A FAST AND LOW-VOLUME PIPETTOR WITH INTEGRATED SENSORS FOR HIGH PRECISION

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ABSTRACT

This paper describes the design and the measurement results of a new micropipetting device for the pipetting of biological liquids. The device has been designed for precise, accurate and fast pipetting in the micro- and submicroliter range. It contains a precision pipetting head with two integrated sensors realised in silicon bulk micromachining to ensure precision and accuracy and a coupled piezo disk-type actuator to fulfil the force requirements of fast pipetting. Measurements in open-loop mode showed pipetting of 0.5 to 2µl with a CV between 1 and 7%. Closed-loop control of the actuator can eliminate the piezoactuator's hysteresis and drift and further increase precision and accuracy.

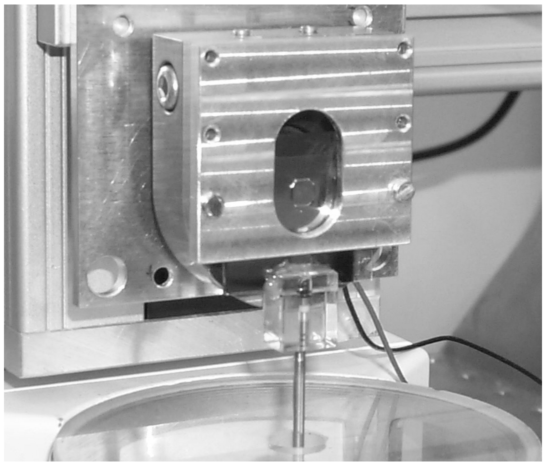

Figure 1: Photograph of the micropipettor

INTRODUCTION

In the environment of the in-vitro diagnostics there is an increasing demand for high precision pipetting of biological liquids (e.g. serum) and reagents in the micro- and submicroliter range. Precision pipetting means controlled and precise aspirating and dispensing of a given volume as compared to dosing where only the dispensing of a liquid is required.

Liquid handling devices proposed so far, however, are mostly concerned with the dosing of liquid. On the one hand, many micropumps and –valves, mostly without sensors [1,2,3, except 4,5] have been reported. On the other hand, many dispensers based on the ink-jet principle have been realised [6,7]. Since the latter does not enable aspirating, [8] has proposed a combination of micro-pumps and ink-jet principle.

We have realised an all-in-one device for aspiration and dispensing with integrated pressure and position sensors (Figure 1). Our micromachined two-way-device can pipette liquid without any valves, hence with no reflux or dead volume. We can aspirate liquid from a primary cup and dispense the same amount onto a liquid surface or into a liquid, e.g. of a water-filled cuvette.

To obtain high precision and to monitor the whole pipetting process, two capacitive sensors have been integrated. One position sensor to measure the displaced volume of a deflected membrane, and one differential pressure sensor to detect malfunctions, e.g. clotting of the tip or to detect highly viscous patient samples.

To fulfil the force requirements for very fast pipetting, a piezo disk-type actuator was used and coupled to the device in a unique fashion. In particular, since the coupling was realised bond-free, it enables a very flexible modular set-up with the coupled actuator on one side and the precision pipetting head on the other. By changing either the piezoactuator type or the geometry of the membranes of the pipetting head, the micropipettor can easily be adapted to fulfil various pipetting requirements. The potential for other volume ranges e.g. was demonstrated in a previous work, where volumes between 190nl and 6µl were pipetted [9,10].

Finally, using the signals of both sensors for closed-loop-control of the coupled actuator will eliminate the piezoactuator's hysteresis and drift and thus further increase precision and accuracy.

DESIGN

Modular Set-up

The micropipettor is basically realised as a modular set-up consisting of two parts: a precision pipetting head fabricated in silicon and an actuation coupling system (Figure 2 and Figure 3). The precision pipetting head contains the structures for the liquid handling and the sensor elements, whereas the actuation coupling system couples the piezo disk-type actuator to the pipetting head.

Precision Pipetting Head

The precision pipetting head is a silicon module encapsulated between two glass plates with a pipetting tip at the front side. The silicon module itself includes an actuator membrane, a sensor membrane, a channel connecting the two membranes and an output channel to the pipetting tip. The actuator membrane also contains a boss to enlarge the displaced volume per stroke length of the piezoactuator. Together with the channels and the pipetting tip, the upper cavities define the fluidic parts of the pipetting head, whereas the lower cavities form the electrode gap for the two capacitors integrated on both membranes. The capacitor on the actuator membrane thereby serves as a position sensor measuring the deflection of the membrane, hence the displaced volume. The smaller sensor membrane serves as a differential pressure sensor giving the pressure difference between atmosphere and the fluidic chambers. With the pressure signal the pipetting process can be monitored and malfunctions, e.g. clotting of the tip, detected. Finally, in the backside glass, a hole was drilled to couple the stroke of the piezoactuator.

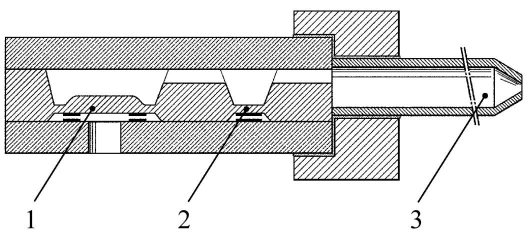

Figure 2: Schematic longitudinal section of the precision pipetting head:
1 actuator membrane with boss,
2 sensor membrane, 3 pipetting tip

Fabrication of the Precision Pipetting Head

From a thermal oxide of 2100nm the membranes are time-controlled etched in KOH through subsequent opening of the oxide layer with several lithography steps. After this, a reoxidation of 50nm is performed to insulate the capacitors from each other. Then, 500nm of aluminium are evaporated on the backside of the wafer and both aluminium and thermal oxide are structured to the final electrode design with two further lithography steps.

On the glass wafer (Corning Pyrex 7740) for the backside, 500nm of aluminium is evaporated and structured. The hole of 4mm diameters was drilled in the 0.5mm thick Corning Glass with the use of a diamond-coated drill bit. Thereby the glass was positioned and fixed by means of a simple gauge.

Finally, the necessary connector for the pipetting tip is epoxy-glued to the pipetting head. Due to its inner thread pipetting tips of various lengths and diameters can be attached.

Actuation Coupling System

For the precise pipetting in the given volume range an actuator with a comparatively large stroke and nonetheless very high resolution is required. Also, for fast pipetting with long pipetting tips as needed for commercial application, comparatively large forces must be applied. To fulfil the mentioned requirements, a piezo-disk type actuator with a stroke of 50µm, sub-nm resolution and a force capacity of 10N was chosen. In contrast to piezostacks, which would also fulfil the specifications, the disk-type piezo furthermore offers a reasonable size. For the coupling of the chosen piezoactuator a special coupling system (Figure 3) to enable accurate positioning and virtually shift-free fixation in the chosen position was developed.

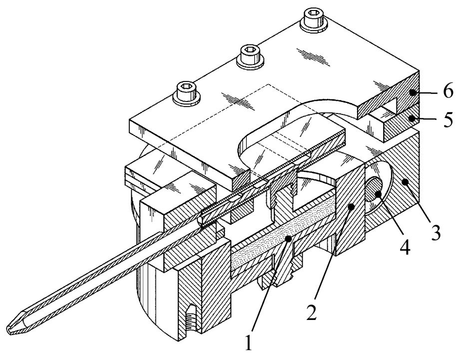

Figure 3: Longitudinal section of the complete micropipettor:
1 piezoactuator, 2 inner cylinder,
3 outer cylinder, 4 bolt with screw (inside),
5 mounting plate, 6 clamping plate

The actuation coupling system consists of two cylinders, which can easily be shifted against one another. Thereby the pipetting head is mounted and clamped on the outer cylinder by means of a mounting and clamping plate and the piezoactuator is fixed on the inner cylinder. Shifting the inner cylinder versus the outer allows aligning of the piezoactuator versus the actuator membrane.

To precisely align the piezoactuator to the actuator membrane, we developed a simple docking station: the outer cylinder can be fixed on the docking station and the inner cylinder is pulled against a spring with screws. The correct position for the piezoactuator is found under an optical autofocus profilometer, which measures the deflection of the membrane boss with a resolution better than 0.1µm. For the results presented below, a preload for the membrane with a deflection of a few micrometers was chosen. It is envisaged, however, to replace the profilometer and to measure the position of the piezoactuator by means of the position sensor integrated in the actuator membrane.

To fix the piezoactuator in the chosen position, the two cylinders must be fixed together. This is done with two bolts in the outer cylinder. The bolts have the same curved surface as the cylinders and are pulled together over an initial gap with a screw. The curved surfaces of the bolts inside the outer cylinder then clamp the inner cylinder and the chosen position is fixed. The docking station with its spring is then removed and the micropipettor as shown in Figure 3 is mounted on the z-axis drive of the measurement set-up.

MEASUREMENT SET-UP

The micropipettor was tested with the measurement set-up shown schematically in Figure 4.

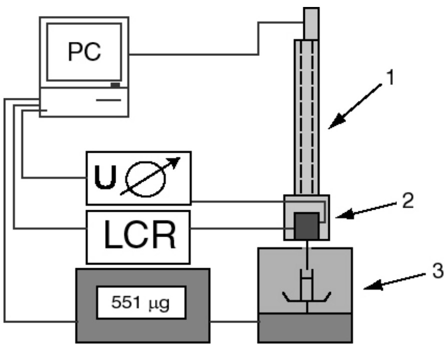

Figure 4: Schematic representation of the measurement set-up: 1 Z-axis drive, 2 Micropipettor 3 Micro-balance with sample cup

For aspiration and dispensing, the pipetting tip of the micropipettor dips in and out of the sample. The sample is placed in a little cup on the micro-balance (Mettler-Toledo MT5). The balance has a resolution of 1µg that corresponds to 1nl of water. It measures the aspirated and dispensed mass, hence the pipetted volume of a liquid with known density. The exact position of the pipetting tip versus the liquid surface level is controlled with a z-axis drive. Its implemented stepper motor has a stepwidth of 12.5µm and a position-reproducibility far better than 0.1mm.

The piezoactuator is controlled with a Source-Measure-Unit and the capacitance changes of the sensors are measured with an LCR-meter. Since the complete measurement set-up is fully PC-controlled, many repetitive cycles can easily be carried out to test the precision of the micropipettor.

In order to prevent evaporation, which at a microliter scale must be taken into consideration, the balance chamber is covered with a glass plate, allowing only the pipetting tip into the chamber through a small opening. Additionally, a wet sponge was put on the bottom of the chamber in order to increase the relative humidity and thus to reduce evaporation. Finally, since the evaporation rate was measured before pipetting, the results were compensated for.

WORKING PRINCIPLE

The results presented below were obtained with the following repeated pipetting cycles. Note that during all cycles, all fluidic parts of the micropipettor except the lower part of the tip are filled with air.

To aspirate a defined liquid volume, the actuator membrane of the precision pipetting head is deflected by the coupled piezoactuator. Then, the pipetting tip is dipped into the sample liquid and the piezoactuator is pulled back. Due to the comparatively strong relaxation forces of the actuator membrane, the membrane is released to its initial position and aspirates the sample. After this, the tip is again dipped into the sample liquid and by actuating the piezo, the membrane is deflected and the sample is pushed out of the pipetting tip back into the cup. In between, the mass in the sample cup is measured with the balance and the differences calculated to give the pipetted mass (Figure 5).

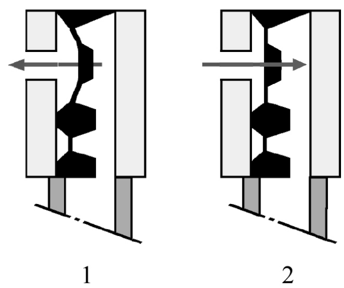

Figure 5: Scheme for aspiration and dispensing (the tip is dipped in the sample liquid): 1 Aspiration: the deflected membrane relaxes and aspirates the sample; 2 Dispensing: the membrane is deflected and dispenses the sample.

RESULTS

The results were obtained with the piezoactuator controlled in open-loop mode and with DI-water as sample liquid. For each cycle, the piezoactuator was given a linear ramp of 3s to reach the given voltage. The maximum deflection of the piezoactuator is achieved at 750V. The tip was dipped 5mm into the sample liquid for aspiration and 0.1mm for dispensing.

As can be seen from Figure 6, we have successfully aspirated and dispensed in the micro- and submicroliter range between 0.5 and 2µl (1µl corresponds to 1000µg of water). Furthermore, the figure also shows a good linear relation between the applied voltage on the piezoactuator and the pipetted mass. Moreover, after a few instable cycles, which were observed at the beginning of a sequence, coefficient of variation was only between 1 and 7% and only a small difference in mass between aspiration and dispensing was observed. As the pipetting tip was always dried with nitrogen flow before a pipetting sequence, these instable cycles may be the result of changing surface conditions inside the tip.

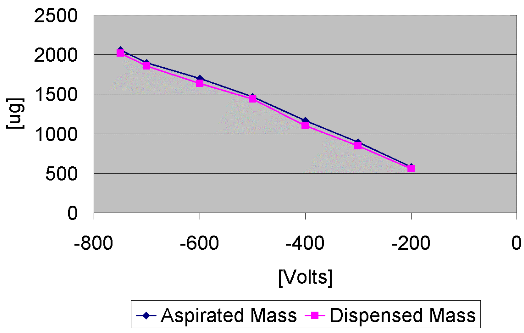

Figure 6: Measured mass of pipetted water versus the applied voltage on the piezoactuator (1µl corresponds to 1000µg of water)

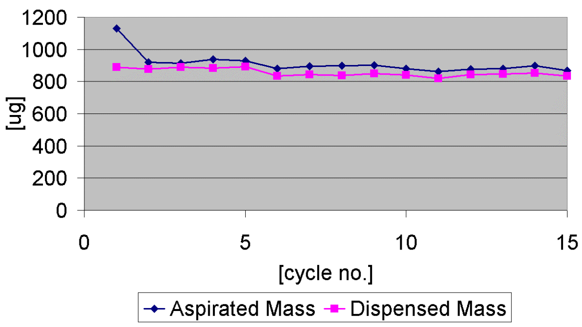

Figure 7: Pipetting precision of 15 cycles with DI-water as sample liquid. At 300V an average of 892µg of water was aspirated and an average of 848µg dispensed.

To illustrate the results of repeated pipetting cycles, a sequence of 15 cycles is shown in Figure 7. In the first cycle the aspirated mass is much higher than the dispensed mass, but then an average of 0.89µl of DI-water with a CV of 2.5% was aspirated, an average of 0.85µl with a CV of 2.6% was dispensed. The piezoactuator was deflected with a voltage of 300V. At each cycle, the aspirated mass was slightly higher than the dispensed mass. In average the difference between the two volumes is only 40nl and can easily be compensated with a slightly higher voltage for dispensing.

Figure 8 finally shows the hyperbolic decrease of the position sensor capacitance over the applied voltage, hence the displaced volume. Since capacitance measurement allows a precise monitoring of the membrane deflection, the signal of the position sensor can therefore be used to define the pipetted volume.

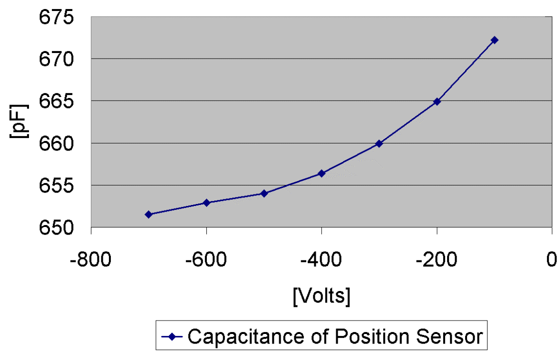

Figure 8: Capacitance of the position sensor integrated on the actuator membrane vs. the applied voltage on the piezoactuator

CONCLUSIONS

We have realised an all-in-one device for aspiration and dispensing with integrated pressure and position sensors for high precision. We could demonstrate successful pipetting in the micro- and submicroliter range with high precision between 1 to 7% CV in open-loop mode. Furthermore, due to the successfully coupled piezoactuator, our system allows fast pipetting through long pipetting tips as needed for commercial application.

Since the coupling of the actuator to the precision pipetting head is bond-free, a modular set-up was realised, which offers maximum flexibility for various pipetting requirements. By adapting either the piezoactuator type or the actuator membrane geometry of the pipetting head, different volume ranges or various force specifications e.g. can easily be fulfilled. Moreover, as different pipetting heads can easily be interchanged on one actuation coupling system, flexibility for the integration in an in-vitro diagnostic system is further increased.

Finally, using the signal of the position sensor, the piezoactuator can be closed-loop controlled to eliminate its hysteresis and drift. Therefore, together with the feedback of the pressure sensor signal, precision and accuracy as well as reliability of the micropipettor will be further increased.

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