Chapter 7

Robotics for Life Science Automation

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“In 1921, Czechoslovakian playwright *Karel Capek* introduced the word *robot* in the play *R.U.R - Rossum's Universal Robots*. The word originated from the Czech word *robota*, meaning work”.

This chapter focuses on the role of robotics in life science industry. Automated biomanipulation techniques and their advantages over conventional cell manipulation techniques are discussed. Implementation of robotics in few of the life science sectors are also presented followed by a brief discussion at the end.

1. INTRODUCTION

Robotics has had a tremendous influence in life science automation ranging from biomanipulation to production of DNA and protein microarray. In this chapter we will briefly describe the various techniques for biomanipulation, cell injection, cell culture automation, high throughput processing of biological samples, and production of DNA and protein microarray. In biomanipulation, we will discuss various techniques such as magnetic manipulation, acoustic energy, micro-electromechanical systems and mechanical manipulation, to name a few. We will also discuss the various robotic systems for cell injection and compare the success rate between robotic and manual techniques. Similarly several commercial systems as well as academic efforts will be discussed in cell culture automation, high throughput processing of biological samples, and production of DNA and protein microarray.

2. CELL MANIPULATION TECHNIQUES

Manipulating individual biological cell is a common process involved in intracytoplasmic sperm injection (ICSI), pro-nuclei DNA injection, gene therapy, and other biomedical areas. The efforts to micromanipulate cells under the microscope date back to the last half of the twentieth century [1]. Conventional methods of manipulating cells have been prevalent in the past. The principles of microinjection were developed by Marshall A. Barber. Barber developed the “pipette method” to isolate bacterial cells. Researchers in the field of biology have conducted experiments using conventional microinjection technique, for example to understand the role of nucleus in embryonic differentiation to suggest that the pronuclei formation from nuclei of a species depends on the activation of egg cytoplasm and developing pronuclei by surgically injecting the egg cytoplasm with a spermatozoa of the same species or different species [2]. However manual manipulation requires long training and the success rate depends on the experience of the operator. Even for an experienced operator, the injection process results in low success rate and less reproducibility. Early efforts have been made to automate the injection process. Capillary pressure microinjection (CPM) is one of the supporting technologies for injecting macromolecules into a single living cell. Injection in nuclei or cytoplasm was performed using an ejection system with pressure levels manipulated by a single button, which requires no learning time and the injection rate obtained can be as high as 70 – 80%. A semi-automatic microinjection system has also been developed to increase the cell survival rate in CPM. The introduction of computer control in manipulating biological cells has improved the efficiency of the process. A computer controlled microrobotic system with 3 DOF was developed for ICSI in mouse [3]. The sperm injection was successfully completed without damaging any of the mouse ova. Later on piezo driven pipette had been used to perform ICSI in mouse, which demonstrated

80% survival rate of sperm- injected oocytes. Few other researchers have proposed piezo actuators for cell manipulation which offers highly repeatable motion and increases the chances of the oocytes survival rate. Different control strategies have also been used to develop a visually servoed microrobotic system. For example Sun et al [4] developed an autonomous embryo pronuclei DNA injection system by implementing a hybrid visual servoing control scheme. In the sections below, we provide a comprehensive overview of the state of the art in biomanipulation, which include various techniques such as: optic and electric micromanipulation, magnetic micromanipulation, acoustic energy for micromanipulation, micro-electromechanical systems (MEMS) and mechanical micromanipulation.

2.1. Optic and Electric micromanipulation

Optical trap is one of the promising methods to manipulate microscopic objects without physical contact. Ashkin [5] was the first to report the acceleration and trapping of micron sized particles by the forces of radiation pressure from visible light. The concept proposed by Ashkin gained importance in the area of cell manipulation. An automated optical manipulator was also developed by Buican [6] for trapping of biological particles. Optical forces are also used for rapid and active control of cell routing on a microfluidic chip. The cell sorter developed by Wang et al [7] uses two lasers: a near infrared laser for the optical switch and a visible wavelength laser for detection and fluorescence measurement. It was demonstrated that the cell sorter can sort 280,000 cells in 44 min.

Apart from optical trapping, another non-contact manipulation technique is electrorotation. Washizu et al [8] proposed dielectrophoresis (DEP) and electrorotation for generating translational and rotational force on living bacteria. Dielectrophoresis involves applying a non uniform electric field to exert an external force on the living bacteria and obtain its force vs. velocity (F-v) characteristics. Electro-rotation involves controlling the phase shift and magnitude of electric fields to exert an external torque on the living bacteria and obtain the Torque vs. speed (T-ω) characteristic. The authors claimed that the characteristics of the biomotor derived will play an important role in understanding it's mechanism. A new technique called "optoelectrostatic" micromanipulation (OEMM) developed by Nishioka [9] combines optical pressure and electrical force to manipulate a single cell. Arai et al [10] proposed a bio-jig, which uses dielectrophoretic force to manipulate a micro-object. Electric field is applied between two pairs of electrodes and analyzed using Finite element method (FEM). An ER (electroreological) joint was proposed and its application in safety mechanism and microgripper was demonstrated. The advantage of the ER fluid is fast response time and the ease of miniaturizing the mechanical component. Later, Arai et al [11] integrated laser scanning manipulator for local position control of the target (cell) and dielectrophoresis for exclusion of other cells around the target, which proved to be an effective method of selective separation.

2.2. Magnetic micromanipulation

The introduction of magnetic energy to manipulate cells resulted in high success rate and increased the viability of the cell as reported by Pesce and Felici [12]. Alenghat et al [13] developed a magnetic tweezer with the goal of applying focused and quantifiable mechanical stress to individual cells in culture. The tweezer is an electromagnet, and ligand coated magnetic microbeads were attached to the cell membrane. This technique allows one to examine cell mechanics of an individual cell which can play a major role in quantifying the material properties of the integrin-cytoskeleton linkages. Cell manipulation system using magnetic techniques to analyze individual cells has also been reported by Boukallel et al [14]. Boukallel et al developed a force sensing manipulator using permanent magnets and diamagnetic material. The approach required no control loop and the manipulator was highly sensitive to measuring forces in the nN range. Manipulating individual cells in a fluid using magnetic and electric fields was developed by Lee et al [15]. Experiments were performed on yeast cells labeled with magnetic beads, which were trapped by a microelectromagnet matrix while the unlabelled cells were trapped by microposit matrix generating electric fields. This set up allowed the possibility of constructing an efficient microfluidic system for sorting cells.

2.3. Micromanipulation using Acoustic energy

The manipulation of microparticles and biological cells by ultrasonic waves has been investigated by a few researchers. The advantage of this technique is that it involves no mechanical contact and is non-invasive and efficient. Kozuka et al [16] used acoustic energy to trap and control the position of micro objects in two dimensions. Polystyrene particles (100µm to 150µm in diameter) were trapped at the nodes of the standing waves which were formed by three ultrasonic transducers placed at an angle of 120^0 to each other in the same plane. The position of the particle was controlled by changing the phase of one of the transducers, which caused the particle to be transported along the sound beam axis of phase shifted transducer. 3D manipulation technique developed by Kozuka et al [17] was realized by using four ultrasonic transducers placed at the corners of a regular triangular pyramid. The movement of each particle was captured by two CCD cameras. The nodes of the standing wave play an important role in manipulating micron sized particles and the position of such nodes can be changed by varying the electronic parameters of the resonator instead of the mechanical parameters [18]. The resonator consists of a fluid filled tube and two piezoelectric transducers. The proposed model of the resonator studied the electronic parameters of piezo devices, and proved its importance in affecting the position of the nodes of standing waves. The advantage of this approach is that there is no mechanical movement which would cause unwanted fluid flow. Kim et al [19] used acoustic forces in an ultrasonic field for concentrating HeLa cells and human mesencymal stem cells (hMSCs).

2.4. MEMS and Mechanical micromanipulation

Micro-electromechanical (MEMS) systems technology has emerged as an important tool to manipulate a single cell or an array of cells. The advent of this technology is proving to be successful in developing micro/nano systems which allows biomanipulation to be more reproducible and efficient. Microrobot was fabricated from conducting polymer like polypyrrole (PPy) by Jager et al [20] to manipulate single cells in an aqueous medium. The fabricated structure has PPy in a bilayer configuration with gold acting as structural layer and electrode. Pillarisetti et al [21] developed a polypyrrole based hexagonal microgripper (LOTUS microgripper) for holding an individual cell. The LOTUS microgripper is comprised of six polypyrrole-gold (PPy-Au) bi-layers. The PPy-Au bi-layer is ideal for biomanipulation because it actuates at low voltages (less than 2V) and can operate in aqueous media at room temperature. Successful actuation of the LOTUS microgripper was demonstrated by performing experiments on zebrafish egg cell. Chan et al [22] took a step ahead and developed a polymer based micro-robotic actuator by estimating the actuation force. The fabricated actuator is trimorph with platinum layer sandwiched between two layers of parylene. The two methods of actuation are: (a) passing current through the resistive heater and (b) changing the temperature of the medium surrounding the actuator. The successful actuation of the device was demonstrated by grasping follicle of zebrafish (Danio rerio) ranging from 500µm to 1mm in diameter. Bronson et al [23] presented concept and design of MEMS "Tensile testing machine" to determine elastic and adhesive properties of cells. Apart from analyzing single cells, MEMS has the advantage of treating an array of cells, thus reducing the time of an operation. Chun et al [24] proposed a micromachined DNA injection system, which presents the detailed fabrication process for an array of hollow microneedles. A review on microneedles for gene delivery is provided in [25]. Microfluidics plays an important role in transporting cells which is an integral part of a cell manipulating system [26].

Mechanical micromanipulation referred to as contact manipulation is the common method adopted to inject sperm/genetic material into oocytes, for example in intracytoplasmic sperm injection (ICSI) and pronuclei DNA injection. Codourey et al [27] discussed the development of two planar 3 DOF mechanisms using piezo actuators. The first one is micro crawling machine and the second one is "Abalone II" which relies on an impact drive. A three degree of freedom micromanipulator incorporating split tube flexure was also developed by Goldfarb et al [28]. The new flexure based revolute joint exhibited no backlash and the range of motion was five times more than a conventional flexure resulting in increased reacheability workspace. It could also withstand roughly 3 times more load than a conventional flexure. One of the new techniques developed by Kallio et al [29] is to use piezohydraulic actuation to construct a three DOF parallel micromanipulator. The basic elements of actuator system are piezoelectric actuator, bellows, and hydraulic oil. The displacement of the piezo actuator is amplified by the actuator system. To have better control on manipulating micron sized objects, Gao and Swei developed a 6 DOF micromanipulator [30]. The piezo electric transducer has high resolution, quick response and the displacement resolution obtained with the system is 10 nm with a natural frequency of 2 KHz. Parallel mechanism, such as the 3 DOF finger module developed by Ohya et al [31] was adopted in designing the actuators due to their high speed, high accuracy, and high stiffness properties. Tanikawa and Arai [32] developed a two fingered micro-hand to manipulate a microscopic object, by simulating chopstick manipulation. A precision parallel micro-mechanism with 6 DOF was developed by Guo et al [33], which consisted of a macro/micro mechanism to position a micro object.

Even though there has been considerable effort to automate manipulation of biological cells, vision has been the only sensing modality. Recently, there have been efforts aimed at sensing the interaction forces to improve the reliability of biomanipulation tasks [34, 35]. Force sensing in addition to vision would make the manipulation process repeatable and accurate. Few researchers have proposed the concept of "bilateral control", which involves a master- slave set up. The bilateral control system takes into account the scaling effect in the macro/micro world and maintains a stable, transparent system. Pillarisetti et al [35] have demonstrated a master-slave teleoperation framework for manipulating egg cells. A force and vision feedback system was also developed by Zhou et al [36] in which the optical microscope provided vision feedback at the micron resolution and an optical beam deflection sensor provided force feedback in the nN range. Three degrees of freedom piezoresistive force sensor was used [37] to measure forces while manipulating the cell of an onion. Ando et al [38] proposed a tele-micromanipulation system with haptic feedback.

Atomic force microscopy (AFM) has also been used to develop a tele-nanorobotics system. In this system, one degree of freedom haptic device has been constructed for tactile sensing. Guthold et al [39] developed a nanomanipulation system consisting of an AFM and a haptic device such as the PHANToM^{TM} (manufactured by Sensable Technologies, Inc.), to provide real time 2D/3D graphics display along with force feedback. To measure real manipulating forces, MEMS force sensors have been developed which offer the advantage of miniaturization. MEMS force transducer have been developed by Lin et al [40] by integrating 3D microstructures and signal processing electronics onto a single chip 2 mm^3 in size. 2-DOF capacitive force sensor developed by Sun et al [34] is capable of resolving forces up to 490μN with a resolution of 0.01μN in the x direction, and up to 900μN with a resolution of 0.24μN in the y direction. The system can be used in aqueous solution where only the probe of the force sensor is immersed in the solution.

3. ROBOTICS IN LIFE SCIENCE INDUSTRY

3.1 Cell Injection

Cell injection involves depositing sperm or genetic material into the cytoplasm/nucleus of a biological cell. Injecting cells is an important process carried out in intracytoplasmic sperm injection (ICSI), pro-nuclei DNA injection, gene therapy and other biomedical areas. ICSI is used to treat male-factor infertility and involves direct injection of a single immobilized spermatozoon into the cytoplasm of a mature oocyte. Transgenic species are produced by injecting deoxyribonucleic acid (DNA) into the pro-nuclei of an embryo. In gene therapy, a normal gene is inserted into the genome to replace an “abnormal”, disease causing gene.

As discussed in the previous section, in the field of molecular biology, conventional pipette technology is used to carry out cell injection tasks and the “pipette technology” was originally developed by Marshall A. Barber. A typical cell injection system is shown in figure 7.1. Conventional methods of injecting cells require the operator to undergo long training (over a period of one year) and the success rates are low (around 10% - 15%) due to poor reproducibility. The fragile nature of a biological cell requires the operator to be efficient; otherwise he/she may damage the cell. There are also chances of contamination due to direct human involvement. The drawbacks involved in conventional methods have motivated the research community to develop robotic based tools to perform cell injection tasks.

Figure 7.1 Cell Injection System

Tremendous improvement in technology over time allowed researchers to develop robotic micromanipulation systems. One of the main features of these systems is to have better control on the movement of the pipette used for injecting cells. A piezo driven micropipette was used to perform ICSI in mouse [41]. A resolution of 0.5 µm was achieved by piezoelectric actuation. The pipette punctured the cell membrane with minimal distortion of the cell (oocyte). Experiments showed that 80% of sperm injected oocytes survived and 70% of them developed into blastocysts using the piezo driven micropipette. By conventional method only 16% of the oocytes survived.

Yanagida et al [42] used piezo micromanipulator to perform ICSI in humans and compared it to conventional ICSI. The results are shown in table 1. Piezo micromanipulation system also allowed a fine resolution of 10 nm for the injecting pipette with repeatable motion. MANiPEN micromanipulator [43] was developed to perform automated capillary pressure microinjection (CPM). The pen-like shape of the manipulator allows simultaneous placement of several manipulators around a microscope. Compared to manual manipulators computer controlled micromanipulators are ideal for cell injection tasks performed at the micro/nano scale.

Type of ICSI	**Survival rate**	**Fertilization rate**	**Pregnancy rate**
Piezo - ICSI	88.1%	79.4%	23.1%
Conventional- ICSI	81.4%	66.4%	14.9%

Table 1 Comparing the results obtained from piezo and conventional ICSI ("From: Yanagida et al, 1998")

Advances in the field of computer vision algorithms facilitated the integration of robotics with visual servoing control schemes. The integration was a boon to the microrobotics community. Pronuclei DNA injection of mouse embryos was performed using an automated microrobot and a hybrid visual servoing control scheme [4]. The injection unit consisted of a high precision 3 DOF microrobot with a step resolution of 40 nm and a travel range of 2.54 cm in x, y & z axes. The microrobot controlled the movement of injection pipette. The movement of the holding pipette was controlled by a three dimensional coarse manipulator (manual). Injection success rate of 100% was achieved with the system. An automatic micromanipulation system (AMMS) used a pair of microrobots and employed real time visual servo control system to perform automatic gene insertion [44]. The system consisted of two manipulators: 3 DOF parallel mechanism and 3 DOF XYZ stage. The parallel mechanism link was driven by piezotranslators and was used to move the injection pipette precisely with an accuracy of 0.1 µm. The xyz stage was driven by a DC motor and was used to move the holding pipette with an accuracy of 1 µm. Microinjection of embryonic stem (ES) cells was carried out efficiently using a semi automated system [45]. The system was composed of two manual micromanipulators for coarse positioning of injecting and holding pipettes and one computer controlled piezo injector for fine positioning of the injecting pipette. Vision based algorithms were developed for the system. A robotic system composed of a pair of 3D hydraulic micromanipulators [3] was used to insert mouse spermatozoon into mouse oocyte. The hydraulic micromanipulators were driven by pulse motors. These motors were regulated by pulse signals initiated by a computer program. With the help of image processing, the computer located the tip of the injecting pipette and the oocyte in the microscopic field. A typical schematic of the cell injection set up is shown in figure 7.2. A fertilization rate of 68.8% was obtained by injecting 143 oocytes.

Figure 7.2 Microscopic view of the cell injection set up

One of the main reasons for low success rates of cell injection tasks is poor control of cell injection forces. Biological cells are highly deformable and excessive force applied by the operator to penetrate the cell membrane may damage the cell. Thus the delicate nature of biological cells posed challenges to the operator. Few researchers addressed this problem. Sun et al [34] used microelectromechanical systems (MEMS) technology to develop a 2 DOF capacitive force sensor (resolution in the range of micro Newton). Thereafter, the robotics researcher used the force sensor to characterize the mechanical properties of mouse oocytes and embryos [46]. Piezoelectric force sensor was used to measure the injection force of zebrafish embryos at various developmental stages [47]. Later on, the group characterized the mechanical properties of zebrafish embryo chorion. Automated MEMS based fruit fly injection system utilized piezoresistive pressure sensor [48]. The efficiency of the system was more than 96%.

3.2 Cell culture automation

Cell culture is a laboratory process used to maintain or grow cells *in vitro.* The process is used to: (a) investigate the normal physiology or biochemistry of cells, (b) test the effect of various chemical compounds or drugs on specific cell types, (c) to generate artificial tissues (tissue engineering), and (d) to synthesize biological entities such as proteins from large scale cultures. Cell culture laboratory has the following equipment: microbiological safety cabinets, centrifuges, incubators, plasticware and consumables. A typical cell culture cycle includes cell growth, harvesting, reseeding, and analysis.

Conventional cell culture is a manual process and involves long hours of repetitive tasks. It requires a diligent technician to maintain sterility and viability of cells under favorable environmental conditions. Moreover, the technician has to undergo rigorous training to carry out the cell culture cycle. Even for an experienced professional there are chances of cell contamination due to direct human involvement. Smooth movement is desired while handling cell culture vessels because rough handling can cause cell damage and halt the cell growth process. Considering the disadvantages associated with conventional cell culture techniques, pharmaceutical companies and researchers have turned to automated cell culture systems. Automated cell culture systems use robots to improve consistency and sterility of the cell culture system, which may not be achievable by even the best technician. Normally, a contamination frequency level of 1 in 50 million is achieved by automated systems compared to 1 in 20 with manual handling. Unattended operation over a period of days or weeks is achieved by introducing automation in the cell culture process.

Robots are integral part of automated cell culture systems. One of the main applications of robots includes handling flasks/tubes carrying biological samples. An automated system was proposed for cultivating mammalian cells [49]. The system used a robot (PA-10, Mitsubishi Heavy Industries) to: (i) move sample tube, (ii) move Cedex (an automated cell counting system, Innovatis Inc) tube, and (iii) move storage tube. The robot was equipped with a camera and a force torque sensor to obtain visual and tactile information respectively. The dual information was used to guide the robot and compensate for uncertainties in positioning the tubes. “RTS life science international” has created “acCellerator”, an automated cell culture system [50]. A unique feature of “acCellerator” is the ability to provide high throughput performance through the use of multiple robots. The use of three staubli robots resulted in parallel processing of flasks for harvesting and plating/passaging. Parallel processing improved the speed, throughput and viability of the cell culture process. Georgia state university automated titration enzyme immunoassay (t-ELISA) for detecting herpes B virus antibodies [51]. The system was custom made by Beckman-Coulter, Inc. and consisted of a Genesis 150 liquid handler (Tecan, Inc) integrated into Sagian Core system (Beckman Coulter, Inc). The system was programmed using Gemini (Tecan, Inc) and Sami (Beckman-Coulter, Inc.) softwares for carrying out ELISA. Genesis liquid handler performed “tube to tube” dilutions of the standard serum. The automated dilution procedure was superior compared to the manual procedure. The performance of robot automated t-ELISA was satisfactory and was within limits of experimental error. The authors stated that the results were the first published report on a fully automated serological laboratory in an academic facility.

Drug discovery efforts were automated by integrated software-robotic system. The Tecan Genesis RSP 200 (Tecan, Inc.) was integrated with software package to automate *in vitro* dose inhibition assays. The workstation has the following key components: (i) Positive identification barcode scanner (The

POSID), (ii) carousel (iii) carousel scanner, (iv) liquid handling arm (LiHa), (v) RoMa (Robotic Manipulator Arm), (vi) Gemini software, (vii) Tecan-db database, and (viii) system liquid. The function of RoMa was to transport bar-coded incubation plates from the carousel and place them into carriers. The tecan robotic system can be operated by pushing a single tool bar icon. The assay results lied within the experimental error and were similar to the data obtained from manual procedures. The main goal in using the system was to minimize human involvement, which reduced the risk of manual error and contamination involved in traditional methods. A robotic system was used for handling human serum samples. The robot CRS 475 (Burlington, Ontario) was mounted on an inverted track and was enclosed in a biosafety cabinet. The purpose of using the cabinet is to protect the operator from infectious agents such as Hepatitis C present in the biological sample. A swapper device maximized the efficiency of the system. It consisted of Packard Multiprobe II pipetting robot (Packard Instrument) with eight swappable shelves. The primary goal was to carry out simultaneous loading/unloading of one shelf by the robot and pipetting on the other shelf by Packard. The robot was controlled by software, consisting of an executable program: BTA.exe and an intelligent algorithm. The empty boxes of disposable tips were replaced by the robot without human intervention, which allowed overnight work. The other parts of the robot cell were refrigerator storage and 2D bar code reading. The Twister II (Zymark Corporation), a laboratory robot was used for handling up to 400 microplates [52]. The microplates were transported to and from plate reader, washer and pipettors. A visual basic application (VBA) interface was developed for controlling Twister II.

An automated robotic system was used for high throughput fermentation for the cultivation of *Saccharomyces cerevisiae* [53]. The system consisted of a screening robot, a rail mounted robotic arm (Sagian ORCA, Beckman-Coulter, Fullerton, CA), a liquid handler (Biomek 2000; Beckman-Coulter, Fullerton, CA). The robotic arm transported microtitre plates between various laboratory devices. The liquid handler transported the liquid medium into 48 well plates and also inoculated the samples from precultures. The automated system permitted 768 micro scale fermentations under high oxygen transfer rates without human involvement. Symex technologies (Santa Clara, CA) developed a flexible automated system called the extended core module (XCM) robotic system [54]. The XCM consisted of a three axis Cartesian robot, pump housing, and deck. The unique architecture of the system allowed addition of appropriate elements to the robot for liquid handling, material handling, moving plate/vial, mixing heads and instrumented probing (i.e. temperature, pH). The XCM also allowed addition of two more robotic arms. The system was controlled by Renaissance Impressionist Software. Thus XCM has the capacity to automate a variety of tasks depending on the need in the life science industry. This feature greatly reduced the time required to reconfigure the workflow for a short term project. The assay services department at Genentech, Inc. implemented two robotic assay ELISA systems (RAS) [55]. Each system comprised of a CRS robotic arm (CRS robotics, Inc.), a

96 well dispensing station, a reagent dispensing station, a plate washer, a plate shaker, a microplate reader and a carousel for holding tips and plates. The robot systems were custom built by Scitec (Wilmington, Delaware). Each RAS was capable of running 10-15 plates per assay run. Assays can run overnight without the presence of laboratory personnel. Over the years, there was an increase in running assays on the robot. In the year 1998, 2 assays were put on the robot. In 1999, 13 assays were running on the robot which increased to 17, in the year 2000. Thus the two robotic systems increased the throughput of assay and reduced the research associate's (RA) bench time by 80%.

"Cellmate", an automated cell culture system (manufactured by "The Automation Partnership Ltd) used a Staubli RX 60 CR robot. The system automated all operations including cell seeding, media change, bottle greasing, and cell sheet rinsing. The robot arm smoothly moved bottles/flasks to a series of cell culture workstations. The system was capable of handling cells up to volumes of 1000 bottles per day. Above all, simple operation was an important feature of "Cellmate". Few of the operations namely loading/unloading, labeling and scraping flasks were performed manually using Cellmate. Bernard et al [56] proposed the integration of three new custom subsystems namely FlaskMaster, FlaskLabeler, and FlaskScraper into the Cellmate robotic system. The FlaskMaster robot provided automated loading and unloading of flasks onto the Cellmate conveyor systems. The FlaskLabeler (subsystem of the FlaskMaster) was capable of printing, applying, and reading labels marked with barcodes on the flasks. The subsystem was used for flask identification. FlaskScraper allowed automated, unattended scraping and was capable of attaining scraping coverage equal to the manual process. The system's performance was promising. The FlaskMaster increased the total unattended batch throughput from 5 to 168 flasks. FlaskScraper provided an FTE (full-time employee) saving of 1.5 days per week. "The Automation Partnership" built "SelecT", an automated cell culture robot. SelecT was built around a horizontally mounted Staubli RX 60 L robot that performed all the handling, incubating and sampling movements on up to 160 T-flasks. The robot was able to handle both the flasks and pipettes. The system was capable of processing small batches of up to 40 different cell lines in parallel. This allowed for immediate analysis of overnight assay plates. The system has the capability to prepare up to 300 assay-ready microtitre plates and can operate unattended outside normal working hours. Later on "The Automation Partnership" upgraded SelecT system to CompacT SelecT. The system was able to automatically manipulate up to 175 T-flasks. Soloveva et al [57] used a robotic team composed of SelecT and integrated laboratory automation system (LAS) (Thermo Electron Corporation) with Dual FLIPR (fluorescence imaging plate reader). The automated system was custom built for Wyeth screening sciences (Collegeville, PA) by Thermo Electron Corporation. Table 2 lists few of the parts of the thermo LAS system along with their functions.

Parts of Thermo LAS	**Quantity**	**Function**

High speed movable belt	1	Moved plate from one part of the system to another (Linear plate transport, LPT).
Flip mover robotic arm	8	Moved plate from LPT to peripheral instruments.
Vertical array loader (VAL)	1	Moved compound source plates from the plate carousels to LPT.

Table 2 Parts of Thermo LAS robotic system ("From: Soloveva et al, 2006.")

Apart from the parts listed in the table, the system has two stericult incubators, a biotek plate washer Elx 40, a Perkin-Elmer flexidrop IV noncontact dispenser, Perkin-Elmer Evolution EP3 pipettor and FLIPR^{TETRA}. The integrated robotic system maintained cell culture conditions according to the protocol and eliminated variable delays between various steps in the protocol. A high throughput screening of 120 plates per day was achieved with the system. The capillarys (multicapillary zone electrophoresis instrument, Seiba Inc) was designed with a three axis robotic system to handle tubes [58]. The robot picked tubes and arranged them on the racks of the capillarys. It was also able to handle racks of tubes. A qualification plan was proposed to validate the automated workcell. It was found that the robot did not influence the capillarys throughput but reduced human intervention in handling cell culture operations.

Few other companies are also involved in cell culture automation. Juan robotics manufactures automated cold storage (Molbank^{TM}), centrifuge (GR4 Auto) and incubator (Autocell) for cell cultivation [59]. The specifications of Autocell incubator and Molbank^{TM} storage system are mentioned in table 3.

Specification	**Molbank^{TM} (Storage system)**	**Autocell 44 (Incubator)**
Storage capacity (number of microplates)	2571	198
Temperature control	-20^0 to + 20^{0}C	30^0 – 50^0 C
Relative humidity	70%	> 95%
Access time	21 to 33 seconds	< 15.3 seconds
Interfaces	RS232, Lonworks network	RS232, V24

Table 3 Specification of automated cell culture components ("From: Triaud et al, 2003.")

The automated incubator was developed for high throughput screening in cell culture operations. Molbank^{TM} automates storage of pharmaceutical compounds. It comprised of a double storage carousel and a gripper to transport plates from the carousels. The stored and retrieved plates were traced by embedded bar code reading. Automated cell culture incubator, Autocell 200 comprised of a carousel and a robotic arm which has shovel at its end. It has an external door which protects the internal door. The internal door allowed visual inspection of the incubator contents without opening the incubator. This set up reduced contamination of biological samples. A robotic gate permitted retrieval of plates without disturbing the internal climate of the incubator. The shovel at the end of the robotic arm was used to load plates on the stackers. The frequency of plate loading and unloading was measured as 150 per hour and 120 per hour respectively. The mean time of the robotic gate opening was measured as 7 seconds.

Experimental results demonstrated no significant difference between the automated and traditional incubator, but authors believed that the Autocell 200 could provide meaningful benefits for cell culture operations. The automated incubator was designed to be integrated in a fully automated cell culture system. A robotic system (manufactured by Cytogration) performed cell culture as well as high throughput screening assays for drug candidates [60]. The cell culture system automated cell production, membrane penetration and/or in vitro screening. The system featured CRS 465 robotic arm in fixed or rail mounted configuration. The fixed arm configuration was used in smaller, feeder systems, and was capable of handling up to 168 plates, with one incubator. The rail mounted configuration was used for all systems namely coater, seeder and feeder. The configuration was capable of handling up to 504 plates, with three incubators. An automated system was developed in collaboration between Organon and Scitec laboratory automation. The system consisted of three CRS robots. One of the robots was capable of performing cellular and non-cellular assays to produce a maximum throughput of 300 (96 well) or 150 (384 well) microtitre plates in 24 hours. The other two robots were involved in: (i) assay plate preparation, (ii) selection of compounds to determine potential drug candidates (cherry picking), and (iii) refreshing storage plates by replacing them with newly prepared plates. The storage and distribution system was kept under controlled temperature (4 – 10^0 C) and humidity (10-15% RH). The system provided high throughput screening and short delays between different operations. REMP store robot achieved high throughput handling of storage racks. The system was flexible and allowed handling of vials, microplate format racks, mini-tube racks, and any of the REMP tube technology formats. Apart from many advantages offered by automated cell culture systems, there are few disadvantages, namely: potential for mechanical failure, need for technical expertise and familiarity with the system. However, automation proved to be beneficial for cell culturing.

3.3 High throughput processing of biological samples

Biological samples contain deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, and other biological materials. These samples are generally involved in DNA purification, RNA isolation, and protein purification. Isolation and purification of DNA from blood samples, biopsy samples, cultured cells, and buccal cells is a key step to investigate genetic contribution to human disease. Isolation of RNA from biological samples is a critical process in many fundamental molecular experiments such as nuclease protection assays, RNA mapping, and cDNA library construction. Purification of protein is desired for protein stability measurements. These measurements study the relationship between protein sequence and stability. Traditional laboratory methods adapted to process biological samples offer many challenges. These methods being manual lack consistency/reproducibility, involve human error, and are time consuming. Robotic workstations on the other hand perform consistently, 24 hours a day/7days a week. Moreover, high throughput is achieved by a robotic system. Janssen research foundation (JRF) started using Zymark robotic system in 1985. Since then different robotic systems have been used by the organization to increase throughput of screening biological samples. The output of automated screening systems increased to six fold from 260,000 to 1,400,000 data points in a five year period (1991 – 1996) which proved that efficiency of the robotic systems increased over years. JRF validated Staccato^{TM} system (Zymark, Inc.) by performing plate replication process and assay formats using 96 or 384 well plates. The Stacatto^{TM} system consisted of a SciClone^{TM}, an automated liquid handling workstation capable of pipetting a variety of liquid solutions with a higher degree of accuracy and precision. The incorporation of Stacatto^{TM} system increased the throughput of cell and enzymatic assays five and ten times respectively.

Companies and researchers are heavily involved in automating purification of DNA, RNA, and proteins from biological samples. The AUTOPURE LS robotic system (Gentra Systems, Inc.) automated the DNA purification process from blood samples in 1 to 10 mL volume range [61]. DNA purified with manual method and the robotic system resulted in equivalent yields of DNA from human blood samples from a single donor. The system was also used to purify DNA from buccal mouth wash samples collected from 16 different donors. The high yield of DNA from buccal samples suggested that the AUTOPURE LS instrument is capable of purifying DNA from difficult samples. The automation of the DNA purification process provided more time for genotyping compared to manual method (71% vs. 36% of the total time). A multipurpose robot (SX-96GC) with Integrated Magtration Units (IMU) was developed for the purification of the DNA sequencing reactants. The purification process is the most tedious step in DNA sequencing. The robotic protocol used biomagnetic beads and was designed to purify 384 samples within 1 hour. The protocol required least technical skills and manual labor. The main features of the automated system were high throughput and adaptability. A fully automated procedure was developed for the purification of polymerase chain reaction (PCR) products using Wizard® Magnesil™ paramagnetic particles and robotic methods [62]. The Magnesil™ purification process has been adapted to a number of robotic platforms. The Beckman

Biomek® FX robotic workstation processed 4 plates (96 and 384 well formats) in an unattended run of 45 minutes. The procedure was highly reproducible and generated PCR products of high purity with minimal loss of target DNA. Purification of BigDye™ Terminator DNA sequencing reactions was also performed using Magnesil™ paramagnetic particles and robotic methods [63]. The Beckman Biomek® processed up to 4 plates in 40 minutes. The Stanford automated sequencing system was capable of processing 10,000 samples per day [64]. The entire system required only 3 technicians to operate at a throughput of 100 plates per day, and less than 500 ft^2 of space. Meldrum et al [65] developed an automated biomehatronic fluid handling system for genome analysis, “ACAPELLA – 1K”. The system was capable of processing 1000 samples in 8 hours for use in molecular biology. One of the goals was to obtain high-throughput DNA sequencing for the Human Genome Project. Later on, “ACAPELLA – 5K” was developed, which was capable of processing 5000 biosamples in 8 hours [66]. The system was tested in the preparation of DNA PCR and DNA sequencing reactions.

Proteomics researchers are depending on automated workstations to purify and prepare protein solutions. Biomek 2000 liquid handling robot (Beckman-Coulter Inc.) was used to purify his-tagged eglin c proteins [67]. The automated workstation increased throughput as well as precision for protein stability measurements. A throughput of about 20 stability measurements per day was attained. Automation reduced protein measurement variability due to contaminants to around 10 % of that obtained by the manual method. Over all, the automated protein purification process was 50-100 times better than manual purification process. “A protein purification robot”, was developed by the “Genomics Institute of the Novartis Research Foundation”. The robot can follow a specific recipe for protein purification and can simultaneously prepare 96 individual samples. The same task would take nearly 30 technicians to complete. Najmabadi et al [68] proposed “Tower-based automation” for automatic execution of various genomics and proteomics protocols. The tower-based configuration has a high throughput to foot print ratio, high scalability, and wide protocol flexibility. The system consisted of a central robotic structure with two arms. One arm is used for transporting accessories (tubes, plates) and the other arm is used for handling liquid. The robotic system performed magnetic isolation of TAP (tandem affinity purification) tagged protein complexes. The electron microscopy proteomic organellar preparation (EMPOP) robot [69] was developed to: (i) perform parallel microscopic screening, (ii) validate subcellular sample purity, and (iii) confirm protein localization for high throughput proteomics. The EMPOP robot consisted of six components: (i) transfer platform, (ii) core mechanism, (iii) cooling platform, (iv) wash station, (v) electromagnetic arm, and (vi) dispensing needle manifold. The robot was able to prepare 96 subcellular fraction samples with high quality. The process was totally automated in less than one working day, and reduced the time and labor by approximately 1000 fold.

Integration of biotechnology protocols with robotic systems is crucial to maximize the efficiency of the purification process. A robotic protocol was developed for high throughput selection of mRNA from total RNA preparations. Presence of ribosomal, transfer, and other RNA species in the sample weakens rare messages in direct and indirect detection assays. The protocol was initially developed on the MultiProbe® II HT EX (Perkin Elmer, Inc.) and Biomek 2000 (Beckman Coulter Inc.) robotic systems. For the proposed protocol, any robotic workstation would take about half hour to process 96 samples with an 8-channel pipetting tool. The automated system removed greater than 99% of the rRNA initially present in the biological sample. The MultiProbe® II HT (Perkin Elmer, Inc.) was used for high throughput isolation of RNA from cultured cells using the RNA aqueous-96 kit (Ambion, Inc) [70]. The run time required to complete the procedure was approximately one hour and twenty minutes. Results indicated that the system provided total RNA for use in the quantitative analysis of target mRNA. An automated platform was developed for liquid-liquid extraction which isolated natural compounds from mixtures [71]. The robotic system composed of a Zymark robot (Caliper Life Sciences), liquid-liquid extraction module, control system, programmable logic controller (PLC) and an operator interface. The operator interface was user friendly and has several control screens developed by the supervisory control software (FIX). A robotic system was used to determine optimal reaction conditions for high purity and high yields of an organic chemical. The system also consisted of a Zymark robot, PAL robot (CTC Analytics) and a solid dispensing system (Autodose S.A.). The Zymark robot integrated the instrumentation of the system. The PAL robot and solid dispensing robot were used for handling liquid and solids respectively. The integrated system was controlled by graphical software and allowed screening of several hundred reaction combinations within short time. Few other companies also developed liquid handling robots. Zinsser Analytic, Inc manufactures Lissy 2002, a robotic system for sophisticated and flexible liquid handling. The system is equipped with up to 16 dispensing tips (8 on each arm). Lissy 2002 is an ideal system for high throughput of 96 and 384 plates. Hamilton Inc manufactures MICROLAB® STAR, a liquid handling robot. Some of the key features of the robot are multiple channels, 0.5 -1000µL volume range, and use of disposable tips. The use of robotic workstations for processing biological samples certainly offer many advantages but at the expense of high budget. Cost, space, throughput and flexibility are the most important parameters for the decision making process in designing or purchasing a robotic workstation.

3.4 Production of DNA and protein microarray

Microarrays are grids of tiny spots of DNA or protein on a microscopic slide. In molecular biology, conventional methods generally involve one gene in an experiment and limit the throughput of the process. The microarray technology allows scientists to analyze expression of many genes in a single experiment quickly and efficiently. The main application areas of this technology are gene discovery, disease diagnosis, drug discovery and toxicological research. The miroarrays can be fabricated by fine pointed pins, photolithography or ink jet technology.

Robotics plays an important role in the production of DNA and protein microarray. In 1995, Schena et al [72] used a high speed robot to spot array of different complimentary DNAs (cDNAs) on the glass surface. The microarrays were used for quantitative expression measurements of the corresponding genes. In 1998, Joseph DeRisi published a document, “MGuide” on the web [73]. The document listed all the necessary parts, suppliers and prices for building a microarray robot. The robot can be employed to print thousands of tiny DNA spots on glass slides. A high precision contact-printing robot was used to fabricate protein microarrays [74]. The robot delivered nanoliter volumes of protein samples onto microscopic glass slides. The proteins were immobilized by covalently attaching them to the slides. A spot diameter of 150 to 200 µm allowed 10000 samples in half the area of a standard (2.5 cm by 7.5 cm) slide. The protein micoarrray was used: (i) to study protein-protein interaction, (ii) to identify the substrates of protein kinases and, (iii) to identify protein targets of small molecules. The probe preparation for DNA microarray was automated by Packard-brand MultiPROBE® II Nuclei Acid Purification Workstation (Perkin-Elmer Life Sciences). The preparation involved plasmid DNA purification, PCR setup and clean up, gel loading, fluorescence DNA quantification, and tracking DNA probes. The robotic workstation provided high throughput production of DNA probes. A pin-based robotic system, termed "SmartPin" was developed for DNA and protein microarray fabrication. The system is based on contactless printing technique. The SmartPin assembly consisted of a fluid reservoir for handling the sample liquid materials, a fluid delivery plunger for dispensing/aspirating liquid and a fiber probe to determine the distance between the fiber tip and the slide. The functions of the smart pin are spot formation, detection of spots, and production of uniform size microarrays. The diameter of the spot varied from 80 to 200 μm. Microarrays of synthetic heparin oligosaccharides were prepared by an arraying robot. The robot delivered 1 nL of carbohydrate containing solutions to create spots with a diameter of ~ 200 μm. An array of 460 spots was generated. The construction of heparin microarrays can be used to rapidly screen heparin protein interactions. A high precision robot was used to print small molecules as microarrays for detecting protein-ligand interactions. The robot picked up a small volume of the compound and delivered approximately 1 nL of solution to defined locations on a glass microscopic slide (~150 slides per print area). Microscopic spot formed on the slides was 200-250 µm in diameter. The immobilized compound spots were probed with a different tagged protein and the binding events were detected by fluorescence linked assay.

Few companies are also actively involved in the production of microarrays. Agilent technologies, Inc. proposed automation of cDNA and protein microarray fabrication using ink jet technology [75]. The goal was to mass produce several microscopic slides containing multiple microarrays of different biological features in a single run of production. The system was used to make cDNA microarrays from a large number of different gene sequences. The size of the microarrays (11,000 or 16,000 features) allowed more tests to be performed with minimal sample amounts. ChipWriter^{TM} Pro (Virtek, Inc) is a high precision robot designed to collect 100 nl of DNA solution to deposit 0.6 nl per spot. The robot was used to spot 21376 gene-specific probes onto a single glass slide, "Drosophilla MicroArray". High quality spots were generated with a spot diameter of 100 µm. The robot controlled the spot morphology, the spot diameter and uniformity. Telechem International, Inc manufactures SpotBot®, a personal microarray robot. The robot can print a maximum of 50,000 spots per chip, with a spot diameter of 100-120µm. Genomic solutions offered production of DNA and protein microarray in 96 and 384 well formats. It was possible using BioRobotics® MicroGrid II and the Gene Machines® manufactured by Genomic solutions. The BioRobotics MicroGrid II has the capacity to hold 16 microplates while the GeneMachines® Omni Grid has the capacity to hold 6 microplates. At least 1000 DNA or protein spots can be printed within individual microplate wells. The arrays can be produced using contact printing. The split pin performed the printing by simple surface tension and adhesion, thereby minimizing the contact between the pin and substrate. This enabled printing on a delicate substrate. The plate array technology allows micro ELISAs, diagnostic arrays and other protein-protein studies in a higher throughput format. Thus, robots are being used in academics as well as industries to produce DNA and protein microarrays.

4. DISCUSSION

Robotics and automation has influenced a wide variety of areas in the life sciences ranging from biomanipulation to production of DNA and protein microarray. There are several promising approaches for biomanipulation. In this chapter, we have covered some of the most common approaches namely optic and electric micromanipulation, magnetic micromanipulation, acoustic energy for micromanipulation, micro-electromechanical systems (MEMS) and mechanical micromanipulation. Each of the above techniques has their own advantages and disadvantages. Optic and electric techniques offer the ability to manipulate single cells without contact; however, these techniques do not readily lend themselves to effective manipulation accomplished by MEMS and other mechanical manipulation techniques. Acoustic energy based micromanipulation is perhaps the most non-invasive approach in biomanipulation. However, this approach can be at best used currently for trapping and holding microparticles in a required position. The research in the use of acoustic energy for micromanipulation is currently in its infancy. Effective manipulation of individual cells has been developed through the use of a bilateral teleoperation framework whereby individual cells can be grasped in place and injected with a genetic material by the operator through a vision and force feedback interface. There are several challenges in micromanipulation. One of those challenges lies in accurately fixating the nucleus to deliver a genetic material accurately within the nucleus.

Since last few decades, robotics had a tremendous impact on life science automation industry. On going efforts in industries as well as academia provide innovative solutions to carry out life science based tasks. Automation certainly improved the efficiency of cell injection task, biological sample purification, cell culture operation and DNA/protein microarray fabrication. For example, the success rate of cell injection tasks increased from 10 – 15% to around 100% using robotics. Such marvelous achievement motivates companies to automate the entire task at an economical price. Robotic methods perform consistently and ensure safe laboratory operation thereby reducing labor costs. There are many commercially available robotic systems to carry out life science based tasks, such as: Sutter Inc, World Precision Instruments Inc, RTS Life Science, The Automation Partnership Ltd, Cytogration, Gentra Systems Inc, Beckman Coulter Inc, PerkinElmer Inc, Zinsser Analytic. Despite of the competition in the market, there is still research going on to implement and integrate new technology in life science based tasks.

For example, integrating “haptics” with the biomanipulation system improved the success rate of cell injection tasks. Similarly MEMS technology is used to develop micro tools to manipulate biological cells, whose size is in μm – nm range. Although haptics based life science systems are yet to be commercialized, it has been proved in academia that haptics plays a critical role in cell manipulation [21]. Robotics can also be integrated with optic and magnetic techniques to manipulate cells without physical contact. On the other hand mechanical manipulation involves physical contact. In certain applications, automation allows technicians to devote more time to tasks like genotyping while the robot processes the biological samples. In summary, robotic techniques for life science automation is currently in its infancy and there are a wide range of applications and engineering challenges that will be addressed in future research.

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