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Short communication

# Webcam camera as a detector for a simple lab-on-chip time based approach

Wasin Wongwilai[a,b], Somchai Lapanantnoppakhun[a,b], Supara Grudpan[c], Kate Grudpan[a,b,*]

[a] Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Research Laboratory for Development of Analytical Instrumentation, Center for Instrumention and Process, Science and Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand
[c] College of Art, Media and Technology, Chiang Mai University, Chiang Mai 50200, Thailand

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ABSTRACT

A modification of a webcam camera for use as a small and low cost detector was demonstrated with a simple lab-on-chip reactor. Real time continuous monitoring of the reaction zone could be done. Acid–base neutralization with phenolphthalein indicator was used as a model reaction. The fading of pink color of the indicator when the acidic solution diffused into the basic solution zone was recorded as the change of red, blue and green colors (%RBG.) The change was related to acid concentration. A low cost portable semi-automation analysis system was achieved.



## 1. Introduction

A cost effective and portable analysis system is of interest for real time on-site studies. As many chemical analyses involve visual detection, the accuracy and precision are normally improved by using a spectrophotometer or a camera as a detection unit. However, a commercial spectrophotometer is rather expensive and bulky. To down size the analysis unit for portability, a built-in camera detector is more suitable. In addition, the image documentation is beneficial for other applications and further studies. For example, the images can be used for counting bacteria [1], for monitoring cell morphology change under certain conditions [2] and for online long distance diagnosis based on visual detection [3].

Various types of cameras such as a TV camera [1,4], digital camera [5] and mobile phone camera [3] have been reported for use as an alternative detector unit for chemical/biochemical analysis systems. The camera detector can replace a more expensive spectrophotometer such as colorimeter, fluorescence and chemiluminescence spectrophotometers [6–11]. In some cases, the camera detector is used together with another detector to provide additional information from the recorded image [2]. These cameras are normally based on either charge coupled device (CCD) or complementary metal oxide semiconductor (CMOS) sensor technology. Both technologies are for capturing images digitally with some similarities and differences in the signal processing, performance and price. Both types of sensors are pixilated metal oxide semiconductors that convert light into electrical charge and then to electrical signals. The difference is that each pixel of CCD transfers charge as an analog signal to be converted to voltage outside the pixel. A CCD requires an external analog-to-digital converter. On the other hand, a CMOS has its own charge-to-voltage conversion system integrated within each pixel and, therefore, the output is a digital signal. In theory, CCD images are superior but CMOS systems are smaller and work faster with a possibility to be produced at cheaper rate, though there have been controversial arguments among distributors of the two systems [12].

The charge coupled device (CCD) camera has been more widely adapted for use as detectors, possibly owing to its earlier and more mature development [13]. A webcam is a common accessory for computer/internet use. As compared to the CCD camera, the webcam is lower cost with the integrated circuit (CMOS) that works more effectively. Its application for immunoassay microplate reader after a detection reaction took place has been reported [14], but so far, the use of a webcam is still rare.

In this current report, another possible application of the webcam is described. This work combines the simple lab-on-chip previously reported by our group [15] and an inexpensive webcam detector in one unit. Here, the webcam was used for continuous monitoring of the chemical reaction that produces a change in color.

* Corresponding author at: Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
*E-mail address:* kate@chiangmai.ac.th (K. Grudpan).

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**Table 1**
Working concept of the control software for the LOC–CMOS system.

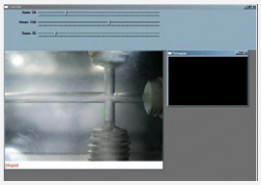
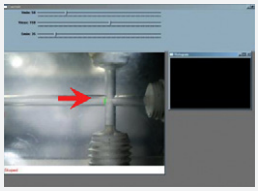
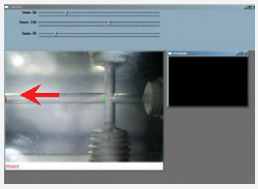
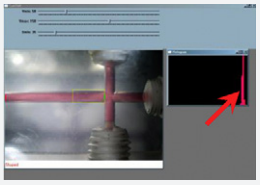
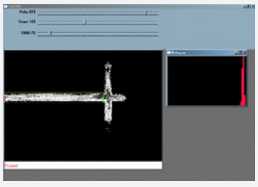
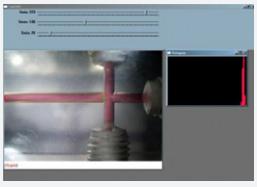
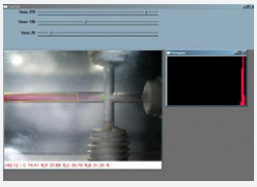
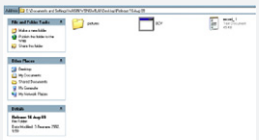
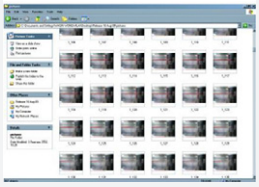
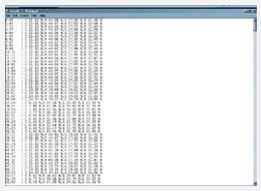
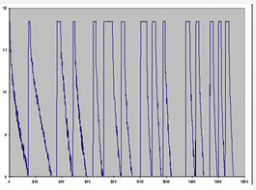
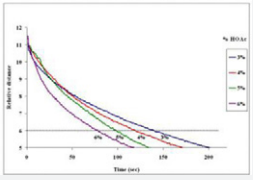
| Step | Sequence | Mode | Remark | Monitor screen |
|---|---|---|---|---|
| 1. | Receive and display photograph as a true picture on monitor screen | Stop | |  |
| 2. | Move cursor to select a starting point (of a channel) | | |  |
| 3. | Move cursor to select an end-point | | |  |
| 4. | By doing (2) + (3) a “relative distance” of a moving zone is defined | | | |
| 5. | Choose color to be performed further (and will be shown in the histogram) | | This is done after introducing indicator into the channel |  |
| 6. | Show black + white contrast (the white will represent the chosen color (in (5)) of the picture on the monitor screen, and then adjust the contrast to cover the “relative distance” | | |  |
| 7. | Change monitor screen back to the color picture: the picture as in (5) is redisplayed | | |  |
| 8. | Start to record the values of relative distance and % of (red, green and blue) every second | Running | |  |
| 9. | Real time display on the monitor screen showing the real color zone moving | | (After introducing acid solution in the channel) |  |

Table 1(*Continued*)

| Step | Sequence | Mode | Remark | Monitor screen |
|---|---|---|---|---|
| 10. | Stop running the test | Stop | |  |
| 11. | The data of relative distance values, values of %(red, green and blue) and pictures are saved in computer hard disc | | |  |
| 12. | The data of relative distance values and %(red, green and blue) are saved as text document file | | |  |
| 13. | The data are plotted and evaluated using an Microsoft Excel program: | | |  |
| | -plot of relative distance vs. real time<br>-plot of overlaid relative distance of each concentration vs. time | | |  |

Real time simultaneous detection of the progress of the reaction could be done. The best data set (i.e. sufficient sensitivity with short analysis time) from all the recorded data could be selected for further use/evaluation. With the control software, specially written for this task, the progress of chemical reaction could be followed from the percentage changes of red, blue and green colors (%RBG) of the reaction zone. The performance of the system is demonstrated using a simple acid–base neutralization reaction by monitoring the fading color of phenolphthalein when concentration of acid solution was increased.

## 2. Experimental

### 2.1. Instrumentation

#### 2.1.1. Lab-on-chip with modified webcam–CMOS system (LOC–CMOS)

A simple chip was made of a 2 cm × 3 cm acrylic piece as described in previous work [15]. Briefly, channels were drilled through its thickness on both the length and width sides using a 1.2 mm drill bit. At the ends of each opening, threads were made to 1/4" size to fit normal FIA nuts. The chip was secured on a plastic tray with super glue. It was placed in a black box made of acrylic plastic to keep the system from outside light. Solenoid pumps, valves, LED and the CMOS circuit with lens from a commercial webcam were arranged in the following fashions, see Fig. 1(a) and (b). The webcam camera (Oker T-45 webcam chat, Taiwan) was secured on the top ceiling of the box, above the chip. Four white high intensity LEDs (NPE, Thailand) were placed at the ceiling of the box to give constant light intensity throughout the experiment. Two solenoid pumps (Biochem valve, USA) were connected to the two openings, one of the longer channel (pump $P_A$) and another of the shorter channel (pump $P_B$). Two solenoid valves (valve $V_A$ and $V_B$, Takasago, Japan) were connected to the other two openings and with waste lines. A fan was placed on the back side of the box to transfer heat out of the box. To be able to control the system in the closed box, control switches for power, pumps and valves were connected to the outside of the box, as shown in Fig. 1(c). The electrical circuit was composed of a 12 V DC power supply that gave electrical current to the diode bridge. Electrical current was passed into the main power switch that distributed power to solenoid valves, pumps, LED and fan through each of their own controlled switches.

#### 2.1.2. Working concept of the control software

The control software was written to automate the time based detection as described in manual operation in the previous work [15]. Briefly, when sample zone came into contact with the reagent zone, diffusion of sample into reagent line occurred. This caused the change in color of the reagent zone. This change could be observed visually as the reaction zone was diffusing toward the detection

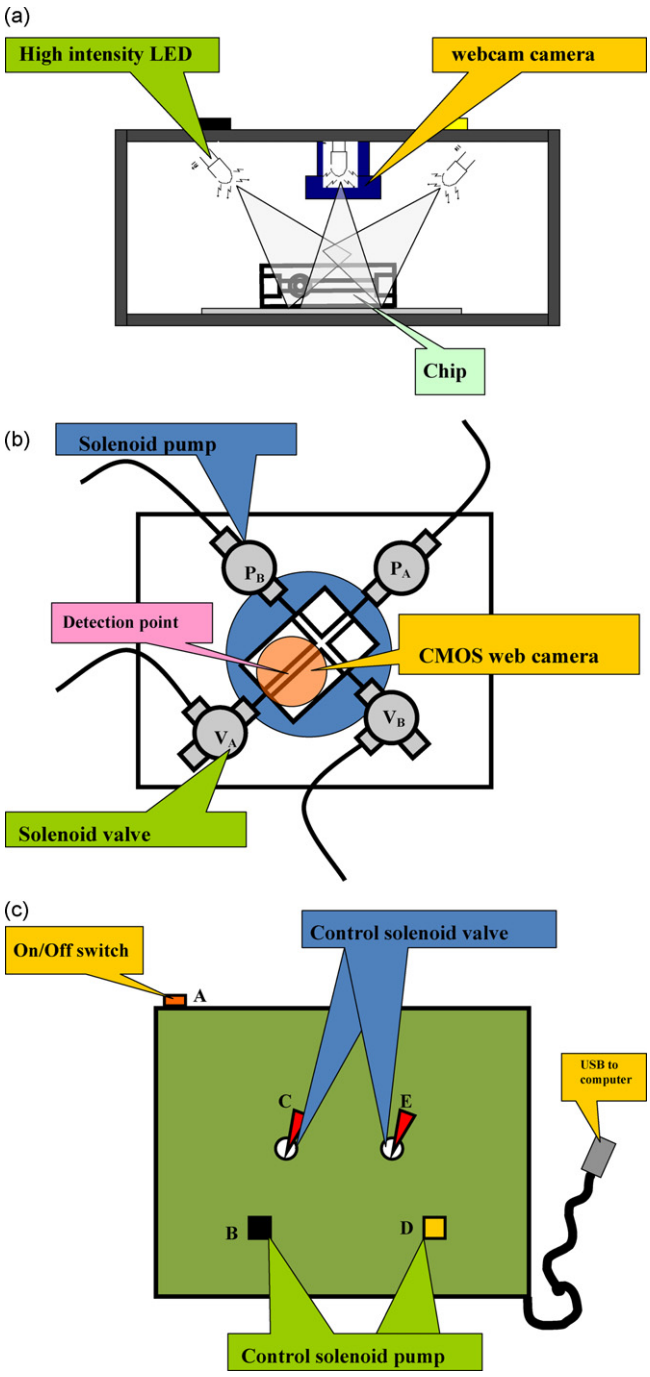



**Fig. 1.** Arrangement of each component in the LOC–CMOS system (a) side view (b) top view and (c) outside view.

point, see Fig. 1(b). Migration time depends on concentration of sample at the fixed concentration of reagent. Real time recording of detection time and observation of color change are achieved with the operation of the software as shown in Table 1.

### 2.1.3. General operational steps for acidity assay

Operation of the flow system on the chip was controlled by pumps and valves. Referring to Fig. 1(b), valve $V_A$ and pump $P_A$ are for controlling the flow in the long channel while valve $V_B$ and pump $P_B$ are for controlling the flow in the short channel. Operational steps are as summarized in Table 2.

**Table 2**
Operational steps for flow system on the LOC.

| Step | operation |
|---|---|
| 1 | The system was washed with water by pumping water with pump $P_B$ while opening both valve $V_A$ and $V_B$. |
| 2 | Valve $V_A$ was closed. Acid solution was pumped into the short channel with pump $P_B$ through the opened valve $V_B$. |
| 3 | Valve $V_B$ was closed. Reagent was pumped into the long channel with pump $P_A$ through the opened valve $V_A$. |
| 4 | Valve $V_A$ was then closed. Valve $V_B$ was opened and pump $P_B$ was switched once to inject standard/sample solution into the system |
| 5 | Reaction occurred and was detected as explained in the software concept |
| 6 | The system was washed before the next run by opening both valve $V_A$ and $V_B$ and DI water was pumped into channels using pump $P_B$. |

### 2.1.4. Chemicals and reagents

Saturated phenolphthalein in sodium hydroxide solution was prepared by dissolving 0.1 g phenolphthalein (Sigma–Aldrich) in 100 ml 0.005 M NaOH (Sigma). Excess phenolphathalein was removed by filtering the solution with filter paper no. 42 (Whatman). Standard acetic acid solutions (Sigma) were prepared at 3–6% (w/w). Dilution of solution and washing of the chip was done using DI water.

### 2.1.5. Vinegar samples

Four samples of commercially available cooking vinegar were obtained from a convenience store. All samples were labeled as containing 5% (w/w) acetic acid. The acetic contents were also assayed by the standard titration with sodium hydroxide [16].

## 3. Results and discussion

### 3.1. Analytical signal from LOC–CMOS

In the time based approach detection, the suitable detection point (distance from the point where sample and reagent come into contact to the point where the timing of migration is stopped) is needed to be optimized so that differences in migration times due to the acid concentrations can be differentiated [15]. Too short migration times would lead to higher %RSDs while longer migration times would lead to lower sample throughput. This optimization step can be omitted when using the LOC–CMOS system. Continuous detection of migration time vs. migration distance (as relative distance) was recorded every 1 s as shown in Fig. 2. The detection point can be chosen from this result which helps to cut down the time consumption in the development of the analysis process. Under the conditions in this experiment, the shortest relative distance that yielded good distinctive migration times

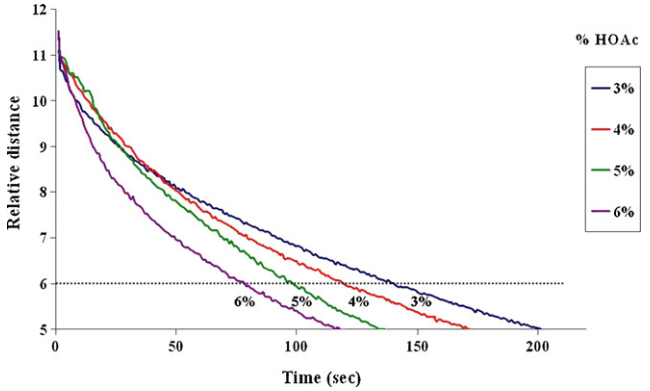



**Fig. 2.** Analytical signals showing continuous changes of migration time with the relative distance of the acetic acid zone at various concentrations.

**Table 3**
Concentrations of acetic acid in vinegar samples using the LOC–CMOS system and standard conventional titration.

| Sample no. | Labeled acetic acid concentration (%, w/w) | LOC–CMOS | | | | | Conventional titration |
|---|---|---|---|---|---|---|---|
| | | migration time (s) | | | Log (%, w/w) concentration of acetic acid | Concentration of acetic acid found (%, w/w) | Concentration of acetic acid found (%, w/w) (triplicate) |
| | | 1 | 2 | Average | | | |
| S1 | 5 | 79.00 | 72.00 | 75.50 | 0.80 | 6.32 | 5.10 |
| S2 | 5 | 89.00 | 78.00 | 83.50 | 0.76 | 5.78 | 5.00 |
| S3 | 5 | 107.00 | 103.00 | 105.00 | 0.66 | 4.54 | 5.30 |
| S4 | 5 | 105.00 | 101.00 | 103.00 | 0.67 | 4.64 | 5.08 |

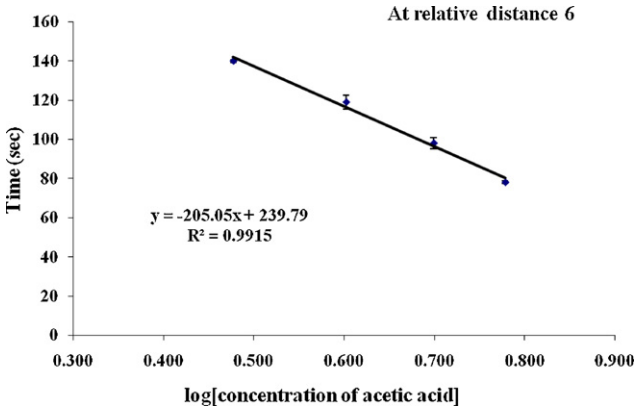



**Fig. 3.** The time based calibration graph obtained from the LOC–CMOS system at the selected relative distance 6.

of various acetic concentrations was selected at relative distance 6.

### 3.2. Calibration graph

Migration times at the selected relative distance were plotted against log concentration of acetic acid. A linear time based calibration graph was obtained ($Y = -205X + 239$, $R^2 = 0.991$) as shown in Fig. 3, where $Y$ is migration time (s) and $X$ is log concentration of acetic acid. The higher the concentration of acetic acid, the shorter the migration time needed to reach the selected relative distance. Therefore, the slope of the calibration graph is negative. This calibration graph was used for estimation of acetic acid concentration in vinegar samples.

### 3.3. Precision

Precision of the system was evaluated by seven replicate injections of 5% (w/w) acetic acid standard solution. Relative standard deviation (RSD) was calculated to be 5.6%.

### 3.4. Analysis of acetic acid in vinegar samples using the simple LOC–CMOS system

The results shown in Table 3 demonstrate the successful performance of the simple LOC–CMOS system for acidity assay.

## 4. Conclusion

A novel application of CMOS webcam camera as a relatively cost effective detection system in chemical analysis was demonstrated. The alternative time based detection of chemical reaction could be performed more automatically with better precision. Continuous monitoring of the change in migration time during the sample zone diffusion into the reagent line helped in better selection of the detection point. Recording of the signal every second is beneficial for "traceability". The collected data can be kept for future use as normally needed in quality assurance in analytical chemistry. The system has a high potential for various on-site chemical analyses.

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