Diaphragm pico-liter pump for single-cell manipulation

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Abstract A pico-liter pump is developed and integrated into a robotic manipulation system that automatically selects and transfers individual living cells of interest to analysis locations. The pump is a displacement type pump comprising one cylindrical chamber connected to a capillary micropipette. The top of the chamber is a thin diaphragm which, when deflected, causes the volume of the fluid-filled cylindrical chamber to change thereby causing fluid in the chamber to flow in and out of the micropipette. This enables aspirating and dispensing individual living cells. The diaphragm is deflected by a piezoelectric actuator that pushes against its center. The pump aspirates and dispenses volumes of fluid between 500 pL and 250 nL at flow rates up to 250 nL/s. The piezo-driven diaphragm arrangement provides exquisite control of the flow rate in and out of the capillary orifice. This feature, in turn, allows reduced perturbation of live cells by controlling and minimizing the applied shear stresses.

Keywords Single-cell manipulation · Micropump · Microinjection · Pico-liter pump · Microfluidics

1 Introduction

Capillary-based cell manipulation using aspiration and dispensing is the most reliable, robust, minimally traumatic, and widely accepted technique for single-cell manipulation and injection (Sanford et al. 1948). A negative pressure applied to the capillary draws medium into the pipette, while a positive pressure dispenses the pipette contents. For many years, mouth pipetting was the most popular method for manipulating liquids and cells, which led to undesirable accidental aspiration of toxic substances in addition to causing accidental infection among laboratory workers (Phillips and Bailey 1964). Due to biosafety concerns, mouth pipetting is strictly prohibited in most laboratories and has been replaced by mechanical methods. Mechanical pipetting methods include the use of simple devices such as hand-held plungers, or rubber bulb syringes, which do not provide high fluid volume and flow control. Commercially available mechanical injectors, including the CellTram[®] microinjector (Eppendorf), are used for accurate manual pressure control and liquid dispensing. Other methods that are used for loading and placement of individual cells include using a Peltier heater/cooler (Wewetzer and Seilheimer 1995), and a syringe pump (Anis et al. 2008, 2010; Lu et al. 2010). The syringe pump used in Anis et al. (2008, 2010) was theoretically capable of aspirating volumes as small as 2 nL at flow rate up to 2 μ L/s. However, fine flow control of a syringe pump is difficult due the low syringe

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stiffness in addition to the compliance of the fluidic tubing.

A number of microfluidic devices have been reported for delivery of small volumes of fluid (Smits 1990a, b; Van Lintel et al. 1988; Van Lintel 1992; Brennan 1992). Manufacture of these devices, often called micropumps, usually employs semiconductor etching and substrate joining technologies such as anode joining methods for connecting glass and silicon substrates. Early micropump designs included those proposed by Smits (1990a, b), Van Lintel et al. (1988, 1992). Smits's pump included valves that contained a diaphragm covered by a single layer of piezoelectric material, which limits the control and total deflection of the valves. Van Lintel developed a single-chamber, reciprocating displacement pump comprised of a thin glass pump membrane, actuated by a piezoelectric disc, and passive silicon check valves to direct the flow. These micropumps are flow rate controlled pumps that have the ability to displace minute fluidic volumes. However, they are continuous-flow type pumps that displace fluid from the source to the sink and they are not optimized for aspiration/dispense applications. Szita et al. presented a micropipetting device for both aspirating and dispensing (Szita et al. 2001). Their device included a precision pipetting head with two integrated sensors and a coupled piezo disk-type actuator and pipetted aqueous volumes in the range of 190 nL to 6 μ L.

In this paper, we present a pump capable of displacing small volumes of fluid, in the pico-liter range, for the manipulation of small particles or biological cells. The proposed pico-liter pump is a displacement pump, where the moving surface is a diaphragm moved by a piezoactuator. This pump is capable of manipulating fluid volumes between 500 pL and 250 nL, at flow rates up to 250 nL/s. The proposed pump is perfectly suited to the manipulation of biological objects due to its compatibility with standard biological and sterilization procedures.

2 System description and operation

The main components of the diaphragm pump assembly are presented in Fig. 1(a) and include the pump body (1) and cap (2), which enclose a flexible diaphragm membrane (3). This flexible diaphragm membrane closes the top of a chamber, a cylindrical cavity in the pump body shown in Fig. 1(b). The chamber (4) connects to a micropipette capillary (9) through a fluidic channel "D", and connects to an external macropump through the inlet channel "A" and

Fig. 1 The diaphragm pump: **(a)** assembly and main components; **(b)** cross-sectional view showing chamber, diaphragm, and piezoactuator

a control valve (8), attached to the pump body. During normal pump operation, the valve port "A" is closed. When the port "A" is closed, a change in chamber volume causes fluid to be aspirated or dispensed from the micropipette capillary.

2.1 Principle of operation

The diaphragm (3) is driven by a piezoelectric stack actuator (5) that pushes against the diaphragm center, causing it to deflect inwards, as shown in Fig. 1(b). The piezoactuator displacement is proportional to the voltage applied to its leads. Therefore a change in voltage causes a change in the diaphragm deflection. System operation entails filling the chamber, channels and micropipette with fluid media. An increase in the diaphragm deflection decreases the chamber volume, increasing the chamber fluid pressure. This causes fluid to be dispensed from the capillary micropipette orifice. A reduction of the flexible diaphragm deflection increases the chamber volume, which creates a negative pressure and causes aspiration of fluid into the micropipette.

2.2 System components

2.2.1 Pump body assembly

The cylindrical pump chamber (4) is connected to a control valve and a micropipette through fluidic channels

“A” and “D”, respectively (see Fig. 1(b)). An O-ring seal (6), inserted into an annular groove in the pump body, and the diaphragm seal the chamber and ensure that fluid does not leak in or out of the chamber. The piezoelectric actuator (5) rests on the diaphragm, centered above the chamber, and is supported by a special housing in the pump cap. The depth of the piezoactuator housing is selected to ensure full piezoactuator-diaphragm contact and to maintain slight downward diaphragm deflection in the absence of any applied voltage. The pump layers are assembled and aligned using eight socket head cap screws (7).

2.2.2 Control valve and connections

A three-way control valve (8) is assembled to the side of the pump body, as shown in Fig. 2. The normally closed (NC) port of the valve is connected to the pump chamber (4) through fluidic channel “A”. The common (COM) port is connected to a bidirectional-flow external pump “macropump” and the normally opened (NO) port is connected to drain. These connections are made to the pump body fluidic channels “B” and “C” using poly-etheretherketone (PEEKTM) tubing, fittings and connectors. When the control valve is OFF, the external pump connects to the drain with the pump chamber perfectly isolated. When the valve is switched ON, the external pump connects to the pump chamber where medium can be pressurized and caused to flow through the capillary micropipette. This is only required during setup and priming of the pump.

Borosilicate glass capillary micropipettes are commonly used for the manipulation of fluid, micro-objects,

and single cells. Available options include homemade or commercially available micropipettes (9). A capillary holding fitting (10) was used to connect the micropipettes to the pump body, as shown in Fig. 1(a). During micromanipulation, it is preferred to orient the pipette with its tip either vertical or horizontal. Here, micropipettes with tips angled at 45° to the main shaft were selected. Rotating the 45° angled micropipette around its main luminal axis enables orienting its tip either vertical or horizontal.

3 Mathematical modeling

In this section, a mathematical model is developed to assist in the selection of the chamber design diameter. That is done by determining the diameter effect on the pump static performance. The relations between the piezoactuator displacement and the diaphragm deflection, manipulated fluidic volumes, and the manipulated particles' positions are derived.

3.1 Diaphragm deflection

The pico-liter pump functions by a piezo-actuated diaphragm applying positive or negative pressure to the media in its main chamber. Here, the relationship between piezoactuator displacement and the volume change in a chamber of diameter D_c is derived. The diaphragm was simplified into a fixed-fixed bending beam of length D_c , shown in Fig. 3. A displacement δ_{mid} , equivalent to that applied by the piezoelectric actuator on the diaphragm's center, acts at the middle of the beam. At steady state, the pressure inside the chamber p equalizes with the atmospheric gauge pressure (i.e., $p = 0$).

Because diaphragm deflections are less than 0.01% of D_c , the diaphragm material can be treated as linearly elastic. The beam deflection δ is a function of the beam position x and is given by Seely and Smith (1932):

$$\delta = -\frac{x^2 \delta_{mid}}{D_c^3} (4x - 3D_c). \quad (1)$$

Fig. 3 Equivalent diaphragm beam deflection actuated by piezoactuator

Fig. 2 Schematic showing control valve (switched off) and common (COM), normally opened (NO), and normally closed (NC) ports

The total volume “ Vol ” displaced by the pump diaphragm (i.e., the reduction in the chamber volume) is the volume of the solid of revolution generated by rotating the deflection curve around the chamber axis:

$$Vol = \int_0^{2\pi} \int_0^{\frac{D_c}{2}} \left(\frac{D_c}{2} - x \right) \delta \, dx \, d\theta, \quad (2)$$

where θ is the angle of revolution.

Combining Eqs. 1 and 2 gives:

$$Vol = 0.3 \, \delta_{mid} \frac{\pi D_c^2}{4}, \quad (3)$$

which shows that the volume Vol is a function of chamber diameter and membrane displacement at the diaphragm center.

3.2 Piezoactuator displacement

Due to compliance in the piezoactuator stack, the diaphragm center deflection δ_{mid} is the difference between piezoactuator displacement δ_{pz} and deformation in the piezoactuator stack δ_{deform} :

$$\delta_{mid} = \delta_{pz} - \delta_{deform} = \frac{\delta_{pz(max)}}{V_{max}} V - \delta_{deform}, \quad (4)$$

where V is the voltage applied to the piezoelectric actuator leads, and $\delta_{pz(max)}$ is the maximum piezoactuator no-load displacement achieved when the maximum voltage V_{max} is applied across the piezo leads. Both $\delta_{pz(max)}$ and V_{max} are specified by the piezoactuator manufacturer.

The piezoactuator deformation δ_{deform} is proportional to the diaphragm retraction force on the actuator. Therefore, δ_{deform} is proportional to δ_{mid} :

$$\delta_{deform} = \frac{k_d \, \delta_{mid}}{k_p}, \quad (5)$$

where k_p is the stiffness of the piezoelectric actuator, k_d is the stiffness of the diaphragm presented by:

$$k_d = \frac{16\pi E w^3}{3(1-\nu^2) D_c^2}, \quad (6)$$

w is the diaphragm thickness, and ν is poisson’s ratio.

Substituting Eq. 5 into Eq. 4:

$$\delta_{mid} = \frac{k_p \delta_{pz(max)}}{V_{max}(k_d + k_p)} V. \quad (7)$$

3.3 Volume displaced

When the voltage changes by a magnitude ΔV , Eq. 3 implies that the change in the chamber volume ΔVol becomes:

$$\Delta Vol = 0.3 \, \Delta \delta_{mid} \frac{\pi D_c^2}{4}, \quad (8)$$

where

$$\Delta \delta_{mid} = \frac{k_p \delta_{pz(max)}}{V_{max}(k_d + k_p)} \Delta V. \quad (9)$$

Equation 8 can be used to determine the change in chamber volume caused by applying a voltage potential to the diaphragm piezoactuator. From Eqs. 8 and 9, the relation between the chamber diameter D_c and the maximum volume change, where $\Delta Vol = \Delta Vol_{max}$, is:

$$D_c = \sqrt{\frac{4(k_d + k_p) \Delta Vol_{max}}{0.3\pi k_p \delta_{pz(max)}}}. \quad (10)$$

3.4 Particle displacement in micropipette

To determine the position of the manipulated particle in the micropipette, the pipette was modeled as m tapered segments. Each segment “ i ” is bounded by two consecutive sections (i) and ($i + 1$), shown in Fig. 4. The first micropipette tapered segment, and the orifice section, are both numbered as zero (i.e., $i=0$). At any section (i), located at a distance $x_{p(i)}$ from the orifice, the pipette has a diameter $D_{p(i)}$. The volume Vol_p is defined as the volume between the micropipette orifice

Fig. 4 Schematic of a tapered segment showing its main dimensions

and the section located at a distance x_p from the orifice. Vol_p was derived and is represented as:

$$Vol_p = \sum_{i=0}^{m-1} Vol_{part} < x_p - x_{p(i)} >^0 < x_{p(i+1)} - x_p >^0 + Vol_{full} < x_p - x_{p(i+1)} >^0, \quad (11)$$

where m is the total number of segments, i is the segment number, and the Macaulay brackets (Macaulay 1919), $< x_p - x_{p(i)} >^0$, $< x_{p(i+1)} - x_p >^0$, and $< x_p - x_{p(i+1)} >^0$, are singularity functions that are replaced by 1 only if the quantities between the angle brackets have positive values; otherwise they are replaced by 0. The volume Vol_{part} is the volume of a tapered segment i when partially filled with fluid while the volume Vol_{full} is the volume of a segment i when fully filled with fluid:

$$Vol_{part} = \frac{\pi}{4} \left(\frac{(x_p - x_{p(i)})^3 (D_{p(i)} - D_{p(i+1)})^2}{3 (x_{p(i+1)} - x_{p(i)})^2} - \frac{(x_p - x_{p(i)})^2 D_{p(i)} (D_{p(i)} - D_{p(i+1)})}{(x_{p(i+1)} - x_{p(i)})} + (x_p - x_{p(i)}) D_{p(i)}^2 \right), \text{ and} \quad (12)$$

$$Vol_{full} = \frac{(x_{p(i+1)} - x_{p(i)})}{12/\pi} \times (D_{p(i)}^2 + D_{p(i)} D_{p(i+1)} + D_{p(i+1)}^2). \quad (13)$$

During the aspiration of a micro-particle, a volume of fluid ΔVol is aspirated into the micropipette capillary. The flow in the micropipette is always steady and laminar (with small Reynolds numbers $Re \ll 2,100$), where the velocity distribution in the micropipette is parabolic. The maximum flow velocity v_{max} occurs at the center of the pipette while the fluid velocity near the capillary walls is zero. The mean flow velocity v_{mean} is half the maximum velocity v_{max} (i.e., $v_{mean} = 0.5v_{max}$) (Munson et al. 1998). The flow moves the particle from its initial position to an end position x_{end} in the capillary. If the particle moves at a velocity v_{mean} , the aspiration volume needed to bring the particle to a position x_{end} is Vol_p , calculated using Eq. 11.

In addition to the pump chamber volume change rate, the actual manipulated particle velocity v_p is affected by other factors. Factors include the particle size and mass, the position of the particle in the flow stream, and the type of fluid medium used. At the steady and

laminar flows applied, the actual velocity v is directly proportional to the mean flow velocity through the equation:

$$v = Rv_{mean}, \quad (14)$$

where R is a parameter that represents the factors affecting the particle velocity, mentioned above, and is determined experimentally. Using Eqs. 8, 11, and 14 the aspiration volume ΔVol , needed to bring the manipulated particle moving at a velocity v , to the micropipette position $x_p = x_{end}$ is derived as:

$$\Delta Vol = \frac{\Delta x}{\Delta x / \Delta V} \frac{Vol_{max}}{V_{max}} = \frac{\Delta Vol_p}{R}, \quad (15)$$

where Vol_{max} is the maximum pump volume change, V_{max} is the maximum piezoactuator voltage change, and $\Delta x / \Delta V$ is the particle displacement per unit voltage.

4 Experiments and results

In this section, experiments performed to evaluate the pump performance and measure the success rate in manipulating individual live cells are described. The pump performance was measured by determining the relationship between the piezoactuator voltage and cell motion in the micropipette.

4.1 Material and component selection

Materials were selected for the pump components. Stainless steel was used for the pump body, while Poly(methyl methacrylate) (PMMA) was selected for both the pump cap and the diaphragm. These materials have the advantages of biocompatibility and resistance to damage during various sterilization techniques including autoclaving, sonication, or washing with alcohol. PMMA has a 3.1 GPa modulus of elasticity, and 0.35 Poisson's ratio. The diaphragm was selected to be 1.3 mm thick. In this work, we desired a maximum volume change of approximately 250 nL. Using Eq. 10, a 22 mm diameter resulted. To use commercially available standard AS568 O-ring seals, the diameter of 0.9 inch ($D_c = 22.86$ mm) was selected, providing a maximum aspiration and dispense volume of 270.2 nL.

The smallest commercially available piezoactuator (PL022.30, Physik Instrumente) was selected. This piezoactuator is a ceramic encapsulated multilayer stack cube with 2 mm sides ($r = 2$ mm) and 27 nF electrical capacitance. Its operating voltage is 0 to +100 V

with nominal displacement of $2.2\ \mu\text{m}$ at $100\ \text{V}$. The stiffness of the piezoactuator k_p is $54.5 \times 10^6\ \text{N/m}$. A commercially available three-way control valve (LHDA-2423111H, The Lee Company) was used.

Commercially-available borosilicate glass micropipettes (MBB-FP-L-45, Humagen), with the dimensions shown in Table 1, were used. The pipette tip is $0.5\ \text{mm}$ long, angled at 45° , and has a $40\ \mu\text{m}$ inner diameter (ID) and a $45\ \mu\text{m}$ outer diameter (OD). These $40\ \mu\text{m}$ ID micropipettes are suitable to manipulate suspended cells of 1 to $25\ \mu\text{m}$ diameters.

4.2 Experimental setup

The pump was incorporated into the six-axis robotic cell manipulation system presented in Anis et al. (2010). The system uses an inverted microscope (TE-2000U, Nikon) with a $10\times$ objective lens (CFI Plan Achromat, Nikon) and brightfield illumination. A high resolution CMOS camera (DCC1645C, Thorlabs) is used to capture images. Figure 5 shows a schematic of the system and its major components. The microscope stage is motorized (MS-2000, ASI) and controls the sample x - and y -direction motion in addition to the microscope objective motion in the z -direction. The pico-liter pump is mounted onto three translation stages (T-LS28-I, Zaber) moving the pump in the x_p , y_p , and z_p directions, as indicated in Fig. 5. The pico-liter pump is connected to an external syringe pump through the use of tubing, fittings, and connectors (Upchurch Scientific). The syringe pump (Versa 6, Kloehn), which uses a $100\ \mu\text{L}$ glass syringe, is only used for the pico-liter pump setup (see Section 4.3). A piezo amplifier, with a voltage noise of $0.3\ \text{mV}_{\text{RMS}}$ at $500\ \text{Hz}$, (E-440-011, Piezosystem Jena) drives the piezoelectric actuator. The piezo amplifier and the hardware components are controlled using LabVIEW (v8.5, National Instruments) and a data acquisition card (PCI-6024E, National Instruments). A graphical user interface (GUI) was developed to control the different stages of the cell manipulation process.

Table 1 The x -position (x_p) and the diameter (D_p) of each section (i) for a $40\ \mu\text{m}$ ID micropipette

Section (i)	$x_{p(i)}$ (mm)	$D_{p(i)}$ (μm)
0	0	40
1	0.5	40
2	7.1	200
3	8.2	307
4	10.3	800
5	55.3	800

Fig. 5 Six-axis robotic workstation schematic

4.3 Pump setup

Objectives for setting up the pump include: (1) sterilizing the pump, (2) wetting the fluidic circuit and (3) filling the circuit with fresh fluid media. Setup is performed with the empty pump vertically oriented (with the pump channel “A” vertical), as shown in Fig. 6. The three-way control valve is first switched *ON* to connect the external pump to the pico-liter pump chamber. Degassed 200-proof ethanol is used for wetting and sterilizing the pump. With the micropipette tip submerged in alcohol, the external pump slowly aspirates alcohol into the chamber until the pump circuit is air and bubble free (see Fig. 6). Two minutes’ exposure to ethanol is sufficient to render the pump sterile. In a similar manner, the circuit is rinsed with distilled water multiple times after which it is filled with the working media. After setup, the control valve is turned *OFF*.

Fig. 6 Pump setup. (a) Empty chamber. (b) Chamber filling

4.4 Cell line information

Barrett's esophagus (BE) cells were used in this work. BE are immortalized human cell lines that are of interest to the Microscale Life Sciences Center (MLSC) (<http://www.lifeonachip.org>). Cells were first released from the cell culture flask and brought into a suspended state in a process that involves rinsing the adherent culture with Phosphate Buffered Saline (PBS), and then with trypsin (Anis et al. 2010). The average diameter of a suspended BE cell is approximately 15 μm . The suspended cells are pipetted into a petri dish with PBS.

4.5 Cell motion in micropipette

To study cell motion inside the micropipette, the micropipette was first aligned with the desired cell. Reducing the voltage on the piezoactuator leads causes aspiration of a cell into the pipette. While the cell is being aspirated, the time (t), voltage (V), and a video of the cell motion were recorded. The cell position in the pipette was determined by analyzing the video. Therefore, complete information about the cell motion was available for the calculations. Figure 7 is a superimposed image which shows a BE cell aspirated at a voltage change rate of -0.25 V/s. It shows that when the voltage changes from V_a to V_b , the cell travels a distance d_{ab} from "a" to "b" in an amount of time that equals $(t_b - t_a)$. The average cell velocity, and the cell displacement per voltage change, can therefore be calculated as $v_{\text{cell}} = d_{ab}/(t_b - t_a)$, and $\frac{\Delta x_p}{\Delta V} = d_{ab}/(V_a - V_b)$, respectively.

Live BE cells were aspirated into and dispensed from a 40 μm inner diameter micropipette at rates of 0.25, 0.5, 0.75, and 1 V/s. For each rate, the cell aspiration and dispense was repeated five times where the cell velocities (v_{cell}) and the displacements per voltage change ($\frac{\Delta x_p}{\Delta V}$) were measured and averaged. Figure 8(a) displays the results and shows that the cell

Fig. 7 Barrett's esophagus cell aspirated into micropipette tip at a rate of -0.25 V/s ($92 \mu\text{m/s}$)

displacement per voltage change was almost constant. Figure 8(b) shows that the relationship between cell velocity and the voltage rate was near-linear with a slope of $441 \mu\text{m/V}$.

The micropipette segment, where the experiments were conducted, had a constant inner diameter D_p . Therefore, the volume ΔVol_p is represented as:

$$\Delta Vol_p = \frac{\pi}{4} D_p^2 \Delta x, \quad (16)$$

Solving Eqs. 15 and 16 and substituting $D_p = 20 \mu\text{m}$, $V_{\text{max}} = 100$ V, $Vol_{\text{max}} = 270$ nL, and $\Delta x/\Delta V = 441 \mu\text{m/V}$, it was possible to calculate the parameter R which represents the factors affecting the particle velocity, including the particle geometry, the position of the particle in the flow stream, and the type of fluid medium used. The parameter R was found to have the value 0.2.

Equations 11 and 15 are used to calculate the aspirated fluid volume ΔVol needed to transport a particle from the orifice to a position x_{end} . The particle end position x_{end} is plotted against the aspirated volume ΔVol in Fig. 9 for a micropipette with the dimensions presented in Table 1. Figure 10 shows the relationship between the voltage V_{end} and the position of a particle aspirated into the micropipette of Table 1.

Fig. 8 Cell motion in micropipette tip of 40 μm inner diameter. (a) Cell displacement per applied voltage is nearly constant for all applied voltage rates. (b) Relationship between cell velocity and voltage rate is linear

Fig. 9 Relation between the aspirated volume and particle position in the capillary micropipette, presented in Table 1

4.6 Cell manipulation

Experiments were conducted using the system described in Section 4.2 to evaluate the performance of the cell manipulation system. A petri dish with BE cells suspended in PBS was mounted on the microscope X - Y stage. The manipulation process starts by visually inspecting cells available in the field of view and manually selecting a target cell based on its size, state (adherent or suspended) and apparent viability. Once a cell has been selected, it is manually positioned proximal to the micropipette orifice using joystick control of the X - Y stage, and the piezoactuator voltage is reduced causing aspiration of the positioned cell. When the cell is seen to disappear inside the pipette, aspiration is stopped and the Z_p vertical stage carrying the pump is instructed to move upwards. The microscope X - Y stage is moved to bring the target location into the

Fig. 10 Effect of aspiration voltage on particle position in the capillary micropipette, presented in Table 1

microscope field of view. In this work the targets were located on another buffer-filled petri dish mounted on the microscope X - Y stage. The Z_p stage was lowered until the pipette came into focus, whereupon piezo excitation was increased at the specified rate until the cell was seen to exit from the micropipette orifice.

The cell manipulation experiment was performed 200 times at a voltage rate of 1 V/s. The average voltage change needed to aspirate a cell was 5 V, corresponding to an aspirated volume of 13.5 nL. Of the 200 manipulation attempts, 197 cells (98.5%) were successfully dispensed to their target locations. The three cell loading failures are attributed to sticking of the aspirated cell to the luminal surface of the capillary micropipette.

5 Conclusions and future work

This paper presents a push-pull diaphragm pump capable of precisely manipulating volumes of fluid in the pico-liter range. The pump is able to aspirate and dispense volumes of fluid in the range between 500 pL and 250 nL at flow rates of up to 250 nL/s. The pump is a macro-scale device that includes interchangeable and low-cost commercially available components. The valve and micropipette connections are integrated into the pump body in the form of fluidic channels, which significantly reduces compliance in the fluidic circuit.

The components of the pump are fabricated of bio-compatible materials, which makes it perfect for the manipulation of biological objects. It can be easily primed and sterilized using standard techniques such as autoclaving, exposure to UV radiation, and alcohol washing. Using a glass capillary micropipette, the pump is used for aspirating and dispensing individual live cells. A mathematical model was developed to determine the effect of both the aspirated fluid volume and voltage change on the cell position in the micropipette. Using the diaphragm pump, high control over the cell position was achieved which resulted in a 98.5% cell manipulation success rate. This exceeds the 92.6% previously reported when using a syringe pump for fluid manipulations (Anis et al. 2010). With the implementation of automation and closed-loop feedback control, the cell loading success rate may reach 100% (Anis et al. 2010).

Future work includes developing a complete mathematical model of the system to study both the static and the dynamic behavior of the pump. This would allow more control over the fluid flow and cell position, enabling the serial manipulation of multiple cells.

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References

- Y.H. Anis, M.R. Holl, D.R. Meldrum, Automated vision-based selection and placement of single cells in microwell array formats, in *2008 IEEE Int. Conf. Automation Science and Engineering*, pp. 315–320 (2008)
- Y.H. Anis, M.R. Holl, D.R. Meldrum, Automated selection and placement of single cells using vision-based feedback control. *IEEE Trans. Autom. Sci. Eng.* (2010). doi:[10.1109/TASE.2009.2035709](https://doi.org/10.1109/TASE.2009.2035709)
- T.M. Brennan, Piezoelectric pumping device, US Patent 5,094,594, (1992)
- Z. Lu, C. Moraes, Y. Zhao, L.D. You, C.A. Simmons, Y. Sun, A micromanipulation system for single cell deposition, in *IEEE International Conf. on Robotics and Automation (ICRA2010)*, Anchorage, Alaska, 3–8 May (2010)
- W.H. Macaulay, Note on the deflection of beams. *Messenger of Mathematics* **48**, 129–130 (1919)
- B.R. Munson, D.F. Young, T.H. Okiishi, *Fundamentals of Fluid Mechanics* (Wiley, New York, 1998)
- G.B. Phillips, S.P. Bailey, *Hazards of mouth pipetting*. Storming Media (1964)
- K.K. Sanford, W.R. Earle, G.D. Likely, The growth *in vitro* of single isolated tissue cells. *J. Natl. Cancer Inst.* **9**, 229–246 (1948)
- F.B. Seely, J.O. Smith, *Advanced Mechanics of Materials* (Wiley, New York, 1932)
- J.G. Smits, Piezoelectric micropump with three valves working peristaltically. *Sens. Actuators* **21**, 203–206 (1990)
- J.G. Smits, Piezoelectric micropump with microvalves, US Patent 4,938,742, (1990)
- N. Szita, R. Sutter, J. Dual, R.A. Buser, A micropipettor with integrated sensors. *Sens. Actuators, A, Phys.* **89**, 112–118 (2001)
- H. Van Lintel, Micropump having a constant output, US Patent 5,085,562, (1992)
- H.T.G. Van Lintel, F.C.M. Van de Pol, S. Bouwstra, A piezoelectric micropump based on micromachining of silicon. *Sens. Actuators*, **15**, 153–168 (1988)
- K. Wewetzer, B. Seilheimer, Establishment of a single-step hybridoma cloning protocol using an automated cell transfer system: comparison with limiting dilution. *J. Immunol. Methods* **179**, 71–76 (1995)