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An approach to enhance self-compensation capability in paper-based devices for chemical sensing

Shih-Jie Lo, Kuan-Hung Chen, Da-Jeng Yao *

Institute of Nanoengineering and Microsystems, National Tsing Hua University, Hsinchu 300, Taiwan

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ABSTRACT

This paper describes a simple design for increasing the tolerance of reagent dislocation on a paper-based platform using a combination of wax-treated paper and a vortex mixer. To date, massive budgetary funds are required in the biotechnological industry to develop new applications; a large part of that cost is attributable to the screening of specific chemical compounds. Here, we propose using a liquid-handling robot to automatically deposit selected reagents on a paper-based platform. We also present a preliminary concept approach for developing a reagent placing device with simple and inexpensive features. A defect of inaccuracy was observed between droplet location and test well location after viewing the performance of the liquid-handling robot on our paper-based platform. Because of dislocation error resulting from robotic reagent placement, we decided to apply an external, rotational force following droplet placement in order to compensate for the distance of reagent dislocation. Note, the largest distance of reagent dislocation was determined by examining the results of altering applied reagent volume, but not concentration, in volumes from 5 μL to 30 μL in a series of experiments. As a result of these experiments, we observed that dislocation was positively affected by an increase in applied volume. A colorimetric assay for nitrite detection was also performed to confirm the feasibility of this method. This work, we believe, can minimize the cost of chemical compound screening for the biotechnological industry.

1. Introduction

Paper-based analytical devices have been developed in recent years for a wide array of detection needs such as monitoring specific molecule concentrations [1,2], diagnosing infectious diseases [3–6], and investigating food safety issues [7,8]. For these issues, well-designed and well-functioning paper-based devices have already been developed [9,10], whereas some studies [11–13] still use paper as a substrate in an array that requires other equipment (i.e., pipettes) to carry out analysis. Paper has become a popular substrate for fabricating point-of-care (POC) diagnostic devices because of its versatility and cost. However, current biotechnological industries still rely on the plastic plate (i.e., 96-well) while screening for specific chemical compounds, and individual tests (for the plastic 96-well plate) require at least 50 μL of reagent for chemical reaction, as opposed to paper-based 96-well plates, which require only 2 μL (even less). The cost of the consumed reagents is a hindrance to drug development, specifically as it relates to screening for specific chemical compounds. We further note that individual placement of reagents by laboratory staff using pipettes is tedious and inefficient, and multichannel pipette devices and/or liquid-handling robots (i.e., Tecan Genesis in our study) have become necessary to improve efficiency. Liquid-handling robots are capable of automatically performing scheduled reagent and wash handling steps for ELISA using plastic plates. Although these robots save human resources in experimental operations, they are expensive and not available to all research and developmental groups. While liquid-handling robots work efficiently in processing plastic plates, the total cost of the consumables in each research project is still high because of the cost of chemical compounds for screening. As Table 1 shows, the cost of each plastic plate is approximately \$1.50, a price one hundred times more expensive than paper (\$0.01) without any chemical treatment. If reagent cost is taken into account, the total cost savings per assay is considerable because paper requires less total reagent, but varies depending on the reagent used. Here, we propose using a paper-based platform to screen for specific chemical compounds in order to reduce the cost of used reagents and consumables by employing the low-cost and reagent-saving characteristics of paper-based devices.

The depositing tips of a liquid-handling robot may become impaired if they incur repetitive hits to plastic well edges.

* Corresponding author.
E-mail address: djyao@mx.nthu.edu.tw (D.-J. Yao).

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Table 1
Comparisons between a plastic 96-well plate and a paper-based 96-well plate.

Plate type		Plastic	Paper
Reagent volume (μL/well)		50–100	2–5
Cost per plate		$1.5	$0.01
Reagent cost per assay	**Cancer-related kinase inhibitor assay**	$3.0–5.0	$0.2–0.4
	Nitrite detection assay	$0.0183–0.0367	$0.0007–0.0018
Total cost per 96-well assay	**Cancer-related kinase inhibitor assay**	$300–500	$20–40
	Nitrite detection assay	$3.2568–5.0232	$0.0772–0.1828
Flexibility		Low (even no)	High
Surface-to-volume ratio		Low	High
Biodegradability		No	Yes

However, multichannel pipette devices and liquid-handling robots are not well suited for paper-based platform use because paper lacks vertical well-wall guides. More importantly, a liquid handling robot operates smoothly with large sample volumes (above 5 μL), but can have problems with small droplet manipulation, because samples can be splashed or unplaced. Splashes may occur when the necessary force to completely expel small droplets is too great, but small droplets remain unplaced if not enough force is applied. One of the main advantages of paper-based platforms is that they spare sample because they require little. Moreover, small solution volumes evaporate quickly and save time.

Yield can be improved by increasing positioning accuracy and reducing what we call the tolerance of reagent dislocation; this decreases cost, reduces production time, and makes liquid-handling robots more compatible with paper-based platforms. The tolerance of reagent dislocation is defined as the capability of substrate to adjust droplet position to the original location if the droplet is undesirably placed. Note, the tolerance of reagent dislocation on a 96-well format patterned paper is better than that for a plastic plate (without surface modification). In sum, we would expect great advantages when screening for chemical compounds with a paper-based platform because such an approach is versatile, reagent-saving, and extremely inexpensive. Using a paper-based approach minimizes the cost of research and design stages and improves efficiency to the benefit of the biotechnology industry [14–16]. The objectives of this study are as follows: (1) investigate dislocated tolerance on a paper-based platform with different volumes of reagent to determine error distance when using a liquid-handling robot; (2) improve tolerance when using a paper-based platform and a pipette by leveraging hydrophobic–hydrophilic interactions and applying externally rotational force via a vortex mixer; and, (3) demonstrate the practicality of this improved method via a colorimetric assay of nitrite concentration. This is expected to benefit microarray yield while developing a suitable liquid-handling machine process for paper-based devices that is relatively inexpensive and easy-to-use.

2. Materials and methods

2.1. Chemicals and materials

Allura Red AC (No.: 458848) was used for observing the experimental performance. The nitrite detecting indicator [17] consists of 50 mM Sulfanilamide (No.: S9251), 10 mM N-(1-Naphthyl) ethylenediamine dihydrochloride (No.: 222488), 330 mM citric acid (No.: 251275). Sodium nitrite (No.: 237213) with 10 μL was used as a standard sample for creating the calibration curve. All the chemical compounds were purchased from Sigma Aldrich. Whatman Qualitative Filter Papers (Whatman grade No. 1 filter paper; No.: 1001-185) and a wax printer (Xerox Phaser 8560DN) were used to fabricate our paper device. A liquid-handling robot (Tecan Genesis Freedom 200) was used to automatically deposit droplets on our paper-based platform. A Vortex-Genie 2 mixer (Scientific Industries; No.: SI0236) was used to apply external rotational force for the droplet dislocation compensation experiments.

2.2. Tolerance analyzing

This experiment was designed to observe the accuracy of droplet placement on a paper-based platform when using a liquid-handling robot and to increase the tolerance of droplet dislocation. We collaborated with Industrial Technology Research Institute (ITRI) in Taiwan, and used a liquid-handling robot to automatically deposit droplets onto our paper-based platform. The distance of the parametric setting between tips and paper-based platform was approximately 3 mm. The droplets were placed onto the paper-based platform at volumes ranging from 5 μL to 30 μL, and the distance of droplet location from the hydrophilic well was altered (distances of 0, 2, 3, and 4 mm to the center of circle-shaped hydrophilic zone were used in the liquid-handling robot experiments; distances of 1, 3, and 5 mm to the edge of hydrophilic zone were used in the experiments examining distance compensation). Our paper-based platform was fixed onto a hollow plastic plate and kept as flat as possible. After determining the coordinate of the first spot (the upper left of wells) and the last spot (the bottom right of wells) and importing the column number for our paper-based plate, the liquid-handling robot was calibrated to automatically deposit droplets onto our paper-based platform. The accuracy of droplets entering into the wells was counted as a step toward increasing the tolerance of droplet dislocation after the robot finished the droplet setting procedure. A paper-based platform, set within the recess of a plastic plate, was fixed on the vortex mixer to increase the compensating probability at an approximate 5° angle. The setting of rotational rate on the vortex mixer from 0 to 10 was converted to revolutions per minutes (RPM) and found to be 600 to 3200 RPM. Droplets were deposited manually via pipette, and different speeds of rotational force were applied to compensate for droplet dislocation. A pulsing action was applied, i.e., three times for one second each time. The compensating percentage of droplet dislocation at different distances was then counted and analyzed.

2.3. Colorimetric assay

Sulfanilamide, citric acid and N-(1-Naphthyl)ethylenediamine dihydrochloride were mixed and used as the nitrite detecting indicator, which was pre-added to our test areas (hydrophilic zone) at a volume of 5 μL. Different concentrations of sodium nitrate ranging from 0 to 500 mM were deposited with a volume of 10 μL at different distances to the test area, and rotational force was externally applied at different speeds in order to compensate for the distance of droplet dislocation.

2.4. Data analysis

Percentage profiles were counted for our paper-based platform, using 48 test zones for the liquid-handling robot application and 30 test zones for the experiment that increased the compensated distance. The colorimetric assay experimental results were scanned using a document scanner (Microtek ScanMaker 5950SD) and the files were saved in 300 dpi JPEG format. Row images were split into three grayscale images representing red, green, and blue channels, and the grayscale image of the green channel was