Nanoliter Dispensing on Pipetting Workstations by Disposable PipeJet-Tips

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Abstract:
We present a liquid handling system for the contact-free application of liquid volumes in the nanoliter range. The droplet generation mechanism is based on the PipeJet™ technology [1, 2] but applies a new kind of disposable pipetting tip, termed PipeJet-tip. A piezo stack driven piston squeezes the lower part of the tip, an elastic tube with an inner diameter of 650 µm, to generate free-flying droplets with volumes down to 20 nl (CV < 5%). This technology is applicable to dispense liquids of a wide range of rheology. Liquids like water, DMSO and 50% glycerol/water mixtures have been dispensed successfully. In addition, even volumes in the microliter range can be delivered by the ejection of multiple droplets at frequencies up to 50 Hz. The PipeJet-tips are fabricated by injection moulding, which enables low cost, disposable products. The actuation unit automatically locks the tips which are loaded into the unit by a pipette handling robot. The parts which are in contact with the liquid are automatically exchangeable. The presented system is fully compatible with existing pipetting workstations

Keywords: Liquid handling, sub microliter, disposable tip, non-contact dosage

Introduction

The need for a precise delivery of fluids in volumes below one microliter increases more and more in the chemical, biological as well as pharmaceutical research and industry. Cross-contamination-free transfer of small liquid volumes on automated workstations with low dead volume and high precision is an important requirement for many applications e.g. in high throughput screening, protein crystallography, cancer research or DNA analysis [3, 4]. Commercially available liquid handling lab robots mostly use steel-needles or air cushion pipettes for dispensing [5]. Steel–needle systems imply the disadvantage of cleaning efforts after usage. Pipette-based systems utilise disposable tips that provide the best safety regarding cross-contamination, but so far are limited to volumes in the microliter range. The presented system combines the advantages of disposable pipette tips and the ability of the ejection of liquid volumes in the low nanoliter range. An autonomic wireless eight-channel dispenser is easy to integrate in existing pipetting workstations.

PipeJet™ Technology

The applied droplet ejection method is based on the PipeJet™ technology (BioFluidix GmbH, [1]). It uses direct displacement by deformation of a liquid filled plastic tube. The extension of a piezo-electric stack actuator (extension length 10 – 25 µm, adjustable by the applied voltage) moves a piston to squeeze the liquid filled tube which in return causes the displacement of the liquid inside. Due to the ratio of the fluidic downstream resistance at the open end (nozzle) and the fluidic upstream resistance at the reservoir $R_{nozzle} << R_{reservoir}$, a well-defined major percentage of the displaced liquid is ejected out of the nozzle, forming a free-flying droplet. The tube refills by capillary forces, initiated by the release of the actuator [1].

Evidently, the deformation of the tube as well as the mechanical mounting has a major influence on the dosage performance. Reproducible dispensing results require a defined tube position relative to the actuation piston. Fig. 1 shows the mechanical mounting of a manually clamped PipeJet-tip.

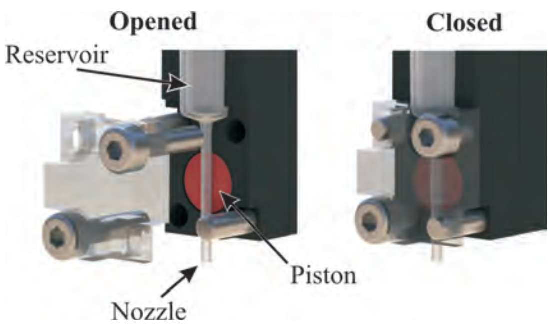

Fig. 1: *Illustration of the clamping of the PipeJet tip. The illustration especially highlights the region of the flexible tube.*

The volume of the ejected droplets can be adjusted by mainly two electrical control parameters: The extension of the piezo (stroke, s) and the velocity of the piezo extension (downstroke velocity, ds). Benefits of the PipeJet^{TM} technology are the very small dead volume and the separation of all liquid containing parts from the actuator.

PipeJet-tips

The new pipetting tips, illustrated in Fig. 2, provide a 300 µl reservoir which is compatible to standard 200 µl pipette heads. The tips can be used for conventional or automated pipetting. Furthermore, these pipetting tips can be applied for non-contact dispensing in the nanoliter range, when driven by the piezo-electric actuation unit as described above.

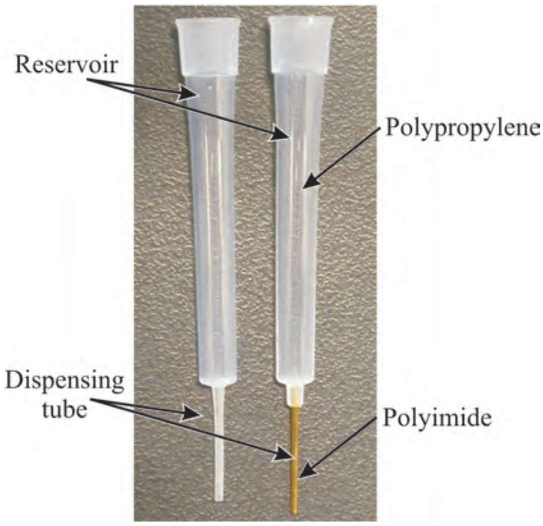

***Fig. 2**: PipeJet-tips: one- (left) and two-component version.*

First prototypes of the PipeJet-tips are fabricated in a two component injection moulding process. Therein a 15 mm long polyimide tube with an inner diameter of 650 µm and a wall thickness of 30 µm are moulded into a 300 µl polypropylene cone (two-component tips). To implement the fabrication of the tips by mass production processes, the tips are also casted in a single component (pure polypropylene) injection moulding process (one-component tips). The inner dimensions of the tips are identical to the two-component version. Due to limitations of the injection moulding process in high aspect ratios, the wall thickness of the tube region had to be increased to 100 µm.

In a so called open setup the liquid meniscus inside the nozzle is hold by capillary forces only. The maximum filling level of the tips is 100 µl for the one-component tips and 140 µl for the two-component version. Those limits are influenced by the liquid properties and the PipeJet-tip material which define the capillary pressure. To use the entire reservoir volume, an additional control of the pressure at the upper end of the tip is required.

Automated actuator

The state-of-the-art actuator of the PipeJet^{TM} technology, e.g. the PipeJet^{TM} P9 module [6], requires the manual loading and clamping of the dispensing tubes (see Fig. 1). This manual process is not suitable for the automated use in high-throughput-screening applications. High numbers of different kinds of applied liquids require a frequent exchange or cleaning of the liquid contaminated parts. Therefore, a mechanism was developed which enables to lock the PipeJet-tips in the actuation unit automatically.

To realize this unit, the required clamping force was investigated experimentally. A specific single channel prototype enabled the adjustment of the clamping force to define the most efficient working condition. The experiment revealed that the clamping force has a strong influence on the dispensing process. The results, using the two-component tips, are presented in Fig. 3. It can be seen that a force of at least 40 N is necessary to reach a high dosage volume and minimize the influence of the clamping process.

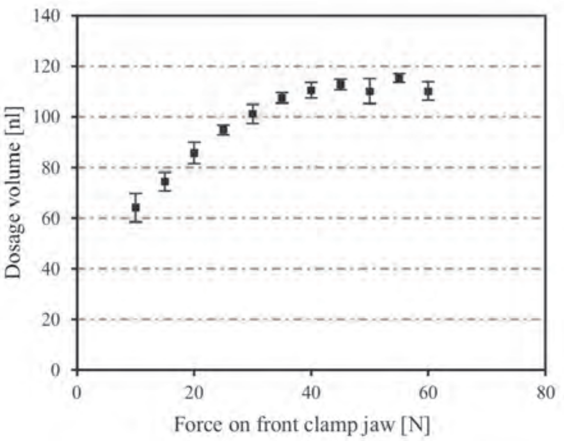

***Fig. 3:** Influence of dosage tube clamping force on dispensing performance.*

With respect to these results, an automated locking mechanism was developed to mount the PipeJet-tips. Fig. 4 shows the first prototype setup based on modified PipeJet^{TM} P9 modules. The modification of the modules comprise the integration of smaller piezo stacks (length of 2 x 13.5 mm instead of 2 x 18 mm) and a smaller piston diameter (4 mm instead of 5 mm) decreasing the module dimensions to enable the design of a small sized eight-channel actuator (see future prospects). The automated clamping mechanism is driven by a brushless DC-motor capable of high torsional moments at a small footprint. The motor rotation is transferred to a linear movement, actuating the locking mechanism by a custom made snail gear. High precision miniature slides are used to guide the parts encasing the dosage tubes in linear direction. The opened and closed positions are defined by limit switches and the whole system is controlled by a custom made electronic circuit and software.

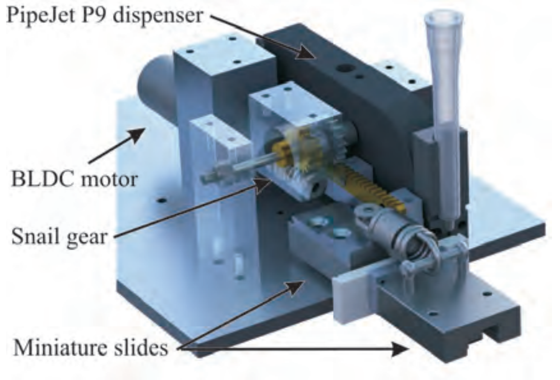

Fig. 4: Prototype of the automated actuator.

To achieve the necessary forces for the clamping mechanism, two different approaches were evaluated. The first approach is based on a pull-spring (40 N at 2 mm expansion), which connects the driving slide and the slide of the clamping mechanism (see Fig. 4). The second approach implements the self-locking ability of the snail gear in combination with the high momentum of the motor.

Evaluation Method

The PipeJet-tip dispensing performance was evaluated by using the actuator setup as described before. The dispensed volumes were measured gravimetrically by a high precision balance (Sartorius SC2). The values are sampled by custom made evaluation software which also triggers the dispensing events, sets the specific actuation parameters and compensates environmental influences like evaporation or vibrations [7].

Evaluation Results

The tips have been loaded fully automatically by a pipetting arm from a tip rack, liquid was aspirated by conventional air displacement and the tip was finally released into the piezoelectric actuation unit that performed the non-contact droplet dispensing onto the weighing balance.

Results generated by the two-component tip in the automated setup are presented in Fig. 5. For each set of parameters (stroke; downstroke) the mean value of 10 single droplets is displayed. By varying the piezo stroke and downstroke velocity, volumes in a range of 11 to 66 nl were achieved with a coefficient of variation (CV) below 9 %. Due to modifications of the PipeJet™ dispensing module mentioned above, the volumes are smaller than in the preliminary experiment presented in Fig. 3.

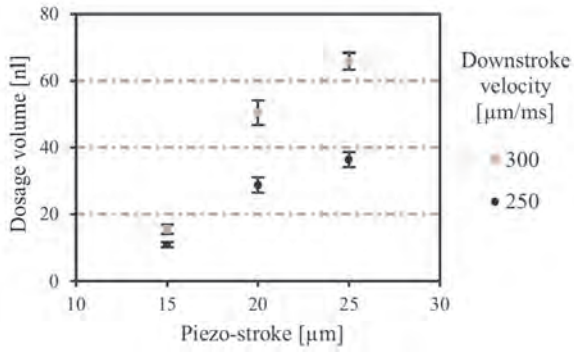

Fig. 5: Characterization of automatically locked two-component PipeJet-tip (average of 10 single droplets, tip loaded once).

Despite the assumption, that the changed material (PP instead of PI) and geometry (wall thickness) in the dosage tube would have a major influence on the dispensing performance, the results of the one-component tips are comparable to the results gained with the two-component tip version. A first experiment using the same setup and parameters as for the two-component version is displayed in Fig. 6. The volume ranges from 14 to 66 nl with CV below 9 %. These are promising results and demonstrate that low cost disposable tips made by injection molding can be applied for dispensing in the nanoliter range.

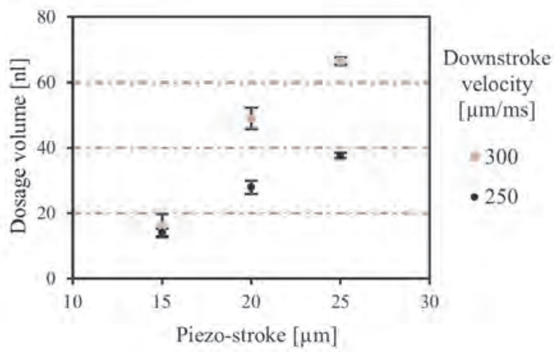

Fig. 6: Characterization of automatically locked one-component PipeJet-tip (average of 10 single droplets).

The dependency of the dispensing volume on the piezo-stroke shows a linear correlation with a correlation coefficient of $R^2 = 0.98$ for both kinds of tips. Both parameters stroke and downstroke velocities enable the easy adjustment of the droplet volume. The experimentally proven possibility to dispense multiple droplets at frequencies up to 50 Hz allows fast and precise dispensing of volumes in the microliter range.

In the experiments, presented in Fig. 7 three different media were dispensed successfully with CVs below 4 %. Besides water (viscosity of 1.0087 mPa·s @ 20°C) as well the widely spread solvent DMSO (dimethyl sulfoxide, 2.14 mPa·s @ 20 °C) and a 50 % glycerol/water mixture

(6.05 mPa·s) were used. These experiments not only prove the applicability of the PipeJet™ technology to liquid properties in a certain range, but also indicate its long term stability. All experiments investigating the media applicability were accomplished by the ejection of more than 1900 single droplets. Each measurement consisted of 15 single droplets considering water and DMSO to reach a target volume of approximately 2 µl. For the glycerol mixture only 10 droplets were required to reach the target volume. The applied droplet ejection frequency was f = 50 Hz.

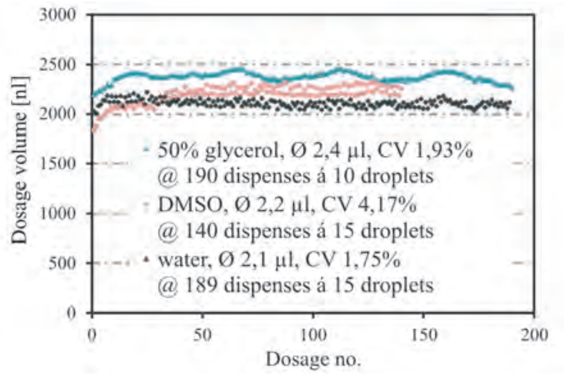

Fig. 7: *Long term stability of two-component tips (version with 800 µm dosage tube inner diameter) for different media. One tip loaded for each medium, stroke 20 µm, downstroke velocity 150 µm/ms.*

Conclusion

The presented liquid handling system based on the PipeJet™ technology enables nanoliter dispensing with automatically exchangeable and disposable tips. Free-flying droplets of various media with volumes in the sub microliter range are ejected at high precision. The system is compatible with existing liquid handling systems.

Future Prospects

Currently an eight-channel actuation system based on the PipeJet-tip technology with automatically exchangeable tips is in development. All necessary electronics are housed in the actuator alongside rechargeable batteries enabling the system to be moved to the desired dispensing position without being tied to cables.

Having only the size of a standard well plate, the automated 8-channel PipeJet-tip dispenser can easily be integrated in existing pipetting robots extending their volume range down to nanoliter. A CAD model of the system is displayed in Fig. 8.

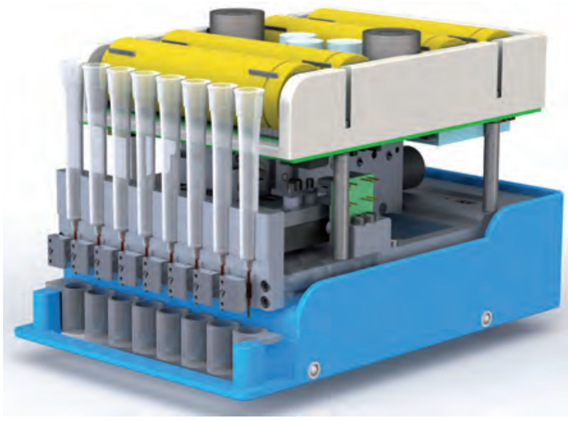

Fig. 8: CAD *model of the 8-channel PipeJet-tip dispenser.*

Acknowledgements

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