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A simple add-on microfluidic appliance for accurately sorting small populations of cells with high fidelity

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Abstract

Current advances in single cell sequencing, gene expression and proteomics require the isolation of single cells, frequently from a very small source population. In this work we describe the design and characterization of a manually operated microfluidic cell sorter that 1) can accurately sort single or small groups of cells from very small cell populations with minimal losses, 2) that is easy to operate and that can be used in any laboratory that has a basic fluorescent microscope and syringe pump, 3) that can be assembled within minutes, 4) that can sort cells in very short time (minutes) with minimum cell stress, 5) that is cheap and reusable. This microfluidic sorter is made from hard plastic material (PMMA) into which microchannels are directly milled with hydraulic diameter of 70 μm . Inlet and outlet reservoirs are drilled through the chip. Sorting occurs through hydrodynamic switching ensuring low hydrodynamic shear stresses, which were modeled or experimentally confirmed to be below the cell damage threshold. Manually operated, the maximum sorting frequencies were approximately 10 cells per minute. Experiments verified that cell sorting operations could be achieved in as little as 15 minutes, including the assembly and testing of the sorter. In only one out of 10 sorting experiments the sorted cells were contaminated with another cell type. This microfluidic cell sorter represents an important capability for protocols requiring fast isolation of single cells from small number of rare cell populations.

1. Introduction

The sorting of select cells from larger cell populations has become a fundamental tool of biochemical research [1]. Technical limitations usually force analyses of larger pools of cells, which can mask the critical behavior of individual cells in biological processes in heterogeneous populations [2]. Experiments involving gene expression, DNA sequencing and proteome analysis from single cells have yielded important information on gene expression, regulation [2–5] and protein expression dynamics [6, 7]. Single cell approaches in the context of newly developed technologies like the single molecule real time DNA sequencing (Pacific Biosciences, Menlo Park, CA), illustrate the need for single cell isolation platforms that are cheap, easy to operate and, most importantly, perturb cells to an absolute minimum extent.

Our goal was to add a cell sorting capability to an extant microscope station for probing single cells extracted from populations of < 1000 cells for genotoxicity and stress endpoints. We considered that there are several methods currently available to sort single cells. These methods offer varying sorting efficiency, rate, and batch size contingent on the physics of the separation method. The most widely used device for cell sorting of large populations is the flow cytometer, based on Fluorescence Activated Cell Sorting (FACS) [8–

10]. Cells, encapsulated in droplets, are sorted by deflection of droplets using applied electrostatic, pneumatic, or piezoelectric mechanisms. Typical cytometry applications deal with the analysis and sorting of $>10^6$ cells derived from large cell pools, with typical throughputs ranging from 300 cells/second to over 10^3 cells/second [8]. Importantly, flow cytometers are not capable of sorting single cells from small cells populations (smaller than 5×10^5 cells) because of cell losses during the sorting process. The physical bulk of a MACS/FACS apparatus was impractical for our application. Further, robust flow accompanying high throughput can induce shear/flow associated injury or stress [11, 12] which would confound stress response studies.

There has been great ferment in the field of miniaturized cell handling modalities, many of which can be mated with a microscope platform. In general, miniaturization can allow for a reduction in cell losses together with a decrease in sorting rate. Several strategies were again considered. Single cells can be sorted manually by a skilled operator using micropipettes, microgrippers or size-based filters [13]. An array of electrophysical techniques are also feasible but also impart stress on the cells (direct physical stress or membrane-depolarization) as they are sorted [13–16][17] and can involve complex setups or special fluid media [18]. In keeping with minimal perturbation of our cells, we sought to employ a physiologically compatible fluid with minimal forces applied to the cells and so chose hydrodynamic sorting in a microfluidic format. A Y-type sorting junction [19] was excluded since cells would come in close proximity of mechanically crushing valves. Inspired by several designs which diverted the flow of cells in channels by indirect valve action [20, 21] we pursued an X-type sorting junction to be employed downstream of a simple gravity-driven vertical loading cassette.

Here we describe the design and the operation of a simple microfluidic cell sorter competent for expanding the capability of an existing microscope found in a cell-biology laboratory which minimizes stress on a sorted population of cells for stress or toximetry endpoints. The design prioritizes 1) single cell sorting capability 2) minimal cell losses commensurate with very small input populations and mHz frequency sorting, 3) obviating extra instrumentation and complexity by employing manual triggering of sort function 4) ease of use, re-use and manufacture and 5) absolute fidelity of sorting commensurate with visual verification.

The resultant design utilizes a hydrodynamically switched X-shaped configuration where a sheath flow is used to divert the cells into one of two output channels, a desired “Select” output compartment or a “Bypass” compartment into which unwanted or clumped cells can be directed. The main flow is driven by gravity in keeping with the minimization of sorter associated devices and mechanical forces acting on cells. The sheath flows, however, were necessarily driven by a syringe pump and controlled with fast solenoid valves (0.5 ms switching time). A loading cassette, comprised of a heparinized capillary tube is added to the input reservoir allowing for controlled admission of small quantities of cells to the sorting junction. The microfluidic channels are sealed with tape, which can easily be removed for cleaning the reusable chip. The advantages of the sorter developed here include ease of production (no clean room or advanced etching techniques), simplicity of operation, and interoperability with standard equipment found in many cell biology laboratories.

2. Concept and Design

Figure 1 shows the flow channel geometry of the cell sorter. The sorter involves the intersection of three inlet channels S1, S2, and I, and two outlet channels O1 and O2. O1 and O2 are interchangeable at the discretion of the operator as “Bypass” and “Select” compartments. Gravity drives a particle-laden solution from the inlet (I) to the intersection.

At this point, cells are deflected to either outlet channel by a sheath flow determined by the state of two valves, located at S1 and S2, which are reciprocally open or closed.

A hydrostatic pressure imposed by a height difference Δh drives cell flow through the main channel, allowing free access with a pipette to the inlet and outlet reservoirs. To allow enough recognition and reaction time for the operator sorting the cells, the target design cell speed is chosen as $V=0.5$ mm/s. Cell diameter varies with cell type. A survey of several trypsinized, detached human lung fibroblasts, human endothelial cells and untreated lymphocytes was conducted using a hemocytometer grid and yielded diameters ranging from 10 to 20 microns. The cross section of the channels was chosen to be $127\text{ }\mu\text{m}$ by $50\text{ }\mu\text{m}$, about 5 to 10 times the cell size so that clogging is prevented, resulting in a hydraulic diameter $D = 72\text{ }\mu\text{m}$. Assuming the cell solution has the volumic mass of water, $\rho = 1000\text{ kg/m}^3$, and a viscosity of $0.001\text{ Pa}\cdot\text{s}$, we obtain a Reynolds number $\text{Re} = \rho UD/\mu$ of about 0.036. The flow is therefore clearly laminar with negligible inertial effects, and cells following streamlines without turbulent oscillations. The pressure difference ΔP between inlet and outlet reservoirs required to drive this flow is given by [22]:

$$\frac{64}{\text{Re}} \frac{L}{D} \frac{1}{2} \rho V^2 = \Delta P = \rho g \Delta h \quad \text{Eq 1}$$

For a travel length in the chip of $L = 20\text{ mm}$, we need a pressure difference $\Delta P = 50\text{ Pa}$, corresponding to a height difference between the reservoirs $\Delta h = 5\text{ mm}$. The height of the inlet and outlet reservoirs are therefore designed to be at least 10 mm high to give more control over the speed of the cells; this height is controlled by the overall thickness of the chip.

The side channel flows can also be provided by gravity flow, and this scenario was tested. It was decided to use a syringe pump, however because it allows an easy way to specify certain flow rates instead of specifying height differences that correspond to certain flow rates. Gravity flow can however be used if a syringe pump is not available.

The target flow speed was chosen as 0.5 mm/s because this speed is slow enough for manual operation (taking into account human reflexes), and fast enough to prevent cells from adhering to the walls. A possible explanation for cells not adhering to the walls can come from the analysis of the Peclet number:

$$\text{Pe} = LV/D = \text{convection/diffusion} \quad \text{Eq 2}$$

Where

$$D = \text{diffusion coefficient} = K_B T / 6\pi\eta r \text{ (Einstein)} = 1\text{e-}14\text{ m}^2/\text{sec}$$

$$V = 0.5\text{ mm/s}$$

$$L = 20\text{ }\mu\text{m} \text{ (half channel height)}$$

The Peclet number in our application is calculated to be 10^6 . The large Peclet number means that convection dominates, and that particles do not diffuse to the walls for adhesion.

In the design process, we used Computational Fluid Dynamics to determine the maximum shear rate experienced by the cells. The finite-element multiphysics software COMSOL was used to simulate the flow at the intersection of three inlet channels and two outlet channels. A 3D mesh was generated by COMSOL with 10 nodes along the z-axis and 200 nodes along the x and y axes, corresponding to XY tetrahedral elements. As a boundary condition, a

pressure difference of 50 Pa, corresponding to the above calculation from Equation 1, was applied between the inlet and outlet reservoirs. The closed valve was modeled as a wall.

Figure 2 shows the outcome of a steady state flow simulation. In the configuration described, the top left valve is closed while the top right valve is open. The average speed of the flow in the simulation is about 0.8mm/s, in good agreement with the design goal of 0.5 mm/s. Trajectories shown in black lines in Figure 2a show that the presence of a sheath flow at the right deflects the particle-laden flow towards the left channel. Reciprocally, figure 2b shows that in the opposite valve configuration deflects the particle-laden flow towards the right channel.

The switching time is dependent on two parameters: the time it takes to accelerate or stop by changing the pressure boundary condition the flow, and the time it takes to redirect the flow from the old outlet to the new outlet. Hydrodynamic theory predicts that the acceleration time depends on the diffusion of the fluid momentum across the channel. This can be expressed as [23]:

$$t_{accel} = \frac{\rho}{\gamma_1^2} \frac{a^2}{\mu} = 0.97ms \quad \text{Eq 3}$$

In the above equation, the symbols a , γ_1 , μ , respectively stand for the hydraulic diameter, the smallest Bessel function root ($\gamma_1 = 2.405$), and the viscosity of the fluid. The time it takes to redirect the flow to the new outlet depends on the velocity and geometry of the channels. It is defined as the time it takes for a particle to cross the characteristic distance between one inlet channel and the opposite outlet channel. For the channel of cross sectional area $A_{cs} = 6.35 \cdot 10^{-9} \text{ m}^2$, a switching flow rate $Q_{sf} = 1 \text{ } \mu\text{l/min}$, and a characteristic length $l = 300 \text{ } \mu\text{m}$,

$$\tau_{redirect} \frac{A_{cs} l}{Q} = 114ms$$

It is clear that the acceleration switching time is smaller than the redirection switching time. An experiment with blue dye flowing from the input and pure water flowing from the side channels was performed to validate this switching time calculation. The valves were connected to a function generator and operated to send the flow in alternating directions at varying frequencies. A high-speed camera was used to image the flow and study the switching time (figure 3). The movie shows that it takes approximately 133 ms to switch the flow from one channel to the other. This corresponds to a theoretical maximum of approximately ~450 complete flow switching events per minute. This rate informs the theoretical maximum of cells sorted by the sorter together with the density of cells in the admitted suspension and the flow rate through the sorter.

The maximum shear stress experienced by the cells corresponds to the largest shear stress in the fluid. The shear stress is defined by [24]:

$$\tau = \mu \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) \quad \text{Eq 4}$$

The maximum shear stress was calculated from the computational fluid dynamics simulation results as the matrix 1-norm of the above tensor. We found the maximum stress occurs along

the walls, with values of 0.042 Pa. This is on the same order as the analytically-estimated average shear stress [25]:

$$\tau \approx \mu \frac{u}{h/2} \approx 0.02 Pa.$$

Typical damaging shear stresses are above 0.4 Pa [25]. Therefore this hydrodynamic switching scheme should be safe for cells. This was assessed in trypan blue staining studies shown in section 4.

3. Manufacturing

The chip was manufactured from a 24.9mm x 29.6mm x 11.9mm block of polymethyl methacrylate (PMMA). Channels 127 μ m wide x 50 μ m deep were surface-machined in an intersecting five-branch configuration using a micro-milling machine (Minimill 3, Minitech Machinery, USA). Figure 4 illustrates the dimensions and major components of the full device. This figure shows the primary chip (A), input reservoir (B), solenoid valves (D), valve-attachment manifold (C), and o-rings (E); details of these components are described further below.

Particles in solution were flowed through a single input branch (Figure 4, B) into either of two output branches. Each of these particle-flow channels terminated in 200 μ l cylindrical reservoirs so that particle solution could be added and extracted via pipette. As described above, the rate of this particle solution flow was controlled by a difference in fluid level between the input and output reservoirs.

Switching was achieved via alternating the open or closed state of two fast (operating frequency up to 2 kHz) solenoid valves (Figure 4, D, from Gyger AG, Switzerland). In this way, a non-stop (particle-free) flow in either of two switching channels diverted the particle solution from the input channel to a specific output channel and reservoir. Transparent adhesive tape was applied to the machined surface of the chip to seal the channels and reservoirs.

The solenoid valves were connected to the chip using a separately-machined PMMA manifold (Figure 4, C); these two components were affixed with a #2-56 socket head cap screw. At the interface, two o-rings (Figure 4, E) sealed the fluid connection between the valves and the chip. A single syringe pump supplied each valve with either isotone buffer or DI water (depending on the sorted particles) via rigid Teflon tubing and a three-way connector.

The valves were actuated using a microprocessor controller (Microchip PIC 16F74) programmed to supply a peak-and-hold current reciprocally to either valve's coil. This signal scheme was necessary in order to maximize the speed of the valves without exceeding their electrical current rating. The controller logic circuit was powered with a 5V DC supply while solenoid coil required 1 amp for 200 μ s to open the valves, and 0.1 amps (indefinitely) to keep the valves open.

4. Results and Discussion

Experiments were conducted to evaluate (1) the performance of the device as a manual sorter using polymer spheres and human fibroblasts, and (2) the stress induced by the device on living human fibroblasts after random sorting. The device was then used to separate

labeled cells that have been mixed with an unlabeled population of the same cells or cell labeled with different fluorescent dyes or fluorescent antibodies.

Priming the channels

To prevent clogging by e.g. undesired air bubbles, sorting began with a filling ('priming') procedure that involved filling all the three initially dry reservoirs with DI water or filtered isotonic buffer, and then initiating a syringe pump flow of the same solution through the switching channels. A function generator was then used to actuate the valves at high speed (~200 Hz) to remove air bubbles attached to the channel walls. During the priming process, the outlet reservoirs were kept filled with liquid. An alternate priming protocol using syringe driven flow through the solenoids while switching at 2 Hz manually was also found to work well and eliminated the requirement for a function generator.

Sorting polymer beads and human fibroblasts

After wetting, the device performance was evaluated by sorting polymer beads and human fibroblasts. First the input reservoir was filled with an aqueous solution of 11 μm polymer spheres (Duke Scientific, 7510A), at a concentration of 33 particle per μl . Gravity caused the particles to flow from the inlet to the outlet chambers. To demonstrate sorting ability and measure throughput, 101 beads were manually sorted to each output reservoir in an alternating manner over a seven minute span. This results in a measured throughput of approximately 14 cells per minute at velocities of ~1 mm/s. The left side of figure 5 shows (top) an incoming bead, (middle) a bead directed to the top outlet channel, and (bottom) a bead directed to the bottom outlet channel. The inlet reservoir was stained with a small concentration of blue dye to visualize the streaklines of the flow and easily predict where the bead would go.

The same experiment was then performed with trypsinized human fibroblasts. First, the priming procedure was performed with isotonic buffer. Then, cells were trypsinized for 5 minutes, washed, and resuspended in isotonic buffer. A coulter particle counter (Model Z1) was used to determine the concentration of cells (60 cells per μl). After the wetting of the chip, 200 μl of the suspended cell solution was added to the input reservoir, and gravity caused the cells to flow between the inlet and outlet reservoirs. The right side of figure 5 shows (top) an incoming bead, (middle) a bead directed to the top outlet channel, and (bottom) a bead directed to the bottom outlet channel. Phase contrast lighting conditions were used to facilitate visualization of the transparent cells, also causing the dark shading on the right side of the images. 30 cells were sorted at velocities of ~0.6 mm/s in less than 3 minutes, resulting in throughputs of approximately 10 cells per minute.

Impact of the shear stress on cells

While the previous tests showed the capability of the device to precisely sort a particle-laden flow, the device was also tested to evaluate the stress on human fibroblasts by the shear of the sorting flow. The cells were prepared in the same manner as the previous section and were flowed through the channels from the inlet to the outlet reservoirs. A function generator was used to switch the flow at a rate of 1 Hz. After randomly sorting cells for a 20 minute period, 20 μl samples were drawn from each output reservoir and a 40 μl control sample was drawn from the input reservoir. Trypan Blue was then used to evaluate the viability of these collected cells under a microscope. The results from this test, shown in Table 1, show a negligible change in the mortality of the sorted cells compared with unsorted cells, indicating an acceptable level of shear stress in the device.

Separation of labeled Fibroblasts from unlabeled Fibroblasts

The microfluidic chip described here was then used for separation of cells expressing GFP or stained with vital dyes from non-stained cells in a scenario typical for bystander effect experiments, where fluorescent cells are plated together with non-stained cells and irradiated with a microbeam [26]. An essential step in these types of experiments is the precise separation of the irradiated cells from the non-irradiated cells for subsequent cell analysis.

Two sorting procedures were used during our utilization of the cell sorter: (a) both types of cells are tagged with different colors of fluorescence, or (b) only the irradiated cells are tagged. In the first case, the irradiated cells are labeled with fluorescent nuclear dye (Hoechst 33342) or GFP, and the bystanders are labeled with vital cytoplasmic dye Cell Tracker Orange, and both can be visualized with a double-pass filter. In the second case, only the irradiated nuclei are tagged and sorted from the non-labeled cells. This method ensures that the tagging does not affect the results from the subsequent analysis, and is fully described below.

Normal human fibroblasts (AG01522 cells) expressing GFP or stained with Cell Tracker Green (Molecular probes, Eugene, OR) were plated in ratio of 1:3 with non-stained cells. After 24 hours the cells were trypsinized, washed and resuspended in Isotone to eliminate small particles that are usually present in unfiltered media. A 100 μ l suspension of cells with concentration of 20 cells/ μ l was placed in the inlet reservoir, mixed by pipetting. This initiated a cell flow at a velocity of about 0.5mm/s. Cells reached the sorting zone at a rate of about 1 cell every 10 seconds, and were observed by the operator under inverted fluorescence microscope by using a combination of filtered fluorescent light (FITC filter) and visual broadband light allowing the user to simultaneously see both fluorescent and non stained cells.

At this stage all cells were driven to the waste “Bypass” chamber by the default state of the controller. Once it was clear that there was a constant flow of cells, the white light was dimmed and cell sorting was performed under fluorescent illumination. When the operator pressed a pushbutton switch, the flow was directed towards the collection “Select” chamber. All fluorescent cells -- once they appeared in the field of view -- were directed to the “Bypass” chamber by releasing the controller’s button. Using a magnification of 4x gives the operator at least 3 seconds to see the fluorescent cells before they reach the cross section of the channels.

It is important to note that successful use of this method ensures that the collection chamber will contain only the non-stained cells of interest, while the waste chamber might contain some non-stained cells, together with the discarded stained cells.

Configuration of the sorter for “Low Numbers” operation

Addition of the cells to the inlet chamber of the sorter did allow for sorting of larger numbers of cells and was competent for sorting of beads to assess the validity of our theoretical work in the design of the sorter. Some beads did fall to the base of the inlet chamber and resuspension by titration was required to admit beads to the suction zone proximal to the channel inlet within the inlet chamber. For work with very small numbers of cells we restricted the volume of the cell suspension while simultaneously insulating the population from perturbation associated with changes and maintenance of the inlet chamber volume. This was accomplished by using an enclosed loading cassette comprised of a heparinized capillary tube that was sealed at one end. The lower end of the cassette was positioned in the inlet chamber close to the inlet flow channel as shown in fig. 6. In these experiments, AG01522 cells were stained with Hoechst 3342 (blue fluorescent dye, emission at 462 nm) at 250 μ M or with Mitotracker Green (green fluorescent dye, emission

at 516 nm) at 500 ng/mL for 1 h at standard culture conditions prior to trypsinization and counting with a hemacytometer. Cells were blended in a 44:55 ratio (Hoechst 3342:Mitotracker Green) and diluted to a final concentration of 44 cells/ μ L. A 4 μ L portion of this final working cell suspension was loaded into a 27 mm long segment of heparinized capillary tubing (StatSpin Technologies, Westwood, MA) which was then capped and plugged at the other terminus with warm moistened parafilm. The sorter was placed on the stage of our inverted fluorescent microscope and the flow was initiated through the primed and wetted cell sorter. The cell loaded cassette was then placed into the inlet chamber at an angle as shown in Figure 6 and examined via 10X objective. The falling cells could be tracked as they fell and sorting was performed by switching from the “Bypass” compartment to the “Select” compartment as the cell entered the inlet channel. Once the desired cell had disappeared from the field of view, valves were switched back to “Bypass” settings.

Ten iterative runs were made in an attempt to separate 2–3 individual blue cells from the mixture. Green cells were effectively screened out of the sort in all but one run where the operator failed to correctly time switching commands to target desired cells to the correct compartment. Small numbers of blue cells were successfully acquired in all but two runs where the close proximity of green cells caused the operator to conservatively allow the cells to pass into the “Bypass” chamber. Success in runs is contingent on the stochastic mixing and evenness of the cell suspension and to some extent on the hand-eye coordination of the operator. Some additional dilution was required to prevent the operator from becoming overwhelmed by the rate of cells falling into the field of view and entering the inlet. This accounts for the somewhat larger number of cells present in the “bypass” compartment of the sorter in the initial run. The first run was the longest at 300 seconds during which modifications to the density of the incoming cell suspension was modified and many more cells were allowed to pass through the sorter into the “Bypass” chamber. Subsequent runs were all less than 5 minutes for a total of no more than 71 minutes allowing for obtaining counts and reloading the cassette with a ready population of cells. During counting, flow through the sorter was stopped and cell counts obtained from the “Selected” compartment in the sorter and the “Bypass” compartment with results shown in Table 2 below.

We have been able to harvest cells from the outlet chambers of the sorter. This is conveniently practical given that the light scattered from the pipette tip coming into the inverted fluorescent microscope’s field of view is visible and forms a confining ring around the cells to be aspirated. Contact with the base of the sorter (against the transparent film) is confirmed when the fluorescent cells are confocal with the pipette tip. As the pipette is filled, cells vanish upward out of the plane of view and into the pipette.

For this sorter, the upper limit of cell density and volume are associated with the capacity of the 27mm length capillary loading cassette and the skill of the operator. The lower limits of operation are theoretically as low as one to two cells in 1 μ L of suspension retained in the loading cassette. The waiting time prior to cells falling into the field of view and the density at which point the user becomes overwhelmed with cells to be sorted with comfortable fidelity are the parameters that must be optimized by the end user.

5. Conclusion

A reusable hydrodynamic cell sorter was designed, manufactured and tested to sort cells mechanically under non-damaging shear stress. Its main advantage is that it can accurately sort extremely small cell populations. The sorter is comprised of a milled block of PMMA, commercially available tubing, two valves, a syringe pump and a controlling Arduino microprocessor. The inlet and outlet chambers are easily accessed by pipette, and the main

cell flow is driven by gravity. The actuation scheme depends on a syringe pump and two fast microsolenoid valves. Computational fluid mechanics was shown to be a useful in assisting the design and predicting device performance. The sorter was tested to sort 101 beads in less than 7 minutes, and 30 cells in less than 3 minutes. Operating the sorter using a fluorescent illumination scheme allowed reliable separation of cells. One limitation of the sorter is a requirement for the fluorescence labeling of the target cell population to be sufficiently intense for the operator to detect. In all experiments we used a basic fluorescence microscope without any additional hardware. Both the assembly and the operation of the sorter are very easy and require minimum training (see supplementary video). The cost of the sorter is very low – it can be built for about \$1000.00 which includes the price of the valves (\$720.00). Taken together, the sorter developed in this body of work is accessible to any laboratory that needs precise cell sorting from very small cell pools. The authors encourage testing of this device in other laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Schematic of cell sorter flow channels. The cells flow from I. Sheath flows from S1 or S2 are used to deflect the cells into the outlets O1 or O2 which can interchangeably function as “Bypass” and “Select” compartments.

Figure 2. COMSOL simulation showing the velocity magnitude (m/s) and streamlines for the two configurations, deflecting the inlet flow to the left and right depending on the state of these side inlets.

Figure 3.

Visualization of the switching time, with ink. Switching time is approximately 133ms, with good agreement to the predicted time of 114ms. Throughput is limited by this time to be approximately 450 cells/min at this velocity.

Figure 4. Major components of the cell sorter chip. Dimensions given in mm. See text for further detail. Adhesive tape is applied to the lower chip surface to seal channels

Figure 5.

(left) images of sorting polymer beads. The red circles indicate 11 micron polymer beads. The top image shows an incoming bead, the middle image shows the bead directed to the top outlet channel, and the bottom image shows the bead directed to the bottom channel. The bead-laden fluid is lightly dyed with ink to clearly show the direction the bead will take. (right) A similar set of images showing the sorting of trypsinized human fibroblasts. These pictures were taken with phase contrast lighting conditions to facilitate the visualization of the transparent cells, but also causing the dark shading on the right half of the pictures.

Figure 6. Configuration of the cell sorter for “Low Numbers” operation. In this configuration, a trimmed capillary tube is filled with a 4uL volume of cell slurry and capped at the opposite end before being added to the inlet well in the sorter.

Table 1

Results of cell stress study

	living cells	dead cells	% mortality
Input	95	10	10.5
Output	30	4	13.3

Table 2

Results of cell sorting runs in “Low Numbers” configuration.

Run	Bypass		Select	
	Blue	Green	Blue	Green
1	21	34	3	0
2	3	6	0	0
3	12	9	2	0
4	4	3	2	1
5	2	3	0	0
6	4	6	3	0
7	6	8	2	0
8	4	3	2	0
9	2	6	2	0
10	3	7	3	0