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Micropipette calibration by differential pressure measurements

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Introduction

Measuring and transfer of small and precise amounts of liquid represented for a long time an important challenge for biology. Generally used, glass pipettes which required laborious cleaning and sterilization procedure were soon replaced by disposable plastic tube. The introduction of plastics opened the possibility of pipetting using calibrated pistons and the concept of today's micropipetting is exemplified by numerous systems all relying on the mechanical displacement of a small piston and aspiration of the liquid in a disposable tip. Whereas the use of such system is limited to low viscosity fluids it is perfectly adequate for most of the biological measurements and is largely diffused in the laboratories. Thanks to the quality of current mechanical precision, these devices allows micropipetting of volumes ranging from a few to more than a thousand of microliters (μL).

However, calibration and regular verification of the pipetting volume remains challenge and the two methods that are accepted today are the gravimetry and dilution with spectrometry measurements. The simplest and most used one is gravimetry: a procedure which consists in weighing, using a precision scale, the amount of water that was pipetted. Whereas gravimetry is rather easy to effectuate in a laboratory which often disposes of a precise weighting instrument, its use can be prone to different errors ranging from the water evaporation, capillarity on the pipette tip, requires appropriate correction for the temperature and is best performed by a well-trained technician. Those limitations are exacerbated for small volume and its reliability is affected for volume below 1 μL range.

Dilution and quantitation using spectroscopy offers an alternative to gravimetry but is also prone to several difficulties, mainly: it can be adequately performed only by trained technician and can be time consuming and requires the use of appropriate dyes. In addition, whereas both gravimetry and dilutions are amenable to automated system or liquid handler, they necessitate adequate calibration programs and well established protocols. The relevance of correctness of the dilution was highlighted in different fields including immunoassays.

The aim of this work is to present a novel calibration alternative based on the physics of perfect gas which exploits the most recent pressure sensor and electronic components that have recently evolved thanks to their wide use in many devices (i.e. smartphone, watches). This differential pressure measurement (DPM) allowed to build an efficient and precise device expected to find a broad usage in laboratories.

The principle

Micropipetting using the positive displacement of a piston implies that the system is composed of a mechanism which displaces a known volume of air that will aspirate or expel the corresponding amount of

liquid in the pipette tip. Aspiration of the liquid in the pipette tip is caused by the lowering of the pressure in the tip and liquid is ejected by applying a positive pressure.

Should it be possible to measure with enough precision the pressure difference occurring during a pipetting cycle, it would be possible to deduce the piston displacement. Whereas this could be easily performed in a system in which the dead volume between the piston and the pipette tip is known constant, such measurement would be insufficient for unknown dead volume. Indeed, different commercially available pipettes present different dead volumes. Moreover, as the volume to be pipetted is adjusted by a displacement of the resting position of the piston, this results in a different dead volume even in a defined system.

Volume measurements using pressure sensitive devices were previously developed but it is only with the technological advances and increased sensitivity that such methods can be adapted to calibration of small volume displacements. The concept is to measure first the pressure increase (or decrease) caused by the piston displacement and introduce at a known timing the opening of an additional and precisely defined volume (*Vref*). Initially described for larger volumes this strategy is applicable, providing a sufficient pressure resolution and stability, to volume as small as a few microliters. The principle of the reference volume is schematized in Figure 1.

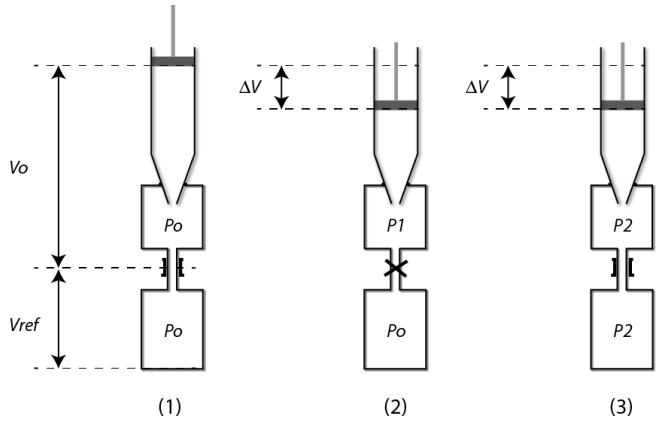

Figure 1: Schematic representation of the pressure measurement principle.
In the initial conditions the pipette is inserted into the port opening and the tip is firmly pressed to seal the gap (1). The pressure inside the upper and lower compartment is equilibrated. The valve separating the two compartments is closed and piston is lowered (2), leading to a difference in pressure between the upper and lower compartments. The valve is open (3) and the pressure between the two compartments re-equilibrate.

To begin, the tip insertion port and the valve of the reference volume (*Vref*) are open and the pressure is equilibrated with the atmosphere P_0. The valve of the *Vref* volume is then closed and pressing the piston causes a reduction of the volume resulting in an increase of the pressure into the compartment V_0. Once the pressure is stabilized and its value captured (P_1), the valve separating the *Vref* volume is open and the pressure P_2 is measured.

$$\Delta V = Vref / [\frac{P_1}{(P_1 - P_o)} - \frac{P_2}{(P_2 - P_o)}]$$

Equation 1 : Pressure changes as function of the reference volume *Vref*.

The difference in volume can be computed from the pressure differences in which P_0, P_1 and P_2 are the pressure values in hPa, *Vref* is the reference volume in µL and ΔV corresponds the volume displaced by the piston in µL.

Simulation of the pressure changes computed for a dead volume of 1000 µL and a reference volume of 400 µL using Equation 1 is illustrated in Figure 2.

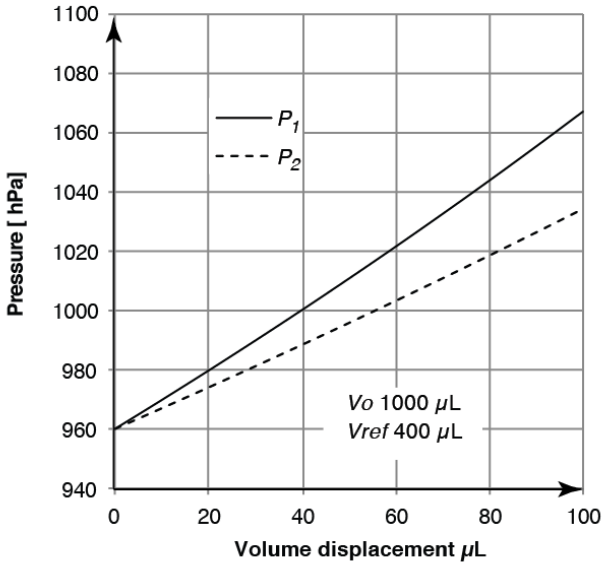

Figure 2: Differential pressure simulation.
Simulation of the pressure increased in function of the volume displacement with a dead volume corresponding to 1000 µL (continuous line). Increasing the internal volume by 400 µL the corresponding of the valve separating the upper and lower compartment reduces the slope of the pressure increase as indicated by the dash line.

Figure 2 illustrates the absolute pressure difference that would be observed for volume displacements ranging between 0-100 µL when the reference volume is closed (continuous line) and upon opening of *Vref* (dashed line). These results illustrate that measurements of reliable changes in volume smaller than 20 µL necessitate a sensitivity of the pressure sensor that is below one hPa. For example, this model predicts that a 4 µL displacement with a dead volume V_0 of 1000 µL and at 960 hPa atmospheric pressure yields a pressure increase of 3.86 hPa. Knowing that last generation of Micro-Electro-Mechanical Systems (MEMS) based pressure sensor commercially available have typical resolution in the range of 0.02 hPa, this indicates that such sensor would be sufficient to offer a reliable reading.

Based on a resolution of the pressure readout better than 1% and assuming that the maximal pressure in the system should not exceed 1200 hPa it can be formulated that the pressure increase ΔP observed when lowering the piston should be comprised between 2 hPa < ΔP < 300 hPa. The relationship between V_0 and ΔP is defined as follows:

$$Vo = \Delta V \left(1 + \frac{P_1}{\Delta P}\right)$$

Where V_0 is the internal volume, ΔV the change in volume caused by the piston displacement, P_1 the initial pressure and ΔP the changes in the internal pressure. This **Erreur ! Source du renvoi introuvable.** highlight the fact that optimal detection is best achieved by adjusting the V_0 between 800 µL for the measurements of small volumes up to about 2000 µL for volume superiors to 500 µL.

Dead volume and precision

The pressure changes caused by the displacement of the piston depend on: (i) the displaced volume and (ii) on the overall dead volume. This dead volume V_0 is the sum of internal volume of the pipette plus the volume of the tip and the internal volume of the DPM device (see Figure 1). Based on the sensitivity and precision of the pressure sensor it is possible to compute the random error for each volume displacement with a given dead volume V_0 and reference volume *Vref*. Results of such computations shown in Figure 3, illustrate the influence of the values of V_0 and *Vref* on the predicted accuracy of the measurement, given a RMS pressure noise on the measurement of 0.02 hPa. The simulations are done for a pipette of 100 µL nominal displacement (P100).

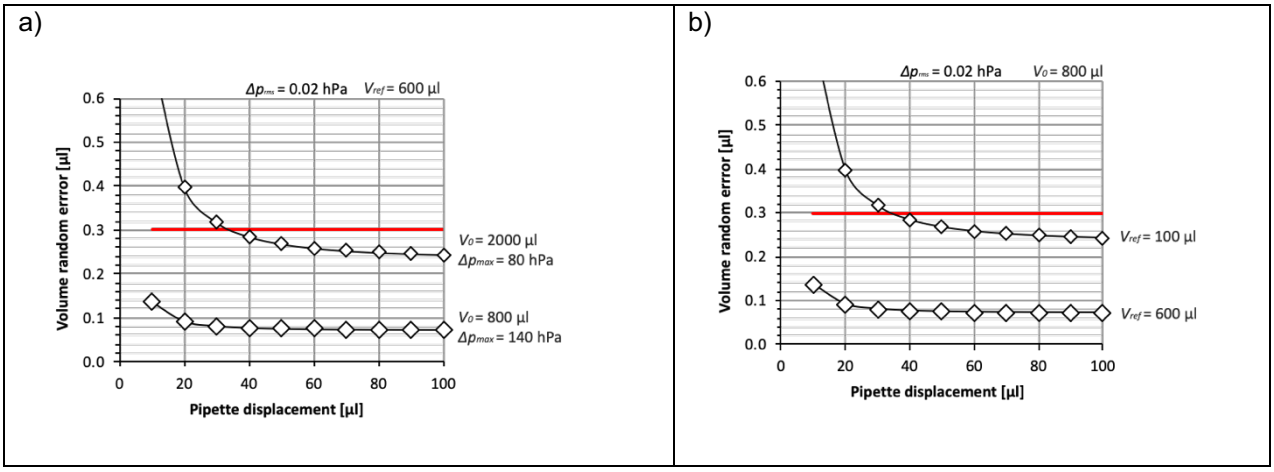

Figure 3: Influence of V_0 and *Vref* on the accuracy.
a) Computation of the accuracy for a fixed random error of the pressure sensor as a function of the pipetted volume and for two dead space volume V_0 with a fixed reference *Vref* or b) for a fixed V_0 and two reference *Vref* (right panel). The ISO-8655 random error limit value for each pipetted volume is indicated by the red line.

Figure 3a shows the volume random error against pipetting volume for two values of V_0. The value of V_0 = 800 µL corresponds to typical V_0 obtained with a P100 pipette on the DPM device. We observe that, within these parameters, the random error remains below 30% of the error allowed by the ISO-8655 norm. For comparison purpose, the error with V_0 = 2000 µL is computed. This clearly shows the influence of V_0 on the errors. Reducing the dead volume provokes a larger working pressure in the DPM device, which may induce leakage in the pipette. Figure 3b shows the effect of changing *Vref* on the volume random error. The typical value of *Vref* used in the DPM system is 600 µL. Reducing this reference volume causes a smaller change of pressure difference P_2-P_1 and consequently increases the measurement error (as shown in the curve with *Vref* = 100 µL).

These simulations indicate that adequate precision can easily be obtained provided a sufficiently large reference volume *Vref* and small dead volume V_0. These results also reveal that even with a dead volume as large as 800 µL it is possible to accurately detect changes caused by 10 µL pipetting provided a sufficiently large reference volume *Vref*. The optimal dead volume and reference volume depend on the nominal pipette size and the tested displacement. However, it can be observed that a single reference volume can be used to cover the pipette range from P10 to P1000, while keeping measurement precision below 30% of the limits of the ISO-8655 norm.

Design of the system

Results presented above reveal that the pressure increase will be defined by the overall dead volume V_0. Whereas its best resolution would be obtained with a minimal dead volume, this might result in a pressure increase exceeding the range for which the pipette was designed. Especially because the leak through O-rings is not a linear function of the pressure as their displacement or deformation might prevent to be contained within the constrains defined by the manufacturer. Whereas estimation of the pressure changes could be deduced from the filling time, tip opening, liquid viscosity and tip capillary pressure, it is interesting to proceed to actual measurements. For such purpose, a pipette tip was modified to include a pressure sensor and readings during aspiration of 1000 µL volume were conducted. Results presented in Figure 4 illustrates the observed pressure change during a standard pipetting of water at room temperature.

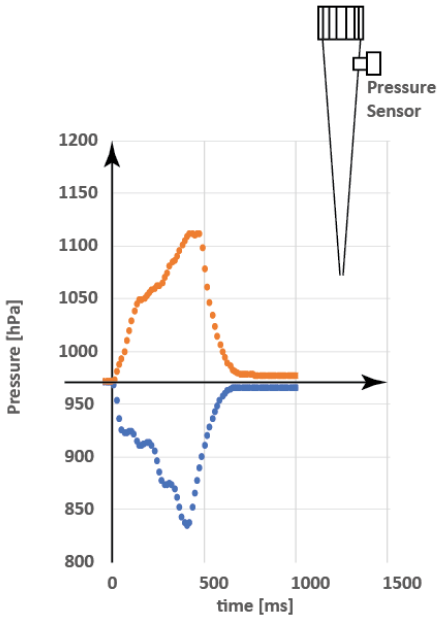

Figure 4: Pressure change during pipetting.
Measurement of the pressure change during pipetting of water was conducted by inserting a pressure sensor into the pipette tip as schematized in the upper right diagram. Aspiration of 1000 µL leads to a pressure change indicated by the orange dots whereas the pressure increase observed during the liquid dispensing is represented by the blue dots. Maneuvering of the pipette was done by hand, using typical procedure conditions at room temperature.

The pressure sensor dispositive used for the determination of the pressure change is illustrated in the inset. Increase and reduction of the pressure relative to the atmospheric pressure observed during 1000 µL pipetting (blue dots) and dispensing (orange dots) is shown as a function of time.

These data illustrate that aspiration or dispensing of 1000 µL within 0.5 s about requires a pressure change of roughly 100 hPa. Larger pressure changes will be expected for more viscous liquids or faster aspiration or dispensing.

To be within the pipette characteristics, the recording system defined above should therefore be designed to perform testing under comparable pressures. This implies an internal dead volume sufficiently large to prevent excess pressure differences either during positive or negative displacement of the piston mechanism but not too large to optimize a detectable pressure change. One obvious solution is to dispose of an additional volume that can be opened by an electro valve or any other electromechanical device. Examination of Equation 1 , reveals that the calculation of the piston displacement volume is independent of the dead volume V_0 but is only defined by the reference volume *Vref*.

This indicates that the total dead volume and the reference volume should be carefully chosen to minimize the method random error as defined by the ISO-8655 norm. Moreover, the *Vref* precision defines the precision of the DPM device and determination of the systematic error (as defined by the ISO-8655 international standard). Because measurements are differential, precision of the pressure sensor will influence only the random error.

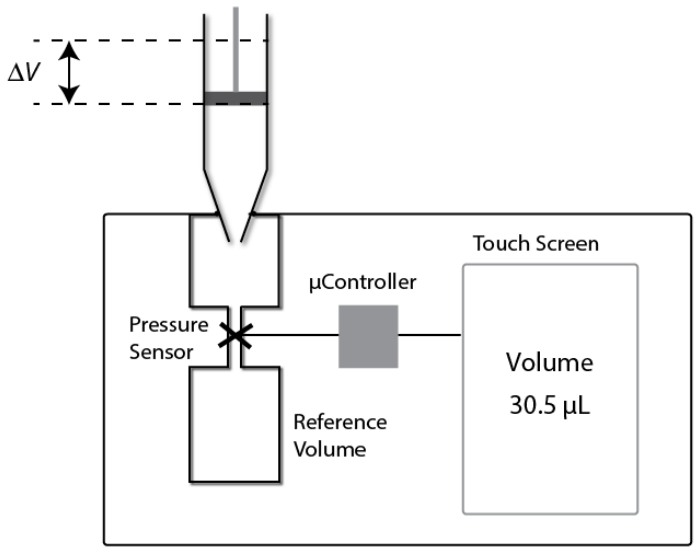

Figure 5: Schematic diagram of the system.
The DPM device is composed of the upper chamber formed by the pipette tip port, the valve which separate the reference volume *Vref*, the pressure sensor monitoring the pressure changes in the upper chamber, a computing device such as a microcontroller, and a touch screen for the user interface.

Results

Comparison of DPM versus gravimetry measurements

Measurements of dispensed volumes were conducted in parallel using standard gravimetry procedure and the differential pressure method. Results obtained for 4 micropipettes of ranging from 10 up to 1000 µL are shown in Figure 6 and summarized in

Table 1. In the DPM, the error caused by pressure sensor noise is significantly smaller and the random error as defined by the ISO-8655 norm. Multiple factors can account for such difference including: i) the pipette manipulation and precision on the stop, ii) mechanical precision of the pipette, iii) placement of the tip inside the port including possible movement during operations and iv) possible thermal gradients between the pipette tip and the different internal volume. Although the gravimetry measurements have a larger random error than the DPM device they are in perfect agreement with values reported by other authors in literature. More importantly, the systematic error of the DPM is comprised in the interval defined by the ISO-8655 norm and using the gravimetry measurement as references.

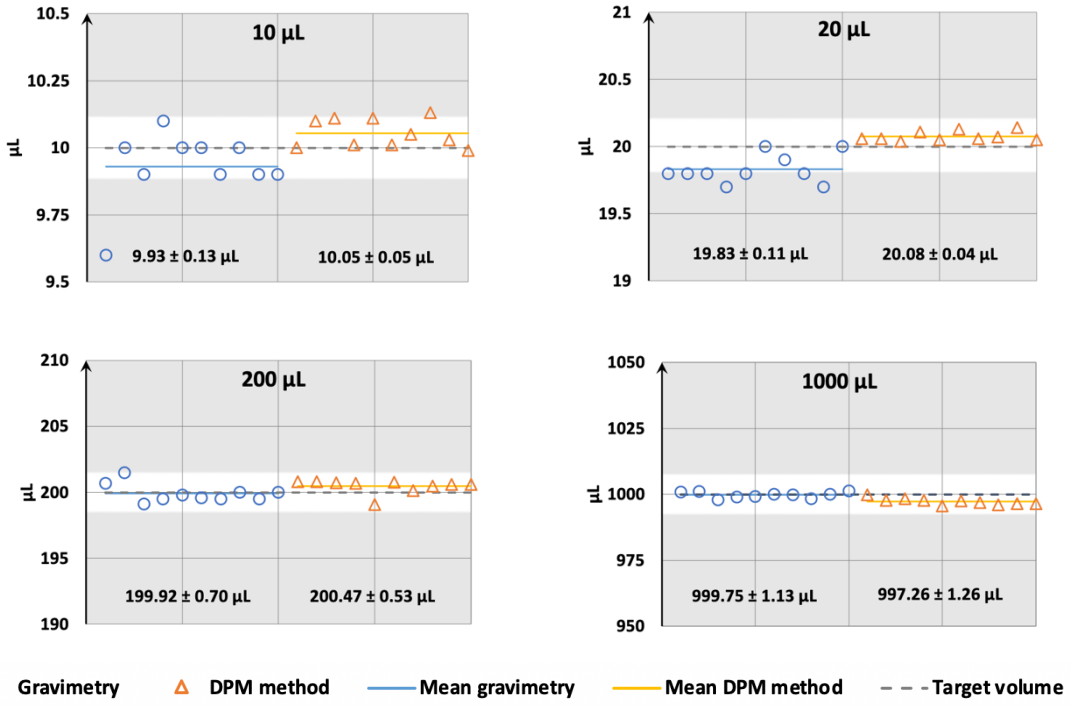

Figure 6: Correlation between gravimetry and differential pressure measurements.
Gravimetric and differential pressure data obtained with pipettes of 10, 20, 200 and 1000 µL respectively. Circles indicate the results obtained by gravimetric measurements using a precision weight scale (AB104-S/Fact from Mettler Toledo, Switzerland). Triangles indicate the results obtained by DPM in the same experimental conditions. Mean values for the gravimetry and DPM are indicated. The white band within the grey background indicates the ISO-8655 systematic error limits for each pipette range.

Spectrometry an alternative to gravimetry

Pipette calibration performed by dilution using a single or dual dye photometric method represents an alternative technique to gravimetry. This method is based on the direct relationship between the light absorption of a dye and its concentration. As shown in Equation 2, measurements of the light absorption of

a dye at a given concentration and after dilution in a known reference volume allows a direct characterization of the volume sample that was diluted.

$$Vx = \frac{C_2 Vo}{(C_1 - C_2)}$$

Equation 2: Determination of the pipetted volume as a function of the concentration.
Where *Vx* is the pipetted volume, V_0 the reference volume used for the dilution, C_1 and C_2 the respective dye concentrations in the stock solution and after dilution.

Whereas it would go beyond the scope of this work to compare the respective qualities and difficulties of each method, suffice to say that comparison of gravimetry and dilution yield comparable results..

Determination of the reference volume *Vref*

To minimize the systematic error of a DPM device it is mandatory to determine with precision the reference volume *Vref*. For this purpose, a special measurement apparatus was designed and is schematically illustrated in Figure 7.

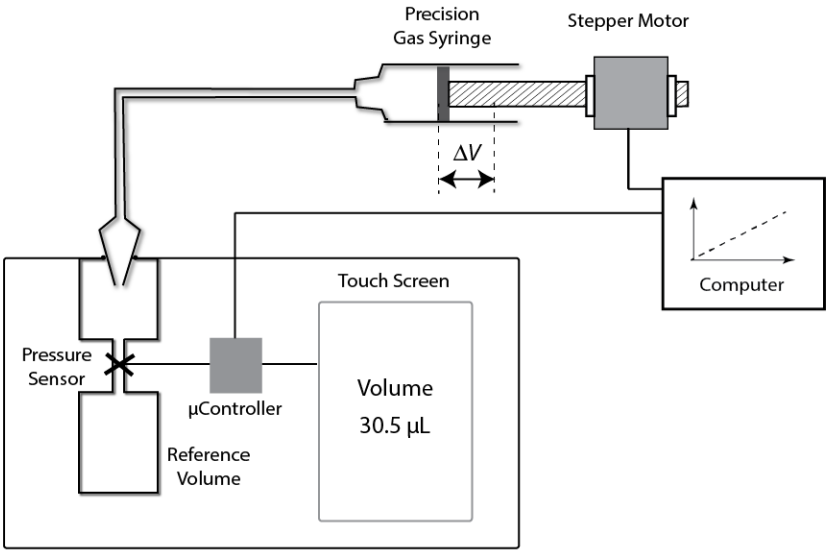

Figure 7: Schematic representation of the apparatus for the *Vref* calibration.
A precision gas syringe driven by a precision lead screw actuated by a computer-controlled stepper motor. Opening and closing of the *Vref* is under control of the main program.

This system is based on the displacement of a precision syringe by a mechanism driven by a high resolution and precision stepper motor mounted with a lead screw and linear guide. The gas syringe is connected to the DPM by a short Teflon tubing with minimal elasticity. Positive displacement of the piston results in an increase of the pressure which is monitored by the sensor of the DPM device. The syringe is then returned to its original position and the pressure is reduced to its initial value. Measurements are then repeated with the reference volume open and the pressure is monitored again.

Plot of the pressures measured in the *Vref* open and closed conditions are illustrated in Figure 8. From the differential pressure measurements, it is possible to compute the apparent V_0 and apparent V_0 + *Vref*, and extract the *Vref* by subtraction between these measurements. The apparent volume (V_0 or V_0 + *Vref*) is obtained using the Equation 3:

$$V = \frac{P_2 \Delta V}{(P_2 - P_1)}$$

Equation 3: Computation of the apparent volume.
Where P_1 is the initial pressure measurement, P_2 is the pressure measured after a small displacement of the syringe ΔV is the delta volume corresponding to the surface of the syringe piston multiplied by the displacement.

With the use of a high precision glass syringe, such as those provided by Hamilton, the curve corresponding to the volume *V* is only limited by the quality of the stepper motor and the lead screw. Precision of this calibration device was independently confirmed using gravimetry. The syringe was connected to Teflon tube disposed horizontally in order to prevent effects of hydrostatic pressure. The tube was was filled with water and positive displacement of the syringe progressively expelled the water. The computer controlling the stepper motor actuating the syringe was also connected to the weighing the precision laboratory scale yielding a series of weights corresponding to the progressive displacement of the piston. Temperature and humidity were controlled. Gravimetry results were analyzed using the recommended equation described in the ISO 4787.

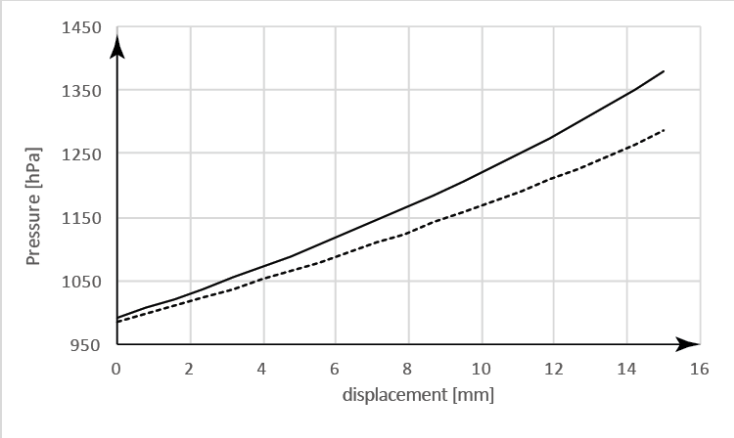

Figure 8: Typical pressure-displacement curve obtained during calibration.
Typical results obtained during the DPM method procedure are shown in Figure 8. The continuous curve was drawn by connecting a series of pressure measurements obtained with the device illustrated in Figure 7 plotted versus the position in mm. The dashed line was obtained for the same displacement of the piston after opening of the reference volume *Vref*.

Repetitive measurements conducted with the DPM device allow to determine the dispersion of the measured volume. As these measurements are conducted with a high precision mechanical device, this eliminates the user associated variations and the dispersion is principally due to the pressure sensor and additional factors associated with the opening and closing of the valve from the reference volume.

The absolute accuracy of *Vref* determines the absolute accuracy of the DPM device (Equation 1). An absolute accuracy of 0.1% of the device requires the determination of the absolute value of *Vref* of 0.1%, or 0.6 µL for typical value of *Vref* = 600 µL used in the DPM device. Assuming that the sensitivity of the pressure sensor remains stable with time, the accuracy of the DPM device is given by the geometry of *Vref*. This guarantee stable operations, independent of the ambient temperature or pressure.

Taking advantage of the differential pressure measurement

Calibrating micropipette designed for µL to sub microliter sampling becomes a true challenge for gravimetric measurements. While typical precision scale often offers a precision of 0.1 mg, the measurement range is basically limited to 1 mg. Measuring with sufficient precision a 0.5 µL volume become therefore not realistic in normal laboratory conditions. The alternative solution that is retained for the calibration of pipette below 1 µL is generally to consider the dilution method.

The differential pressure measurement (DPM) limits are defined by the precision of the pressure sensor and by the combined internal volume of the pipette and the device. Assuming a dead volume V_0 of the ensemble of 10 µL a change of 0.1 µL would yield a pressure change of 9.75 hPa which is clearly above the 10 fold of the pressure sensor resolution. Increasing the volume displacement to 0.5 µL would yield a pressure change of 50 hPa. These data indicate that a significant pressure change can be obtained even for very small volume displacement, the challenge of such design is to minimize the combine internal volume of the pipette and the device. To assess the feasibility of such measurements and illustrate the advantage of the method, a specially designed embodiment minimizing the dead volume was realized. Determination of the optimal *Vref* volume can be done using Equation 1 and results from this computation reveal that with an internal volume of 10 µL and a reference volume of 40 µL would yield a differential pressure of 9.85 hPa for a pipetting of 0.5 µL.

A potential caveat to this hypothesis is that the internal volume of the micro pipette and the pipette tip add a non-negligible dead volume. Measurements of the internal volume of tips typically used for pipetting volumes in the microliter range revealed that the additional dead volume is at least 20 µL. Measurements conducted with a standard micropipette and a device in which the internal space was minimized yielded an overall internal volume in the sixty microliters range. Nonetheless effective pressure changes of 10.29 ± 0.1 hPa (n = 10) were measured for pipetting volume of 0.5 µL.

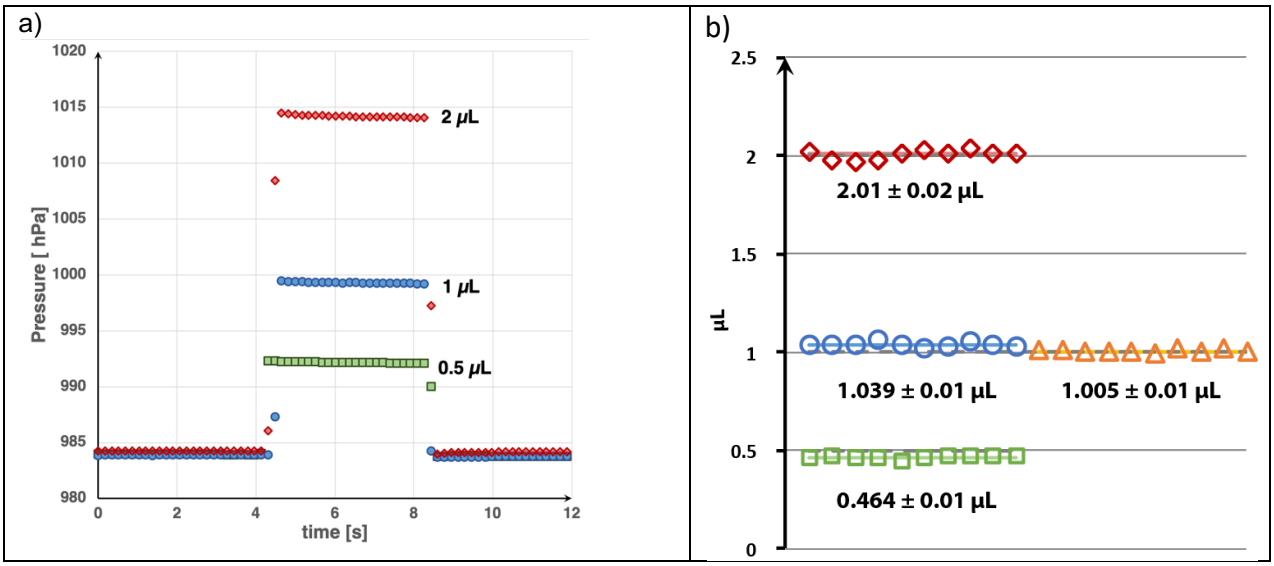

Figure 9: Pressure change measured for pipetting volume ranging between 0.5 to 2 µL.
a) pressure changes determined for a precision pipette with adjustable volumes of 0.5, 1 and 2 µL are shown as a function of time. To assess the stability of the pressure changes during the base line and piston displacement, recordings were performed over 4 seconds for each period. b) are the results obtained for series of 10 measurements conducted at 2, 1 and 0.5 µL. Blue circles and orange triangles illustrate volume measurements obtained for two pipettes; one variable volume set at 1 µL and one fixed 1 µL volume (see also Table 2).

Comparison of the data obtained with a minimized dead space with the values proposed by the manufacturer of a fixed 1 µL pipette reveals an average value of 1.005 ± 0.01 µL (n = 10) obtained by pressure changes versus a value of 1.005 ± 0.007 µL given by the fabricant (see Table 2).

Discussion

Today's standard calibration method for laboratory pipette is mainly based on the precise weighing of the dispensed liquid on a laboratory precision scale. While this method is perfectly adequate it requires precision and skill to conduct appropriate measurements. Moreover, care should be taken to adapt measurements in function of the temperature and to minimize evaporation.

For the gravimetric measurements, examination of table B.1 from the ISO_8655-2_2002 presents the influencing parameters resulting in error measurements further reveal that reuse of the tip with liquid or straightness of the pipette tips can induce errors ranging to 4 and 10% respectively. Thus, even for a pipetting device that is in good conditions and has passed the calibration tests proper manipulation and tip quality are evident sources of discrepancies.

As an alternative to gravimetry calibration can be done by dilution of a dye and the most common system uses differential measurements of two dyes having different absorption wavelength for the solute and the compound that is diluted. Whereas such method can be efficient and is often used for robotic pipetting it can only be done by a skilled person and necessitate the use of a precise photometric measurement.

Both gravimetry and dilutions methods imply the manipulation of liquid and therefore cannot be performed as a control using the same tip before a specific measurement is realized.

The development of a differential pressure device presented herein alleviates many of the difficulties encountered in gravimetric or dilution measurements and can be conducted by anyone without specific skill or training. Moreover, because the differential pressure device works in absence of liquid, measurements are independent of factors such as humidity or temperature.

Pressure measurements realized during a typical pipetting cycle, such as those illustrated in Figure 4 reveal a non-negligible pressure difference between the chamber of the pipette and the atmospheric pressure. Should the pipette present some leakage only above a certain pressure difference, this will result in inadequate pipetting volume only in conditions presenting sufficient pressure differences.

Measurements of the pressure changes observed during a steady state condition allows to determine the leakage of the pipetting device. Unless it is sufficient to alter the amount of liquid aspirated and dispensed by the device the pipette leakage at the piston level or at the tip connection cannot be characterized using gravimetry or dilution methods. Using the DPM method, the user can check immediately prior to his/her experiment the adequacy of the pipetting volume and air tightness off the micropipetting and tip device.

As exemplified by the simulations presented in Figure 3, appropriate accuracy can be achieved, using the appropriate dead volume V_0 and reference volume *Vref*, over a broad range of measurements. Providing adequate design this method can be extended either to small volume ranging below one μL, which are extremely difficult to measure using gravimetric methods. This is best exemplified by results obtained with two series of measurements shown in Table 2 that were conducted with a) a variable volume device and b) a fixed volume pipette. As shown in this table differential pressure measurements yielded results that are in good agreement with those given by the manufacturer. Repeatability determined using the DPM device further illustrate the stability of the pressure measurements and its expected low random error.

While gravimetric measurements can be transposed in different system and even in automated liquid handling, they necessitate the incorporation of a precise weight scale and appropriate mounting to avoid vibration effects. To avoid such cumbersome approach calibration of liquid handler are often conducted using photometric calibration with dual dyes. A clear limitation of both methods, resides in the fact that the calibration cannot be conducted on-line and that the liquid handling tip must be replaced before the liquid manipulation of interest.

In contrast, measurements using air displacement, such as those presented herein can be done prior to the sensitive liquid handling with the very same pipette tip and conditions as those used for the experiments. Direct reading of the pressure sensor by the internal computation unit minimize the development efforts and results can be transmitted to the liquid handling device for quality control before any specific operation.

Whereas data presented herein were done with a DPM device having a single tip port and single pressure sensing channel this system can readily be extended to height (8) or twelve (12) multichannel

pipetting system. Simultaneous reading of multiple heads will offer a significant advantage for the verification of multi-pipettes either for manual use or in robotic platforms.

Table 1: Summary of gravimetry and DPM device results

	10 µL		**20 µL**		**200 µL**		**1000 µL**	
	Grav.	DPM	Grav.	DPM	Grav.	DPM	Grav.	DPM
	9.6	10	19.8	20.06	200.7	200.82	1000.9	999.78
	10	10.1	19.8	20.06	201.5	200.82	1001	997.79
	9.9	10.11	19.8	20.04	199.1	200.73	998	998.43
	10.1	10.01	19.7	20.11	199.5	200.7	998.9	997.81
	10	10.11	19.8	20.05	199.8	199.09	999.1	995.66
	10	10.01	20	20.13	199.6	200.78	1000.1	997.56
	9.9	10.05	19.9	20.06	199.5	200.12	999.8	996.78
	10	10.13	19.8	20.07	200	200.46	998.4	995.97
	9.9	10.03	19.7	20.14	199.5	200.61	1000	996.38
	9.9	9.99	20	20.05	200	200.6	1001.3	996.39
Average [µL]	**9.93**	**10.05**	**19.83**	**20.08**	**199.92**	**200.47**	**999.75**	**997.26**
Random error [µL]	0.13	0.05	0.11	0.04	0.70	0.53	1.13	1.26
Systematic error	-0.70	0.54	-0.85	0.38	-0.04	0.24	-0.03	-0.27
Random error %	1.34	0.53	0.53	0.18	0.35	0.26	0.11	0.13

Table 2: Measurements for pipettes in the low µL range

µL	Volume [µL]										Average	Rnd	Sys %
0.5	0.46	0.47	0.46	0.46	0.45	0.46	0.47	0.47	0.47	0.47	0.464	0.007	-7.2
1	1.04	1.04	1.04	1.06	1.04	1.02	1.03	1.05	1.04	1.03	1.039	0.011	3.900
1*	1.01	1.01	1.00	1.00	1.00	0.99	1.02	1.00	1.02	1.00	1.005	0.010	0.500
2	2.02	1.98	1.97	1.98	2.01	2.03	2.01	2.04	2.01	2.01	2.006	0.023	0.3

*Note that by comparison for the second pipette the calibration given by the manufacturer was 1.005 ± 0.007 µL. Rnd = random error and Sys = systematic error was computed according to the Centre Suisse de Contrôle de Qualité (CSCQ) which defines the $Error = (\frac{(Cm-Cn)*100}{Cn})$ where Cm is the average measured values [µL] and Cn is the nominal value of the pipette [µL].