, appearing good linearity trend between the voltage values and number of the *E.coli*. This study successfully expressed the concept of measuring ATP directly in the urine to quickly and accurately detect UTI on a microfluidic chip.**

## I. INTRODUCTION

The urinary tract is the most common organ system to experience bacterial infections [1]. Urinary tract infections are the most common infections in both hospitalized and community patients [2], which are common infects causing serious morbidity and significant expenditures in healthcare dollars and lost wages [3].

Use of bioluminescence as a urine screen was first described in 1944 [4]. Application of the luciferase enzyme and luciferin to the detection and quantitation of bacteria was originally described by Chappelle and Levin, who used a luminometer to measure bioluminescence (as relative light units [RLU]) [5]. Firefly lantern bioluminescence is produced by a biochemical reaction in which the luciferase enzyme utilizes ATP in the oxidation of luciferin to adenyl-oxyluciferin, with the concurrent release of photons in proportion to the amount of ATP that is converted to AMP. The mechanism of the bioluminescent reaction occurs [6] as follows:

$$\text{ATP} + \text{luciferin} + O_2 + \text{luciferase} \rightarrow \text{AMP} + \text{oxyluciferin} + CO_2 + \text{PPi} + h\nu \quad (1)$$

Luciferase produces cold light (~560 nm) by the ATP-dependent oxidation of d-luciferin. Consequentially, the amount of ATP is directly associated with the amount of emitted light by luciferase, which in turn represents the amount of cells in the solution.

Although an incubation step is required, urinary ATP concentration luminance analysis method can provide the rapid and reliable result. Several problems including the presence in urine of free nonbacterial ATP, the intracellular ATP contained in somatic cells, and luciferase inhibitory substances and the variation in ATP content among bacterial species appears in initial attempts to apply this methodology to the enumeration of bacteria in clinical urine specimens, all of which compromised the quantitative aspect of the assay [7-8]. These problems were efficiently solved by using buffers and reagents for the release and destruction of nonbacterial ATP in clinical urine specimens. The reported threshold for positivity was $10^5$ CFU/ml, with a sensitivity of 86 to 95%, a specificity of 75 to 82%, a positive predictive value of 36 to 77%, and a negative predictive value of 88 to 95% [9-10]. It is reported that urinary ATP concentration analysis is useful for determining urinary tract infection and renal damage caused by drugs and by means of the firefly luciferin-luciferase method, reference value was established as $1.77 \times10^{-10}$ M to approximately $7.70 \times10^{-9}$ M. [11]

Another paper reported that using the microfluidic device with ATP standard solutions, the bioluminescence intensity was linearly correlated with $2\times10^{-12}$ M to $2\times10^{-8}$ M of ATP [12] , which was more accurate, however, it was not applied for detecting UTI. Thereby, in this paper, I combine the usage of microfluidic device to detect the ATP as assay, it can not only quickly get the accurate result ranging from $2\times10^{-12}$ M to $2\times10^{-8}$ M but also prevent the poor stability and high cost of luciferase and d-luciferin in bioluminescent reactions .

Photomultiplier tube (PMT) based detection module is also installed for quantify weak signals of the bioluminescence reaction (quorum sensing) [13]. It has been instantaneously recording the maximum bioluminescent light signal emitted from the whole reaction process by measuring the changes of the voltages on the Labview™. Usage of PMT by measuring wavelength to count luminance photons one by one had been studied and would be used in the future researches, which has better sensitivity and seems promising. Considering the structure of one chip [14] which is quite suitable for the firefly luciferin-luciferase method and PMT

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T. Dong (IEEE member: 90889664) is with the department of Micro and Nano Systems Technology (IMST, TekMar), Buskerud and Vestfold University College (HBV). (PhD T. Dong is the corresponding author, e-mail: Tao.Dong@hbv.no; phone: +47 3303 7731 fax: +47 3303 11 03).

S.L. Feng is with the department of Micro and Nano Systems Technology (IMST, TekMar), Buskerud and Vestfold University College (HBV), Norway. (E-mail: shilun.feng@student.hbv.no).

Z.C. Yang is with the department of Micro and Nano Systems Technology (IMST, TekMar), Buskerud and Vestfold University College (HBV), Norway. (E-mail: Zhaochu.Yang@hbv.no )

detection, I applied this chip for the application of detecting UTI. The chip is composed of two counter-flow micromixers, a T-junction droplet generator and time delay channels (TD-Cs). Urine sample which had been pretreated and nucleotide releasing buffer were imported into the first micro mixer at rate of 1μL/min where the ATP would be totally released. And then the mixed solution and *Luciferin* were imported into the second micromixer at the rate of 1.8 μL/min, after which the droplet generator encapsulated them inside aqueous droplets separated by air. Air flow was the disperse medium, which could guarantee sufficient oxygen supply for the cells in droplets and also control the time that solution stayed in the channel of TD-Cs. The TD-Cs is a round spiral channel which can provide the platform for the detection of the PMT. The system showed high reliability and stability through numerical and experimental investigations. In the microfluidic domain, the analyzer is based on continuous flow, using syringe pumps. The system includes the flow in the chip, the PMT photon detecting and electrical circuit to connect to the PC, and the software including Labview™ to visualize the data. Continuously, it had been tested by the ATP standard solution. After which, fresh normal Urine samples had been mixed with different amounts of *E. Coli* cells and tested, which proved feasibility of detecting UTI.

This paper have successfully given out the concept of using microfluidic chip as a platform providing bioluminescent reaction happening and using the PMT system detecting the luminance photons emitted and finally getting the signals for detecting UTI accurately and quickly.

## II. MATERIALS AND METHODS

### *A. Preparation of biological samples*

ATP Assay Kit (119107-1KIT) was purchased from Merck Chemicals Ltd. (Padge Road, Beeston, N G9 2JR Nottingham, United Kingdom). The reagent is constituted of one vial of Luciferase ATP Monitoring Enzyme, Enzyme Reconstitution Buffer, a bottle of Nucleotide Releasing Reagent, one vial of ATP Standard, and a user protocol. The assay is based on the firefly luciferase-catalyzed oxidation of D-luciferin in the presence of ATP and oxygen, where the amount of ATP is quantified by the amount of light produced.

Prepare the ATP standard solution by dissolving 1mg ATP into 1ml of $H_2O$, and store it in the fridge at -20℃, which will be used to detect the whole system. Remove different volumes of normal urine medium and $10^4$-$10^6$ *E.coli* cells cultured in Luria Broth (LB) medium with 10g/L Tryptone, 5g/L Yeast extract and 10g/L NaCl as inlet 1, nucleotide releasing buffer as inlet 2, they are mixed at first Micromixer for about 5min at room temperature. Reconstitute ATP Monitoring Enzyme with 2000 μ L of the Enzyme Reconstitution Buffer freeze at -70℃ for future use and then use 1ul ATP monitoring enzyme being diluted with 49ul Nucleodtide releasing buffer as inlet 3 for the chip. ATP and enzyme solutions were pre-treated by Multifunctional sample preparation kit [15] and injected into the microfluidic chip at different flow rates through 3 inlet ports separately.

### *B. Installation of the microfluidic chip*

In this study, a designed chip [14] as shown in Fig. 1,

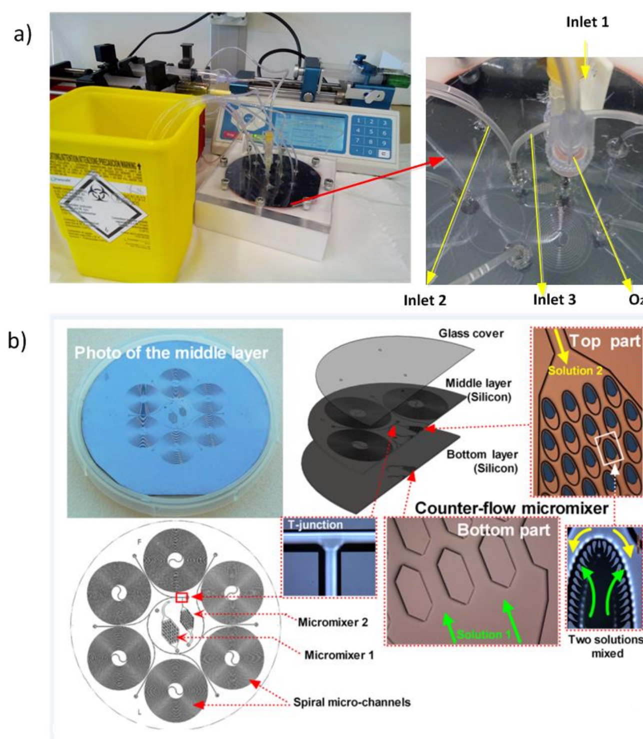



Figure 1 Description of the cell-based LOC device; (a).Application of LOC chip; inlet 1 for the samples, inlet 2 for the nucleotide releasing buffer and inlet 3 for the mixed ATP monitoring enzyme; (b).Construction of the cell-based LOC device [18]; The chip has three layers, but the three major structures are in the middle layer, including two counter-flow micromixers, a T-junction droplet generator and six spiral micro-channels. A photo of the device is shown in the top left corner. The schematic and micrographs of the counter-flow micromixer are illustrated at the top right part, which houses the counter-flow units (on the middle layer) and the inlet channels (on the bottom layer). The tiny inlet port is located in the center of the counter-flow unit. The sample, buffer solution and the cell suspension are mixed and used to form droplet within the air flow.

composed of two micromixers, a T-junction droplet generator and six TD-Cs was used. The two micromixers recreate optimal environmental conditions for the release of the ATP from constant cells while the droplet generator encapsulates them inside aqueous droplets separated by air. The droplet flow had been investigated through numerical simulation since it represents an innovative two-phase flow that allows to oxygenate living cells and to operate at lower pressure than the common two-phase flow. The concentration of ATP in different levels of E. coli for different urine samples can be measured by quantifying the light intensity at the TD-Cs.

### *C. Building of the detecting system*

The whole system is shown in Fig.2, which includes the microfluidic chip, the PMT photon detector and read-out circuit connecting to a PC with the software of Labview to visualize the data. Form the voltage appearing on the Labview which reflects the intensity of the light, getting the relationship of the voltage with the concentration of the ATP. With the decrease of the concentration of the ATP that applied, we can detect the lowest detection concentration. After the whole system being built, different concentration of *E.coli* in the urine sample was transported to the chip system; after going through the chip, the signal was recorded with the PMT detecting system. After that, the signals were visualized by

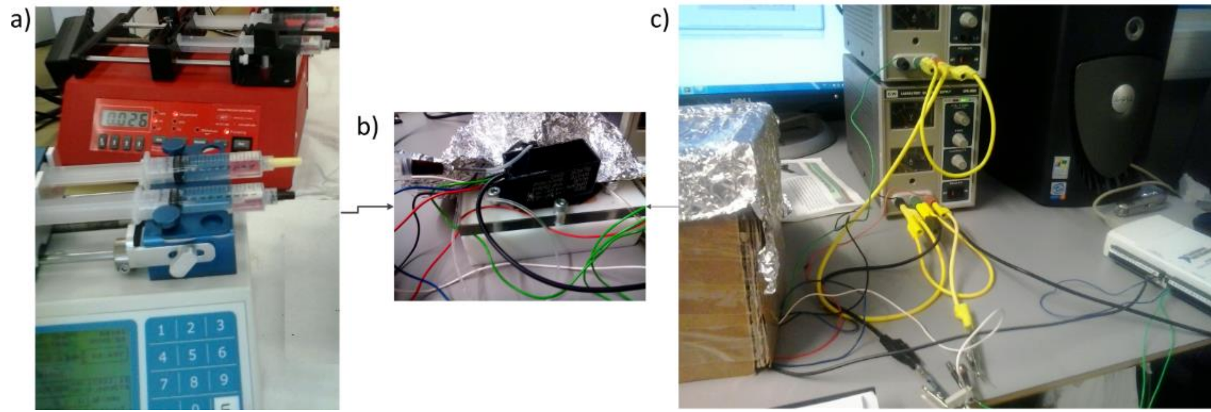


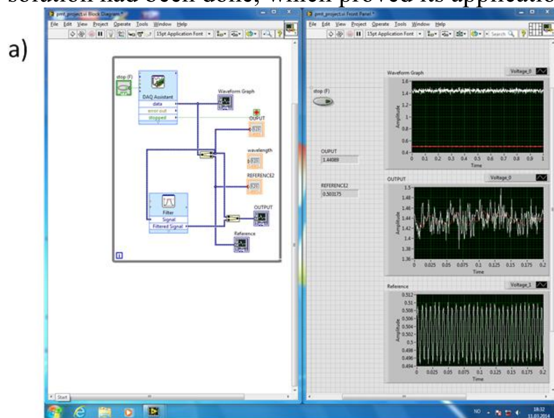

Figure 2 Testing of the whole system; (a).the flow of the chip. (b).the PMT photon detecting; (c). electrical circuit to connect to the PC and the software including Labview™ to visualize the data

Labview on the PC. the data of UTI can easily observed and shared [16].

## III. RESULTS AND DISCUSSION

### A. Testing of the whole system

Software of this system was designed, by measuring the output which is linearly corresponding with signal intensity. Hardware of this system was also designed, using two power supplies, one constant 4.7kΩ resistor, one 1kΩ potential resistor, several wires to connect and DAQ National Instruments. Both the testing of the software and hardware of the PMT and basic measurement of the voltage reflecting the bioluminance light intensity emitted from the ATP standard solution had been done, which proved its application.



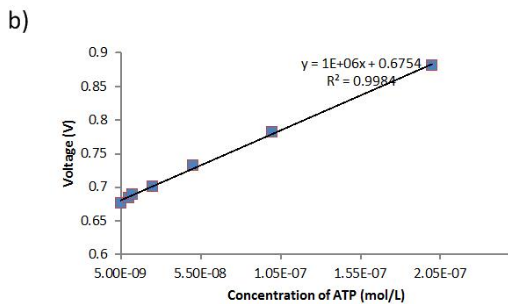




Figure 3 Testing of the whole system; (a) Labview™ was used to visualize the data by measuring the change of the voltage; (b) ATP standard solution was used to calibrate the whole system.

### B. Measurement of UTI

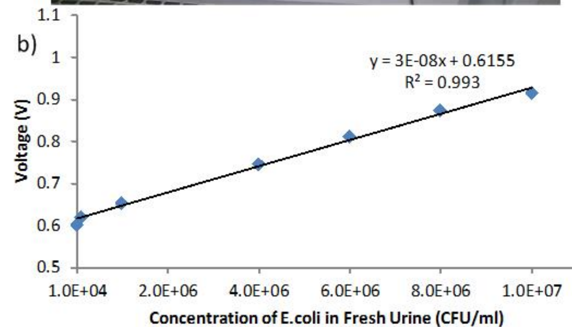



Figure 4 Detecting of the Urine with different amounts of E.coli

Pick up constant number of the *E. coli* growing in Luria Broth (LB) medium with 10g/L Tryptone, 5g/L Yeast extractand 10g/L NaCl and then dilute them in the constant volume of fresh urine and then transport them into the system separately. As Fig. 4 shown, the voltage has the linear trend with the concentration of the *E. coli*, which proves feasibility of detecting the UTI. *E.coli* cells were kept for 8 hours in the fridge at 4℃，however no signals were detected, which prove that the system can only detect the fresh samples.

### C. Testing the mixture efficiency of the microchip

Several experiments had been done for the microfluidic features of the chip including the refining microfiltration [17] and different inlets rates determine the mixture effect. Finally when urine sample form the inlet1 being at rate of 1μl/min, nucleotide releasing buffer solution flowing at 1μL/min from

inlet 2, and mixed with mixed ATP monitoring enzyme flowing at 1.8μL/min from inlet 3 and mixed solution was capsuled by the Air flow at the rate of 1.5μL/min, which provided a stable flow and meeting with the optimal condition for chemical reaction and detection.

### *D. The simply and more precise PMT detection system*

The aim of this paper is to apply the PMT detecting system to read the photons of the ATP luminance. A lot of investments have been done, however, what the experiments had been down is to have built a system using Labview™ visualized the dates form the luminance accumulated light by measuring the voltage appearing. In the latter work, improvement of PMT detection system will be done, the light signal can be measured by photons one by one, thereby creating the highest sensitivity and other methods will also be tried, including polycarbazole photodetector [18-19].

### *E. The efficiency of the detecting UTI on lab-on-chip*

Combing with the ATP assay and the microfluidic chip is designed to detect the UTI, which has the advantages of both fast reliable detecting and more precisely with very lower limit detections. Using LOC also prevents the poor stability and high cost of *luciferase* and *d-luciferin* in bioluminescent reactions. And it can also reused, which makes the less expenses. Moreover, because of lab-on-chip which is small and portable, more related experiments could be investigated based on this platform and it may render the complicated immuno-NASBA assays convenient to common users without special training [20].

## IV. CONCLUSION

ATP luminance method to detect UTI was applied on the microfluidic chip. One ATP commercial assay kit and one designed microchip had been combined and with the help of the PMT detecting system, the UTI could be quickly and precisely detected by reading the visualized data from the luminance photons emitted from ATP firefly luminance reaction. This combined method is promising because microchip can protect the ATP away from polluting to get an accurate result, moreover, by reading photons, the lower concentration can be figured out without urine culture, which provides the quick detection and saves high cost of *luciferase* and *d-luciferin* in bioluminescent experiments. In this paper, only simple experiments have been done for this promising conception, the more precise experiments will be done in the future.

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## REFERENCES

[1] Nicolle, L. 2001. Epidemiology of urinary tract infection. Infect. Med. 18153-162.

[2] M. Marschal, M. Wienke, S. Hoering, I. B. Autenrieth, and J.-S. Frick, “Evaluation of 3 different rapid automated systems for diagnosis of urinary tract infections.,” Diagn. Microbiol. Infect. Dis., vol. 72, no. 2, pp. 125–30, Feb. 2012.

[3] D. J. Schwartz, V. Kalas, J. S. Pinkner, S. L. Chen, C. N. Spaulding, and K. W. Dodson, “Positively selected FimH residues enhance virulence during urinary tract infection by altering FimH conformation,” vol. 110, no. 39, pp. 15530–15537, 2013.

[4] Min, J, “17β-Estradiol-stimulated eNOS gene transcriptional activation is regulated through the estrogen-responsive element in eNOS promoter,” Biotechnol.Bioprocess Eng, vol. 12, pp. 446-449, 2007.

[5] Chappelle, E. W., and G. V. Levin. 1968. Use of the firefly bioluminescent reaction for rapid detection and counting of bacteria. Biochem. Med. 241-52.

[6] V. Ivancic, M. Mastali, N. Percy, J. Gornbein, J. T. Babbitt, Y. Li, E. M. Landaw, D. a Bruckner, B. M. Churchill, and D. a Haake, “Rapid antimicrobial susceptibility determination of uropathogens in clinical urine specimens by use of ATP bioluminescence.,” J. Clin. Microbiol., vol. 46, no. 4, pp. 1213–9, Apr. 2008.

[7] W. W. Nichols, G. D. Curtis, and H. H. Johnston, “Analysis of the disagreement between automated bioluminescence-based and culture methods for detecting significant bacteriuria, with proposals for standardizing evaluations of bacteriuria detection methods.,” J. Clin. Microbiol., vol. 15, no. 5, pp. 802–9, May 1982.

[8] J. C. Kolbeck, R. a Padgett, E. G. Estevez, and L. J. Harrell, “Bioluminescence screening for bacteriuria.,” J. Clin. Microbiol., vol. 21, no. 4, pp. 527–30, Apr. 1985.

[9] L. Selan, F. Berlutti, C. Passariello, M. C. Thaller, and G. Renzini, “Reliability of a bioluminescence ATP assay for detection of bacteria.,” J. Clin. Microbiol., vol. 30, no. 7, pp. 1739–42, Jul. 1992. 12. Semeniuk, H., J. Noonan, H. Gill, and D. Church. 2002.

[10] M. Pezzlo, “Detection of Urinary Tract Infections by Rapid Methods,” vol. 1, no. 3, 1988.

[11] H. Poor, An Introduction to Signal Detection and Estimation. New York: Springer-Verlag, 1985, ch. 4.

[12] T. Fukuba, Y. Aoki, N. Fukuzawa, T. Yamamoto, M. Kyo, and T. Fujii, “A microfluidic in situ analyzer for ATP quantification in ocean environments.,” *Lab Chip*, vol. 11, no. 20, pp. 3508–15, Oct. 2011.

[13] C. M. Waters and B. L. Bassler, "Quorum sensing: cell-to-cell communication in bacteria," Annu Rev Cell Dev Biol, vol. 21, pp. 319-46, 2005.

[14] X. Zhao and T. Dong, “A microfluidic device for continuous sensing of systemic acute toxicants in drinking water.,” Int. J. Environ. Res. Public Health, vol. 10, no. 12, pp. 6748–63, Dec. 2013.

[15] X. Zhao and T. Dong, “Multifunctional sample preparation kit and on-chip quantitative nucleic Acid sequence-based amplification tests for microbial detection," Anal Chem, vol. 84, pp. 8541-8, Oct 16 2012

[16] X. Zhao and T. Dong, "Design and Experimental Approach to the Construction of a Human Signal-Molecule-Profiling Database," International Journal of Environmental Research and Public Health, vol. 10, pp. 6887-6908, 2013.

[17] N. M. M. Pires and T. Dong, "Recovery of Cryptosporidium and Giardia organisms from surface water by counter-flow refining microfiltration," Environmental Technology, vol. 34, pp. 2541-2551, 2013.

[18] N. M. Pires, T. Dong, U. Hanke, and N. Hoivik, “Integrated optical microfluidic biosensor using a polycarbazole photodetector for point-of-care detection of hormonal compounds, ” Journal of Biomedical Optics, vol. 18, p. 097001, Sep 2013.

[19] N. M. M. Pires and T. Dong, "Ultrasensitive opto-microfluidic immunosensor integrating gold nanoparticle–enhanced chemiluminescence and highly stable organic photodetector," Journal of Biomedical Optics, vol. 19, 030504, 2014.

[20] X. Zhao, T. Dong, Z. Yang, N. Pires, and N. Hoivik, "Compatible immuno-NASBA LOC device for quantitative detection of waterborne pathogens: design and validation," Lab on a Chip, vol. 12, pp. 602-12, Feb 7 2012