

Directed Cellular Manipulation Using Polymer Microgrippers

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Introduction

Individual cell manipulation has been gaining prominence in a wide range of applications including stem cell sorting, gene and molecular delivery, cellular diagnostics, and single cell-based assays. Direct, physical cell manipulation offers much more precise selection and understanding of cell properties than data-averaging over a population of cells. Manipulation of cells is a challenging task, as it requires not only a precise, controllable manipulator set-up but also a suitable end-effector which can be actuated to perform desired tasks without damaging the cells. Major considerations for performing manipulations (like gripping and moving of isolated cells) are biocompatibility of the end effector, the precision of positioning the end-effector, and gentler handling force.

The Zyvox L200 Nanomanipulation System is a highly versatile manipulation platform comprised of independently-controllable nanopositioners which provide sub-micron resolution for precise and accurate motion control. Combined with suitable end-effectors, one can use the L200 to manipulate biological samples — ranging from biopolymers to cells. Polymer microgrippers made of SU-8, an epoxy-based negative photoresist, have been fabricated and mounted as end effectors onto the L200 in order to perform manipulation of cells in suspension. This application note presents a brief description of the polymer microgripper, and a demonstration of the manipulation of suspended cells in aqueous media using these microgrippers.

Cell Manipulation – Overview

Directed, controllable manipulation of cells in an aqueous medium is attracting interest for biomedical applications. Various manipulation techniques have been developed to achieve controlled manipulation of cells. These techniques can be broadly classified into contact and non-contact techniques.

Non-contact techniques predominantly employ optical principles; examples include opto-electronic tweezers

and laser tweezers. A major disadvantage of these tweezers is the potential damage they can cause to biological systems, resulting in a decrease in the amount of active lifetime of a system — limiting the time that can be spent studying a system. Other non-contact techniques utilize electric and magnetic fields. These methods, though capable of trapping single and multiple cells, are typically cell-specific and require complex electrode or magnetic arrangements.

The most generally-used contact technique employs vacuum technology for holding cells. This technique, capable of holding cells for micro-injection, doesn't give sufficient control for cell sorting and isolation. Another contact technique for manipulating biological specimens involves the use of micro-fabricated devices such as microgrippers and microprobes. Technologies used in micro-electromechanical systems (MEMS) enable the fabrication of microscale devices which provide a wide range of options in terms of design, performance, and material compatibility. Direct motion control of microgrippers enables controllable cell manipulation and multi-functional capabilities, including cell sorting, cell isolation, and cell positioning. Recent developments in fabrication technologies for polymeric micro-devices have been a significant factor in the growth of research activities in microscale, biocompatible tools. Microgrippers can be easily and securely mounted onto the L200 as robust end-effectors for performing biological manipulation.

Intracellular pH Sensing

Biological manipulation tools require end-effectors which are biocompatible, operate at physiological temperature, and have gentle handling forces. In addition, such tools should also be capable of being integrated with manipulation systems to achieve precise, controllable motion. Microgrippers made of metal or silicon, and those actuated with electrostatic or piezoelectric mechanisms, do not meet one or more of the above mentioned criteria.

Electrothermally-actuated polymer microgrippers have been developed specifically to serve as end-effectors for manipulating biological specimens. SU-8, an epoxy-based negative photoresist has been used as the structural layer, while nickel is used as the heating layer for the electrothermal actuators. The properties of SU-8 (such as structural rigidity, high coefficient of thermal expansion [$\sim 52 \times 10^{-6}/^{\circ}\text{C}$] and ability to make high aspect ratio structures) have been utilized to achieve grippers with low power consumption and, consequently, low operating temperature.

The fabricated microgripper is shown in **Figure 1a**. The microgripper consists of one movable arm and one stationary arm. Long, bent beams, connected in series, act as the electrothermal actuator. As compared to a normal chevron-type beam, this design has higher resistance (and consequently lower current flow), resulting in a lower operating temperature. The electrothermal actuators (made of nickel) are resistively heated by passing current. Heat is then transferred onto the bulk SU-8 layer which expands, producing the required lateral displacement at the gripper tips. In order to avoid undesired out-of-plane displacement, the ratio of SU-8-to-nickel thickness is kept high ($50 \mu\text{m}: 2 \mu\text{m}$).

The grippers are photolithographically patterned resulting in highly-repeatable manufacturing. Once patterned, the sacrificial layer between the SU-8 and the substrate is removed, leaving behind the grippers which are suspended via tethers. Finally, the grippers are completely released from substrate (by breaking the tethers) and are packaged onto a heat dissipating sub-mount (shown in **Figure 1b**).

L200 Setup for Biological Manipulation

Figure 2 shows the set-up for biological manipulation using the L200 installed on an inverted microscope. The L200 fits onto the microscope stage using a mounting ring which allows for arbitrary placement for each of the independently-controllable positioners. The joystick control of the L200 enables precise X, Y, and Z manipulation of the end-effector. The mounted microgripper is loaded as

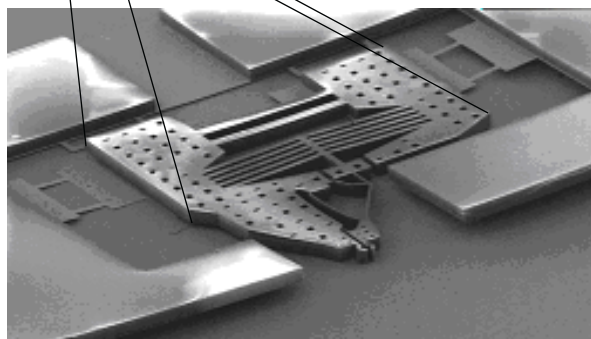
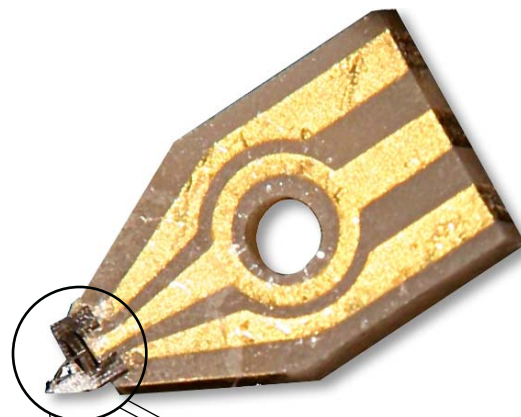


Figure 1a (top) Zyx NanoEffector[®] microgripper sub-mount.

Figure 1b (bottom) Polymer microgripper suspended to pads using tethers, prior to mounting on end-effector.



Figure 2 Compact L200 control cabinet, 27" H x 21" W x 26" D (left). L200 installed on a Nikon TE2000 inverted confocal microscope (right).

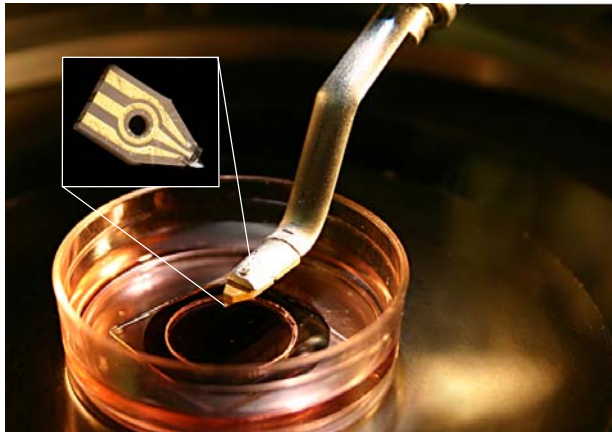


Figure 3 Polymer gripper mounted as end-effector for biological manipulation. Insert shows the ceramic gripper mount with electrical pads.

an end-effector onto the L200 system using a dedicated adaptor as shown in **Figure 3**.

Manipulation of Cells in Aqueous Medium

In order to demonstrate single-cell manipulation, normal rat kidney (NRK) cells, suspended in phosphate buffered saline (PBS) solution, are dispensed into a petri dish and placed on the microscope stage. The microscope objective is focused onto the desired cell to be moved. The nanopositioner mounted with the polymer microgripper is controlled using the joystick so that the gripper is brought directly above the desired cell (**Figure 4a**). Due to the working distance of the objective lens, the microgripper is out of the focal plane.

The gripper is normally in the closed position, with the gap between the arms designed to be similar to the desired cell diameter. Once a DC voltage is applied, the gripper opens and remains stable in that state until the voltage is turned off. The actuated gripper is slowly brought down using the joystick control so that the gripper tips enclose the cell (**Figure 4b**). The DC voltage is then cut off and the gripper closes and holds the cell (**Figure 4c**). An advantage of this method is reduced agitation in the solution during the gripper actuation and de-actuation. The gripper holding the cell is then slowly moved to the desired location in the solution, actuated again, and the cell is slowly released (**Figure 4d-f**).

Conclusion

Manipulation of individual cells is desired for single-cell sorting and cell-based diagnostics. In order to effectively

manipulate cells while maintaining cell viability, precision manipulators and biocompatible end effectors are necessary. The L200 Nanomanipulation System mounted on an optical microscope, and fitted with a polymer microgripper, can perform *in vitro* cellular manipulation with sub-micron precision and repeatability enabling a range of applications where gentle handling and directed manipulation of living cells are critical.

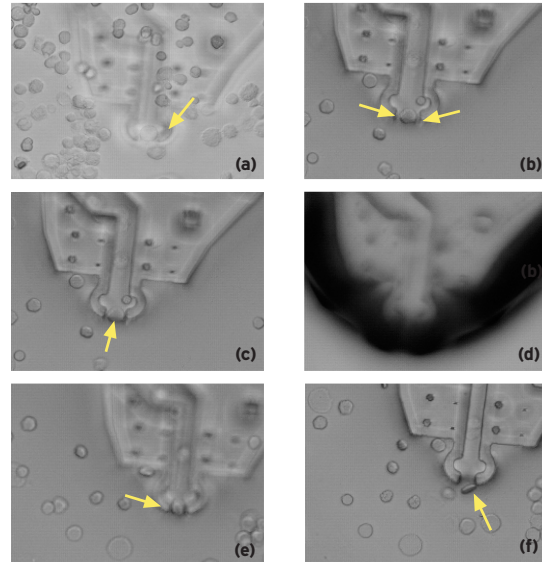


Figure 4 Sequence of single cell manipulation.

References

1. Lee, H., et al. *Electric and Magnetic Manipulation of Biological Systems*. AIP Conference Proceedings, 2005. 772(1): p. 1583-1584.
2. Yi, C., et al. *Microfluidics technology for manipulation and analysis of biological cells*. Analytica Chimica Acta, 2006. 560(1/2): p. 1-23.
3. Alexandra, B.F., et al. *Electronic sorting and recovery of single live cells from microlitre sized samples*. Lab on a Chip - Miniaturisation for Chemistry & Biology, 2005. 6(1): p. 121-126.
4. Han, S.W., et al. *A molecular delivery system by using AFM and nanoneedle*. Biosensors & Bioelectronics, 2005. 20(10): p. 2120-2125.
5. Matsuoka, H., et al. *High throughput easy microinjection with a single-cell manipulation supporting robot*. Journal of Biotechnology, 2005. 116(2): p. 185-194.
6. Ferrari, E., et al. *Biological samples micro-manipulation by means of optical tweezers*. Microelectronic Engineering, 2005. 78-79: p. 575-581.