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ONLINE LIQUID CALIBRATION TECHNOLOGIES

D. Liang[1], *T. G. Muniyogeshbabu*[2], *L. Tanguy*[2], *A. Ernst*[2,3], *R. Zengerle*[1,2,4], *P. Koltay*[2,3]
[1] HSG-IMIT - Institut für Mikro- und Informationstechnik, Georges-Koehler-Allee 103,
79110 Freiburg, Germany
[2] Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering,
University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
[3] BioFluidix GmbH, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
[4] BIOSS - Centre for Biological Signaling Studies, University of Freiburg, Germany

ABSTRACT

Three online liquid volume calibration methods – image processing, flow sensing and capacitive droplet detection – are simultaneously used to measure the volume of single nanoliter sized droplets. Good precision (imaging method CV<2.7%, flow sensing method CV<3.2%, capacitive droplet sensing method CV<4.1%) and reasonable accuracy in comparison to a gravimetric reference method have been observed in 192 dispensing experiments. Besides the quantitative performance comparison, the three methods are qualitatively benchmarked. The benchmark shows all of these methods to be consistent and to enable equally non-contact measurement of liquid volumes with CV better than 5% in the volume range 12 to 54 nl.

KEYWORDS

Online volume calibration, droplet detection, capacitive sensor, flow sensor

MOTIVATION

The miniaturization of liquid-handling devices is requiring new calibration technologies for volumes in the nanoliter range. In comparison to standard offline calibration technologies like gravimetric [1] or photometric [2] methods, where the liquid used for calibration is wasted, non-contact online methods have big advantages such as the continuous monitoring, the possibility to apply closed loop control mechanisms and a smaller volume detection limit. Based on previous studies [3-5] a multi-principle calibration system has been built. With this system single liquid droplets in the range from 2 nl to 70 nl can be characterized simultaneously with three online methods – the imaging, the flow sensing and the capacitive sensing method. A comprehensive study and benchmark of the three online calibration methods is presented.

EXPERIMENTAL SETUP

In order to compare the performance of the three online liquid calibration methods with the same nanoliter droplet, the experimental setup shown in Figure 1 has been used. A PipeJet P9 dispenser (Biofluidix GmbH, Germany) is employed to generate the droplets. The dispenser was selected because of its high reproducibility and the open reservoir that does not require a pressure source to generate the droplet. Thus, the reservoir inlet could be connected to a flow sensor (HSG-IMIT, Germany), which measures the air flow into the reservoir during dispensing. Form the air flow the liquid volume can be determined by integration of the flow signal over time [3]. In addition a capacitive droplet sensor is directly mounted below the nozzle of the dispenser. This sensor detects the change in capacitance when a droplet passes through the electrodes like described in previous work [4].

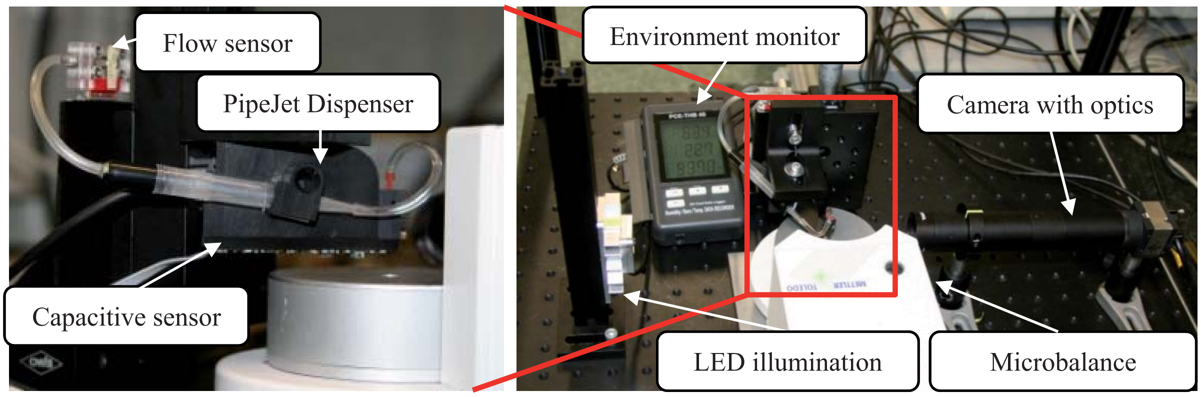


Figure 1: Experimental setup of the multi-principle liquid volume calibration station

Below the nozzle of the dispenser a microbalance XP2U (Mettler-Toledo, Switzerland) is placed in 5 mm distance for gravimetric reference measurements according to the GRM-R method [1]. This distance allows a stroboscopic camera to record the droplet in flight. With the imaging processing as described below the volume of the captured droplet can be precisely detected. The experimental setup includes environmental monitoring of humidity, temperature and pressure. Whenever necessary corrections with respect to the environmental factors have been made to achieve comparable results referring to T = 20 °C.

FLOW SENSING METHOD

The investigated flow sensing method [3] is realized by mounting a flow sensor at the opening of the dispenser reservoir and detecting the air reflow into the reservoir during droplet ejection (Figure 2(a)). The flow sensor involved in the presented method is an OEM air flow sensor from HSG-IMIT based on calorimetric measuring principle. In contrast to direct measuring the liquid flow signal the presented strategy with indirect measuring the air reflow can detect the volume independently of the fluid properties like density, viscosity and specific heat. A specific calibration for each fluid is therefore not required.

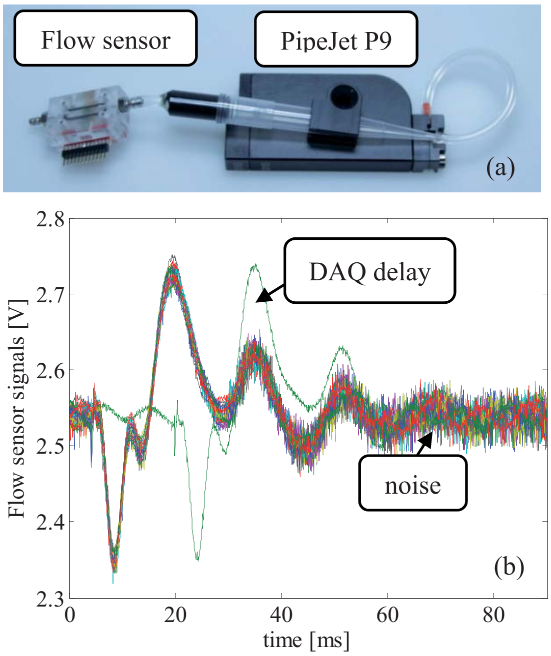


Figure 2: (a) Mounting of the flow sensor at the opening of the dispenser reservoir. (b) Typical flow sensor signals (24 measurements with 36 nl droplets).

The typical flow sensor signals gathered during dispensing are shown in Figure 3. Due to the working principle of the PipeJet dispenser, which creates a short backflow into the reservoir before droplet ejection, a negative peak can always been observed at the beginning of the data acquisition (DAQ) of the flow sensor signals. The volume is calculated from the flow by equation:

$$V_{flow} = F_1 \cdot \int U_{flow} dt + F_2 \qquad (1)$$

Where V_{flow} is the measured volume of the flow sensing method. U_{flow} is the flow sensor signal. F_1 and F_2 are calibration constants that have been experimentally determined. In this work F_1 and F_2 were calibrated by two experiments with 29 and 36 nl droplets.

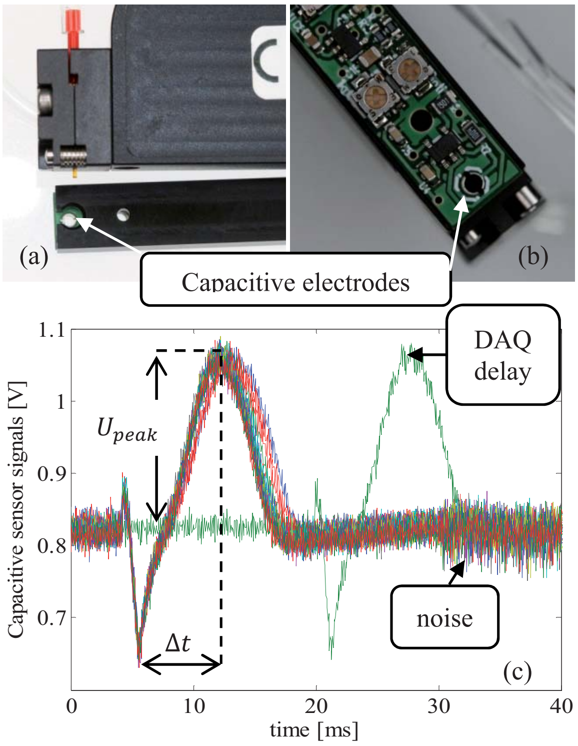


Figure 3. (a) Zoom view of PipeJet nozzle and capacitive sensor (b) Inside view of capacitive sensor (c) Typical capacitive sensor signals (24 measurements with 36 nl droplet).

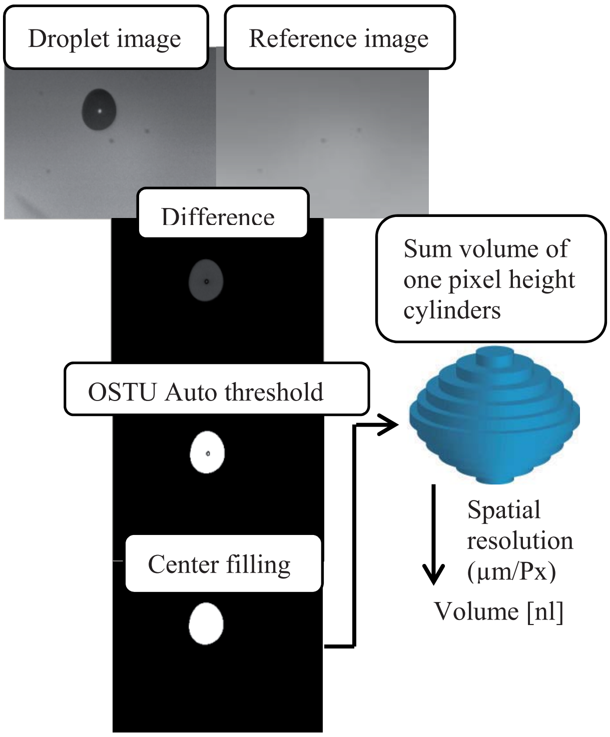
CAPACITIVE SESENSING METHOD

The presented capacitive sensing method is realized through a capacitive sensor prototype from BioFluidix (Figure 3(a), (b)) described in [4]. The typical signal when a droplet passes through the capacitive electrodes is shown in

Figure 3(c). The signal begins with an obvious negative peak that is caused by capacitive coupling effect when the ejected droplet is still coupling with the grounded nozzle. The droplet volume V_{Cap} has a linear relation to the positive peak value U_{peak} and the droplet velocity, which is proportional to $\frac{1}{\Delta t}$ described the equation [4]:

$$V_{Cap} = \mathrm{C}_1 \cdot \mathrm{U}_{\mathrm{peak}} + \mathrm{C}_2 \cdot \frac{1}{\Delta \mathrm{t}} + \mathrm{C}_3 \qquad (2)$$

Where $\mathrm{U}_{\mathrm{peak}}$ is the positive voltage peak value of capacitive sensor signal. $\Delta\mathrm{t}$ is the time between the negative peak and positive peak. C_1, C_2, and C_3 are calculation constants that needs to be calibrated. In this work C_1, C_2, and C_3 were calibrated by three experiments with 26 nl, 36 nl and 54 nl droplets.



IMAGING METHOD

Figure 4: Working principle of the imaging method for volume reconstruction from droplet image

In stroboscopic imaging the droplet is photographed during flight with short shutter time (9µs). The captured droplet image is then firstly compared in greyscale value pixel by pixel with a reference image without droplet. With the Otsu auto-threshold algorithm [6] and a simple corresponding center filling algorithm the droplet is separated very clearly from the background (Figure 4). The volume calculation from the droplet contour is described in [5]. The droplet is regarded as the stack of many cylinders, which have the height of one pixel each (Figure 4). The droplet volume is then the sum of the volume of all these small cylinders.

In order to get accurate volumes in nl the magnification of the used camera and optics had to be calibrated to yield a spatial conversion parameter. This calibration was experimentally carried out with a positive 1951 USAF test target which has traceability to NIST. The spatial conversion parameter was 1.986 µm/pixel.

EXPERIMENTS AND RESULTS

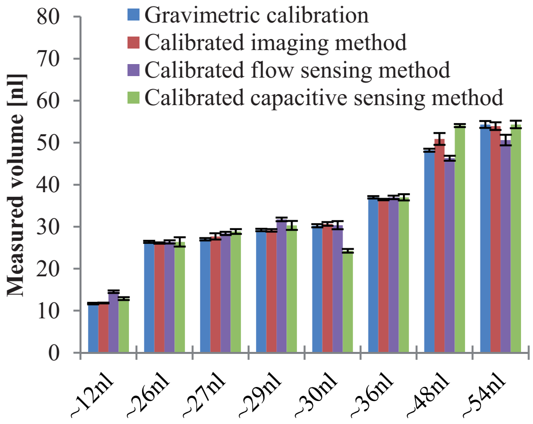


Figure 5. Benchmark results for all three methods

The results of 192 benchmark experiments for 8 different droplet volumes (24 dispenses for each volume) are presented in Figure 5. For all experiments double distilled water was used. The imaging method always provides the best repeatability in terms of the coefficient of variation (CV). It remains consistent with the gravimetric reference, except at 48 nl, where non-spherical shaped droplets have been observed as possible reason for this. The negative bias of the flow sensor results compared to the gravimetric reference at 48 nl and 54 nl may be caused by insufficient data acquisition time (90ms in experiments). Except experiments at 30 nl (electronic error) and at 48 nl (dispenser disturbed) the capacitive sensor has shown high consistency with the gravimetric reference.

DISCUSSION

Besides the quantitative proofs of the performance, the three methods have been compared in a qualitative manner concerning Signal-to-Noise-Ratio (SNR), user-friendliness, working range, integration size and liquid suitability (Figure 6). Due to the best SNR and simple calibration (better user-friendliness) the imaging method may have less risk to achieve reliable results than the other two methods. However, the imaging method as used here cannot deliver reliable results for liquid jets instead of droplets, which limits its application range to small volumes. High precision imaging can detect droplets down to picoliters but always needs special camera and optics, which increases the costs and the equipment size.

In contrast, the flow sensing method can be applied for liquid jet measurement as well, which extends its upper working range. In case of allowing modification of the reservoir as presented above, the size and the integration of the flow sensor can have advantages over the imaging method. In the presented experimental conditions the flow sensing method has lower SNR than the imaging method, which limits its application at very small volumes. The calibration of the flow sensor is also more complicated than for the optics and camera.

The most innovative method – the capacitive sensing – has unfortunately similar calibration difficulties and lower SNR, but it provides the smallest integration size as well as lowest costs. With some modifications the capacitive sensor might also be used for jet measurement under specific conditions. In our experiments the capacitive sensing method had almost the same lower limits of working range as the flow sensing method. All the three methods have in principle no limitation regarding the testing liquid.

CONCLUSION

Three online liquid calibration technologies - the flow sensing, the capacitive sensing and the imaging method - have been benchmarked in both quantitative and qualitative manner. The quantitative evaluation of the three methods was carried out by measuring the same droplet with a special designed multi-principle liquid calibration station. The results show all of these methods to be consistent and to enable measurement of liquid volumes with CV better than 5% in the considered volume range (12-54 nL). The qualitative benchmark consists of the comparison of the three calibration methods in SNR, user-friendliness, working range, integration size and liquid suitability. Such a comprehensive experimental study of three different liquid calibration methods executed on the same droplet has never been reported so far. All the three methods have been found to have good potential in further development of calibration standards or for smart integration of online sensing in micro fluid liquid handling devices.

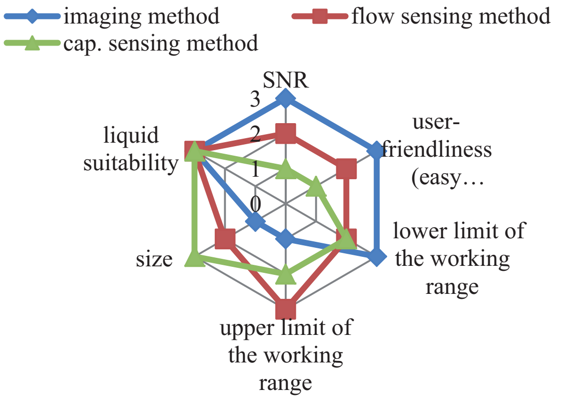


Figure 6. Qualitative comparison in terms of SNR, user-friendliness, working range, size and liquid suitability. Higher numbers show relative advantage.

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REFERENCES:

[1] D. Liang, et al, “Novel gravimetric calibration Method for nano liter liquid handling devices”, Proc. MFHS Conference, 2012

[2] J. Bradshaw, et al, “Multichannel Verification System (MVS): a Dual-Dye Ratiometric Photometry system for performance verification …”, JALA, 2005.

[3] W. Streule, et al, “Kontaktfreie und medienunabhängige Volumenbestimmung in Nanoliter Dispensern”, Proc. MST-Kongress 10.-12. October 2005, Freiburg, Germany, 2005

[4] A. Ernst, et al, “Numerical investigations on electric field characteristics with respect to capacitive detection of free-flying droplets”. Sensors 2012, 12(8), 10550-10565

[5] K.Thurow, et al, “An optical approach for the determination of droplet volumes in nanodispensing.” J. of automated methods & management in chemistry, 2009

[6] N. Otsu. "A threshold selection method from gray-level histograms". IEEE Trans. Sys., Man., Cyber. 9 (1): 62–66, 1979.