Virtual Reality Training for Micro-robotic Cell Injection

by

Syafizwan Nizam Mohd Faroque
BSc (Hons), MEd

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

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I am the author of the thesis entitled

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Abstract

Micro-robotic cell injection is a procedure requiring a high degree of precision to manoeuvre a motorised micropipette and deposit a small quantity of material at a particular location inside a cell. Performing the procedure requires three-dimensional movement of the micro-robot with individual or simultaneous control of the three normal Cartesian axes.

Currently the micro-robotic cell injection procedure is performed manually by an expert bio-operator where lengthy training and a great deal of experience are required to become proficient. In order to reduce the training cost, duration and ethical issues surrounding the use of real cells for training, this thesis introduces two virtual reality micro-robotic cell injection training platforms. The first is a desktop-sized haptically-enabled virtual reality training system which supports portability and flexibility and employs common personal computer or laptop peripherals. A reconfigurable multipurpose haptic interface was also developed as part of the platform in order to provide convenience in setting up and mobility. The second platform provides a large-scale virtual reality platform for micro-robotic cell injection training. It utilises three large screens which are arranged in different display configurations to provide immersive virtual environment. The three display configurations project a large virtual replication of a micro-robotic cell injection setup in two-dimensional, three-dimensional and three-dimensional.
immersive displays respectively. The large display area of the two-dimensional large-scale display can provide a very detailed representation of the environment. The three-dimensional large-scale display, in addition, can deliver depth perception which contributes to an increased level of immersion and presence in the virtual environment while the three-dimensional immersive display provides views from three different angles in the virtual environment in order to enhance user’s spatial and orientation understanding.

In order to achieve the required high precision movement of the micropipette an appropriate input device and control method are required. This thesis investigates the use of three distinct input devices for control of the micro-robot. The first two input control methods are the computer keyboard and the Phantom Omni haptic device utilised in the desktop-sized virtual reality training system introduced in this thesis. The keyboard is a commonly used interface due to its low-cost and simplicity. From an ergonomics perspective the keyboard control method presented in this thesis is advantageous in that the bio-operator’s hand/arm can be easily supported by the table or desk surface. The Phantom Omni haptic device as an input control method is then considered. A positional mapping between the haptic device stylus and the tip of the micropipette was considered to provide an intuitive control as if the bio-operator is holding a handheld needle insertion device. In addition, haptic feedback is provided to the bio-operator as guidance during injection. The haptic guidance provided in two forms, virtual fixtures and
force feedback. Three virtual fixtures, conical, axial and planar, were evaluated in their ability to guide the bio-operator in achieving the suitable penetration and deposition points. The force feedback is provided to assist the bio-operator in estimating appropriate force during penetration of the cell membrane in order to increase the survivability of the cell. In order to achieve this, a gradual repulsive force is provided to the bio-operator’s hand during penetration attempt to simulate the contact between the micropipette and the cell membrane. Additionally a sudden drop of force will occur immediately when the cell membrane is penetrated to indicate the rupture as indication to the bio-operator to arrest the force exerted. The third input control method is the large workspace haptic device, INCA 6D, utilised in the large-scale virtual reality training system introduced in this thesis. Utilising the large workspace haptic device coupled with the large display provides several benefits such as delivering an immersive virtual environment and enhancing user’s spatial awareness through intuitive handling. Additionally the utilisation of user’s gross motor skill when manipulating the large workspace haptic device can provide several advantages such as less responsive to insignificant or unintentional movements like vibrations, hand tremors and minor deviations, so that consideration can be focussed on more important criteria such as user’s spatial awareness and understanding of the three-dimensional environment.

The approaches provide an alternative method by which to train bio-operators comprised of two different scales of virtual reality platforms. The
interface for the systems is achieved using computer keyboard and haptic devices which provide the bio-operator with effective and intuitive control. It is also suggested that the acquired skills, knowledge and understanding from the virtual training such as the spatial awareness, depth estimation and hand-eye coordination can be transferred into physical micro-robotic cell injection or similar real tasks.

The key contributions of this thesis are: 1) presenting two different scales of haptic VR system for micro-robotic cell injection training, 2) introducing and evaluating a new input control method using a computer keyboard for the desktop-sized VR system, 3) considering the usability and effectiveness of using the Phantom Omni haptic device as an input control method for the desktop-sized VR system, 4) developing and considering the training effects of a large-scale haptic VR system providing three distinct display configurations with a large workspace haptic device as an input control method.
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Chapter 1  Introduction

Cell injection is a procedure where a small amount of material, such as protein, DNA, sperm or bio-molecules is injected into a biological cell. Since the introduction of enabling technology early last century, cell injection technology has been widely applied to areas such as cellular biological research, transgenics, in vitro fertilisation (IVF), drug development and toxicology [1]. For example in the intracytoplasmic sperm injection (ICSI) application, the technology is used to inject an immobilised sperm into the centre of a mature egg to stimulate fertilisation. Another widespread cell injection application is in drug development where researchers inject drugs into a cell and observe the effects.

Mechanical microinjection is a versatile approach to material deposition and a micro-robot manoeuvred micropipette is common practice. Compared with non-contact methods discussed in Chapter 2, microinjection is superior in flexibility and cell viability rates [2]. In this approach a micropipette is attached to a high-precision motorised micro-robot which moves to achieve the required precise movements. Aside from the ability to manoeuvre the micropipette at the micro-scale, employing a micro-robot enables functionality such as removal of operator hand tremor.
To perform the microinjection process, it is necessary to approach an immobilised cell with a micropipette, puncture the chorion, penetrate the cytoplasm and then stop at a suitable location inside the cytoplasm for deposition. The success of an injection can be characterised by various metrics including injection accuracy, trajectory and speed.

Despite the use of micro-robots capable of high precision movement, successful injection is often not accurately reproducible, being a contributor to high failure rates even among the experienced bio-operators [3]. The movement of the micro-robot is normally controlled in a human-in-the-loop manner by the bio-operator using input controllers such as rotary encoders or a joystick for each the $x$, $y$ and $z$ axes. Automatic cell injection systems, removing the human-in-the-loop from controlling the micro-pipette, have been proposed [4] however have the limitation in terms of its flexibility to deal with the large variety of cell type of different properties, e.g. size, shape, morphology, etc. As such, the development of accurate automatic cell injection system remains a challenging topic among researchers.

Given the need for the human-in-the-loop to provide human level judgement and intuition, adaptability and flexibility in the cell injection process, it remains common practice [5]. It does however have several major drawbacks in terms of its speed, precision, throughput and reproducibility [4, 6].
Micro-robotic cell injection is normally performed by an expert human bio-operator who has extensive training and experience. The procedure requires delicate operations such as positioning the micropipette accurately in order to penetrate the cell membrane and inject the foreign material appropriately. Given this, it is widely acknowledged that a high level of skill is required to perform the cell injection procedure successfully. As such this research was carried out to fill the gap within existing knowledge on the approaches to improve the bio-operator training for the procedure which is outlined in the next sections in this chapter.

1.1 Research Significance

Conventionally, cell injection is performed manually by an expert bio-operator using a micro-robot for manoeuvring the micropipette. Aside from the widespread benefits of the procedure, there remain challenges to be overcome. Amongst these are the lengthy training and extensive hands-on experience required in order to become proficient at the task. Another challenge is the significant amount of required access to injection equipment which can be costly and also makes the equipment vulnerable to excessive use as well as accidental damage by inexperienced bio-operators. Additionally, practice injection cells can only be used once requiring a new cell for each practice attempt.

To contribute to overcoming these challenges, this research proposes two versions of haptically-enabled virtual reality (VR) micro-robotic cell
injection systems which provide flexible training approaches for the bio-
operator. The motivation is that VR has the capability to provide an effective
learning and practice environment and have advantages over the real-life
training in terms of cost, portability and flexibility. Meanwhile, haptic
technology has been playing a significant role in assisting in applications such
as motor skills training since its introduction in the last two decades.
Currently, there is significant growth in the development the haptically-
enabled VR systems designed to efficiently train humans for various physical
tasks including biomedical applications.

The proposed VR training systems provide a virtual replication of the
physical system where it is suggested that bio-operators can train offline in a
similar way to which they would with the physical system. It is also
anticipated that after receiving adequate training, bio-operators can transfer
their skills to the similar physical system. Such training systems offer several
benefits including reduced training costs and low maintenance.

The haptically-enabled VR micro-robotic cell injection training systems
presented in this thesis provide bio-operators with an immersive virtual
environment. Aside from the interactive virtual environment of a micro-
robotic cell injection setup, the systems also provide haptic feedback to bio-
operators as guidance and to add to the sense of immersion. Three virtual
fixtures (VFs) of different shapes and functions are provided to guide the bio-
operator in achieving ideal trajectory and improving accuracy. In addition to
the VFs, force feedback is provided to assist the bio-operator in estimating the appropriate injection force aside from delivering sense of immersion during cell membrane penetration.

The two introduced systems provide very different scales of interaction within a VR environment. The first system utilises a desktop haptic device which provides a small-scale, portable and low-cost input control method. The desktop haptic device, Phantom Omni, provide an intuitive control method through its stylus where users can manipulate it as if they are using a handheld insertion tool. The low maximum exertable force of the Phantom Omni enables user to override the haptic guidance provided by the haptic device and retain full control of the movement. This feature is important to prevent passive learning where users spend less to no effort correcting mistakes, even when the mistake is noticeable. The desktop haptic device needs only be connected to a normal personal computer or laptop in order to run the VR system, making it a flexible and portable training tool for micro-robotic cell injection training. This research also introduces a reconfigurable multipurpose haptic interface which combines the required peripherals into a portable compartment to support mobility.

The second system provides a large-scale VR training system utilising large workspace haptic device, INCA 6D and large display delivering an immersive virtual environment to users. The INCA 6D is a cable-driven haptic device inspired by SPIDAR™ technology offering up to 6-DOF haptic
feedback where the actual workspace is configurable based on the motors and cables positions. Manipulating the large workspace input control can afford several benefits such as less responsive to insignificant or accidental movements like hand tremors and small deviations, so that consideration can be focussed on user’s spatial awareness and understanding of the three-dimensional environment.

It is apparent that the utilisation of haptics in VR can offer significant benefits in the skills training. This research introduces two haptically-enabled VR systems specifically for micro-robotic cell injection skill training. The systems were designed to provide a flexible approach to micro-robotic cell injection training enabling bio-operators to practise the important skills such as accuracy, trajectory and force control and improving their understanding of the three-dimensional space environment such as position, orientation and estimation of the depth of the micropipette.

1.2 Research Problems

There are several requirements needing consideration in biological manipulation such as the cell injection. The cell as the manipulated object and the micropipettes as the tool are both extremely small and the associated contact force is only within the $mN$ to $\mu N$ range [7]. Therefore skills such as precise positioning, puncturing and penetrating are crucial. The injection accuracy, trajectory, speed and force are also significantly important in determining the success of an injection [3, 8].
In order to achieve a successful injection the micropipette tip should be positioned precisely at an appropriate penetration point at the cell membrane. Once the penetration point is achieved appropriate force should be carefully exerted with the micropipette to penetrate the cell membrane. The amount of force applied during the penetration should be carefully considered. The cell membrane will not able to be pierced when the force applied is insufficient while excessive force can increase the risk of overshooting the target location. After penetrating the cell membrane the micropipette is required to be manoeuvred slowly in straight path within the cytoplasm towards the centre of the cell. The micropipette’s movement should be stopped when its tip has reached the desired deposition target which often located at the cell’s centre. The deposition of foreign material will take place after the micropipette tip has reached the target location.

As such this research considers the development of virtual training systems considering the above important skills where bio-operators can train offline and then transfer their acquired skills to the real task.

1.3 Research Objective

This research investigates the utilisation of haptically-enabled VR environments for training in micro-robotic cell injection. The objective is to provide a bio-operator with an intuitive and effective method to manipulate a virtual micromanipulator while receiving appropriate force feedback from the
virtual environment. In order to attain this, a realistic, scalable, and flexible approach is required.

1.4 Thesis Layout and Contributions

The key contributions of this thesis are as follows:

**VR Haptic Research Platforms:** Two haptically-enabled VR platforms are presented for micro-robotic cell injection training. The first platform is a desktop-sized system designed to be a portable, flexible and cost effective training tool utilising two input control methods using the computer keyboard and Phantom Omni haptic device. The second platform is a large-scale system developed to provide high quality display and immersive virtual environment for training utilising a large workspace haptic device, the INCA 6D.

**Keyboard Control Method for VR Micro-robotic Cell Injection Training:** A new input control method through use of a computer keyboard for the desktop VR micro-robotic cell injection platform is introduced and evaluated. The evaluation results demonstrate a minimum success rate of 80% suggesting significant performance. The use multiple axes which gradually increase throughout the experiments suggests skills transfer from using the common computer keyboard. As such the keyboard can be utilised as a low-cost, simple and feasible method to control the virtual micro-robot.
Intuitive Haptic Device Control Method for VR Micro-robotic Cell Injection Training: The usability and effectiveness of using the Phantom Omni haptic device as input control method for VR system is considered. User evaluation experiments were designed and conducted to evaluate participants’ performance using the input control method and their performance improvement after undergoing the training was analysed. Results demonstrate the ability to improve participants’ performance by 6 and 63% for success rate and accuracy metrics respectively, suggesting the Phantom Omni as a practical, intuitive and effective input control method.

Large-scale Haptic Device Control Method for VR Micro-robotic Cell Injection Training: The large-scale haptic VR system was developed to provide three distinct display configurations presenting high quality visual display through combination of three human-sized screens. The first display configuration provides increased visibility through detailed two-dimensional images with a wide viewing angle. The second display configuration projects three-dimensional images to improve users’ sense of immersion and depth perception. The third display configuration provides higher immersion where the user is surrounded by a three-dimensional VR environment in a Cave Automatic Virtual Environment (CAVE™) arrangement. Three different viewpoints of the virtual environment
are provided through three large screens to assist user in estimation of the depth of the micropipette and improving their spatial awareness. The usability and training effect of using the INCA 6D as an input control method for the large-scale VR system are considered based on evaluation using human participants. Results demonstrated that participants achieved significant success rates between 73 to 100% across the experiments and 24 to 27% performance improvement for accuracy.

1.5 List of Publications

The publications associated with this thesis are as follows:


Chapter 2  Literature Review

This chapter presents a comprehensive review of VR and haptic technology relevant to micro-robotic cell injection training and discusses the feasibility of developing such training system. A brief explanation of cell injection and the challenges associated with the procedure is first presented. Important skills, such as accuracy, trajectory, speed and applied force, which need to be mastered by the bio-operator in order to achieve successful injection, are then discussed. Then an overview of various types of haptic feedback and approaches is presented. This is followed by discussion on the application of haptics to skills training across various fields including medicine and cell injection. Then a discussion of approaches to cell modelling and the haptically-enabled virtual training systems evaluation is presented. Finally, conclusions are then presented to support the contributions of this thesis.

2.1 Introduction

Cell injection is the process of inserting a small volume of material, e.g. protein, DNA, sperm or biomolecules, into a specific location of suspended or adherent cells. The technology has been widely adopted in drug development, toxicology, cellular biology research, transgenics, and in vitro fertilisation [1]. The technology can, for example, enable researchers to observe at the cellular
level the implications of injecting material or drugs into a cell. It is also extensively used in intracytoplasmic sperm injection (ICSI). ICSI is performed by injecting a sperm into cytoplasmic of a mature egg to enable fertilisation (see Figure 2.1).

![Figure 2.1: Sperm injected into an egg cytoplasm in the ICSI procedure](image)

In general, the micromanipulation of cells can be achieved through non-contact manipulation such as laser trapping and electro-rotation techniques. In the laser trapping technique, a tightly focused laser beam is used to generate force appropriately for micromanipulation tasks. The electro-rotation technique on the other hand uses electric fields and dipole moments to generate torque useful in cell positioning tasks. Both techniques however have been reported to have distinct drawbacks in terms of their applicability to the cell injection procedure. According to Sun and Nelson [3], the high energy light used in laser trapping can potentially damage the cell, while electro-rotation technique does not have sufficient force to hold the cell to be injected.

There are also several non-contact methods specifically aimed at material deposition into cells such as the nanovector-based delivery [9], and
electroporation [10]. Despite the advantage of being able to introduce materials into multiple cells, both techniques suffer from problems with instability making them unsuitable for the cell injection procedure focused on in this thesis. For example in transgenics, the delivered material may become an isolated plasmid or be endocytosed in the intracytoplasmic vesicle which can prevent a stable transfection to happen [11].

Contact manipulation relates to methods where physical contact is made with the cell using appropriate bio-apparatus. Mechanical microinjection is a versatile approach to material deposition and a micro-robot manoeuvred micropipette is common practice. Compared with non-contact methods, microinjection is superior in flexibility and cell viability rates [12]. In this approach a micropipette is attached to a high-precision motorised micro-robot which moves to achieve the required precise movements. Aside from the ability to manoeuvre the micropipette at the micro-scale, employing a micro-robot enables functionality such as removal of operator hand tremor.

Conventionally, micro-robotic cell injection is carried out manually by a qualified bio-operator. The task requires the bio-operator to perform operations such as moving the micropipette appropriately in order to penetrate the cell membrane, and requires a high level of skill. It is common for the procedure to be performed by experts who have undertaken extensive training and developed years of experience in order to be proficient at the task. However, despite extensive training, success rates can still remain low [3, 13].
One reason for this is that successful injection is not necessarily repeatable. Also, given the nature of the process, manual, or human-in-loop cell injection, is inherently limited to low speed and poor precision [6], and low throughput and reproducibility [4].

The term haptics refers to the human’s sense of touch [14]. Haptics can assist in training users to perform physical tasks and has been used in applications requiring motor skills training such as medicine, sport and aviation.

The integration of haptics has shown to improve training against metrics including speed [15], accuracy [16], and the time taken to master a task [17]. Researchers have proposed simulators for cell injection procedures such as for ICSI [18], and also for other procedures including cell indentation [19] and heart myoblast cell injection [20]. None of these works however focus on bio-operator training efficacy for cell injection.

2.2 Cell Injection Skills

In the biological cell injection procedure, the bio-operator is required to appropriately move the micropipette towards the immobilised cell, puncture the cell membrane at a suitable location, insert the micropipette’s tip into the cell and then deposit the specific material in the cell [21]. Cell diameters can range from 1 to 100\(\mu m\) [22] and the contact force exerted by the micropipette during cell injection is in the range of \(mN\) to \(\mu N\) [7]. Successful cell injection can be determined based on cell survivability and is related to injection
accuracy, trajectory and speed [3], as well as the force applied to the cell membrane during penetration [8, 23].

It is worth mentioning that applicable methodologies and technologies may differ for suspended and adherent cells and therefore careful considerations should be made prior to development of a cell injection system. For example, one of the most apparent differences is that the holding or suction micropipette is not normally required for adherent cell injection because this type of cell will naturally adhere to the dish. Unlike adherent cells however, suspended cells typically need to be immobilised and held by a suction micropipette. There are several developed microinjection systems which specialise in either suspended [4] or adherent [6, 24] cells and accommodate the inherent properties of each cell type. Although suspended and adherent cells have different properties, such as size and morphology, the steps to perform injection of both types of cells are very similar. In this chapter, the works discussed are applicable to both cell types and the essential microinjection parameters common to both cell types are discussed in Sections 2.2.1 and 2.2.2 below.

2.2.1 Accuracy and Trajectory

Injected material needs to be deposited within the cell’s nucleus, and as such the boundary of the nucleus needs to be identified [25]. Given the small size of biological cells, as well as the large variety of cell types, the injection micropipette requires precise positioning [24]. To achieve successful injection,
the tip of the micropipette should first be positioned to be able to penetrate at a suitable point on the cell membrane. Then, by moving the micropipette, force should be applied to pierce the cell membrane. The micropipette then needs to move through the cytoplasm, stop at the required deposition point (e.g. the nucleus), and then deposit the desired material [5]. The micropipette’s trajectory needs to be carefully considered in order to prevent damage to the cell and micropipette. For example, when the penetration point on the cell membrane is too far from the centre the interaction can cause a torque which can rotate the cell, the micropipette can fail to penetrate the cytoplasmic membrane and potentially collide with the cell holding micropipette. Once the membrane has been penetrated, the optimal micropipette trajectory is along the cell’s diameter line passing through and eventually stopping at the centre of the cytoplasmic membrane [26]. Ammi and Ferreira [27] discussed how to remove the micropipette once the deposition has been made. To increase the likelihood of success, the micropipette should be extracted back along the path of insertion.

2.2.2 Insertion Speed and Force

A significant challenge in enabling haptic feedback in a cell injection training system is the dynamic nature of the micro-scale cells making it difficult to determine necessary cell parameters and behaviour in order to develop suitable systems to simulate the cell injection procedure.
The cell membrane is delicate and can be easily damaged by excessive insertion force and is a critical parameter in survivability of the injected cells. Accurate force must be applied to the cell membrane during penetration. While insufficient force can result in the micropipette failing to puncture the cell wall, an even slightly excessive insertion force can damage the cell membrane. Being able to measure and control the force arising during cell injection can enhance the functionality of cell injection systems [28]. It is also important, that once the cell membrane has been penetrated, the exerted force be rapidly reduced to avoid overshoot which can cause damage to the opposite wall. Pillarisetti et al. [29] described a force feedback interface which reflected the insertion force to the user. The force change before and during insertion were recorded, which includes the sudden loss of membrane reaction force once puncture has occurred.

Aside from insertion force, the speed of insertion is important to successful cell injection. Different cell types have different physical properties and it is important that the micropipette is inserted and withdrawn at a speed appropriate for the particular type of cell. Determination of the appropriate injection speed is normally based on an bio-operator’s observations and experience [30]. After successful deposition of the desired material, the micropipette must be retracted as quickly as possible to minimise damage to the cell during removal [27].
2.3 Overview of Haptic Feedback and Approaches

Due to recent technological advancement VR has been widely utilised for skills training across various fields and applications. In order to develop the VR training system for a particular application, it is essential to consider the important skills to be mastered, such as the skills discussed in previous section for cell injection. Aside from that it is also important to employ appropriate feedback and approaches for a particular system. Integration of haptic technology in VR systems has become prevalent among developers nowadays. Haptic feedback can facilitate the user’s sense of touch and feel in virtual training and this section discusses different types of haptic feedback and devices relevant to virtual training.

2.3.1 Haptic Feedback

Haptic feedback refers to the display of information through the human’s haptic modality and can include force and tactile interaction. Force feedback provides kinaesthetic information, while tactile feedback systems emulate cutaneous sensations. Force and tactile feedback and their application to different manipulation tasks are discussed in the following subsections.

2.3.1.1 Force Feedback

Force feedback stimulates the human’s kinaesthetic system, which perceives sensations originating in muscles, tendons and joints. In the medical domain, force feedback has been employed in robotic surgery applications enabling the
surgeon to perceive forces such as those exerted by the robot during surgery [31, 32].

2.3.1.2 Tactile Feedback

Tactile feedback interacts with the human’s cutaneous system, which responds to sensations on the skin’s surface. These sensations are generated by mechanoreceptors within the skin and are sensitive to mechanical stimuli [33]. Mechanoreceptors can be categorised by their receptive field size and adaptation rate. Type 1 are small and with well-defined borders, and type 2 have large borders which are not well defined. In terms of adaptation rate, there are slow adapting and fast-adapting. Table 2.1 illustrates the classification of the mechanoreceptors in human hand skin based on receptive field and adaptation rate properties.

Table 2.1: Characteristics of the four types of mechanoreceptors – Adapted from [34]

<table>
<thead>
<tr>
<th>Mechanoreceptor type</th>
<th>Rapidly adapting type 1 (RA)</th>
<th>Slowly adapting type 1 (SA1)</th>
<th>Rapidly adapting type 2 (PC)</th>
<th>Slowly adapting type 2 (SA2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afferent ending</td>
<td>Meissner corpuscle - small receptive field</td>
<td>Merkel cell - small receptive field</td>
<td>Pacinian corpuscle - large receptive field</td>
<td>Ruffini complexes - large receptive field</td>
</tr>
<tr>
<td>Effective stimulus</td>
<td>Skin motion</td>
<td>Texture (edges, points, curvature)</td>
<td>High frequency vibration</td>
<td>Skin stretch</td>
</tr>
<tr>
<td>Frequency range</td>
<td>1-300 Hz</td>
<td>0-100 Hz</td>
<td>5-1000 Hz</td>
<td>0-7 Hz</td>
</tr>
</tbody>
</table>

There are range of simple input devices which utilise tactile feedback which include the 3D mouse [35], touch screen CRT [36], touch pad panel [37], and keyboard [38]. Kuchenbecker et al. are active in this field and their work includes a haptically-enabled oral presentation timing notification system.
2.3.2 Approaches to Haptic Feedback

There are three different approaches normally used for incorporating haptic feedback for performance and training. These are (i) haptic VFs, (ii) record/replay strategy, and (iii) the shared control paradigm [43]. To overcome the passive learning problems associated with the application of these three approaches, progressive haptic guidance has also been proposed by researchers and is discussed in Subsection 2.3.2.4.

2.3.2.1 Haptic VFs

VFs were first introduced by Rosenberg [44-46] as a perceptual overlay used to enhance telepresence and assist the operator in controlling a robot in a remote environment. VFs are passive guides able to assist the operator in following an ideal trajectory or surface, or from moving past a predetermined geometrical area and moving into a prohibited zone [47]. Haptic VFs have demonstrated to enhance performance in path following tasks against metrics such as speed and precision [16, 48, 49]. The benefits of using VFs for guidance in a training environment was investigated by Kuang et al. [50] where participants used a haptic device to hold a virtual object and then bring it to a target location at the end of a maze. The results of the work indicated
promising learning outcomes against time taken and path length for the performed task.

2.3.2.2 Record/replay Strategy

The record/replay strategy is used for training where the interaction with a haptic device by an expert is recorded and subsequently played back to the learner. Using such an approach the learner can feel the ideal motion and conform to it, and then later attempt to perform it without guidance from the haptic device. Yokokohji et al. [51] focused on human-to-human skills transfer systems using haptic and visual representation. Despite having proven to be effective in skills transfer and training, a limitation of the record/replay strategy is that the learner is passive while undergoing training due to the absence of corrective feedback. The work developed a prototype of a WYSIWYF (‘What You See Is What You Feel’) display employing the record/replay strategy. Their experiments involved manipulating and moving a simple virtual cube on a flat table, however were later considered by the authors as not challenging enough to obtain significant results. The paper does however provide useful information and ideas regarding the feasibility of utilising the record/replay strategy for skills training. A later study by Lu et al. [52] applied the record/replay strategy to a virtual tank gunnery skills training system. In one of its two system modes, the expert would move a handle to draw a virtual sine path. The path was recorded and played back using a proportional-derivative (PD) controller. Although this experiment showed that
the PD controller can provide comparable replication of the expert’s movement, the actual effectiveness of the system for skills training and acquisition requires further investigation.

2.3.2.3 Shared Control Paradigm

The shared control paradigm provides automatic intervention to the user’s control of a system [53]. In a training system shared control can provide corrective feedback to the user by dynamically intervening during training. In a series of studies, O’Malley et al. demonstrated that shared control can enhance performance [54] and training outcomes [55] in a dynamic targeting task. They implemented a modified Fitts’ Law [56], which has been widely used for measuring human hand-eye coordination performance, to examine performance and learning improvement when controlling an under-actuated slave system. In the studies, subjects were instructed to manoeuvre the end-effector to hit a fixed pair of targets, alternating on each repetition. Four pairs of targets were used with only one individual target being active at any time. If performed correctly this should typically produce rhythmic movement similar to that of controlling a yo-yo. The results showed that the haptic guidance assisted in improving task performance.

2.3.2.4 Progressive Haptic Guidance

As mentioned earlier progressive haptic guidance has also been proposed by researchers to overcome passive learning associated with the application of the
approaches discussed in the above three subsections. When learning passively, learners are likely to expend less effort on correcting their movements even when making noticeable mistakes, and as a result it is possible that passive visuo-haptic training may achieve efficacy no better than visual training alone [57].

To overcome an over-reliance on error-correction and guidance features, Huegel and O’Malley [58], and Li et al. [59] presented a progressive guidance training system where guidance gradually reduced as the trainee’s performance improved. The works by O’Malley et al. [43] and Li et al. [53] both developed a virtual mass-spring-damper system to study the efficacy of an error-reducing guidance scheme. In the experiments, participants were asked to control an underactuated mass-spring-damper system so as to alternately hit the given pair of targets during 20 – second intervals. The results demonstrated that progressive haptic guidance can improve training of a dynamic task.

The ability to employ progressive haptic guidance, where the level of guidance to the user decreases as the user’s performance improves, and even where the complete removal of guidance once a performance threshold has been reached, has significant potential for a haptically-enabled cell injection training system. Such an approach could allow users to train using the training system and then once adequately trained, move to a real-world system, which may not have haptic guidance to perform cell injection.
2.4 Haptically-enabled Skills Training

Over the past two decades, haptic technology has been considered for enhancing human motor skills training in applications such as weapons handling [60, 61], vehicle manoeuvring [62, 63], sporting [64, 65], medical operation [66, 67], and handwriting and calligraphy [15, 68-73]. This section discusses existing approaches to haptically-enabled skills training and their relevance to a VR cell injection training system.

2.4.1 Haptics in Motor Skills Training

According to Singer [74], ‘motor skill’ refers to “an activity of a person involving a single or a group of movements performed with a high degree of precision and accuracy.” (Singer, 1980). Fitts and Posner [75] suggest that the learning process is sequential and that there are three different phases when learning a new skill:

1. Cognitive phase: identifying and developing the skill components including construction of corresponding mental images.
2. Associative phase: relating the skill components to a refined action including training and reflection to achieve perfection.
3. Autonomous phase: developing an automatic action where minimum awareness or attention required when performing the skill (only certain performers are able to achieve this stage) [75].
Research by Solis et al. [76] demonstrated that haptic feedback can be used to improve learning in the first two phases. In the study, reactive robot control was used to replicate Japanese characters and a Hidden Markov Model based recognition system was used to evaluate users’ stochastic performance. The users’ performance significantly improved when both visual and haptic cues were supplied.

### 2.4.2 Haptics in Medical Skills Training

The use of haptic technology in virtual simulation for medical training has received significant interest over the past 15 years [77]. Coles et al. [78] presents a detailed discussion of the role of haptic technology in virtual medical training applications. Surgical training is an important area benefiting from the application of haptic technology.

Most medical procedures require fine motor skills such as precise movement (mainly focused on the coordination of wrist, hands and fingers), and control of applied forces. These skills are similar to those required to perform cell injection. There are also similarities in the types of tools used in both procedures such as the injector (syringe and pipette), and the grasper and holder.

Researchers have presented various studies employing haptically-enabled medical skills training systems for procedures such as manual surgery [79-83], telerobotic surgery [84, 85] and dentistry [86], as presented in Table
2.2. These studies provide valuable knowledge and insight related to the
development of a haptic cell injection training system.

Table 2.2: Utilisation of haptic technology in medical skill training

<table>
<thead>
<tr>
<th>Application area</th>
<th>Application</th>
<th>Training focus</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>General surgical procedures, e.g. stapedotomy and cochleostomy</td>
<td>Virtual drilling simulation</td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>Eye cataract surgery, i.e. replace a clouded lens with an artificial lens</td>
<td>Cataract surgery simulation</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>Cardiac muscle palpation for cardiac surgeon</td>
<td>Training system consists of virtual beating heart and haptic device</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td>Virtual surgery</td>
<td>Virtual surgical system consists of virtual scalpel</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td>Wrist arthroscopic surgery</td>
<td>Computer-based training simulation</td>
<td>[83]</td>
</tr>
<tr>
<td>Telerobotics</td>
<td>Telerobotic surgical training</td>
<td>Surgical simulation using THUMP console</td>
<td>[84]</td>
</tr>
<tr>
<td>surgery</td>
<td>Telerobotic spine surgery</td>
<td>VR simulation system</td>
<td>[85]</td>
</tr>
<tr>
<td>Dentistry</td>
<td>Common dental procedures, e.g. drilling, caries removal and cavity preparation</td>
<td>VR simulation system with master-slave control structure</td>
<td>[86]</td>
</tr>
</tbody>
</table>

2.4.3 Haptics in Micro-manipulation Training

Cell injection can be considered as micro-domain manipulation due to the small value of the parameters (sizes, forces, etc.) involved in the procedure. There are both similarities and differences to macro domain manipulation which should be considered in developing an effective micro-manipulation system [87]. Amongst these considerations are the fabrication and actuation of the micro-mechanism so as to ensure mechanical performance, as well as the effects of operating at the micro-scale where forces such as electrostatic and Van der Waals become significant. Also, the three-dimensional integration
between micro-size parts can make the development of 3D micro-manipulation systems difficult [88].

Given the challenges inherent to using current micro-manipulation systems, realisation of a VR training system offers significant promise. Moreover, by employing haptics, these VR training systems can provide the user with additional information and physical guidance such as through VFs, record and replay, and shared control. This subsection reviews some of the related work.

In the field of rational drug design, Sourina et al. [89] proposed a haptically-enabled virtual biomolecular docking system for studying helix-helix interactions. It was proposed that the system can be used for e-learning in subjects such as physics and chemistry. Haptics for the docking process was also considered by Persson et al. [90] where a Chemical Force Feedback system was developed and experimental validation with twenty three Biological Chemistry and Biotechnological Engineering undergraduate students demonstrated that it assisted students in understanding important information related to ligand-enzyme interaction.

Marchi et al. [91] evaluated an educational haptic system for studying nano-scale physical phenomenon (approach-retract phenomenon). Forty postgraduate Physics students took part in the experiments and the results showed improvement in students’ understanding and skills acquisition. Jones et al. [92] investigated the use of a haptically-enabled web-based learning tool for
improving students’ knowledge regarding viruses, microscopy, and nanometer scale. Based on assessment of fifty high school students, it was demonstrated that comprehension of micro-scale, virus morphology and dimensionality had improved as a result of using the web-based tool.

Given the demand for optical fibre by the industrial sector, Luo and Xiao [93] developed haptically-enabled VR models for micro/nano optical fibre assembly tasks. Based on the experimental results, it was argued that the developed models and simulation could be valuable for micro/nano-scale skills training and automated assembly designs.

In the area of cellular studies, Minogue et al. [94] explored the advantages of haptic feedback augmentation for a VR program for middle-school Science instruction. Eighty students participated in experiments which examined the cognitive and affective impact of haptic technology on students’ knowledge of an animal cell’s structure and functions. The paper presents important theoretical and practical fundamentals to be considered in the development and implementation of haptically-enabled instructional VR programs in terms of the system’s impact on students.

### 2.5 Haptics in Cell Injection

When performing cell injection, the human bio-operator mostly depends on visual information via a microscope which is prone to errors such as slippage, overshoot, hand tremor and excessive contact force which can easily damage the cell or micropipette. This section considers studies which propose haptic
technology for cell injection. Haptics can be applied for real-time cell injection assistance, such as the haptic display of cell injection forces, or for training using a virtual environment, using for example cell models for providing a virtual representation of a cell to be injected. Results of user evaluations carried out to investigate the performance improvement of utilising haptic technology in existing haptically-enabled cell injection setups are presented in Subsection 2.5.5.

Figure 2.2: Techniques proposed for cell injection

Common techniques for employing haptics for cell injection are by using physical polyvinylidene fluoride (PVDF) force sensors, image analysis, cell biomechanical models and VFs - as described in the subsections below. These techniques have been utilised to aid bio-operators both in real-time operation and offline cell injection training and simulation. Force sensors and image analysis are mainly applied to real-time applications whereas cell biomechanical models can be used as the basis for real-time estimation of cell injection forces or for representing virtual cells in virtual training and
simulation. Like cell biomechanical models, VFs have been employed in both real-time applications and virtual training.

2.5.1 Force Sensors

PVDF sensors can be used to measure forces during real-time cell injection procedure which can then be displayed to the bio-operator so as to assist them during the procedure. Additionally the data recorded from the sensor during injections could also be useful to formulate realistic cell biomechanical models. Studies by Cho and Sim [95] and Kim et al. [96] were amongst the earliest work using haptic augmentation for cell injection. Both studies introduced systems aimed at overcoming the problems associated with the conventional cell injection procedure. Micro end-effectors were developed by attaching a PVDF sensor to the micropipette tip enabling measurement of contact and penetration forces. This force information was then displayed by a haptic device enabling the bio-operator to feel the injection force. The papers demonstrated the capability of the systems to measure injection force with high signal-to-noise ratio, stability, linearity and repeatability. The usability and practicality of the system however requires further research. Pillarisetti et al. [29] then proposed the integration of visual and haptic feedback in a semi-automatic cell injection system. The work successfully implemented and calibrated a force sensor and integrated a force feedback interface to display forces to user during the cell penetration process. Experiments performed on two types of egg cells, salmon and flying fish, demonstrated that the user
could easily feel the puncture of the cell membrane based on the sudden drop of force felt through the haptic device. This system relies on access to a suitable force sensor, and the authors suggest that the system may not be able to be generalised to cells smaller than 50μm. This is a significant limitation considering that plant and animal cells can have a diameter as small as 1μm [22]. In their later work, Pillarisetti et al. [13] developed a cell injection system with visual and force feedback able to measure force within the μN range. Evaluation involving forty novice subjects performing injection of trepan blue dye into zebrafish egg cells demonstrated that providing both types of feedback simultaneously can lead to higher injection success rates (see Subsection 2.5.5). However a comparative study of the subjects’ performance against other parameters such as trajectory and accuracy would prove a useful benchmark for the feasibility of haptic technology for the procedure.

2.5.2 Image Analysis

To aid bio-operators during physical cell injection, in 2005 Ammi and Ferreira [27] developed a user interface providing a combination of visual and haptic feedback. Rather than a physical PVDF force sensor, a vision-based biomembrane pseudo-force technique which estimates the applied force was used. Based on the force information, a VF in the form of a cone-shaped attractive haptic force was used to assist the bio-operator. Aside from the complexity and lacking commercial availability of PVDF cell force sensing, the vision-based approach enables estimation of forces in different areas of the
cell in contrast to using a PVDF sensor attached to a micropipette which can only measure at the single point of contact. The paper however does not evaluate the impact of providing the haptic information to bio-operator performance. Later work by Ammi et al. [97] presents a 3D pseudo-haptic rendering system through integration of visual tracking data of cell deformation and a mass-spring-damper model to estimate interaction forces. The paper also demonstrated convincing experimental results showing the practical efficacy of the multimodal system (see Subsection 2.5.5).

**2.5.3 Cell Biomechanical Models**

The modelling of cell’s biomechanical properties is challenging. While the works discussed in Section 2.6 later in this chapter present underlying concepts and approaches to cell biomechanical modelling, this subsection focuses on cell biomechanical models developed specifically for haptically-enabled cell injection systems. Several works discussed in this subsection present the application of cell biomechanical models discussed in Section 2.6 for virtual micro-robotic cell injection environments. The dynamic modelling of cytoplasm and cytoskeletons using the finite element method (FEM) with a mass-tensor model and viscoelastic Kelvin–Voigt elements was proposed by Ladjal et al. [98, 99]. The model was used to simulate cell deformation during the perforation process. Both studies aimed mainly at developing a virtual environment with a visual and haptic interface to assist in training and simulation of the cell injection procedure. Their later work [100] described the
development of a computer-based training system for simulating ICSI in a virtual environment. The haptic and visual feedback elements of the system make it applicable to bio-manipulation training.

To reduce training and maintenance costs, Horan et al. [101] presented an offline training system by developing a virtual replication of their haptic cell injection system. The training system has the ability to augment the virtual environment with training aids and other information. In order to virtually represent the cell deformation and penetration force in a realistic manner, the particle-based cell model introduced by Asgari et al. [102] was implemented. The work by Horan et al. also discusses two different approaches to the development of the virtual training environment. Firstly, the virtual environment was developed using Webots simulation software [103], however satisfactory real-time interaction with the virtual cell could not be achieved due to the update rate of the software. Secondly, a virtual training environment was developed using C++ and DirectX and demonstrated improved graphics and cell rendering. The preliminary work may contribute to future work into more comprehensive studies development of haptically-enabled cell injection training systems.

2.5.4 Virtual Fixtures (VFs)

An alternative to existing autonomous and semi-autonomous cell injection systems is presented by Ghanbari et al. [21, 104] where haptic devices are used to intuitively command [105] and control [106] a micro-robot. The papers
describe a micro-robotic system which guides the bio-operator during the cell injection procedure using haptic VFs. The system guides the bio-operator to appropriately manoeuvre the micropipette towards the cell membrane for penetration and then after penetrating the cell membrane, to terminate the micropipette’s movement at a deposition target location inside the cytoplasm. The papers utilise cone and paraboloid-shaped force-field haptic VFs. These VFs provide haptic forces to bio-operator’s hand as guidance so to move the micropipette tip along a desirable trajectory to an appropriate penetration point at the cell membrane. Apart from the guidance VFs, the papers also introduce a planar forbidden region VF [47] to stop the bio-operator from commanding the micropipette tip beyond the deposition target location within the cell.

Ghanbari et al.’s work [5] is perhaps one of the latest published papers concerning the implementation of haptic technology for cell injection. One of the main contributions is the realisation of a haptically-enabled micro-robotic system for assisting bio-operators in performing real-time cell injection. In order to guide the bio-operator to the appropriate penetration point, the same VFs concept as introduced in their earlier works [21, 104] was utilised. A new neiloid-shaped force-field VF was introduced and the three volumetric (neiloid, cone and parabolic) VFs were then compared against each other in order to evaluate their performance in terms of success rate and completion time. Another significant contribution of the paper is the implementation of a virtual training environment to replicate the haptically guided cell injection
system. This allows the bio-operator to perform offline training and later on apply their acquired skills to the actual cell injection system. To better utilise this innovation, detailed studies are required to analyse the efficacy of the approach in terms of skills acquisition and motor learning.

2.5.5 User Evaluations of Haptics for Micro-robotic Cell Injection

The works discussed thus far in this section have considered the technical aspects of employing haptics for micro-robotic cell injection. Included amongst them, are two studies which have undertaken user evaluation in order to investigate the performance improvement when haptic feedback is provided. Both studies observed better performance when haptic feedback was provided, as shown in Table 2.3.

Table 2.3: Performance results for haptically-enabled micro-robotic cell injection

<table>
<thead>
<tr>
<th>Haptic Technique(s) Utilised</th>
<th>No. of Subjects</th>
<th>Results</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force sensor for measurement and display of cell injection forces</td>
<td>Forty novice participants</td>
<td>Higher success rate with haptic feedback No significant improvement of completion time</td>
<td>[13]</td>
</tr>
<tr>
<td>Image analysis and cell biomechanical model for interaction force estimation and VFs for haptic guidance</td>
<td>Thirteen participants (experts, students and technicians)</td>
<td>More stable micropipette motion with haptic feedback Lower execution time with haptic feedback Higher participant appreciation with haptic feedback</td>
<td>[97]</td>
</tr>
</tbody>
</table>

The study by Pillarisetti et al. [13] (discussed in Subsection 2.5.1) found that 30 of 40 participants’ achieved higher success rates when haptic feedback was provided compared with that of visual feedback alone. In the experiments participants were asked to perform five trials with visual feedback only and then five trials with combined visual and haptic feedback. Freshly harvested
zebrafish eggs with diameter range between 600 – 700μm and trepan blue dye were used for injection. Two scenarios were considered in the experiments: transparent and non-transparent (simulated by prohibiting participants from seeing the material deposition process) cells. For the non-transparent cell injection trials, the results showed that participants achieved an average success rate of 37% subject to visual feedback only and 81% for visual and haptic feedback combined. For the transparent cell injection trials the average success for using the visual feedback only was 75% and 89% for the visual and haptic feedback combined.

In the work by Ammi et al. [97] (discussed in Subsection 2.5.2), thirteen participants from different backgrounds (experts, students and technicians) were evaluated. Execution time and participants’ appreciation were among the parameters considered for two different scenarios. In the first scenario participants were asked to perform cell injection with and without haptic feedback. The results showed that all participants improved their execution time when haptic feedback was provided. Twelve of the thirteen participants rated higher appreciation for the haptic feedback. The second experiment scenario was designed to consider the rectilinear VF proposed in addition to the haptic feedback. The results demonstrated significant improvement in execution time when the VF was provided for both with and without haptic feedback, compared to when only haptic feedback was provided. The
appreciation rates were at their highest when both haptic feedback and VF provided simultaneously.

2.6 Cell Modelling Approaches

Accurate biomechanical cell models are required for a virtual cell injection training environment. The approaches to cell modelling discussed in this section provide some insight into modelling techniques that may be utilised to represent the cell interactions within a virtual cell injection training system.

As depicted by Figure 2.3, most of the models developed for representing the biomechanical properties of living cells can be classified into three main approaches: (i) continuum; (ii) energetic; and (iii) micro/nanostructural.

![Figure 2.3: An overview of approaches for the mechanical modelling of living cells – Adapted from [99, 107]](image_url)
A detailed review of the continuum approach can be found in the work by Lim et al. [107]. Using such an approach, cells are considered as continuum materials with fluidic, elastic, viscoelastic or solid properties. As shown by Figure 2.3 there are different modelling techniques which can be classified as continuum approaches.

The Newtonian liquid drop model considers a cell as a uniform liquid core encapsulated by a cortical shell [108]. The technique has been applied and investigated in a variety of works to achieve various research aims [109-112]. Some researchers have discussed the non-homogeneous characteristic of the cell’s inner region and that the nucleus has greater stiffness and viscosity than the surrounding cytoplasm [113-116]. For this reason, the compound Newtonian liquid drop model employs a more complex structure using three major cell layers [113, 117, 118]. Each of the three layers; the plasma membrane, cytoplasm and core, have their own mechanical properties. The outermost layer is the plasma membrane with an approximate thickness of 0.1$\mu$m [119] and under constant tension. The cytoplasm is the middle layer and has the smallest viscosity value. The innermost layer, the core layer, represents the nucleus of the cell and surrounds the cytoskeleton. It has higher viscosity but smaller volume than the middle/cytoplasm layer. The compound Newtonian liquid drop model also considers some other additional parameters and according to Lim et al. [107] is more proficient in modelling the actual cell and representing some of the non-linear events which cannot be achieved.
by using the homogeneous model. As opposed to both of the earlier discussed Newtonian models, the data from Tsai et al.’s [110] experiment demonstrated non-Newtonian behaviour of the neutrophil cytoplasm. To characterise this non-Newtonian behaviour of the cytoplasm the mean shear rate was estimated based on numerical simulation. The paper describes the shear thinning behaviour of the neutrophil cytoplasm and suggests that the power-law fluid model is a more suitable model. Using the shear thinning liquid model the cell cytoplasm is modelled as a power-law liquid surrounded by a cortex with constant tension and offers benefits for representing the large deformation of the human neutrophils. While the shear thinning liquid model can effectively represent large cell deformation, there is also the need for a model which considers the small deformations likely to occur during the initial phase of micropipette aspiration. The Maxwell liquid drop model was employed to examine the deformations of partially aspirated cells into a small micropipette, as well as the recovery of cells after the expulsion from full aspiration into a large micropipette [120]. It was observed that the model is able to replicate the experiment results of using a micropipette for rapid small deformation during the initial entry and gradual recovery after undergoing a large deformation.

In addition to liquid-like cell models, some researchers have also considered the solid behaviour of certain types of cells such as chondrocytes and endothelial [121, 122]. Using solid models the entire cell is assumed to be homogeneous and unlike the cortical shell-liquid core models, there are no
multiple layers such as the cortical and cytoplasm layers. Elastic solid models, which are fundamental to the below discussed viscoelastic solid model, are constructed based on experiments on cells using micropipette aspiration [123], cell poker [124], magnetic twisting cytometry [125] and atomic force microscopy [126]. The viscoelastic solid model was first proposed for evaluating the rheology and mechanical properties of leukocytes in micropipette aspiration experiments [127]. It was determined that the solid viscoelastic model is capable of precisely characterising the small strain deformation of the leukocytes.

Later research by Fabry et al. [128] inferred that the cytoskeleton undergoes a sol-gel transition, where it exists in the form of liquid and then a solid in the sol and gel phases respectively. Based on experimental data, the authors identified that rather than behaving as the assumed gel, the investigated cells exhibit soft glassy material (SGM) behaviour, existing close to a glass transition. As such the power-law structural damping model was proposed because the viscoelastic models are not suited to represent the rheology of SGMs.

Single-phase models such as those discussed above often fail to represent certain essential cell behaviours such as volume variation as the result of mechanical or osmotic loading, mechanical foundation due to viscoelasticity, and the association of mechanical, electrical and chemical attributes inside the cell [129]. Multiphasic (biphasic and triphasic [130])
models were designed to take into consideration the relationship between solid, fluid and, in certain instances ionic phases, of cells. The fundamental basis of biphasic theory is that articular cartilage and chondrocytes are treated as a mixture of fluid and solid [131, 132].

Unlike continuum approaches which consider cells to contain certain continuum material properties, energetic approaches consider the contribution of the cell structure to the energy budget of a cell. Energetic approaches are based on percolation theory and polymer physics models [133, 134] and provide the advantage of being independent of the coordinate system selection and cytoskeleton structure [99]. However the approach has the drawback that it requires large cell deformation and disregards the extracellular matrix attributes of cell mechanics [102].

The third category of cell models considered are micro/nanostructural approaches which focus on the inner molecular structure of cells as the key factor for determining biomechanical properties. One such model is the spectrin-network model developed for examining the role of the spectrin-network and cell membrane in large deformation of red blood cells. This model assumes that the mechanical properties of the cell membrane are influenced by the intrinsic elasticity and topology of spectrin within the skeleton [135]. The basis for the approach was introduced by Discher et al. [136-138] who discussed a spectrin-based model which exists in between continuum and atomic scales. While the spectrin-network model was
developed for suspended cells, it remains unclear as to whether it is suitable for modelling adherent cells.

The tensed cable network model on the other hand was proposed to enable prediction of adherent cells’ mechanical response. In a study by Coughlin and Stamenović [139], actin cytoskeletons of adherent cells were modelled as a network of pre-stressed elastic cables. Simulations of adherent cell deformation were performed to emulate the measurement techniques of cell poking, magnetic twisting cytometry and magnetic bead microrheometry. While the model was not able to fully represent cell response for magnetic twisting cytometry and magnetic bead microrheometry, the simulations demonstrated that filament tension is a key determinant of the response of the model.

Another microstructural modelling approach is the tensegrity model introduced by Ingber and Jamieson [140, 141]. The approach is based on the tensegrity architecture introduced earlier by Fuller [142] and represents an adherent cell by a network of pre-stressed cables connected to sets of rigid struts. The pre-stressed cables represent the microfilaments and intermediate filaments while the rigid (compression-resistant) struts represent the microtubules of the cytoskeleton. The premise of this model is that the cytoskeletal mesh holds initial stress (pre-stress) before the application of any external loading, and the pre-stress is balanced by the compression of the microtubule (strut) and extracellular matrix adhesion [143].
In order to replicate the mechanics of endothelial cells, an open-cell foam model [144] was developed by applying the theory of foam [145]. The authors observed that the endothelial cell cytoplasm is filled by a network of cross-linked F-actin - distributed cytoplasmic structural actin (DCSA). The developed model considers DCSA as having a foam-like microscopic structure. Based on computation and comparison with experimental data, it was determined that implementation of the theory of foam is useful for modelling the DCSA network to determine endothelial cell mechanical properties.

It is apparent that a cell’s biomechanical properties vary according to the cell’s type. As such, different techniques are required for modelling different cell types. The models presented in this section, with modification as required, can be used to represent the biomechanical properties of cells within a virtual environment. These properties such as viscosity, elasticity, etc. can then be displayed to the user by the haptic device.

2.7 Haptic System Evaluations

The use of haptic technology, especially in skills training, requires a combination haptic hardware with other computer-based technologies which may provide different sensory information, e.g. visual and sound. The system as a whole, which is the combination of these technologies, can provide a VR environment. As such another topic worth discussing is the application of VR in providing skills training. Before being able to develop an effective virtual
training system, thorough investigation of the components involved for specific skills training should be made in order to achieve the desired results of using such a system. For example, it is important to take into account components such as hand trajectory, speed of movement, location accuracy and applied force in performing successful cell injection. However, even if the system has been carefully designed to consider all of the necessary skills, the efficacy of the system as a tool for assisting users in mastering the skills requires extensive evaluation. Realism of the VR, long-term skills acquisition and retention, and transferability of skills all need to be considered.

According to Samur et al. [146], there are two commonly employed evaluation methods for haptically-enabled VR systems, the haptic interface performance and the user perceived haptic feedback. The first method is generally performed using algorithm validation and comparison based on the rendering realism [147], while the second, involves evaluation of psychophysical factors to measure feedback perceived by users [148]. Various human factor analyses have evaluated the haptic systems performance in sensory-motor control tasks, in terms of both the haptic interface and the feedback perceived by users [146, 149, 150]. Apart from these, there are also several works which propose different methodologies for haptic system evaluation. Among the recent work in this area has been of the work by Jia et al. [151-154] which introduced a Multidimensional User-centred Systematic Training evaluation (MUSTe) method for haptically-enabled VR training.
systems. The method was designed to overcome the limitations such as the reliability of the expert-based evaluation methods inherent in the previous user-centred evaluation method [155]. The work provides a significant contribution towards a better understanding of the important aspects in VR training system efficiency and their influence on the end results of virtual training.

2.8 Conclusions

Studies have shown that haptic feedback can assist in the training of a wide range of motor skill tasks. In order to be of practical use for micro-robotic cell injection training, a thorough investigation of the specific skills such as the ability to execute accurate trajectories, speeds, and forces is presented in this chapter. Determining these required skills needs to be in the context of a pragmatic system design otherwise it may not feasible for real-world training operations. These important skills are considered in Chapter 3 where the design of the VR micro-robotic cell injection platforms is presented. Problems associated with skills acquisition and motor learning curves should be considered as presented in Chapters Chapter 4 to Chapter 6 of this thesis.

In order to develop an effective haptically-enabled virtual training system for micro-robotic cell injection, careful consideration was made to investigate suitable commercially available hardware and devices in the context of the skills to be trained, or to some extent to upgrade, modify or develop new hardware. Given the acquisition of the appropriate haptic
hardware, the techniques and methodologies for displaying haptic information were also discussed.

A portable cell injection training system may prove more useful in terms of access and time constraints. Therefore, utilising portable platforms such as the reconfigurable multipurpose haptic interface [156] is beneficial. The interface can provide several kinematic configurations (as discussed in Chapter 3). To optimise this capability further investigation on the integration of the interface with compatible applications is recommended. Previous work such as the virtual cell injection training environment [157] and the 5-DOF haptic stylus [158] are among applications which have already utilised the interface.

Additionally, utilising a large workspace interface can provide more benefits with the premise that the large-scale interaction may assist the user to better understand the spatial relationship. As such in Chapter 3 a large-scale VR micro-robotic cell injection training system which utilises a 6-DOF large workspace haptic device, the INCA 6D based on Spidar™ technology, is introduced. Aside from enhancing users’ spatial and orientation understanding through intuitive handling, the large workspace haptic device also utilises users’ gross motor skill which can provide several benefits such as insensitive to insignificant movements such as vibration and tremors.

As discussed earlier, there are three main approaches to incorporating haptic feedback to the user in terms of training and performance enhancement,
i.e. VFIs, record/replay and shared control. In developing a system specifically for micro-robotic cell injection training, the applicability of each of these approaches was considered in order to determine the suitable approach for displaying haptic information to trainees.

Despite the level of sophistication of a micro-robotic cell injection training system, its level of effectiveness in supporting skills acquisition and performance improvement is the most important characteristic pertaining to the usefulness of the system. There are methods developed for evaluating haptic systems by considering both the user-centred evaluation processes and outcomes as the one proposed by Jia et al. [159]. However there are no specific evaluation methods thus far developed for VR micro-robotic cell injection such as utilised in this thesis, so that the skills acquired through use of the haptically-enabled system can be evaluated.

Overall, the works considered in this chapter suggest that it is feasible to use haptic feedback for a haptically-enabled VR micro-robotic cell injection training system. The integration of haptic technology to the virtual training system can provide extra guidance and realism to the system, thereby leading to better training outcomes. However, relevant problems have been identified and discussed and need to be mitigated when developing a haptically-enabled training system for cell injection. In the next chapter the design of a VR micro-robotic cell injection training systems is discussed considering their specific requirements such as the interface, input control methods and displays.
VR system platforms are introduced, the desktop-sized and large-scale VR training system for micro-robotic cell injection. Different technologies and methodologies are utilised in each platform mainly aimed at assisting users to enhance important skills such as trajectory, accuracy and injection force.
Chapter 3  VR Haptic Research Platforms

Based on the extensive review of the related work presented in Chapter 2 it is suggested that the utilisation of VR and haptic technology is a feasible approach for providing effective motor skills training in various domains. As such this chapter discusses the development of the platforms employed in this research which were specifically designed for evaluation of haptic and VR micro-robotic cell injection skill training. An introduction to a reconfigurable multipurpose haptic interface as utilised in this thesis is presented in Section 3.1. The developed interface features a portable and low-cost setup which was utilised with the desktop VR micro-robotic cell injection training system which discussed in Section 3.2. In the section, the design of the VR training system based on waterfall software development model is discussed so as to provide details of the principal and concept of the system. Finally the design and development of a large-scale micro-robotic cell injection VR training platform is discussed in Section 3.3. The large-scale VR training platform provides a selection of three large display configurations: 2D, 3D and CAVE-like to achieve a more immersive VR training environment.
3.1 Introduction

In this study the earlier developed reconfigurable multipurpose haptic interface [156] was utilised in order to achieve a portable interface for the VR training system. The work introduced a low-cost haptic interface providing four different kinematic configurations. The different configurations were achieved using two Phantom Omni [160] haptic devices combined with a series of clip-on attachments. Aside from the flexibility to easily reconfigure the interface, three of the four configurations provide functionality which is either not readily available or is cost prohibitive for many applications.

The interface was achieved using two Phantom Omni haptic devices, a linear-rotary stage, and a series of low-cost attachments. The two Phantom Omni haptic devices can be linked to each other by a set of special clip-on attachments to provide a 5-DOF control and feedback. Generally the roll of the micropipette is the least important axis to consider in a cell injection procedure since it is not usually necessary to adjust it during an injection. As such being able to provide a 5-DOF interface can be a prospect for an intuitive control of the micro-robotic cell injection procedure. In order to reduce the cost and complexity of the interface, a feasibly minimum number of Phantom Omni haptic devices, i.e. two, to achieve a 5-DOF interface is suggested. To be able to connect the clip-on attachments, the Phantom Omni haptic devices require minor modification to remove the stylus jack. Changing between configurations is simply a matter of disconnecting the magnetic clip-on
attachments and then reconnecting the required ones. Depending on the configuration it may also be necessary to slide and/or rotate the Phantom Omni haptic devices.

Figure 3.1: The four possible configurations for the reconfigurable multipurpose haptic interface
From top to bottom: Two 3-DOF Phantom Omnis, Dual-point gripper, 5-DOF wand, and 5-DOF stylus

To support portability and to reduce the amount of external ancillary hardware, a computer and power supply were installed within the base of the
system. Therefore, to use the system, only a display, keyboard and mouse need to be connected. Figure 3.1 shows the four different configurations.

The first is the two Phantom Omni haptic devices, able to be rotated and moved using the linear-rotary stage, suitable for bimanual 3-DOF haptic interaction. The second is the refined version of a haptic gripper [161] providing independent 3-DOF Cartesian forces to each finger. A 5-DOF wand configuration is then shown and was inspired by a similar approach using two Novint Falcon devices [162]. The final configuration is the 5-DOF stylus [158] which uses a similar approach to the pen-based haptic virtual environment [163]. The 5-DOF stylus can be used in applications where haptic feedback about the stylus’ longitudinal axis is not required.

### 3.2 Desktop-sized VR Micro-robotic Cell Injection Training Environment

This section discusses the design of a VR training system for micro-robotic cell injection. An overview of the system development based on waterfall model is first presented. This is followed by discussion on the skills required by the bio-operator to achieve successful injection, such as accuracy, trajectory and applied force. The design of the VR system which includes details of the visual display, input control methods, mapping strategies, haptic guidance and output data is then presented. Initial evaluation of the VR system is then presented which includes analysis and discussion based on conducted user evaluations.
3.2.1 System Development

A low-cost, portable and flexible VR micro-robotic cell injection training system [164] is proposed in this section. The training system employed haptic interaction to provide force-based guidance and learning assistance according to different metrics. To develop the VR training system, the waterfall model (derived from [165]) was utilised. The model is a conventional software development model which has five sequential stages where each stage must be completed before the next stage can be executed as depicted by Figure 3.2. The literature suggests that the waterfall model is best suited for small project development where the requirements are explicitly recognised [166, 167]. This is the case for the development of the VR micro-robotic cell injection training system where requirements are clearly defined so as to assist the bio-operator in improving their cell injection skills. These skill requirements are a function of performance against identified metrics such as injection trajectory, force and accuracy. This section presents the development of the VR system focusing on the first four stages of the waterfall software development model, i.e. requirement, design, implementation and verification.

![Figure 3.2: Waterfall software development model](image)
3.2.2 Requirements Analysis

As discussed in Chapter 2, injection trajectory, accuracy and force are important parameters which relate to the success of an injection [3, 8]. The skills necessary to be able to accurately control these parameters need to be mastered by the bio-operator in order to become proficient at the task and the developed VR micro-robotic cell injection training environment was designed to enhance these skills.

In order to obtain useful information about the real micro-robotic cell injection procedure, an expert in the area, Dr Mulyoto Pangestu who is a lecturer within the Department of Obstetrics and Gynaecology, Monash University, Australia, were consulted. Among the inputs gathered from Dr Pangestu were related to the challenges in performing and training of the procedure, important skills required to become proficient and the correct technique for a successful injection. This information is discussed in several necessary locations throughout this thesis including in this chapter. Dr Pangestu also has been actively involved as one of the co-authors in publications related to this research.

3.2.2.1 Trajectory

The ability for bio-operators to be able to execute a precise trajectory is important. To perform cell injection, the micropipette’s tip needs to be manoeuvred to an appropriate penetration point on the cell membrane. Moving
the micropipette along an optimised trajectory, from the starting location to the penetration point, will improve the chance of success and may reduce the time taken for completion. After piercing the cell membrane the micropipette needs to be moved in a straight line path along the longitudinal axis of the micropipette within the cytoplasm towards the suitable deposition point (e.g. the nucleus) [5]. This is because movement deviating from this path after piercing the cell membrane will cause slicing of the cell.

3.2.2.2 Accuracy

The penetration point needs to be accurately determined when performing the cell injection procedure. An inappropriate penetration point, e.g. too high or too low from the cell centre, can generate torque causing the cell to rotate compromising the penetration attempt [26]. It is very important to make sure that the micropipette’s tip accurately stops at a suitable deposition point inside the cell, e.g. the nucleus which commonly located at the centre of a cell and carries important information such as DNA [6]. Given the relatively small size of the cell, achieving acceptable injection accuracy can be extremely challenging. To achieve an ideal injection in real procedures, the micropipette tip has to be positioned at the centre of the cell before performing material deposition. As such it is suggested that the distance between micropipette tip stop point and the centre of the cell is considered as a measure of users’ performance for accuracy.
3.2.2.3 Injection Force

It is important for the bio-operator to be able to control the force exerted by the micropipette when penetrating the cell membrane. Even a slightly excessive force may damage the cell membrane, while insufficient force may not allow the micropipette to penetrate the cell membrane [28]. It is also important to stop the motion of the micropipette at a suitable deposition point inside the cell to prevent overshoot which can damage the opposite cell membrane or even the injection equipment.

3.2.3 VR Training System Design

The VR micro-robotic cell injection training system was designed to cater to two distinct requirements, being the provision of an appropriate level of immersion and the ability to provide haptic guidance. An overarching objective was to achieve low-cost implementation and portable operation. The system’s development considered five main elements; visual display, input controllers, mapping strategies, haptic guidance and output data.
3.2.4 System Implementation

The system was implemented based on the design presented in Subsection 3.2.3 which comprised of five main elements; visual display, input controllers, mapping strategies, haptic guidance and output data. This subsection discusses the implementation stage of the system according to the waterfall software development model.

3.2.4.1 Visual Display

The VR environment utilised was developed in C++ and using the OpenHaptics® toolkit designed for the Phantom® range of haptic devices, and Direct3D and OpenCV for graphics programming. The virtual environment displays a virtual cell injection setup consisting of a cell to be injected and bio-manipulation equipment including a microscope, micromanipulator, injection micropipette, holding micropipette and cell holding dish. The setup is placed...
on top of a rectangular table in the virtual environment. To improve the visualisation, a three-dimensional view of the environment is presented to the bio-operator with the option to zoom in to focus on areas of interest.

The virtual micromanipulator replicates the actual MP285 micromanipulator by Sutter Instruments. The MP285 provides three motorised degrees of freedom (DOF) motion typically controlled using rotary optical encoders or a joystick. It can be manipulated in three axes \((x, y, z)\) and can also provide an artificial fourth axis to achieve diagonal movement from the combination of any two of the three axes. One of the rotary encoder’s dials needs to be used to control the movement of the micromanipulator along the fourth axis.

The cell in the virtual environment is displayed as a sphere that deforms in response to contact made with the micropipette. In order to achieve the replication of the cell, a suitable cell bio-mechanical model was employed. Aside from representing visual deformation, the model also provides an estimate of interaction forces which can be displayed haptically to the bio-operator as discussed in Section 3.2.4.4.

### 3.2.4.2 Input Controllers

Two choices of input controllers can be used with the system which are the keyboard and haptic stylus. These input controllers are used to manipulate the virtual micropipette in three-dimensional space. The first input controller, the keyboard was chosen due to its ubiquitous use in daily computing applications.
In VR systems, the keyboard is commonly used due to its simplicity and low-cost. The evaluation discussed in Chapter 4 suggests that skills obtained from other keyboard applications such as computer use and gaming are transferrable to the operation of the system. As shown in Figure 3.4, the buttons of the numeric/directional keypad were pre-determined to control the virtual micromanipulator.

The second input controller is achieved using the stylus of a Sensable Phantom Omni® (now known as Geomagic® Touch™) haptic device. Aside from being able to provide force feedback to bio-operators, the haptic stylus also allows bio-operator to control the micropipette intuitively in 3D using a similar method to that of handheld needle insertion.

### 3.2.4.3 Mapping Strategies

For keyboard control, the mapping was designed in such a way that the virtual micropipette moves at constant velocity in response to keystrokes. As such, bio-operators can achieve gross and fine control by holding down and tapping the key(s) respectively. The direction of the movement is based on which pre-determined key is pressed by the bio-operator (shown in Figure 3.4). For haptic stylus control, the position-to-position mapping framework [104] was utilised. Using the framework, movement of the haptic device’s haptic interaction point (HIP) is mapped to the virtual micropipette’s tip. The bio-operator can perform the cell injection procedure by controlling the haptic device stylus in order to move the virtual micromanipulator appropriately.
Figure 3.4: Mapping strategy between the input controllers and the tip of the virtual micropipette

3.2.4.4 Haptic Guidance

The haptic guidance provided to the bio-operator is in the form of VFs, comprising conical, axial and planar VFs (see Figure 3.5). The conical VF assists the bio-operator to move the micromanipulator along an optimised trajectory. The large opening of the conical VF allows the micropipette tip to simply enter the guided region, and then guide the user to advance the micropipette’s tip to the penetration point located at the apex. Once the cell is penetrated, the axial VF guides the bio-operator to not deviate from the micropipette’s longitudinal axis. The deposition point is located on the surface of the planar VF attempting to prevent the bio-operator from overshooting the target location.
Since the maximum exertable force of the Phantom Omni haptic device is low enough to be overridden by the bio-operator, the VFs only provide guidance and ultimate control of the movement is retained by the bio-operator. The haptic guidance and visual overlay of the VFs can be enabled/disabled as required by the bio-operator. In addition to the VFs, the bio-operator is also provided with a simulated injection force during penetration which includes the sudden force drop immediately after the cell membrane is penetrated. The simulated injection force aims to add more realism to the virtual environment as well as guiding the bio-operator to know when the cell membrane is penetrated so to arrest the momentum of the micropipette. In order to achieve the simulated injection force, a particle-based cell model as also utilised in other work [101] was employed. The cell model is able to estimate cell
interaction forces to be displayed haptically to the bio-operator along with the visual representation of cell deformation.

It is worthwhile to mention that the VF s only serve as an assistance to the user and even with the assistance provided it is still significantly challenging for the user to achieve a perfect trial. Although inverse kinematics can be applied to the control so that a best trial can be achieved in each trial, as discussed in Subsection 2.3.2.4, this may lead to passive learning where the user significantly relies on the guidance therefore less effort made to correct mistakes.

3.2.4.5 Output Data

The system was designed to be able to record the data during evaluation. The position coordinates of the micropipette tip is recorded at sampling rate of $60Hz$. The recorded data is saved in a spreadsheet file able to be accessed and analysed using appropriate computer applications.

The output data used for the evaluation in this thesis are in comma delimited form consisting of four columns. The first column represents the time where each row equals to $1/60 seconds$. Meanwhile the second, third and fourth columns represent the position of the micropipette’s tip in $x, y$ and $z$ axes respectively.
3.2.5 Validation of the Research Platform

According to the waterfall model, verification logically follows system implementation. To verify that the implemented system achieves its requirements a set of user evaluations were conducted. Thirteen participants (nine males and four females) took part in the experiments. They were screened to ensure that they had no prior experience in cell injection activities. As illustrated by Figure 3.6, participants were divided into two groups, Group 1 and 2 which comprised of three and ten participants respectively. Each group underwent different training sequences. Group 1 undertook the experiments exclusively for evaluating the keyboard control method and for this reason was a smaller group. Each experiment comprised three sessions, i.e. pre-training (PRE), training (TRA) and post-training (POS). In PRE and POS sessions, participants were asked to perform ten trials using keyboard control. As presented in Chapter 4, the keyboard control method, aside from providing a simple and cost effective method for micromanipulator control, can be used as a benchmark for evaluating performance progress after training using more sophisticated input controllers such as the haptically-enabled control device. The TRA session was held in between the PRE and POS sessions in which the participants underwent a systematic training programme. In TRA session thirty trials were conducted using the input controller(s) assigned to the group. Group 1 underwent training using only keyboard control for all thirty trials while Group 2 performed ten trials each using passive stylus (stylus with no
haptic feedback), keyboard and haptically-enabled stylus consecutively. The selection of the input controller(s) for each group was in order to consider the performance progress after undergoing two different training regimes.

Data such as time and positions of the virtual micropipette tip were recorded during the experiments. Two parameters were analysed in order to measure performance, i.e. success rate and injection error. In real procedures, the micropipette is required to be moved towards the cell, penetrate the membrane and then stop at an appropriate deposition point inside the cell. The most ideal deposition point is usually at the centre of the cell. An injection is considered successful when the bio-operator managed to penetrate the cell membrane and stop the micropipette tip inside the cell. An ideal injection was considered to be when the micropipette tip was positioned at the centre of the cell to perform deposition. As such the injection accuracy is considered as how close is the micropipette tip stop point to the ideal deposition point. Inversely the injection error is measured by the distance between micropipette tip stop point and the centre of the cell, where smaller distance reflects lesser error and vice versa. Therefore it is worthwhile to note that in this evaluation the injection accuracy was considered as the inverse of the injection error where the smaller the error value, the better the accuracy.
As shown in Figures 3.7 and Figure 3.8, the success rate of Group 1 deteriorated from 93% in PRE to 90% in POS while the error remained unchanged at 0.53 units. Group 2, on the other hand, showed more consistent results where performance improvement was observed against both parameters. The success rate improved from 87% in PRE to 93% in POS and the error declined from 0.89 units to 0.56 units.
Based on the results it is observed that by utilising the haptic stylus as supplementary to keyboard in training resulted in better bio-operator performance. This may be because of the nature of the haptic stylus where its usage can provide more immersive training and enhance bio-operators’ spatial awareness. The success rate drop observed for keyboard training was less significant and may be due to demotivation after excessive performance of the repetitive task. It is worth mentioning that after completion of the verification stage, the system will go through the maintenance stage which involves
troubleshooting such as finding bugs and resolving defects as well as non-corrective maintenance such as upgrades.

3.3 Large-Scale VR Micro-robotic Cell Injection Training Environment

In the previous section a desktop-sized VR system for micro-robotic cell injection training was introduced [157, 168]. One of the aims of the study is to investigate the role of the VR system in providing skill training for micro-robotic cell injection. The VR system presented in the study can be used with two different input control methods, either a computer keyboard or Phantom Omni (now known as Geomagic Touch [160]) haptic device. Apart from low-cost application, the virtual environment also designed for portability where it was suggested to be used with the earlier introduced platform, the reconfigurable multi-purpose haptic interface [156]. The haptic interface can be used with up to two Phantom Omni haptic devices for various types of applications. It also has a computer and power supply attached inside the base compartment to support portability.

The setup discussed in this section is extended beyond the earlier work discussed in the previous section where different scale of display configurations and interface were utilised. Instead of using desktop monitor as in the previous version of VR micro-robotic cell injection training setup, this section introduces a large-scale display achieved through up to four large projection screens. Three of the screens, i.e. left, right and centre, are known as the wall screens while the other one is known as the floor screen. A roller is
fitted underneath the left and right screens allowing them to be folded in/out up to 90 degree angle. As such the screens can be set to several arrangements, mainly the CAD wall and CAVE™ (see Figure 3.9), to suit the desired applications. A total of four projectors designated to each of the screens. The three wall screens are rear-projected while the floor screen is overhead-projected (downwards) and all four screens have the dimensions of $3.2m \times 2.4m$.

The setup utilises four Barco Galaxy NW-12 stereoscopic projectors which can provide active three-dimensional stereo display with active Infitec® technology. The active stereo features and the active Infitec technology based on ‘Infitec Excellence’ filters support stereoscopic images to be displayed with very fine detail. Each projector supports high light output of 12000 lumens enabling significantly high performance even in a bright environment. Other basic specifications related to the projectors’ display are such as WUXGA $1920 \times 1200px$ resolution, up to 2,000:1 contrast ratio, three-chip DLP and $2kW$ xenon lamp.

Multi-projector integration optimisation can be achieved through edge blending, DynaColor™ and linked constant light output (CLO), and warping (geometry correction) techniques. The edge blending technique produces seamless images from a multi-projector system. Fundamentally it formulates a mechanism to eliminate the visible band at the overlap region between two projected images. DynaColor™ and linked CLO are utilised to obtain
consistent light and colour levels across the multi-projected image. The DynaColor™ by Barco is an algorithm which aligns the digitally set primary and secondary colour coordinates of each projector to a common colour gamut in order to achieve colour matching throughout multi-projected images. Meanwhile the Linked CLO constantly control the light output of each projector to the lowest required value regardless of their lamp life. This can ensure same light outputs across all projectors independent of lamps’ lumen depreciation over time allowing mixed lamp lives usage and individual lamp replacement. Finally the image warping technique precisely adjust the projected image to be displayed across different screen surfaces or shapes and from different projection angles. In this section a set of large-scale virtual environments for micro-robotic cell injection were considered. The VR training environment development was rationalised based on the hypothesis that a large display can assist user to improve performance on spatial orientation tasks and provide better sense of presence [169]. Three large-scale display configurations were developed for the VR micro-robotic cell injection training environment. The first and second display configurations provide large-scale two-dimensional and three-dimensional display respectively, while the third configuration provides a more immersive three-dimensional virtual environment achieved through the CAVE system. The CAVE was first introduced in 1992 by Cruz-Neira et al. [170] from the University of Illinois featuring a room with projected images across its walls, ceiling and floor
surrounding the viewers. In this evaluation, the CAVE located at the CADET VR Lab, Deakin University, Australia was utilised which consists of four large screens, i.e. left, centre, right and floor. Aside from the large-scale display, every display configuration is also haptically-enabled in order to provide guidance to the bio-operator.

As depicted in Figure 3.9 there are two screen arrangements used for in this evaluation, the CAD wall and CAVE. The 2D and 3D display configurations utilised the CAD wall arrangement where the left, centre and right screens combined to form a large flat display. Meanwhile the CAVE-like display configuration utilised the CAVE screen arrangement. In this display configuration, the left, centre and floor screens were utilised where the left screen is folded in to be perpendicular to both the centre and floor screens. While in the 2D and 3D display configurations only one viewpoint of the virtual environment is displayed to the combined three screens, the CAVE-like display configuration provides three different viewpoints of the virtual environment across the three screens. The multiple viewpoints can provide higher immersion and better understanding of the orientation and movement of the virtual objects in the three-dimensional virtual environment.
For the 3D and CAVE-like display configurations, Volfoni® EDGE™ 1.2 active 3D glasses were utilised in order to view the three-dimensional virtual micro-robotic cell injection environment during the injections. The glasses provide high-quality images from all viewable angles through a pair of fast-response liquid crystal lenses. Performance-wise, the glasses offer 38% optical transmission, > 500:1 stereoscopic contrast, and 170 – 115 field of vision (H,V) which are fully compatible with the display system and able to achieve optimised projected images.
3.3.1 Virtual Environment

The virtual environment displays a replication of a cell injection setup. It consists of a virtual cell and basic bio-manipulation equipment such as microscope, micromanipulator, micropipette, cell holding dish, etc. The user will have a view of the environment and able zoom in to concentrate on the areas of interest.

The virtual training environment was built on Vizard VR software by Worldviz. In order to replicate the cell interaction in a virtual environment, an appropriate model is required. The model should be able to provide realistic visual and haptic responses to contacts made by the virtual micropipette. For this purpose a particle-based cell model [101] was utilised. The virtual cell is modelled to visually deform in response to micropipette contacts as well as providing interaction force estimations. These force can be haptically displayed to the user while performing the procedure.

3.3.2 Display Configurations

This subsection introduces a large-scale micro-robotic cell injection training system which provides an immersive virtual environment through three large screens.
There are three types of large-scale display configuration introduced in this study as shown in Figure 3.10. The first is a 2D display configuration which projects a two-dimensional image across three large screens arranged in CAD wall. The user is provided with a magnified top view similar to what can be seen from a microscope during an injection. Similarly the second display configuration, 3D, provides a large-scale three-dimensional image across the three screens in CAD wall. Thirdly the CAVE-like display configuration provides a three-dimensional multiple viewpoints of the virtual environment across three large of the four screens in the CAVE arrangement. The front screen displays a magnified top view similar to the view provided by the 2D and 3D display configurations. Meanwhile the left screen displays a view from
behind the micropipette and the floor screen displays a view from side of the micropipette. With this display configuration user is able to obtain a more immersive virtual environment where the display from three different angles are provided simultaneously while injection is performed. In contrast to providing images in three desktop screens the CAVE-like display configuration projects large-scale displays where user can move on the floor screen to obtain more sense of presence and increased spatial awareness of the virtual environment. In addition, the seamless display with high resolution image provided by the CAVE system can increase the quality of the representation.

### 3.3.3 Large Workspace Input Controller

An INCA 6D haptic device by Haption [171] was employed in the experiments to interact with the virtual environment. The INCA 6D is a cable-driven haptic device based on Spidar™ technology introduced by Sato [172]. The haptic device can provide up to 6-DOF (3 translational and 3 rotational) force feedback to user within large workspace (actual workspace depends on the motors and cables positions). The haptic device is capable to simulate heavy object handling realistically with up to $37.5N$ force feedback displays. It has $2mm$ positional resolution to be mapped to its representation in the virtual environment. The interface was achieved by mapping the orientation and position between the haptic device and the virtual micropipette as shown in Figure 3.11. As such the virtual cell injection procedure can be performed
by holding the haptic device handle to control the virtual micro-robot which holds the micropipette.

Given the mapping strategy implemented to the interface user is able to experience an intuitive handling of the micropipette as if they are holding the micropipette as opposed to the traditional rotary encoders. When performing the procedure with the haptic device user have the options to either enabling or disabling the haptic guidance. The haptic guidance provided in this VR training system is discussed in the next subsection.

![Mapping strategy between INCA 6D haptic device and virtual micromanipulator](image)

Figure 3.11: Mapping strategy between INCA 6D haptic device and virtual micromanipulator

In order to replicate real micro-robotic cell injection procedure, the virtual micropipette is fixed to a 30 degree offset (tilted) in the $x$-axis and its movement is limited to the translational $x$, $y$, $z$-axes (Figure 3.11). As such to perform an ideal injection user should move the haptic device as straight as possible towards the deposition point.
3.3.4 Haptic Guidance

When the haptic feedback is enabled, the VR training system provides two forms of haptic guidance, VFs and force feedback. There are two VFs provided by the system that serves as physical guidance of user’s control of the micro-robot so that the micropipette tip achieved the appropriate penetration and deposition point at the membrane and centre of the cell respectively. The first is a cone-shaped VF which guides the user to follow ideal trajectory where it allows the micropipette to move inside its conical guided region. Once the micropipette entered the guided region the conical wall prevents the micropipette from going through it. As such, as the user commands the micropipette to approach the cell, the conical VF encourages them to follow an optimised trajectory to the penetration point on the cell membrane, where the apex of the cone is. Once the micropipette’s tip has reached the penetration point, and the user attempts to pierce the cell membrane they will feel a simulated reaction force, followed by a sudden force drop representing the rupture and penetration of the cell membrane. The user then needs to move the micropipette in a straight line along its longitudinal axis towards the deposition point inside the cell. To guide the user’s movement along this path an axial VF is provided. In order to prevent the user from overshoooting the deposition point, which can cause damage to the cell, a planar VF is provided. The planar VF attempts to prevent the user’s control of the micropipette from passing the deposition point at the cell centre.
Once the micropipette’s tip reaches the deposition point, the virtual cell becomes inactive and the system can be reset to perform a new trial. For the planar VF, it is important to remember that it only provide haptic guidance, and given the maximum amount of force set to be displayed by the haptic device, the user remains in full control of the injection process. The VFs can be used with or without visual overlay and can be turned off based on user’s preference.

3.4 Conclusions

This section presents the development process of two VR training systems for micro-robotic cell injection. Section 3.2 introduced a desktop-sized VR micro-robotic cell injection training system considering its requirements, design, implementation and verification. Important skills relating to injection trajectory, accuracy and force are considered. The VR training system was developed based on five major elements, i.e. visual display, input controllers, mapping strategies, haptic guidance and output data. Based on the initial evaluation carried out it is suggested that the VR micro-robotic cell injection training system is feasible as an alternative to the physical system training. A comprehensive user evaluation is presented in Chapters Chapter 4 and Chapter 5 in order to validate the usability and effectiveness of the desktop-sized VR training system. Further exploration on optimising haptic technologies to provide more immersive representation and guidance to bio-operator is recommended as upgrade of the existing system design. As such the large-
scale version of the VR training system was developed and introduced in Section 3.3.

The large-scale VR micro-robotic cell injection environment presented in Section 3.3 provides an immersive, in-depth, attractive and motivational approach for motor skill training by utilising state-of-the-art technology available. One of the advantages of using large displays is it provides more detailed image representations which can increase sense of presence and understanding of the virtual environment. In addition, the multiple viewpoints of the virtual environment provided by the CAVE-like display configuration are beneficial in improving users’ understanding of the three-dimensional space within the environment especially to estimate the depth of the micropipette which is very problematic in a two-dimensional view such as from the microscope. Finally, with the utilisation of gross motor skill when using the large workspace haptic device, INCA 6D as the input controllers provides several benefits such as less sensitive to unintentional and insignificant movements such as vibration, tremors and minor deviations. Therefore the VR training can be a platform for bio-operators to improve the important skills such as the trajectory and accuracy of an injection. In order to validate the usability and effectiveness of the large-scale VR training system a user evaluation was carried out and discussed in Chapter 6.
Chapter 4  Keyboard Control Method

VR offers significant potential for skills training applications. In the previous chapter two VR platforms for bio-operator training for the micro-robotic cell injection procedure were introduced. This chapter presents the evaluation of the keyboard control method for VR micro-robotic cell injection training. Firstly in this chapter some of the related work carried out to investigate the effectiveness for various VR training systems are presented. This is aimed to provide the idea of what and how the evaluation of the VR systems implemented by other researchers and to justify the significant of the evaluations made throughout this thesis.

The interface between the bio-operator and the system can be achieved in many different ways. In the next section of this chapter the input control method to be evaluated, the keyboard control method, is discussed. The computer keyboard is ubiquitous in its use for everyday computing applications and also commonly utilised in VR systems. Based on the premise that most people have experience in using a computer keyboard, as opposed to more sophisticated input devices, this chapter considers the feasibility of using a keyboard to control the micro-robot for cell injection.
The next sections discuss about the overview, design and results of the evaluation. In the study, thirteen participants underwent the experimental evaluation. The participants were asked to perform three simulated trial sessions in a virtual micro-robotic cell injection environment. Each session consisted of ten cell injection trials and relevant data for each trial were recorded and analysed.

Finally the conclusions of the evaluation are presented in Section 4.6. It is worth noting that the results and analysis discussed in this chapter have been presented and published in [173].

4.1 Introduction

The use of haptics technology, especially in skill training, requires combination of the technology itself with other computer-based technology which can provide different sensory information (e.g. visual and sound). The whole system, which is the combination of these technologies, is known as virtual reality (VR). In other words, haptics are commonly the subset of the VR system where visual and/or sound cues are also blended into the system. Consequently, another topic that is worth discussing is the application of VR in providing skill training. Prior to development of any virtual training system, thorough investigation of the components involved in specific skills should be made in order to achieve the desired results in using such a system. It is crucial to take into account components such as hand trajectory, speed of movement, location accuracy and force applied in performing successful cell injections.
Even if the system has been carefully designed by considering all the skills comprised, the efficacy of the system as a tool in assisting users in mastering the skills is still a subject of extensive debate among researchers. Issues such as realism of the system, long-term skill acquisition, transfer of skills acquired to a real environment and user improvement after undergoing VR training are among the highly reviewed topics regarding the usage of VR in providing skill training. The research about the matter is now so intense that only a few references will be included in this section.

In the medical field, VR training has been validated for its effectiveness in several areas of surgical skill, such as laparoscopic [174, 175], basic endoscopic [176, 177], bronchoscopic [178] and vitreoretinal [179], to name but a few. The results of these studies have shown that subjects who have underwent VR training achieved better performance than the untrained subjects. The performance of both groups (trained and untrained) was assessed by observing the qualities of specific procedures performed either in the operating room (OR) [174, 175, 178, 179] or on a virtual simulator [176, 177]. The qualities observed are the crucial parameters that determine the success of the specific surgery. The time to complete the procedure and expert’s evaluation were also recorded as general appraisals of effectiveness.

Apart from comparing subjects who have underwent VR training with the untrained, there is also a need to show the superiority of VR to the conventional training. This is important to explore the capability of particular
virtual system to replace the traditional practice, thus reducing the reliance on the usage of human patients, animals, cadavers or physical models which require constant replacements. Besides, there are also other issues associated with the conventional training that have led to the development of virtual training systems such as ethical concerns [180], fewer mentoring opportunities [181], subjective competency evaluation [182], time constraints [183], risks of frequent exposure to radiation and the costs accompanying real medical facility consumption [184].

Experiments were conducted in several studies [185-187] to compare the pre- and post-training performance of two randomised groups: (i) subjects provided with only standard training; and (ii) subjects provided with a combination of standard and VR training. Results from these experiments clearly indicated more significant performance improvement achieved by the subjects in the latter group. This could be the rationale of using VR training as a reliable supplement to existing methodologies in order to obtain better training outcomes.
<table>
<thead>
<tr>
<th>Simulator / Evaluation Method</th>
<th>Observed Parameters</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIST-VR [174]</td>
<td>Procedure completion time, Error score, Economy of movement score</td>
<td>VR trained surgeons demonstrated significantly superior performance improvement than the untrained surgeons in the OR</td>
</tr>
<tr>
<td>LapSim [175]</td>
<td>Specific task completion time, Expert evaluations: Using a 9-step differential rating scale to assess overall performance of the participants</td>
<td>VR trained participants demonstrated significantly superior performance than the untrained participants for all the tasks set out in the study</td>
</tr>
<tr>
<td>Simbionix URO Mentor [176]</td>
<td>Procedure completion time, Guidewire insertion time, Incidence of mucosal trauma, Number of perforations, Fragmentation time, Expert evaluations: Using a global rating scale, Self-evaluation</td>
<td>VR trained students demonstrated significantly superior performance improvement than the untrained students in the post-test</td>
</tr>
<tr>
<td>GI-Mentor [177]</td>
<td>Insertion time for gastroscopy and colonoscopy, Percentage of correct pathologies identification, Number of adverse events during gastroscopy and colonoscopy, Virtual skill test performance (Endobasket and Endobubble)</td>
<td>VR trained participants demonstrated significantly superior performance than the untrained participants in the post-test</td>
</tr>
<tr>
<td>AccuTouch® flexible bronchoscopy simulator [178]</td>
<td>Intubation completion time, The number of times the bronchoscope tip hit the mucosa, Mucosa viewing time, Percentage of airway viewing time</td>
<td>VR trained residents demonstrated significantly superior performance improvement than the untrained residents in the fiberoptic intubation procedure</td>
</tr>
<tr>
<td>Computer-assisted training system consisting of a computer-based medical workstation for simulation of pars plana vitrectomy (University of Mannheim, Germany) [179]</td>
<td>Amount of vitreous removed, Amount of retinal detachment, Number of retinal lesions during vitrectomy and foreign body removal, Foreign body removal time, Expert evaluations: Using a subjective scale, assessed after each surgery</td>
<td>VR trained participants demonstrated significantly superior performance than the untrained participants in the pars plana vitrectomy procedure</td>
</tr>
<tr>
<td>MIST-VR [188]</td>
<td>Expert evaluations: Bowel grasping, Electrocoagulation of vessels, Bowel loop ligation, Ligature cutting, Bowel dividing</td>
<td>No significant performance distinctions between the VR trained and the untrained medical students, MIST-VR could be used as a reliable assessment tool to predict real surgical results</td>
</tr>
</tbody>
</table>
Although additional exercise on VR over standard training leads to better performance, it will correspondingly increase time consumption and costs. Furthermore, other issues associated with standard training as stated above will not be optimally surmounted because such training still needs to be performed as part of the programme. Consequently, performance comparison should be made between subjects who were trained solely by either standard or VR training. The findings of such comparison can be the indicator to determine the practicality of using VR training as a substitute for standard training. Hamilton et al.’s [189] is among the studies which have conducted this kind of comparison. This study found out that VR training significantly improved residents’ performance of laparoscopic surgery in the OR. The improvement observed was even better than that of subjects who received training on a video trainer, a conventional form of training for laparoscopic surgery. Another study by Kothari et al. [190] discovered no significant difference of improvement percentage between both groups. Hence, it was concluded in this paper that the virtual system used in the study (named MIST-VR) was comparable to the conventional method (named Yale Skills Course) for training the advanced laparoscopic skill of intracorporeal suturing.

There are also some other comparison studies performed by other researchers, in which a group of untrained subjects were included in addition to the VR and standard training groups. Similar results were obtained from those studies [183, 191, 192] where the VR training yielded significant skill
improvements, equivalent to those achievements derived from conventional training. Both trained groups showed superior post-training performance compared to the untrained group.

In contradiction to the aforementioned findings, however, there are some studies that discovered the inferiority of a virtual system to the standard practice in phlebotomy skills [193] and real-world tasks [194] training. In addition, experiments conducted by Ahlberg et al. [188] did not establish any significant difference in performance between medical students with and without MIST-VR training. These findings indicate that MIST-VR training did not improve laparoscopic surgical skill of the users. However, these results should not be generalised as to reflect the inefficiency of all VR training systems because it has been argued that different training tasks have different learning curves [195-197]. Those studies are in fact among the occasional cases which probably resulted from inappropriate experiment implementation. In Ahlberg et al.’s [188] experiments, for example, the subjects were only trained for 3 hours in which the plateau in their learning curve may not have been reached [174]. This argument was based on a previous study [198] that demonstrated the plateaus in the learning curve of beginner laparoscopic surgeons were reached after the seventh repetition of all six MIST-VR tasks, which requires considerably more than 3 hours of training.
**Table 4.2: Experiments performed to investigate VR training effectiveness: Standard training versus Standard and VR training combination**

<table>
<thead>
<tr>
<th>Evaluation Method</th>
<th>Observed Parameters</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- and post-test of basic ureteroscopic tasks performance on URO Mentor [185]</td>
<td>Objective: - Procedure completion time (bladder neck, ureteral orifice, cannulate orifice, calculus, total ureteroscopy time) - Number attempts at cannulation - Number of times subject reoriented - Rate of complications - Number of urothelial petechiae Subjective: Expert evaluations: Using a modified endourological global rating scale</td>
<td>Students who received additional VR training (i.e. using URO Mentor) demonstrated significantly superior performance improvement than the student who received only standard training (i.e. teachings and demonstrations from expert urologist) for both objective and subjective measurements</td>
</tr>
<tr>
<td>Cavity preparations and restorations performance in practical exams [186]</td>
<td>Exams score: Cavity preparations and restorations quality</td>
<td>Students who received additional VR training (i.e. using DentSim®) demonstrated significantly superior performance improvement than the student who received only standard training (i.e. traditional laboratory-based instruction) throughout the first and final exams of the year</td>
</tr>
<tr>
<td>Pre- and post-test of gallbladder excision performance [187]</td>
<td>- Procedure duration - Number of errors - Economy of diathermy</td>
<td>Residents who received additional VR training (i.e. using MIST-VR) demonstrated significant laparoscopic cholecystectomy performance improvement in the OR</td>
</tr>
</tbody>
</table>

**Table 4.3: Experiments performed to investigate VR training effectiveness: Standard training versus VR training**

<table>
<thead>
<tr>
<th>Evaluation Method</th>
<th>Observed Parameters</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- and post-test of laparoscopic cholecystectomy performance on video trainer (VT), MIST-VR and OR [189]</td>
<td>- MIST-VR task performance: Completion times, errors, economy of motion and diathermy - VT task performance: Average time elapsed Expert evaluations: Global assessment tool</td>
<td>- VR trained (i.e. using MIST-VR) residents demonstrated significantly superior performance improvement than the standard trained (i.e. using VT) residents in laparoscopic cholecystectomy in the OR - VR trained residents is more likely to have superior crossover improvement (from VT to MIST-VR, vice versa) than the standard trained residents - Both VR and standard trained residents effectually improved their psychomotor skills</td>
</tr>
<tr>
<td>Pre- and post-test of intracorporeal knot performance [190]</td>
<td>Intracorporeal suturing times</td>
<td>No significant performance improvement distinctions between the VR trained (i.e. using MIST-VR) and the standard trained students (i.e. using Yale Skills Course)</td>
</tr>
</tbody>
</table>
Further verification of the advantages of using VR as a tool in medical skill training can be found in quite a number of papers published in various forms such as reviews [199-202], surveys [182] and meta-analyses [203]. These papers have presented more comprehensive research regarding the effectiveness and applicability of the VR training system. Because of this, it

<table>
<thead>
<tr>
<th>Evaluation Method</th>
<th>Observed Parameters</th>
<th>Findings</th>
</tr>
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</table>
| Pre- and post-test of laparoscopic surgery task performance on glove and tube models [183] | - Number of hands movements  
- Hands travel distance  
- Task completion time  
- Economy of hand movement  
- Expert evaluation: Number of errors | Standard (i.e. using box trainer) and VR (i.e. using LapSim) trained groups equally demonstrated significant performance improvements than the untrained group in all assessed parameters |
| Pre- and post-test of tasks performance on a real steadiness tester [191] | Number of errors | Standard (i.e. by real training) and VR (i.e. using dVISE) trained groups equally demonstrated significant performance improvements than the untrained group in real world post-test |
| Pre- and post-test of laboratory-based laparoscopic surgery tasks performance on a real laparoscopic trainer [192] | Imperial College Surgical Assessment Device (ICSAD):  
- Time consumption  
- Distance travelled by instrument  
- Number of movements  
- Speed of instrument movements | Standard (i.e. standardised minimal-access training drills) and VR (i.e. using MIST-VR) trained groups equally demonstrated significant performance improvements than the untrained group in the post-test |
| Real-world task performance [194] | Response times of a pick-and-place sequence | - Standard trained (i.e. by real training) group demonstrated significantly superior performance than both the VR trained (i.e. using Dataglove™ and HMD Eyephone(s)™) and the untrained group  
- No significant difference between the VR group and the untrained group |
may be assumed that VR will have a bright future in training and also indicates good prospects in the adoption of this technology into the cell injection training system.

As discussed in this section, many research have been carried out to evaluate the feasibility of VR for various training applications. However it is important for any VR training system to be evaluated exclusively in order to consider its specific objectives and targets. Therefore the in the next sections and chapters the evaluation of the VR micro-robotic cell injection systems focusing on their usability and effectiveness is presented. In this chapter an overview and evaluation of the keyboard control method utilised in one of the VR training systems in this thesis is discussed.

4.2 Keyboard Control Method

As discussed in Chapter 3, two input control methods can be utilised to interact with the desktop VR micro-robotic cell injection environment. This section discusses about one of the two input control methods for the VR micro-robotic cell injection system, the keyboard control. The computer keyboard is ubiquitous in its use for everyday computing applications and also commonly utilised in VR systems. Based on the premise that most people have experience in using a computer keyboard, as opposed to more sophisticated input devices, this chapter considers the feasibility of using a keyboard to control the micro-robot for cell injection.
The virtual micro-robot replicates the MP-285 micromanipulator from Sutter Instruments which provides three actuated degrees of freedom usually controlled using a rotary optical encoder or joystick. In addition to the three axes, \((x, y, z)\), the micromanipulator also provides an artificial fourth (diagonal) axis of movement which is a combination of any two of the three axes to move along a pre-determined angle. When using the fourth axis, one of the rotary encoder’s dials is used to control the movement of the micromanipulator. Modifications were made to the earlier mentioned VR micro-robotic cell injection system to facilitate keyboard control. This was achieved through mapping the movements of the virtual micro-robot to the pre-determined keyboard buttons. The buttons of the numeric/directional keypad shown in Figure 4.1 were used for control of the micro-robot. The numeric/directional keypad provides keys in easy finger reach for the operator.

The mapping was implemented such that the micro-robot moves at constant velocity in a particular direction in response to key presses by the operator. This allows the operator to implement both gross and fine control based on how long they hold down the respective key(s). For example, fine control can be achieved by tapping a particular key, whereas gross control can be achieved by holding down the key for a longer time. The following section presents the experimental evaluation of the keyboard control of the micropipette for VR cell injection training.
Figure 4.1: Keypad to virtual micro-robot mapping

$x$, $y$ and $z$ are the directions of the Cartesian axes $(x, y, z)$, $d$ is the artificial diagonal axis $(d)$

4.3 Experiment Overview

The objective of the experiments discussed herein is to evaluate the feasibility of the proposed keyboard control method for VR micro-robotic cell injection. The research was approved by the Human Ethics Advisory Group (HEAG), Faculty of Science, Engineering & Built Environment, Deakin University.

Thirteen participants (nine male and four female) took part in the experiments. All participants were screened to ensure that they had no prior experience in cell injection activities.
4.4 Experimental Design

As illustrated by Figure 4.2, participants were divided into two groups, Group 1 and 2, which underwent different training sequences. In addition to practice with the keyboard control method, Group 2 (ten participants) also underwent practice with a haptic device, as the performed experiments were part of the larger work discussed in the next chapters. Group 1 undertook the experiments for the sole purpose of evaluating the proposed keyboard control method and for this reason was a smaller group with three participants.

Each experiment was conducted over three sessions, numbered 1 to 3. In each session, participants performed ten trials, while data such as time and position, and motion of the virtual micropipette were recorded. In between sessions, participants were given a break to rest followed by a practice session where the participants were required to perform ten additional cell injection practice trials. This allowed the participants to train and plan their strategy for the next session with minimal pressure. Group 1 participants used only keyboard control during both practice sessions. Group 2 participants however used the haptic device for the both practice sessions. The haptic device with and without haptic guidance were used in the first and second practice sessions respectively. As mentioned above, the reason for this is to consider skills acquisition in the context of the larger work discussed in Chapter 5, however possible impact to their learning should be considered. The data for the trials during the practice sessions were not considered in the following analysis.
After all sessions had been completed, participants were asked a set of questions, so as to gain feedback regarding their experience using keyboard control for performing virtual micro-robatic cell injection trials. Participants were also video recorded during their trials to gain useful qualitative observations.
The micropipette’s position in the VR environment was logged at 60Hz. In real-world applications cell diameters range from 1 to 100μm [22]. In this analysis the virtual cell diameter is assumed to be 2μm which is considered a small cell. The virtual cell was 2 units in diameter where a spatial unit in the virtual environment relates to 1μm in the real world. The selection of a small cell size is useful in order to access performance accurately. The virtual cell had a radius of 1μm and centred at the origin (0,0,0). An injection was considered successful when the operator penetrated the cell membrane and stopped the micropipette tip inside the cell. In an ideal injection, the micropipette tip is positioned at the centre of the cell for deposition. As such, injection error was defined as the distance between micropipette tip stop point and the centre of the cell.

For this analysis, the injection error, E and success, S, were considered as two performance metrics. During a virtual cell injection, participants would determine when they believed they had reached the target location to the best of their ability. This final position of the micropipette tip, F was compared with the actual target location to determine E and S.

An injection was considered successful if the position of F was located within the cell (indicating penetration of the cell membrane), when the participant declared they believed that they had penetrated the cell and reached the target location (centre of the cell). For this evaluation purposes an injection can be considered ideal, if the micropipette’s tip is stopped at the cell’s centre.
which can be denoted by \( C \). Based on this, the magnitude of error, \( E \), was defined as the difference between the micropipette’s final position, \( F \) and the ideal deposition target, \( C \). Given this, an injection was considered success only when the magnitude of \( F \) is less than 1\( \mu m \) given by

\[
\text{if } |F| = \sqrt{x_F^2 + y_F^2 + z_F^2} < 1; S = 1
\]

\[
\text{if } |F| = \sqrt{x_F^2 + y_F^2 + z_F^2} \geq 1; S = 0
\]  

(1)

It is worthwhile noting that once the cell membrane has been penetrated, in order to avoid damage to the cell a second perforation of the cell membrane, in any direction, should not occur. Therefore observations were made to verify that no multiple penetrations were made in either inward or outward directions. Multiple penetration can be identified when the distance between the micropipette tip and the cell centre becomes greater than the cell radius after it has decreased to below the value of the cell radius for the first time.

Accuracy is one of the most important parameters relating to the survivability of the injected cell. Herein accuracy is considered the opposite of error, where lower error corresponds higher accuracy. For an ideal injection, i.e. perfect accuracy and no error, the deposition point achieved would be at point of the cell centre, \( C \). As such \( E \) is determined by the distance between \( F \) and \( C \) which is given by the magnitude of \( F \) as follows
4.5 Keyboard Control Method Training Outcome

This section presents the data analysis and results of the experiments mentioned in the previous section. Figure 4.3 and Figure 4.4 show the overall performance of all participants for each session in the experiments. In terms of success rates, five participants demonstrated significant improvement after the three sessions, while two participants showed a decrease in performance. The performance improvements are likely due to the natural learning curve which occurs as the participants gain more experience with the environment and task. The keyboard is a commonly used device, and as such it is expected that the improvements in performance of the keyboard control observed within this chapter can be used a benchmark by which to compare other methods. Four participants’ performance levels remained unchanged and two participants’ performance was inconsistent. In terms of injection error, three participants improved their accuracy from first to the third sessions, two participants decreased and nine demonstrated inconsistent performance. No steady trend, e.g. increasing or decreasing, was observed between the keyboard control usage over the three sessions.

For Group 1, one participant showed 10% improvement in success rate over the three sessions and one participant demonstrated 20% decrease. Another participant showed inconsistent performance where their success rate
varied between 80 and 100% over all sessions. In terms of mean injection error, two participants showed inconsistent performance ranging from 0.51 to 0.81μm and one participant demonstrated steady improvement over the sessions. Meanwhile for Group 2, four participants improved their success rates by 10 to 30% over all sessions, one participant’s performance decreased by 40% and one participant was inconsistent with success rates varying between 80 and 100%. Aside from that, one participant’s success rates remained unchanged at 90%, and another three participants achieved 100% consistently across all trials. These participants were consistent in achieving their maximum performance in their first trial. The mean injection error showed that three participants improved, one participant decreased in performance and six participants had inconsistent performance. Only three participants demonstrated a significant change in injection error between sessions, and these three participants improved significantly after the first practice session.

Figure 4.3: Mean success rates for all participants over the three sessions
Considering both groups combined, it can be observed that participants’ mean injection error improved from 0.80 to 0.55\(\mu m\) from the first to third session. This can be considered as a relatively significant improvement in the context of this virtual system application considering the 0.25\(\mu m\) improvement will increase the accuracy to 25\%, given the cell radius of 1\(\mu m\). Likewise, the mean success rates also improved from 88 to 92\% from the first to third session. This is a significant result given the success rate for actual procedures range from 20 to 80\% [3]. The mean injection error for the participants of Group 1 decreased from 0.53 to 0.65\(\mu m\) between Session 1 and Session 2, and then improved to 0.53\(\mu m\) in Session 3. The 0.12\(\mu m\) of difference in injection error between sessions can be considered as small and the improvement and decrease are not significant.

The mean injection error of the participants in Group 2 steadily improved from 0.89\(\mu m\) in Session 1, to 0.57\(\mu m\) in Session 2 and then finally to 0.56\(\mu m\) in Session 3. In terms of success rate, Group 1 showed a decrease
from 93% in Session 1 to 80% in Session 2, followed by an increase to 90% in Session 3. Meanwhile participants in Group 2 achieved a success rate of 87% in Session 1, which improved to 95% in Session 2 and dropped to 93% in Session 3.

Figure 4.5: Mean injection error for Group 1, Group 2 and both groups combined for all three sessions.

Lower value denotes better performance

Figure 4.6: Mean success rates for Group 1, Group 2 and both groups combined for all three sessions
It is interesting to observe that there were participants who naturally used multiple axes simultaneously during the cell injection trials. The results show that despite no training or instruction regarding the ability to control multiple axes simultaneously five participants significantly did so throughout the experiments. In practice this is achieved by holding down multiple keys at the same time. It is worth noting that participants were only instructed on how to use a single axis at a time and no information about the ability to control multiple axes simultaneously was provided. As such it is assumed that these actions were self-discovered or intuitive. The artificial fourth axis provided by the MP-285 micromanipulator could be used through a pair of keys just the like other synthetic axes. Participants were briefed about this, unlike the case where multiple arbitrary axes were employed through pressing multiple keys simultaneously. Thus it can be assumed that the latter case was self-discovered and intuitively used and considered herein as an important finding. Furthermore it can be observed that the percentage multiple axes usage increased as participants’ progressed through the sessions, and as such it may be deduced that the behaviour was intentional.

It was also observed that the simultaneous use of multiple axes only occurred for the $x$ and $y$ axes combination. This suggests that participants may have identified the more direct straight line path, based on the two-dimensional view provided by the microscope in the $x$ and $y$ planes, to move the micropipette to the penetration target and then tried commanding the $x$ and
y axes simultaneously. This suggests positive learning curves occurred after training sessions where the participants realised and utilised a more efficient way to manoeuvre the micropipette.

The use of multiple axes, considered for all participants from both groups, gradually increased from 0.23% to 1.23% from the first session to third sessions respectively. It is suggested that this is the result of skills transfer from using the common computer keyboard which is ubiquitous and widely used. Participants could have developed this intuition and skills from many possible experiences such as general computer operation, computer gaming, and technical skills.

Figure 4.7: Usage of multiple axes as a percentage of the total time across all three sessions
Those participants who employed multiple axes, constituted five of the six participants who identified themselves as competent computer gamers. This supports the above suggestions that the use of this functionality was intuitive and intentional. It is logical to assume that computer gaming can improve players’ 3D manipulation skills through repetition and familiarisation of coordination between the control device and corresponding movements on the computer screen. From a psychological point of view, it has been reported that computer gaming experience correlates to spatial ability, the capacity to recognise and retain the spatial relativity between objects [204, 205]. Gaming experience can also improve executive control skills, i.e. the ability to control additional cognitive activities by manipulating and reconfiguring the other tasks’ parameters to respond in accordance with the current task [206], which usually benefits the performance of multiple tasks simultaneously [207]. There are also studies which report on bio-manipulation performance improvement.
through unrelated digital gaming experience [208, 209]. In this evaluation however no significant relation between the participants’ self-considered gaming competencies and their performance (success rates and accuracy) was observed. One possible reason for this is the way gaming competencies are self-rated and this type of measurement is usually subjective, inconsistent and perception-based. On the other hand, the increased multiple axes usage frequency throughout training sessions suggests that participants realised a more efficient way to manoeuvre the micropipette by mean of moving it in the $x$ and $y$ axes simultaneously.

Finally based on the interview conducted during the experiments it was found out that all participants did not face major difficulty in controlling the virtual micro-robot using the keyboard control method. Furthermore the Group 2 participants who experienced haptic stylus control during the break, the feedback was that they found the keyboard control to be the more convenient of the two methods.

4.6 Conclusions

The introduced keyboard control method is mechanically robust, low-cost, and exploits the vast array of experience operators are likely to have in operating computer keyboards for many other possible applications.

Results demonstrate that the approach lends itself to the intuitive control of multiple axes simultaneously, as evidenced by five of thirteen participants (across both groups) who controlled multiple axes simultaneously despite no
instruction on how to do so. Aside from demonstrating the dexterity with which the keyboard control method can be used, this also supports the notion that transferrable skills relating to the use of the keyboard can be employed.

Although this evaluation does not focus on the use of the haptic device control method used by Group 2, it is worth acknowledging that Group 2 did demonstrate better progress in performance than Group 1. It is known that haptically-enabled virtual environments can offer immersive and advanced training features and it is possible that this is reason for the better performance of Group 2. As such, the presented keyboard control method, can be considered to provide a simple and cost effective method for micro-robot control. The method can also be used as a benchmark for evaluating performance improvement when using a haptic input control method such as discussed in the next chapter.
Chapter 5  Intuitive Haptic Device Control Method

This chapter extends the experimental evaluation of the VR micro-robotic cell injection training system as presented in the previous chapter to consider the usability and effectiveness of the proposed intuitive haptic device control method. Aside from being a robust, economical and simple input controller, the keyboard control method also can be used as a benchmark to consider other input control methods. User training evaluation experiments were designed and conducted to evaluate participants’ performance when using the different input control methods, the keyboard and haptic device, and their performance improvement after undergoing training. Thirteen participants took part in the virtual cell injection experiments using the different input control methods.

This chapter evaluates the different input control methods and the introduction, experimental design, data analysis and results of the evaluation are discussed in the following sections.

5.1 Introduction

As mentioned above, this evaluation considers the proposed intuitive haptic device control method for VR micro-robotic cell injection training. The evaluation aimed to investigate more into detail the usability and effectiveness
of the system, focusing on the effects of using the different input control methods provided. The evaluation design is discussed in the next section. Terminologies used such as group and session names are defined.

Chapter 4 only considered the keyboard control as a method for controlling the micro-robot. Herein, the evaluation of the keyboard control method serves as a basis for comparison with the multiple input control methods, i.e. keyboard, haptic device with guidance disabled, and haptic device with guidance enabled, considered in this chapter. The experimental evaluation discussed in this chapter concentrates on the effectiveness of each input control method as training tool by considering participants’ performance improvement after undergoing training. The evaluation also considers the usability of each input control method by analysing participants’ performance when using the control methods.

5.2 Design of User Training Evaluation

Specifically there are two main aims of this evaluation. The first is to study the performance improvement of the participants after training sessions. The second is the evaluation investigating participants’ performance level for using different input control methods in their trials.

Experimental evaluation was designed in order to consider the usability and effectiveness of the system in training users to improve their performance against a set of defined metrics. The evaluation first considers performance improvement of the participants using the keyboard control method. Aside
from ubiquitous use of the keyboard, where utilisation of transferable skills is possible, it provides a simple and low-cost way of controlling the micro-robot. As such, the keyboard control method is considered as a benchmark for evaluating the introduced haptic device control method. Secondly, the evaluation considers the usability of the system by analysing participants’ performance in using the two different input control methods. Usability is defined by the level to which a system can be utilised by users to accomplish its aims with effectiveness, efficiency and satisfaction for specific usage circumstances [210]. Herein, as suggested by [211], binary task completion and accuracy are considered as measures of effectiveness. To that end, binary task completion and accuracy were quantified by the success rates (success/fail) and magnitude of error (opposite of accuracy) respectively. There are two different configurations for the haptic device control method, i.e. guidance disabled and guidance enabled, and as such three groups were considered, i.e. keyboard, haptic device with guidance disabled, and haptic device with guidance enabled.

The evaluation was granted a human research ethics approval by the Human Ethics Advisory Group (HEAG), Faculty of Science, Engineering and the Built Environment, Deakin University. Thirteen participants (nine males and four females) were recruited for the experiments and all were screened to ensure that they had no prior experience with cell injection processes. Their demographic data were also obtained to be considered in the analysis.
As shown in Figure 5.1, participants were divided into two groups, the keyboard and haptic device groups, comprising of three and ten participants respectively. The keyboard group undertook the experiments using only the keyboard control method for all the evaluation and training sessions, rather than alternating through the more complex haptic guidance disabled-enabled sequence for both the evaluation and training sessions, and for this reason was a smaller group. Participants underwent the experimental evaluation in three evaluation sessions, i.e. pre-training (PT), post-training 1 (PT1) and post-training 2 (PT2).

Before undertaking the evaluation participants were given an initial briefing where they were shown a comprehensive range of angles and sizes (by rotation and zooming) of the environment so as to provide a better understanding of the VR operating environment. Participants were also instructed to move the micropipette appropriately towards the cell, to penetrate the membrane and then stop the micropipette tip as close as possible to the cell centre for deposition, and without multiple penetrations occurring.

In each session, participants were asked to perform ten virtual injections while data such as the position of the virtual micropipette tip were recorded. The position of the virtual micropipette was considered rather than the haptic device for two reasons. Firstly, the system was designed to work with multiple input control methods, i.e. the keyboard and haptic device, which operate very differently. So as to standardise the measurement for the purpose of
comparison, the position of the virtual micropipette’s tip, which is common to both input control methods, was recorded. Secondly, the virtual micropipette, not the haptic device, is considered to make virtual contact with the cell and VFs in the virtual environment, and thus more important to consider for determination of the parameters such as accuracy and success.

Figure 5.1: Evaluation process flowchart
Each group undertook two training sessions, T1 and T2 in between the three evaluation sessions, PT, PT1 and PT2. The keyboard group used only the keyboard control method for both T1 and T2. The haptic device group however used the haptic device control method in two different configurations, the Phantom Omni with haptic guidance disabled and enabled for T1 and T2 respectively. In the manner implemented, the haptic device, irrespective of whether haptic guidance is enabled or not, serves as a 3D input device to control the virtual micro-robot. In this method the participant can move the micropipette intuitively using 3D position to position mapping.

For the haptic device group, PT1 and PT2 were undertaken after training with the haptic device. PT1 was conducted after the haptic device training (with guidance disabled) in T1 while the PT2 conducted after haptic device training (with guidance enabled) training in T2. When haptic guidance is disabled, the participant is benefitting from the ability to intuitively control the micropipette using 3D position-to-position mapping, and this can be observed through comparison of the performance of PT1 and PT2 as discussed in Section 5.3. Participants’ performance for all evaluation and training sessions was measured. Participants were also video recorded and interviewed during the experiments in order to obtain useful qualitative data.

In the first part of this evaluation the two metrics explained in Chapter 4, magnitude of error, $E$ and injection success, $S$, given by equation (1) and (2) respectively, were analysed. Additionally, a performance factor, $P_F$ is
introduced to provide a combined metric which considers whether the injection was successful, the observed magnitude of error and the completion time, given by

\[ P_F = S/ET \]  

(3)

where the value for \( P_F \) will be zero if the injection is not successful regardless of the values of \( E \) and \( T \). For a successful injection \( S \) is equal to 1 thus the \( P_F \) will have a reciprocal non-linear relationship with \( E \) and \( T \). For the purpose of this particular metric an errorless injection, \( E = 0 \) is assumed never achieved by any of the participants in any of their injections. Since \( T \) represents completion time that will always be greater than 0, the denominator of the \( P_F \) function \((ET)\) will always be greater than 0. \( P_F \) values were only used for the purpose of ranking and comparison between injections and the non-linear characteristic of the function does not affect this type of comparison. For example, an injection with \( P_F = 10 \) represents better performance compared to injection with \( P_F = 1 \) despite a non-linear relationship where the former is not 10 times better than the latter. For the purposes intended in this chapter this was considered adequate.

The starting location for each injection was constrained to the region outside the cell membrane in the positive \( x \)-axis direction, as shown in Figure 3.5 (Chapter 3), thus given by \( x \geq 1\mu m \) since the cell has \( 1\mu m \) radius. This is
the usual practice of the real procedure where bio-operator needs to set the position of micropipette to be able approach the cell laterally.

In order to cater for different starting points for each injection, and to allow for fair comparison, $T$ represents the normalised value of the injection’s actual completion time, $T_{trial}$. The normalised completion time was calculated such that if the straight line distance from the starting point to the centre of the cell, denoted by $B_{trial}$, is less than the average starting point of the experiment, $B_{\mu}$ additional time will be added to $T_{trial}$. In such a case the participant is penalised since the distance to the cell centre, $C$ which is the target, is shorter than the average. In contrast, there will be reduction of the actual time if $B_{trial}$ is further from $C$ than $B_{\mu}$. $T$ is given as follows

$$T = T_{trial} + \left( \frac{B_{\mu} - B_{trial}}{B_{\mu}} (T_{trial}) \right)$$

(4)

5.3 User Training and Performance Analysis of Input Control Methods

This section presents the results and analysis from the implemented evaluation with human participants. The main aims of the analysis are to study the effects of training using each input control method and the usability of the input control methods by examining participants’ performance.
5.3.1 User Training Outcome

The previous chapter considered the keyboard alone and investigated whether it is a viable input control method. It was concluded that the keyboard can serve as a practical, straightforward and economical method to control a micro-robot for cell injection.

To consider the effects of training on performance in the task using each input control method, participants’ performance was recorded before and after each training session. Recall that PT, PT1 and PT2 are the pre-training, post-training 1 and post-training 2 evaluation sessions, respectively. The two training sessions, T1 and T2, were carried out between PT and PT1, and between PT1 and PT2 respectively. The mean success rates for the keyboard group for PT, PT1 and PT2 can be observed in the upper row of Table 5.1 as well as in Figure 5.2.

Table 5.1: Mean success rates and magnitude of error for the keyboard and haptic device groups for PT, PT1 and PT2

<table>
<thead>
<tr>
<th>Group</th>
<th>Success Rate (%)</th>
<th>Magnitude of Error (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PT</td>
<td>PT1</td>
</tr>
<tr>
<td>Keyboard</td>
<td>93 / 0.53</td>
<td>80 / 0.65</td>
</tr>
<tr>
<td>Haptic device</td>
<td>87 / 0.89</td>
<td>95 / 0.57</td>
</tr>
</tbody>
</table>
Figure 5.2: Mean success rate for the three sessions, PT, PT1 and PT2 for the keyboard and haptic device group

Success rates were specified as the average of successful injections performed by all participants of a particular group across a session. As discussed in Section 4.4 (Chapter 4) an injection is considered successful when the micropipette tip is stopped within the cell and no multiple cell penetrations were made during the injection. The end of the attempt occurred once the participant acknowledged that they believed they had successfully performed the injection by pressing a designated button representing stop/deposit.

As can be observed, the average success rates of the keyboard group fluctuated more significantly over the three sessions, with the performance in the final evaluation session PT2 actually lower than for the initial pre-training session. The average success rate was initially 93% for the pre-training, PT, then decreasing to 80% in PT1, after the first keyboard training session. In the second post-training evaluation session, PT2, the average success rate increased to 90% however remaining lower than for pre-training. Qualitative
participant feedback suggests that the increase-decrease characteristic of the group’s performance may be due to demotivation after repeatedly performing the same task with the same and familiar input control method, i.e. the keyboard, for the entire evaluation.

Despite the increase-decrease characteristic where the final result was lower than the initial performance, the outcome can be considered positive based on the fact that no instruction was given to the participants other than the initial briefing and the lowest achieved performance level across the evaluation sessions was 80%. It is valuable to compare this result with that of a study by others where novice participants were required to inject colour dye into a cell and, success was determined if dye remained inside the cell and the cell did not collapse on removing the micropipette [13]. In the study participants used the conventional method for controlling the micro-robot and micropipette where three rotary encoders, one for each axis, are used. Their study reported success rates ranging from 37 to 75%. Comparing this with the result of this evaluation where at least 80% of attempts were successful, and participants used only a computer keyboard, the approach suggest significant promise. Furthermore, this minimum success rate was achieved in the first session participants performed the injection process, i.e. Pre-Training, which consisted of 10 injections (refer Figure 5.1).

Furthermore, the keyboard group demonstrated improvement against the performance factor, discussed later in this section, which aside from success
rate also takes into account other important parameters such as the magnitude of error and completion time. These results suggest that the keyboard control method is viable as a method for micro-robotic cell injection.

The haptic device group, however, demonstrated performance improvement in both post-training sessions when compared to the pre-training session. In PT, participants achieved a mean success rate of 87% followed by 95% and 93% in PT1 and PT2 respectively.

As mentioned in the previous section, the haptic device group, undertook PT1 after pre-training, PT, and after the training with the haptic device without guidance, T1. Meanwhile the PT2 session was undertaken after the PT1 and following haptic device training with guidance, T2. Given this sequence, the results of PT1 and PT2 can be considered to evaluate the impact on participants' performance without and with haptic guidance respectively.

As observed in the haptic device group results above, participants demonstrated a 2% better success rate in PT1 compared to in PT2. There was a higher improvement of 8% from PT to PT1, where no haptic guidance was provided in the training between the two evaluation sessions. Based on this it appears considerable performance improvement occurred as the result of being able to intuitively control the micropipette using the stylus and 3D position to position mapping.

The haptic device group demonstrated a better overall success rate of 92% as well as significantly improved performance in both post training
sessions. There was a slight decrease in performance (2%) in PT2 compared to PT1, however both remained significantly higher than pre-training (87%), at 95% and 93% respectively. The ten participants in these groups each performed ten injections in each session, making a total of one hundred injections per session. Therefore on average it can be deduced that the participants achieved eight and six more successful injections in PT1 and PT2 respectively, compared to PT. This is compared to an overall success rate of 88% for the keyboard group who demonstrated a decrease-increase characteristic across the sessions. Both groups however can be considered to have achieved significantly high performance based on the analysis made. Given that the haptic device group demonstrated both better performance and performance improvement than the keyboard group across the sessions, it is suggested that after undergoing the haptic device training participants can achieve better controllability than they would with the keyboard control. It should be acknowledged that even the keyboard control method appears to achieve higher performance compared to a similar evaluation where the conventional input control method of using rotary encoders was utilised [13] as was discussed earlier.

When observing the success rates for both groups a decrease-increase characteristic for the keyboard group and increase-decrease for the haptic device group was discovered. As mentioned earlier, qualitative feedback suggests that demotivation amongst the keyboard group was responsible for
decreasing performance from PT to PT1, and the fact that the performance for PT2 was actually lower than for the initial PT session. Participants stated that the demotivation was due to using the same keyboard control method across the entire evaluation, and it is suggested based on the fact that the keyboard is a ubiquitous everyday technology, it is less motivating than using the haptic device. When comparing the initial success rates for PT across both groups, however, it can be observed that the keyboard group scored higher (93/87%). Despite higher overall achievement of the haptic device group, they demonstrated initial success rates lower than the keyboard group. As such, it is interesting to observe the haptic device group made a relatively better progress after the training using haptic device despite lower initial success rates. This suggests the haptic device control method as a viable approach for micro-robotic cell injection training.

Before undertaking the experiments all participants were screened to have no prior experience in any cell injection activity so were considered novice users. It is important to note that prior to the PT session the participants were given an initial briefing where they were given a demonstration on how to perform a successful injection using the keyboard. The characteristics of a good quality cell injection were also explained, including moving the micropipette appropriately towards suitable penetration point at the cell membrane, stopping as close as possible to the cell centre for deposition and avoid multiple penetrations. It was observed from the questionnaires that all
the participants are regular computer users and very familiar with keyboard. The initial briefing was provided as to close the gap between varying levels of keyboard handling skill among the participants. As such, it is suggested that both groups initially have the same level of knowledge of the system based on the fact that all participants have no prior exposure to the system, or to cell injection processes, and were given the same briefing and demonstration before undertaking the experiments. Based on the questionnaires undertaken by the participants it was observed that two of three participants (67%) in the keyboard group and three of ten participants (30%) in the haptic device group considered themselves as competent computer gamers. Chapter 4 discussed how computer gaming experience had influenced participants’ strategies to using keyboard control when performing injections through the usage of multiple axes. As such it can be assumed that the computer gamers may have more developed skills relevant to controlling the micropipette using the keyboard. Since the success rates were the average success score for each group, it should be acknowledged that some of the better initial performance of the keyboard group may be attributed to the fact that they had a higher proportion of members identified as competent computer gamers (67%).

The haptic device control method utilises 3D position to position mapping whereby movement of the haptic device stylus in 3D space results in corresponding motion of micropipette. The keyboard control method however requires the use of keyboard key pairs to move the individual axes of the
micropipette. It is worthwhile to note, that as mentioned in Chapter 4, despite no instruction on how to do so, nine participants intuitively controlled multiple axes simultaneously. Moreover it was also observed that the use of multiple axes continually increased throughout the sessions for both groups suggesting deliberate action made by the participants. Given this, combined with the higher success rate for the initial session, PT, it is suggested this is related to transferrable skills relating to the keyboard is a common input device.

The overall success rates of 92% and 88% suggest that participants using either control method achieved relatively high overall success rates compared to the study mentioned earlier [13] which reported success rates between 37 to 75% for forty novice participants. Participants in both groups performed thirty injections in total across the three evaluation sessions. Therefore on average it can be deduced that the participants achieved more than twenty six successful injections out of the thirty total injections. Based on observation, participants spent less than two hours to complete the entire evaluation process comprising a total of fifty injections for the five evaluation sessions, i.e. two training and three evaluation. On average this can be considered as less than 2.4min taken per injection which is considered a very reasonable amount of time given the nature of the task.

For this study, participants used only the keyboard control in all evaluation sessions. As such based on the promising results obtained above, it is suggested the keyboard control is a viable, low-cost and straightforward
method to be used in micro-robotic cell injection procedure. On the other hand, study of participants’ performance when using haptic device control is considered later in this section. Without haptic guidance, the haptic device is suggested to be utilised as an intuitive method for controlling the micropipette where the user manipulates the stylus as if holding a handheld needle for insertion. This can enhance users’ spatial awareness and understanding of the movements in the three-dimensional space. In addition, integration with appropriate image processing and force sensor may potentially provide physically guided micro-robotic cell injection procedure where the cell contact force and augmented VFs can be rendered haptically to user as proposed in earlier work by other researchers [5, 21, 29, 95, 97].

Success rates are derived from a binary measure of whether participants inserted the tip of the micropipette into the cell without multiple penetrations or exited the cell after penetration. Further investigation of participants’ performance can be achieved by considering the magnitude of error, as determined by the distance between micropipette tip’s final position and the cell centre. The magnitude of error is a parameter which represents a measure of intensity between the two scores. For example $E = 0.1$ is twice as accurate than $E = 0.05$. The average rates of $E$ for all sessions are depicted by Figure 5.3.
Figure 5.3: Mean magnitude of error of the three sessions, PT, PT1 and PT2 for the keyboard and haptic device groups

Figure 5.3 shows that, in a similar manner to the keyboard group’s success rates, the magnitude of error did not continuously improve across the sessions. The magnitude of error for the keyboard group was 0.53μm for PT, then increasing to 0.65μm in PT1 then decreasing to 0.53μm. Given that the magnitude of error for the last post-training session, PT2 was the same as the pre-training session, PT this suggests no significant improvement in accuracy occurred. The difference in accuracy across PT, PT1 and PT2 is 0.12μm, corresponding to 6% of the diameter of the virtual cell, and approximately 3 key presses incrementing the position of the virtual micropipette. Considering that a key press for a given axis corresponds to 0.05μm movement of the virtual micropipette, in order to perform injection on average participants need to perform in the order of 208 incremental key presses. Given that 3 out of 208 key presses is quite minor, it is suggested that despite no significant improvement across the sessions, participants achieved very high precision.
This may be partially attributed to the fact that distinct key presses corresponds to incremental movement of the micro-robot and it is possible for participants to adopt a press and observe approach, less likely to result in undesired commanded motion.

Unlike the keyboard group, the haptic device group did demonstrate continuous improvement across all sessions. The magnitude of error for pre-training by the haptic device group was lower than that of the keyboard group, however participants then demonstrated significant magnitude of error reduction in PT1 and PT2. The magnitude of error declined from 0.89\(\mu m\) in PT to 0.57\(\mu m\) and 0.56\(\mu m\) in PT1 and PT2 respectively. It is interesting to note that the observed reduction in magnitude of error, i.e. improved accuracy, from PT1 to PT2 was despite a reduction in success rate (Table 5.1 and Figure 5.3). This would suggest that despite the drop in success rate PT2, participants did manage to stop closer to the cell centre than in PT1 meaning they are closer to achieving a successful injection.

The reduction in magnitude of error of 0.01\(\mu m\) between PT1 and PT2, corresponds to 0.5% of the diameter of the virtual cell and would correspond to less than 1 key press if incrementing the position of the micropipette using the keyboard control method. Given this relatively small distance, with respect to both the cell diameter (0.5%) and the minimum distance commandable using the keyboard control method, this suggests high accuracy was achieved using the haptic control method.
From Table 5.2, it can also observed that the standard deviation in both PT1 and PT2 for the haptic device group decreased from $1.50\mu m$ in PT to $0.28\mu m$ and $0.30\mu m$ respectively. The reduction of standard deviation can be interpreted as more consistent accuracy for the session. Meanwhile for the keyboard group, the standard deviation for both post-training, PT1 and PT2 increased compared to the results in PT. This decrease in consistency even despite the fact that this group had more chance to practice, correlated to the two previously discussed parameters, success rate and magnitude of error, which showed inconsistent performance of the group. However despite the inconsistent performance, it was observed that the success rate, accuracy and consistency of the keyboard group did improve from PT1 to PT2. Therefore it is can be suggested that the participants in the keyboard group should be given more training on the keyboard control to produce more significant outcome. Meanwhile, the haptic device group showed significantly more consistent performance after the training sessions which is an important finding to prove the benefit of using the Phantom Omni as an input controller.

Table 5.2: Standard deviation for keyboard and haptic device group in PT, PT1 and PT2

<table>
<thead>
<tr>
<th>Group</th>
<th>Standard Deviation of Error ((\mu m))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PT</td>
</tr>
<tr>
<td>Keyboard</td>
<td>0.28</td>
</tr>
<tr>
<td>Haptic device</td>
<td>1.50</td>
</tr>
</tbody>
</table>
On the hand, the overall magnitude of error for the keyboard group is 0.57\(\mu m\) lower than 0.67\(\mu m\) for the haptic device group (see Table 5.1). The overall accuracy performance is likely to also depend on some other influences such as participants’ demographical background, e.g. experience, education, etc. This can be observed when considering the mean accuracy of each participant where the five highest scoring participants across both groups have significant previous experience in digital graphics such as computer aided design (CAD), animation, image editing, etc. Given this, and because this chapter focuses on the introduced tools for training and performance improvements, overall accuracy is not the primary parameter to be considered.

The boxplots in Figure 5.4 and Figure 5.5 were used to compare the variation in the scores, i.e. the magnitude of error, and from the data, six scores were determined as outliers by the following equation

\[
MO = Q3 + 1.5IQR \\
EO = Q3 + 3IQR \\
IQR = Q3 - Q1 
\]

where \(MO\) and \(EO\) denote mild and extreme outliers respectively, and \(Q1\) and \(Q3\) denote quartile 1 and quartile 3 respectively.

Outliers are usually indicated as a dot or cross in the boxplot diagram, however to avoid large scaling of the axes in this analysis the six outliers were shown in a separate table. The outliers occurred in a session of each group, i.e. PT2 of the keyboard group and PT of the haptic device group. In this analysis,
the outliers could resulted from human error. Extreme outliers can happen when the participant presses the deposition button (F key) extremely far from the ideal deposition target, the cell centre. Based on careful observation, there was no deliberate keypress of the button for deposition made by any the participants which could cause an extreme outlier. Based on that, it can be assumed that the extreme outliers occurred unintentionally. Mild outliers however are small in value and likely to occur due to participants’ action and, therefore are included in the analysis as failed attempts.

Figure 5.4: Magnitude of error distribution for keyboard group
From the boxplot, it was observed that the median value for all injections in the keyboard group are of comparable values (PT=0.53μm, PT1=0.52μm & PT2=0.52μm), indicating that approximately 50% of all the injections scored below those median values. Likewise, the haptic device group also has similar median values for all sessions (PT1=0.54μm, PT1=0.55μm & PT2=0.54μm). Despite the score being lower than that of the keyboard groups’ score, positive performance improvement can be observed when considering Q3 of each session. The Q3 specifies that 75% of the injections had scored below its value. In the haptic device group, the Q3 levels for PT, PT1 and PT3 were 0.84μm, 0.80μm and 0.77μm respectively. This
improvement provides more compelling evidence of the effectiveness of using haptic device as a training tool for micro-robotic cell injection along with the success rate and magnitude of error improvement discussed earlier in this subsection.

It was observed that both groups recorded comparable median values for all sessions ranging from 0.52 to 0.55\(\mu\text{m}\). Since the range is below 1\(\mu\text{m}\), it can be concluded that at least half of the attempts made by the participants are successful. Moreover, the trend of performance improvement demonstrated by the haptic device group throughout the sessions indicates that the haptic training can produce better training outcome than the keyboard training.

From the boxplots it can be clearly observed that inconsistency in performance exists in the keyboard group across all sessions. This once again aligns with the previous analyses such as success rate, magnitude of error and consistency.

The performance factor, \(P_F\) was derived from the recorded data of error and time to compare the performance among the participants in more detail than using success rate alone. The completion time and accuracy (as opposed to error) are two main parameters to be considered in evaluating cell injection performance. Figure 5.6 below depicts the mean \(P_F\) of each session categorised by group.
From the chart it can be observed that both groups exhibited significant improvement over the sessions. The keyboard group which has the higher $P_F$ for all sessions scored 0.08 in the PT session. The score then increased to 0.13 and 0.21 in PT1 and PT2 respectively. While for the haptic device group, the score of the pre-training session, PT, was 0.06. After undergoing training with the haptic device with guidance disabled the score increased to 0.10 in PT1. Finally in PT2 which was recorded after training with the haptic device with guidance enabled, the score increased further to 0.13. Based on these results it is suggested that both groups demonstrated positive performance improvement after undergoing the training. Presumably the higher performance increase demonstrated by the keyboard group is due to the participants’ increasingly familiarity with the system over time. This was considered in Chapter 4 which discussed how participants intuitively learned how to control the keyboard better over time and achieved performance improvement. The haptic device group however used only the keyboard control method during the three
evaluation sessions, PT, PT1 and PT2, and haptic device control method in the training between those sessions. Interestingly despite less time with the haptic device, than the keyboard group had with the keyboard, the haptic device group still showed significant performance improvement over the sessions.

It is interesting to acknowledge that some of the participants discovered the ability to use multiple axes simultaneously in order to command the micropipette motion in 3D space. The main advantage arising from this use of multiple axes of motion is that it can reduce the completion time by progressing to the desired location quicker and with shorter distance. Given that in reality operators may need to perform many injection processes in a given sitting, the reduction in completion time may contribute to reduction in fatigue which can lead to error and stress as well as increasing throughput.

5.3.2 Performance of Input Control Methods

As mentioned earlier, the second evaluation primarily focused on studying the performance of the participants during the training sessions. The second evaluation discussed in this subsection only considers the haptic device group which underwent training using both input control methods. The sessions considered for the evaluation were the PT, T1 and T2. In the PT session the participants perform an initial test using keyboard control. Even though there are two other sessions, i.e. PT1 and PT2, which used the keyboard control, the PT session was selected to eliminate familiarisation factor since it was the first session undertaken by the participants. For T1 and T2, participants underwent
a training session using haptic device with guidance disabled and enabled respectively.

Figure 5.7 shows the success rates of each participant during the training sessions. In PT, there were four participants which achieved 100% success rates, two participants achieved 90%, two participants achieved 80% and another two participants each achieved 70% and 60% success rates. In T1 two participants achieved 100%, two participants achieved 90%, one participant each achieved 80% and 70%, two participants achieved 60% and one participant each achieved 40% and 20%.

![Figure 5.7: Participants' success rate during training](image)

It can also be observed that five of the ten participants achieved the same success rates as in their PT session where two of them achieved maximum performance level of 100% success in all sessions. There are however four participants which demonstrated poor performance in T1 compared to PT with differences ranging from 20 to 80%. The significant number of participants
who achieved lower success rates in T1 is consistent with the qualitative data obtained where the participants felt that the T1 was the most difficult session to perform. According to the participants the main difficulty encountered was the sensitivity of the haptic device stylus where even slight movement or tremor results in movement of the micropipette. Despite this difficulty however, all the participants, based on the interview conducted during the experiments, agreed that the haptic device stylus did help in improving participants’ spatial awareness and understanding of the micropipette orientation in three-dimensional space which can be otherwise difficult to grasp, and is known as a challenge in training in the task.

Interestingly, it can be directly observed from the chart that all the participants achieved 100% in the PT2 session. This suggests that the haptic feedback in the form of VFs is beneficial in guiding the participants to achieve successful injections. Additionally the proposed haptic guidance can potentially later incorporate feedback of cell injection forces once reliable and practical sensors become available.

In terms of error, all the participants reached the ideal target, i.e. the cell centre, $C$ during session T2. Recall that the magnitude of error is measured as the distance between the final position of the virtual micropipette, $F$, and the centre of the cell, $C$. Therefore the injection error of all the participants for T2 session is zero since $F$ was the same as $C$ for all injections in the session. This occurred because in the guidance enabled mode, the participant is given haptic
guidance in the form of several VFs and what this indicates is that despite the
device only able to exert a maximum of $3.3N$ of force, this is adequate to
guide users as to desired motion. This is a particularly valuable result,
indicating that the relatively small amount of maximum force displayable,
equivalent to $0.33kg$, from this commonly obtainable and low-cost haptic
device is sufficient to guide the participants. It is also important to recall that
the VFs, discussed earlier in Chapter 3, are intended as guidance where the
user is able to override the VFs if deemed appropriate. This support for the
human in the loop control of the micropipette’s motion allows the user to
remain in complete control and able to execute independent decisions but still
able to benefits from the haptic VF indicating the likely correct paths.

![Figure 5.8: Magnitude of error for keyboard and 3-DOF training sessions](image)

Figure 5.8: Magnitude of error for keyboard and 3-DOF training sessions

Figure 5.8 depicts the magnitude of error of the participants in PT and
T1. Note that the score for the T2 is not included in the chart since all the
participants achieved zero error in the session. Results show that only one
participant scored lower magnitude of error in T1 compared to PT with
The other nine participants however demonstrated higher magnitude of error in T1 with differences range between 0.07 and 0.97 μm. These results show that 90% of the participants demonstrated lower accuracy performance when using the haptic device with guidance disabled compared to using the keyboard.

The lower accuracy performance of the T1 group discussed above shows that participants achieved lower performance when using the haptic device with guidance disabled. The results also aligns with the information gathered from the interview conducted during the experiments where all the participants indicated that they faced difficulties when performing the injections using the haptic device control method with haptic guidance disabled. User’s training using the haptic device with guidance disabled demonstrated significant improvement over the sessions. As such, although the haptic device with guidance disabled is more difficult to use, it is suggested as an effective training tool which can provide more intuitive way of handling and better understanding of the micropipette’s movement and orientation in the three-dimensional workspace.

It is interesting to observe that despite lower accuracy performance of the participants when using the haptic device with guidance disabled in T1, as discussed in Subsection 5.3.1 the outcome of the training still showed significant improvement better than of the keyboard group. Therefore it is suggested that although the haptic device with guidance disabled is more
difficult to use for performing cell injection, it is suggested as beneficial to be used as an effective training tool which can provide more intuitive way of handling and better understanding of the micropipette’s movement and orientation in the three-dimensional workspace. Therefore it is suggested that aside from the benefits mentioned above, with the utilisation of haptic guidance, the haptic device control method also leads to an improved training outcome in terms of accuracy and success rate.

Table 5.4 summarises the results reported in Subsections 5.3.1 and 5.3.2 and the next section presents conclusions based on the results.

Table 5.4: Summary of results

<table>
<thead>
<tr>
<th>Performance Improvement</th>
<th>Table/Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter: Success rate, S</strong></td>
<td>Table 5.1, Figure 5.2</td>
</tr>
<tr>
<td>- keyboard group – inconsistent performance</td>
<td></td>
</tr>
<tr>
<td>- haptic device group – improved performance</td>
<td></td>
</tr>
<tr>
<td>- overall – better performance of the haptic device group</td>
<td></td>
</tr>
<tr>
<td><strong>Parameter: Magnitude of error, E</strong></td>
<td>Table 5.1, Figure 5.3</td>
</tr>
<tr>
<td>- keyboard group – inconsistent performance</td>
<td></td>
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<tr>
<td>- haptic device group – improved performance</td>
<td></td>
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<tr>
<td>- overall – better performance of the keyboard group</td>
<td></td>
</tr>
<tr>
<td><strong>Parameter: Consistency (standard deviation)</strong></td>
<td>Table 5.2</td>
</tr>
<tr>
<td>- keyboard training – no better consistency</td>
<td></td>
</tr>
<tr>
<td>- haptic device training – better consistency</td>
<td></td>
</tr>
<tr>
<td><strong>Parameter: Score variation (boxplot)</strong></td>
<td>Figure 5.4</td>
</tr>
<tr>
<td>- keyboard group – inconsistent performance</td>
<td></td>
</tr>
<tr>
<td>- stylus group – improved performance</td>
<td></td>
</tr>
<tr>
<td>- both groups – comparable median values for all sessions</td>
<td></td>
</tr>
<tr>
<td><strong>Parameter: Performance factor, P_F</strong></td>
<td>Figure 5.6</td>
</tr>
<tr>
<td>- both groups – improved performance</td>
<td></td>
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<tr>
<td>- overall – better performance of the keyboard group</td>
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</table>

<table>
<thead>
<tr>
<th>Control Methods’ Performance</th>
<th>Figure 5.7</th>
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</thead>
<tbody>
<tr>
<td><strong>Parameter: Success rate, S</strong></td>
<td></td>
</tr>
<tr>
<td>- keyboard – good performance</td>
<td></td>
</tr>
<tr>
<td>- no guidance haptic device – worst</td>
<td></td>
</tr>
<tr>
<td>- with guidance haptic device – best</td>
<td></td>
</tr>
<tr>
<td><strong>Parameter: Magnitude of error, E</strong></td>
<td>Figure 5.8</td>
</tr>
<tr>
<td>- keyboard – good performance</td>
<td></td>
</tr>
<tr>
<td>- no guidance haptic device – worst</td>
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<tr>
<td>- with guidance haptic device – best</td>
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</tbody>
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5.4 Conclusions

The findings presented in this chapter indicate that the utilisation of haptic device which provides an intuitive control method for VR micro-robotic cell injection leads to performance improvement of the participants, especially in improving the success and accuracy of the injections. It was also learnt that the participants achieved more consistent performance after undergoing training using the intuitive control method.

The intuitive control method can enhance participants’ understanding of micropipette’s position and orientation which is very important when performing the micro-robotic cell injection procedure, especially when only the top view, as obtained from the microscope, is available. From the feedback of the participants, it was learnt that the main difficulties faced by them in order to obtain an efficient trajectory and accuracy are the lack of spatial awareness which is the ability to be aware oneself in space which also involves understanding the relationships between objects when there is a change of position. Given the two-dimensional view provided to the bio-operator through the microscope and the three-dimensional manipulation of the micropipette, it is extremely challenging task to estimate depth of the micropipette. Additionally, the projection of the micropipette is hard to be imagined since it actually tilted at 25 degrees which is visible as a straight line from the microscope. As such it is an essential advantage of the intuitive control method which can assist to improve spatial awareness among
participants which is an important skill for estimation of distance and depth of
the micropipette in relative to other objects around it.

Additionally, the haptic guidance offered with the intuitive control
method can serve as an efficient training tool for bio-operators. The three VFs
provided are useful in assisting them to achieved appropriate trajectory and
reaching the ideal penetration and deposition point. The gradually repulsive
force provided when the bio-operator attempts to penetrate the cell membrane
with the sudden drop of the force once penetrated can assist them to estimate
the injection force to be applied. It is suggested that after sufficient training the
bio-operator’s dependency to the haptic guidance will reduce and the skill
obtain can be transferred to the real procedures.

However there were also concerns raised by five participants regarding
the force feedback provided especially for the cell’s response to injections.
The force feedback was felt as if the cell is too stiff to be penetrated requiring
the participants to apply excessive force which is difficult to arrest once the
cell membrane penetrated. If the momentum of the micropipette is not
immediately arrested once penetrating the cell membrane overshooting can
occur and this will jeopardise the injection success. As such this issue was
taken into consideration in later chapter, the large-scale VR micro-robotic cell
injection system, introduced in this thesis.

Considering the participants’ performance of using different input
control methods, it was obvious that the participants achieved perfect results in
terms of success rates and magnitude of error when using the haptic device with the haptic guidance enabled. It is suggested that this can be a good justification to focus on similar haptically-enabled physical cell injection system.

It is also worth mentioning that although the keyboard control showed inferior results, it is suggested that this input control to be used as a benchmark to study the training progress based on its similarity to the existing manual cell injection setup. It also has other main advantages compared to other input methods such as robust, low-cost, simple, widely used and easily learned.

The next chapter presents a large-scale VR training system for micro-robotic cell injection which provides large set of displays and utilising a more sophisticated large workspace input control method is presented.
Chapter 6  Large-scale  Haptic  Device  Control Method

Previous chapters discussed the development and evaluation of a desktop-sized VR system for micro-robotic cell injection training. The VR system provides a portable, low-cost and flexible training approach providing either a computer keyboard or haptic device to control the micropipette for cell injection. The computer keyboard is a robust and ubiquitous interface while the haptic device, Phantom Omni, provides an economical, portable and intuitive way of interacting with the virtual environment. In Chapter 3 the large-scale VR training system for micro-robotic cell injection was introduced. The system was developed utilising the state-of-the-art facilities available in the CADET VR Lab at Deakin University, Australia. The setup consists of three large 3.2m wide and 2.4m tall screens able to be configured to three different display configurations, 2D, 3D and CAVE-like. A cable-driven haptic device, INCA 6D was utilised as the input controller for the large-scale VR training system.

This chapter aims to evaluate the effectiveness of the different display configurations and the input control method employing the INCA 6D device. The first section presents an overview of different kind of haptic devices currently available or being developed. This can provide insight and reference
for developing application specific VR skill training systems such as the proposed large-scale VR micro-robotic cell injection training system. The subsequent sections present the evaluation of the large-scale VR training system for micro-robotic cell injection based on the experiments conducted.

6.1 Introduction

Haptic devices are mechatronic systems enabling haptic interaction with a human user. The choice of haptic devices and hardware is a critical consideration to the effectiveness of a haptically-enabled training system. A complete survey of all available haptic devices extends beyond the scope of this thesis, rather herein devices relevant to the development of a haptically-enabled micro-robotic cell injection training system are discussed. For a wider reaching survey of haptic devices in general, the following works are a good starting point: Hayward et al. [212, 213], Biggs and Srinivasan [214], Stone [215] and Fisch et al. [216].

Haptic devices can be categorised into two types: ground-based or body-based [217]. Ground-based devices refer to those where they are attached to the ground or a point in the environment, e.g. a desk or wall. Ground-based devices have the ability to have their mass supported by the ground, and being able to provide grounded forces to the user. Devices in this category include passive devices (without force feedback) such as computer mice, joysticks, steering wheels and flight yokes, and active devices (force reflecting), such as the commercially available Geomagic Touch (previously Phantom Omni)
range of devices [160]. Given their nature, ground-based devices are inherently limited to a restricted working area.

Researchers have proposed approaches aiming to enhance the capabilities of common grounded Commercial-off-the-Shelf (COTS) haptic devices. One such work is the low-cost 5-DOF haptic interface presented by Isaksson et al. [158]. The interface employs two Phantom Omni devices, which each offers 6-DOF positional sensing and 3-DOF force feedback, to provide a low-cost 5-DOF haptic interface while maintaining the Phantom Omni’s stylus interaction. A similar approach was introduced by Shah et al. [162] which uses two Novint Falcon devices to build a very low-cost 5-DOF haptic wand. Both approaches provide the user with 3-DOF Cartesian forces and 2-DOF pitch and yaw torques. While COTS haptic devices offering 5-DOF or more are available, it should be acknowledged that they can be expensive and potentially cost prohibitive for the proposed virtual training system.

There are also researchers who proposed approaches modifying the functionality of commercial haptic devices to better suit specific applications. One example is the Reconfigurable Multipurpose Haptic Interface [156] introduced in Chapter 3 providing a low-cost mobile platform and four kinematic configurations achieved by using two Phantom Omnis and customised detachable end-effectors. Apart from desktop VR micro-robotic cell injection platform introduced in Chapter 3 of this thesis, there are some of
the applications which have already utilised this interface such as the above mentioned 5-DOF haptic stylus [158] and the multi-point haptic grasping [218]. Further details of this interface can be found in Chapter 3 of this thesis.

Body-based devices are attached to and supported by the human body. Examples include gloves, suits and exoskeletons which generate haptic sensations. Some well-known commercially available devices are CyberTouch [219] glove which provides vibrotactile feedback to the palm and fingers, the HapticGEAR [220] worn like a backpack and the WearableMaster [221] which is mounted on the user’s forearm. Unlike ground-based devices, these provide a theoretically large workspace. This however comes at the cost of the user needing to sometimes support bulky and heavy hardware. This can be especially troublesome if the user needs to use the device for a prolonged period of time. The INCA 6D by Haption [171] utilised in this study falls into the body-based devices category. The style of device was inspired by Sato [172] and the user holds the handle of the device linked to eight actuators, each of which are driven through a mechanical cable, providing up to 6-DOF force feedback. The specifics of the INCA 6D haptic device are discussed in Chapter 3.

6.2 Large-scale Haptic VR User Training Evaluation

Chapter 3 introduced the large-scale VR micro-robotic cell injection system. The system provides a large display of the virtual environment and a large workspace haptic device to be used as the input controller. The large display
provided can be reconfigured to a variety of configurations, three of which are used in this evaluation, i.e. 2D, 3D and CAVE-like. The three configuration each present a different level of immersion and presence. The cable-driven INCA 6D haptic device provides large workspace manipulation with or without haptic feedback. When enabled, the haptic guidance provides the user with VFs and force feedback to provide physical assistance during injection. When the haptic guidance is disabled, the device can be used as a 3D input control device for commanding the micropipette where VFs and force feedback are not provided. However in both haptic guidance modes, the INCA 6D is set up to provide a locking force around the roll, pitch and yaw axes to prevent rotations. This means that the input controller is haptically locked to $x, y, z$ axes regardless of the haptic guidance mode provided. The haptic lock was designed in order to replicate the real micro-robotic cell injection setup where the angle of the micropipette is not usually adjusted during an injection, rather it is pre-set based on suitability. In the large-scale VR system introduced herein the micropipette is fixed to a 30 degree angle, as shown in Figure 6.1, which is assumed as suitable for this particular application.
Haptic lock is provided in the roll, pitch and yaw axes to constraint micropipette movement in x, y, z axes.

This section considers evaluation of the effectiveness of the large-scale VR system as a training tool for micro-robotic cell injection. Data for the evaluation were gathered through a set of experiments with human participants conducted at the CADET VR Lab, Deakin University where participants’ performance improvement against metrics such as success rate and magnitude of error was considered in the evaluation. The first part of the evaluation considered the success rates and learning curves of six groups of participants, each of which performed injections with a different display configuration and haptic guidance mode combination. The participants’ performance when using each display configuration, 2D, 3D and CAVE-like, were then compared in the second evaluation. To obtain a fair comparison, the six groups were assigned into two clusters. The first cluster comprised the haptic guidance disabled groups, 2DN, 3DN and CAN, and the second cluster the haptic guidance enabled groups, 2DH, 3DH and CAH. It is anticipated that the participants
who are provided with haptic guidance will achieve better performance than those who are not. This is also supported by the results discussed in Chapter 5 where the participants who utilised the haptic device with haptic guidance during injection performed significantly better than other participants who utilised the keyboard and haptic device without haptic guidance. As such the analysis were performed separately for each cluster to distinguish the performance level of participants who were provided with different haptic guidance modes. For example, participants’ performance for the first cluster were only compared to each since they all performed the injections without haptic guidance but each group in the cluster utilised a different display configuration. The third evaluation compared the performance between the haptic guidance enabled group and the haptic guidance disabled group for each display configuration. Therefore, three clusters were formed where each cluster consisted of both haptic guidance enabled and haptic guidance disabled groups for a particular display configuration. The first, second and third clusters consisted of 2DN and 2DH groups, 3DN and 3DH groups, and CAN and CAH groups respectively. Each cluster was considered separately in order to investigate the effects of providing haptic guidance to users as both groups in a cluster were provided with the same display configuration. For example, both groups in the first cluster, 2DN and 2DH, were provided with 2D display configuration and the latter group also provided with haptic guidance during injection. Finally the fourth evaluation considers participants’ performance
improvement after undergoing training with the haptic guidance provided. For the purpose of the fourth evaluation a series of additional injection trials, categorised as training and post-training sessions, were conducted for selected participants as discussed in the next subsection. The magnitude of error metric between the pre-training and post-training sessions was compared to investigate the participants’ performance progress in terms of accuracy.

The evaluations were granted low risk human research ethics approval by the Human Ethics Advisory Group (HEAG), Faculty of Science, Engineering & Built Environment, Deakin University. Eighteen participants (eleven males and seven females) were recruited for the experiments. All participants were screened to ensure that they had no prior exposure to any physical cell injection activity including the experiments discussed in previous chapters. This was in order to obtain a set of participants who have the same entry level experience with the procedure as new people being trained in the procedure. Their demographic data were also obtained to be used in the analysis. Participants’ performance across the sessions are discussed in the next section. Participants were video recorded and interviewed during the experiments in order to obtain useful qualitative data.

6.2.1 Experimental Design

As depicted by Figure 6.2, participants were randomly divided into six groups, i.e. 2DN, 2DH, 3DN, 3DH, CAN and CAH. Each group had a specific combination of display configuration and haptic guidance mode, and
comprised three participants. Participants were asked to perform ten injections and the time and position of the virtual micropipette tip were recorded at a sampling rate of 50Hz. The 2DN and 2DH groups performed virtual cell injection using the 9.6m wide and 2.4m tall two-dimensional display with haptic guidance disabled and enabled respectively. Likewise, the 3DN and 3DH groups also performed injections with haptic guidance disabled and enabled respectively, however with the large display providing a three-dimensional view of the environment. The participants in the CAN and CAH groups were provided with three-dimensional display with three different viewpoints of the virtual environment across three of the four large screens in CAVE™ arrangement as shown in Figure 3.10 (Chapter 3). The CAN group performed injections with haptic guidance disabled whereas the CAH group did so with haptic guidance enabled. The premise for the provision of the large visual display is that the large human size visual display will contribute to better understanding of the micropipette’s orientation as well as improving participants’ spatial awareness.

In addition to the ten injections performed by all groups, the participants in the 2DN, 3DN and CAN groups also underwent subsequent training and post-evaluation sessions. To achieve with the purpose of this evaluation the ten injections for the three groups were redefined as a pre-training session. The participants of the three groups then undertook a training session which consisted of an additional ten injections with haptic guidance enabled in the
same display configuration as their pre-training session. Finally, a post-
evaluation session was undertaken where participants performed ten more
injections with haptic guidance disabled. Pre-training, training and post-
training sessions, as the name of the sessions imply, were used to evaluate the
performance of the participants before, during and after the training with
haptic guidance enabled respectively. Doing so provides the basis to evaluate
the effectiveness of the training with haptic guidance.

As was the case for the desktop haptic VR training system discussed in
Chapters Chapter 4 and Chapter 5, in this evaluation two performance metrics
were considered, i.e. magnitude of error, $E$ and success, $S$. As was the case for
the previous two chapters, the success and magnitude of error of an injection
were considered based on the final position of the micropipette tip, $F$. The
final position, $F$, was determined by the participant, through pressing a button
on the haptic device when they believe to have reached the best deposition
point and are ready for deposition. For this evaluation, an injection was
considered successful when $F$ is located inside the cell, indicating that the
participant managed to penetrate the cell membrane and stop inside the cell for
deposition. In an ideal injection, the micropipette tip is positioned at the cell
centre, $C$. As such the error, $E$, was defined as the distance between $F$ and $C$. 
Figure 6.2: Flowchart of the experiments implementation
For the purpose of this evaluation the diameter of the virtual cell was assumed to be $2 \mu m$ which is considered to be a small cell based on the fact that real cell diameters range from 1 to $100 \mu m$ [22]. The relatively small sized virtual cell was chosen intentionally on the basis that smaller cells present a more difficult scenario for the operator and therefore a more valuable study. As such the virtual cell had a radius of $1 \mu m$ and was centred at the origin $(0,0,0)$ of the virtual environment.

An injection is considered successful only when the magnitude of $F$ is less than $1 \mu m$, i.e. the radius of the cell, determined as follows

\[
if |F| = \sqrt{x_F^2 + y_F^2 + z_F^2} < 1 ; S = 1
\]

\[
if |F| = \sqrt{x_F^2 + y_F^2 + z_F^2} \geq 1 ; S = 0
\]  (6)

Additionally, once the cell has been penetrated, so as to avoid damage the micropipette should not allowed to be moved (retracted or pushed forward) in any direction beyond the cell membrane. As such, for each injection observation was made to verify that multiple penetrations in any direction had not occurred. Aside from direct observation of each injection, the position data were also examined to ensure no multiple penetrations were made during an injection. This is achieved by analysing whether the magnitude of $F$ becomes greater than $1 \mu m$ (outside cell membrane) after it crossed the threshold to be less than $1 \mu m$ (inside cell membrane).
As identified in Chapter 2, accuracy is one of the most important parameters relating the survivability of the injected cell. Herein accuracy is considered as the inverse to error where high accuracy corresponds to low error and visa-versa. Given that an ideal injection is achieved when the micropipette tip ends at point $C$, the error, $E$ is determined by the distance between $F$ and $C$ which can be obtained by the magnitude of $F$ as follows

$$E = |F| = \sqrt{x^2 + y^2 + z^2}$$  \hspace{1cm} (7)

### 6.2.2 Participant Training Results

In order to investigate the usability and effectiveness of the VR training system for micro-robotic cell injection procedure, a set of experiments were conducted. The data collected from the experiments were then analysed through four evaluations. The data analysis was performed to accomplish four major aims. The first was to study the success rate and learning curve of the six groups of participants where each group performed ten injections using different combinations of haptic guidance mode and display configuration. The second aim was to analyse the performance between the 2D, 3D and CAVE-like display configurations for both with haptic guidance enabled and haptic guidance disabled modes (2DN vs 3DN vs CAN and 2DH vs 3DH vs CAH). Next an analysis was carried out to evaluate the performance between the haptic guidance disabled and haptic guidance enabled modes for each display configuration (2DN vs 2DH, 3DN vs 3DH and CAN vs CAH). Finally
the learning effects of the participants after undergoing training with haptic
guidance enabled were investigated by analysing the performance before and
after the training session (2DN_{pre} vs 2DN_{pos}, 3DN_{pre} vs 3DN_{pos} and CAN_{pre} vs
CAN_{pos}).

For the first evaluation a binary completion task parameter was
measured to consider the success rate of every group. The success of an
injection, \( S \) was a binary completion time where it involved a pass/fail option
based on the final position of the micropipette, \( F \). As discussed in Subsection
6.2.1 an injection is considered successful, \( S = 1 \) when the magnitude of \( F \),
\(|F| \) is less than 1μm. The success for every injection in a group were
accumulated to calculate the success rate in terms of percentage as depicted in
Figure 6.3.

![Figure 6.3: Success rate of every group](image)

All six groups demonstrated promising results in terms of their success
rate where four of the six groups, 2DH, 3DH, CAN and CAH achieved 100%.
Meanwhile another two groups, 2DN and 3DN achieved 90\% and 73\% respectively. From the total of thirty injections for each group, the 2DN and 3DN groups achieved twenty seven and twenty two successful injections respectively. Despite not achieving 100\% success rate, the two groups are considered to demonstrate favourable achievement based on the fact that participants in both groups received no haptic guidance and other instruction than initial briefing before the session started. Furthermore, the two success rates can be considered relatively promising results when compared to similar study by other researchers [13] which considered novice participants injecting colour dye into a cell. Injection success in the study was determined if the injected dye remained inside the cell after the micropipette removed from the cell. Unlike the approach in this platform which used the INCA 6D haptic device as an input control method, participants in the study used traditional three axes rotary encoder for controlling the micro-robot and micropipette. The study reported success rates ranged from 37 to 75\% and comparing to the success rates of both groups, the INCA 6D approach suggest promising application. For the haptic guidance enabled groups, 2DH, 3DH and CAH their 100\% achievement mainly because of the provided haptic guidance leads to a very high chance of success and accuracy even for inexperience user, as also discussed in later evaluations. The promising results demonstrated by all the participants suggest the usefulness of the large-scale VR system for the applications such as the micro-robotic cell injection training. One of the main
contributors is the large workspace interaction using the INCA 6D which helps to provide better spatial relationship understanding beneficial in improving participants’ performance. In addition it can also be attributed to the utilisation of large displays which enhance participants’ sense of presence, and support better performance in spatial orientation [169] and 3D virtual navigation tasks [222].

Interestingly, the CAN group who performed injections in CAVE-like display configuration with haptic guidance disabled also achieved 100% along with the three haptic guidance enabled groups. This is a promising results given that the CAN group were not provided with haptic guidance during injections and only provided with visual feedback. The visual feedback provided however consists of a multiple viewpoints of the virtual environment to enhance users’ understanding of the three-dimensional virtual space. For example in 2D and 3D display configurations only the top view of the cell and micropipette, as if looking from the microscope is provided where estimating the depth of the micropipette is one of the biggest challenge. The CAVE-like display configuration however provides two additional viewpoints of the virtual environment along with the top view in order to assist user in understanding the micropipette’s orientation and movements during injection. Given that in real task the bio-operator still able to see the orientation of the micropipette, this display configuration is suggested as the closest imitation of the real environment. Although the bio-operator is not able to see the contact
between the micropipette and the cell in real task, being able to see the micropipette physically can yield significant difference especially in terms of understanding the orientation and estimating the depth of the micropipette.

The success rate provides evidence of the usability of the proposed micro-robotic training system. Apart from the success/fail parameter considered so far, this evaluation investigates how the participants progress across the session by considering their learning curve. For this evaluation each participant’s progress throughout the ten injections undertaken in terms of their magnitude of error, $E$, was projected as shown in Figure 6.4. From the mean curve of all six graphs, it can be observed that 2DN, 3DN, 2DH, 3DH and CAH groups demonstrated a fairly consistent performance trend. It was also observed that the 2DH, 3DH and CAH groups provided with haptic guidance enabled mode in 2D, 3D and CAVE-like display configurations respectively, demonstrated a significantly lower mean $E$ value than all other groups. This provides an early impression that the haptic guidance can provide assistance to user in achieving better accuracy.
Despite a consistent performance levels demonstrated by the five groups, the CAN group otherwise showed a positive learning curves where the mean of $E$ declined steadily across the sessions. The results suggest that the immersion within the three-dimensional virtual environment provided by the CAVE-like display configuration assisted the participants in achieving better accuracy. This is an interesting outcome where the group also showed superior performance level compared to the other two groups for the same haptic
guidance mode, 2DN and 3DN. Although the mean $E$ range for the CAN group was relatively higher than the other three groups, 2DH, 3DH and CAH, given the fact that no haptic guidance provided during injections, this can be interpreted that the immersion provided by the CAVE support better controllability and spatial understanding to participants leading to improved accuracy.

For the haptic guidance enabled mode, the results were very similar for the three groups involved. Given that the participants were provided with haptic guidance during injections the chance for making error was minimum. This can be observed through the relatively low magnitude of error demonstrated by the three groups since the initial evaluation, and maintained throughout the sessions.

The second analysis considers the participants’ performance for each display configuration. In this analysis the magnitude of error mean for each display configuration were compared to each other based on the haptic guidance mode provided (2DN vs 3DN vs CAN, 2DH vs 3DH vs CAH). Table 6.1 shows the overall score of magnitude of error and standard deviation for all groups. For the haptic guidance disabled groups it was observed that CAN demonstrated best performance than the other two groups, 2DN and 3DN. Additionally it was observed that all three participants in CAN were most consistent in their injections across the session. Participants’ consistency is
reflected by the standard deviation of the scores where lower values denote better consistency.

Table 6.1: Overall scores for all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Haptic Guidance Disabled (µm)</th>
<th>Haptic Guidance Enabled (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnitude of Error</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>2DN</td>
<td>0.555216</td>
<td>0.197158</td>
</tr>
<tr>
<td>3DN</td>
<td>1.063526</td>
<td>0.362136</td>
</tr>
<tr>
<td>CAN</td>
<td>0.468659</td>
<td>0.091834</td>
</tr>
</tbody>
</table>

Among all the groups, 3DN has obtained the highest magnitude of error mean which suggests the lowest performance level among all the groups. Based on the individual score it was observed that two of the three participants in the 3DN group achieved the highest magnitude of error mean among all participants. Demographic data of the two participants revealed some obvious similarities between them, such as gender, education background, gaming experience and prior experience with haptic technologies. Both were the only participants with non-technical backgrounds (management and education). Referring to the bottom five participants it was observed that four of them, including the two in 3DN group, rated themselves as non-competent computer gamers with no experience playing 3D computer games and spending less than two hours a week playing any kind of digital game. Therefore it can be concluded that education background and gaming experience may have
correlation with participants’ performance. Meanwhile for the haptic guidance enabled groups it was observed that the 3DH performed better than the other two groups, 2DH and CAH. However despite lowest magnitude of error mean, 3DH actually was the least consistent group compared to the other two while CAH group was the most consistent. The superior standard deviation demonstrated by both CAN and CAH suggests that the CAVE-like display configuration can assist participants to obtain consistent injections regardless of the haptic guidance mode provided. This once again suggests the benefit of having an immersive visual environment to assist the bio-operator in understanding the spatial relationship between the micropipette and cell, i.e. orientation of the micropipette and estimation of depth between the micropipette tip and the cell centre.

It is also demonstrated that the CAVE-like display configuration provides more benefits in the haptic guidance disabled mode where participants showed significantly lower magnitude of error mean than the other groups. Meanwhile for the haptic guidance enabled mode the differences between scores were significantly small in the range of $10^{-5}$ to $10^{-2} \mu m$. Given that the cell diameter was $2 \mu m$ in this evaluation, this range is only around 0.0005% to 0.5% of the cell diameter which represents significant consistency. As discussed in the next evaluation the similar performance level for all the three groups in the haptic guidance enabled mode was as expected
given that the participants were provided with haptic guidance which minimise the chance for error.

In the third evaluation the performance between haptic guidance disabled and enabled groups was considered. The magnitude of error for three pairs of groups were compared based on their display configuration (2DN vs 2DH, 3DN vs 3DH, CAN vs CAH). As is apparent from Table 6.1 all three haptic guidance enabled groups, 2DH, 3DH and CAH, scored significantly lower magnitude of error mean than their counterparts, 2DN, 3DN and CAN respectively. Furthermore results for the third evaluation show superior performance of the haptic guidance enabled groups both in accuracy (inverse of magnitude of error) and consistency (based on standard deviation). Therefore it is suggested that the haptic guidance provided assisted the user in achieving better performance. This is an expected result given the participants were provided with VFIs and force feedback during injections making less chance for error. Therefore in order to investigate the benefits of the haptic guidance provided for training, the fourth evaluation was designed and carried out. In terms of the display configuration, it is found that the CAVE-like provides a valuable method for presenting the virtual environment effectively. It was demonstrated that the CAN group performed better than the other two haptic guidance disabled groups, 2DN and 3DN both in terms of accuracy and consistency. All three haptic guidance enabled groups, 2DH, 3DH and CAH
demonstrated satisfactory performance with significantly very small difference between them.

For the fourth evaluation, two additional sessions were assigned to all three haptic guidance disabled groups, 2DN, 3DN and CAN. The two sessions consisted of consecutive training and post-training session with haptic guidance enabled and disabled respectively, both in the same display configuration with their first session. For example, the 2DN group underwent the training and post-training sessions in 2D display configuration and 3DN group underwent both sessions in 3D display configuration. As such for the purpose of this evaluation, the first session for 2DN, 3DN and CAN groups is considered as pre-training session and the groups’ name generalised according to their display configuration, 2D, 3D and CAVE-like respectively. In all three sessions participants performed ten virtual micro-robotic cell injection procedures.

The results for this evaluation is shown in Table 6.2. Interestingly it can be observed that all three groups, 2D, 3D and CAVE-like demonstrated significant performance improvement after undergoing training with haptic guidance enabled mode. The 2D group demonstrated highest improvement of 27% followed by 3D and CAVE-like groups with 25% and 24% improvement respectively. Overall the participants demonstrated 25% performance improvement in the post-training session.
The final evaluation was conducted to consider the learning effect after undergoing the haptic guidance enabled training. From this evaluation it is shown that all three display configuration groups demonstrated significant improvement from 24 to 27% for their accuracy. Despite different initial performance levels, all three groups then showed significant improvement which suggests that the haptic guidance provides better understanding of the three-dimensional spatial relationship within the virtual environment.

Table 6.2: Results for pre-training, training and post-training for each display configuration

<table>
<thead>
<tr>
<th></th>
<th>Pre-training (μm)</th>
<th>Training (μm)</th>
<th>Post-training (μm)</th>
<th>Improvement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D</td>
<td>0.56</td>
<td>0.34</td>
<td>0.40</td>
<td>27</td>
</tr>
<tr>
<td>3D</td>
<td>1.06</td>
<td>0.32</td>
<td>0.80</td>
<td>25</td>
</tr>
<tr>
<td>CAVE-like</td>
<td>0.47</td>
<td>0.34</td>
<td>0.36</td>
<td>24</td>
</tr>
<tr>
<td>Mean</td>
<td>0.70</td>
<td>0.33</td>
<td>0.52</td>
<td>25</td>
</tr>
</tbody>
</table>

Although the CAVE-like group demonstrated the least performance improvement, as discussed in the first evaluation participants in this group already achieved relatively high performance in the pre-training session. The group then achieved even better performance in post-training where the magnitude of error mean is just 0.02μm higher than their training session where haptic guidance was provided.

6.3 Conclusions

This chapter presents a large-scale VR micro-robotic cell injection training system which utilises three different display configurations and
employs an INCA 6D haptic device for input control. The haptic device can be used either with or without haptic guidance in the form of VFs and force feedback. Haptic guidance provides physical support to user in following the ideal trajectory towards appropriate penetration point at the cell membrane, apply accurate force for penetration and then stopping at the suitable deposition point at the cell centre.

The three display configurations, 2D, 3D and CAVE-like provide immersive graphical representation of the environment each of which is at different level of technological complexity. The 2D display configuration provides large and high quality two-dimensional graphics with wide viewing angle for an increased visibility. The 3D display configuration provides more sense of immersion and depth perception through the presentation of three-dimensional graphics. Lastly, the immersion provided by the CAVE-like display configuration can benefit in improving users’ spatial awareness, ability to estimate depth and to understanding spatial relationships such as objects’ orientation, movement and position in the virtual environment.

Consistent with the findings of the evaluations in previous chapters, correlation between participants’ computer gaming experience and performance was observed. As expected digital games can improve players’ spatial cognition [223], and it is suggested that the skills obtained from the experience such as spatial awareness and visuo-motor coordination were transferrable to the micro-robotic cell injection manipulation.
The usability and training effect of using the INCA 6D as an input control method for the large-scale VR system were considered in this chapter. Results demonstrated that participants achieved significant success rates between 73 to 100% across the experiments demonstrating strong performance levels for the micro-robotic cell injection task. It was also observed that the participants’ accuracy improved between 24 to 27% after undergoing training with haptic guidance enabled mode. The findings of this study indicate that the large-scale VR micro-robotic cell injection training system introduced herein, specifically using a large workspace haptic device, INCA 6D as the input control method can benefit bio-operators, especially to better understand spatial relationship of the virtual environment. It is also suggested that the acquired skills, knowledge and understanding from the virtual training such as the spatial awareness, depth estimation and hand-eye coordination can be transferred into physical micro-robotic cell injection or similar real tasks.
Chapter 7  Conclusions

In this chapter, the contributions and findings of this thesis are summarised and concluded. Then a discussion on future directions and other potential research problems is presented.

Among the challenges of the conventional cell injection training is it usually conducted in a designated location, e.g. laboratory, which only provides limited accessibility. Additionally real cells used in the training are not reusable after an injection attempt. The lengthy training process requires numerous practice attempts performed by trainees, therefore this approach can be costly ultimately. Finally the sophisticated and expensive equipment utilised in the physical micro-robotic cell injection are also vulnerable to damage caused by excessive use and mishandling by inexperienced users during training.

To contribute to surmounting these challenges, this thesis proposed two haptically-enabled VR systems to aid bio-operators in micro-robotic cell injection training. VR has the potential to deliver an effective learning and training environment and provides several advantages over the physical training in terms of flexibility and cost. The immersive VR environment utilised in the systems provides great flexibility where the virtual objects such
as the cell and equipment can be modelled and remodelled according to
requirements. VR can also significantly reduce the cost where virtual
equipment and cells are used to eliminate the risk of damage and real cell cost.
The systems enable intuitive control of virtual micro-robot through mapping to
different input control methods and technologies. The haptic technology
employed as interface in the systems offers significant benefits in assisting in
motor skills training applications such as by providing force feedback to
enhance user’s sense of presence and spatial awareness.

The first is a desktop VR system being a portable, low-cost and flexible
tool for micro-robotic cell injection training which utilises customary personal
computer or laptop peripheral devices. Additionally a complementary
reconfigurable multipurpose haptic interface was also developed to afford
convenience in setting up and mobility. As such the system is ready to be
utilised for micro-robotic cell injection training at any time and location
convenient to users. By providing this great portability and accessibility it is
suggested that the system is capable to significantly reduce the amount of
required training duration. The VR environment displays a replication of a
micro-robotic cell injection setup. It consists of a virtual cell and basic bio-
manipulation equipment including microscope, micro-robot, micropipette, cell
holding dish, etc. The bio-operator is provided with a 3D view of the virtual
environment, and is able to change the viewing angle and zoom in to
concentrate on the areas of interest. To interact with the virtual environment,
the system provides two different input control methods, being the keyboard and Phantom Omni or other haptic devices capable of 3D motion input. Trainee bio-operators using the system have the option to activate either of the two input control methods.

The keyboard control method is designed to provide control of the three axes $(x, y, z)$ as well as the fourth artificial axis $(d)$, which is the combination of any two of the three axes. One set of button pairs is used for controlling each of the three axis and is considered a similar method to that of the MP-285’s rotary encoders. The keyboard control method uses a mapping strategy between the virtual micro-robot and a set of pre-determined numeric/directional keypad button pairs as shown in Figure 4.1 (Chapter 4). The numeric/directional keypad button pairs were chosen based on their accessibility and easy finger reach by the bio-operator. The mapping was realised in such way that the virtual micropipette will move at constant velocity in a corresponding direction in response to a key pressed by the bio-operator. This way the bio-operator can implement gross and fine control by holding down and tapping the key(s) respectively.

It was deduced that the keyboard control can provide a practical, simple and cost effective method to manipulate the virtual micro-robot. Based on these advantages the keyboard control method can be considered a viable alternative to more sophisticated and expensive input controllers. It was also observed that, despite no specific instruction on how to do so, multiple axes
were intuitively controlled suggesting the skills transfer from other keyboard-based applications had occurred. This evaluation also reported a positive relationship between computer gaming experience and participants’ keyboard control strategies when performing injections through multiple axes movements. The main benefit of using multiple axes is that it supports optimised movement by advancing to the target quicker and with shorter distance which can reduce the completion time. Given that in reality operators need to perform substantial amount of injections at a time, the completion time reduction can indirectly minimise fatigue which can lead to error and stress.

It was also suggested that the keyboard control method can be used as the benchmark for comparing participants’ performance improvement after training sessions using different input control methods such as ones using the haptic device. Results from this study suggest significant benefits of keyboard control method. The keyboard group demonstrated performance improvement, in which key parameters such as the magnitude of error and completion time were considered. Based on the results it is also suggested that the keyboard is a viable input controller for micro-robotic cell injection since the participants achieved reasonably high precision using keyboard control. This may be partially attributed to the adoption of a press and observe approach, minimising unwanted commanded movements.

Another way to control the micro-robot is by using the Phantom Omni haptic device as introduced in Chapter 3. Using this input control method the
bio-operator can perform the procedure by moving the stylus of the haptic device to control the virtual micropipette. The position-to-position kinematic mapping framework, as shown in Figure 3.4 (Chapter 3), can provide intuitive control of the virtual micropipette in similar way to handheld needle injection. Aside from facilitating 3D position-to-position mapping and intuitive input control, the Phantom Omni can also provide haptic feedback to the bio-operator. In this system haptic feedback is provided to the bio-operator in form of VFs as guidance of movements, and force feedback to deliver immersive sensation during cell penetration. The haptic guidance is also displayable to the bio-operator if desired.

The main roles of the integrated haptic feedback are to provide guidance and sense of immersion to the bio-operator. In terms of guidance, three VFs of different shapes and functions are provided, i.e. conical VF, axial VF and planar VF, as shown in Figure 3.5 (Chapter 3). The conical VF guides the bio-operator to follow the optimised trajectory towards a suitable penetration point on the cell membrane. Meanwhile the axial VF is provided to guide the bio-operator along the straight line inside the cell, coinciding with the micropipette’s longitudinal axis, towards the deposition target. When the micropipette tip has reached the deposition target, the planar VF will provide haptic force attempting to prevent the bio-operator from penetrating the planar surface and hence exceeding beyond the deposition target location. In addition to the VFs, gradually increasing force feedback is provided during penetration
of the cell membrane. The magnitude of this force is determined based on the Discrete Element Method (DEM) where the spherical cell membrane is divided into discrete particles using a meshing algorithm. Then each particle is linked to its adjacent particles by spring-dashpots. Once the exerted force has exceeded that which would result in rupture of the cell membrane, the micropipette’s tip passes into the virtual biological cell and the force feedback representing penetrating the cell membrane drops immediately.

Training sessions provided using the haptic device have resulted in participants’ performance improvement in terms of success rate, accuracy and consistency. Considering these results, it can be deduced that the haptic device training produces promising outcome and the intuitive control similar to handheld injection needle can promote bio-operator’s spatial awareness especially in estimating relative distance, depth and orientation of the micropipette.

Both participants who underwent training with keyboard and haptic device were considered to have achieved significantly high performance based on the analysis made in Chapter 5. The overall success rates of 88% for keyboard group and 92% for haptic device group suggest that participants using either input control method achieved relatively high overall success rates compared to the study mentioned earlier [13] which reported success rates between 37 to 75% for forty novice participants. As such it is suggested that
both input control methods are useful training tools for the VR micro-robotic cell injection system.

The second system introduced a large-scale micro-robotic cell injection training system providing an immersive virtual environment through three large screens and interacted with INCA 6D haptic device. There were three display configurations presented in this study. The first is a 2D display configuration (Figure 3.10 – Chapter 3) which displays two-dimensional images across the three large screens. As discussed in Chapter 3 for this display configuration the screens were set to CAD wall arrangement which combines the left, centre and right screens to yield a large (9.6m × 2.4m) flat screen. The virtual environment consists of a magnified top view similar to a typical view from a microscope during real injection procedure. Utilising large-scale display offers several benefits such as a more detailed virtual environment can be created and clearly visible to users. The second display configuration, 3D, provides the same viewpoint of the virtual environment to that of the 2D display configuration and also utilises the CAD wall arrangement. However in 3D display configuration, large-scale three-dimensional images is projected across the three screens, instead of two-dimensional images such as provided in the 2D display configuration. The three-dimensional images provide more immersive environment through perception of depth. On the other hand, the third display configuration, CAVE-like provides a three-dimensional display with multiple viewpoints of the
virtual environment across the three large screens. The screens for this display configuration were set to CAVE™ arrangement, however only the centre, left and floor screens were utilised. The centre screen displays a magnified top view of the virtual environment similar to the 2D and 3D display configurations. The other two screens, left and floor, display a viewpoint from behind and side of the micropipette respectively. The CAVE-like display configuration provides a high immersive virtual environment where the real-time display from these three different viewpoints of the virtual environment are projected simultaneously to each of the screens. As opposed to using three desktop screens, the large display utilised in the CAVE-like display configuration provides more sense of presence where users can step on the floor screen as if they are inside the environment. It was observed from this study that the CAVE-like display configuration supports participants’ performance improvement to achieve consistently higher success rate and better accuracy. It is suggested that the improvement can be attributed to the increased spatial and orientation understanding among users when provided with higher immersion by way of the multiple viewpoints of the virtual environment in CAVE arrangement, as opposed to single top view provided in 2D and 3D display configurations where the orientation and estimation of the depth of the micropipette in relation to the cell are more challenging.

From all six groups, each of which performed injections in specific display configuration and haptic guidance mode, the CAN group, which
performed injections in CAVE-like display configuration with haptic guidance disabled, has demonstrated steady improvement learning curve while the other five groups demonstrated fairly consistent results throughout the session. The results suggest that the higher immersion within the three-dimensional environment by way of the multiple viewpoints provided by the CAVE-like display configuration assisted the participants to achieve consistently better accuracy.

It is found that the CAVE provides a promising way of presenting the virtual environment effectively. It was demonstrated that the CAN group performed better than the other two haptic guidance disabled groups, 2DN and 3DN both in terms of accuracy and consistency. While all three haptic guidance enabled groups, 2DH, 3DH and CAH demonstrated decent performance with significantly very small difference between them.

A cable-driven haptic device based on Spidar™ technology, INCA 6D was employed to interact with the virtual environment. The INCA 6D provides up to 6-DOF (3 translational and 3 rotational) force feedback within large workspace. With up to 37.5N force feedback display, INCA 6D is able simulate the handling of heavy objects realistically. A mapping strategy was designed between the INCA 6D and the virtual micropipette with 2mm positional resolution as shown in Figure 3.11 (Chapter 3). As such the virtual cell injection procedure can be performed by holding the haptic device handle to control the virtual micro-robot which holds the micropipette.
Given the mapping strategy implemented to the interface, user is able to experience an intuitive handling of the micropipette as if they are holding the micropipette as opposed to the traditional rotary encoders. When performing the procedure with the INCA 6D user has the options to either enabling or disabling the haptic guidance. In order to replicate real cell injection procedure, the virtual micropipette is fixed to a 30 degree offset (tilted) to x-axis and its movement is limited to the translational x, y, z-axes as shown in Figure 3.11 (Chapter 3). As such to perform an ideal injection user should move the haptic device as straight as possible towards the deposition point.

In order to investigate the usability and effectiveness of the large-scale VR training system for micro-robotic cell injection procedure, experiments using human participants were conducted. The data collected from the experiments were then analysed through four evaluations. The first evaluation considered the learning curve or progress of the participants in each group, after repeated VR micro-robotic cell injection executions. For the haptic guidance enabled mode, the results were very similar for the three groups involved. It was observed that all the three groups achieved relatively low magnitude of error across the sessions, even for the earlier sessions. These results were as expected given that the participants in all three groups were provided with haptic guidance during injections minimising the chance of making mistakes. Additionally, from another evaluation, it was observed that the haptic guidance enabled groups had demonstrated superior performance.
both in accuracy and consistency. These results provide significant indication that the haptic guidance provided can assist users to achieve better performance. The final evaluation was conducted to consider the learning effect after undergoing the haptic guidance enabled training. Results of the evaluation demonstrated significant participants’ accuracy improvement ranging from 24 to 27%. It was also interesting to observe that all three groups showed significant improvement regardless of their initial performance level, which suggest that the haptic guidance provides better understanding and awareness of objects’ position, distance, movement and orientation in the 3D space of the virtual environment.

7.1 Future Work

Based on the work carried out in this thesis several areas of future work are suggested. It is suggested that the keyboard control method is a feasible, low-cost and robust input control method for the VR micro-robotic cell injection system. As such it would be valuable to investigate the implementation of the keyboard control to the physical micro-robotic cell injection setup and consider its efficacy against important metrics such as accuracy, completion time and success rate.

The use of a 5-DOF haptic device providing pitch and yaw torques and 3-DOF Cartesian forces, offers significant potential for a desktop virtual training system for cell injection. The 3-DOF forces can be used to represent the interaction of the injection micropipette in 3D Cartesian space, while the
pitch and yaw torques can represent the micropipette’s orientation. Given the nature of the cell injection process, and that of the micro-robot used to move the micropipette, representing the roll rotation of the micropipette is of lesser importance and given the cost for which the 5-DOF system can be achieved using approaches such as that Isaksson et al. [158] and Shah et al. [162], a 5-DOF solution represents a valuable trade-off when compared with the cost of purchasing a 6-DOF device. As such investigating the utilisation of such low-cost 5-DOF haptic interfaces can be a good prospect for future research endeavours in the area.

Based on participants’ feedback discussed in Chapter 5 the main difficulty encountered when manipulating the haptic device was the sensitivity of the device where even slight movement or tremor affected the micropipette’s movement. As such to increase the efficiency of the input control method, it is suggested that an approach of filtering or methods for reducing the impact of tremors can be incorporated to the system.

It was suggested that spatial awareness is one of the most important abilities to be developed in order to become competent cell bio-operator. Future work can revolve around aiding bio-operators in improving their spatial awareness. The correlation between visual workspace size and bio-operator’s performance can be studied, as well as investigating different potential input control methods.
The proposed haptic guidance can be one of the prospects for future physical micro-robotic cell injection application. Reliable and practical sensors can enable the bio-operator to obtain force feedback of the contact between cell and micropipette providing the sense of immersion and aiding in injection force estimation. Additionally given the availability of appropriate imaging and rendering techniques, the VFs proposed in this work can be useful to be applied to the real world setup.

To develop a comprehensive haptically-enabled cell injection training system which includes cell specific haptic information, further research is recommended for developing a database of different cell biomechanical properties. This includes information relating to factors including types, shapes, sizes, subcellular locations, stiffness, viscosity and elasticity. The database should also include realistic images for visually representing the cells to trainees. The realisation of such a database will enable the training system to call upon information pertaining to a particular cell type as required.

It would greatly benefit the research to have some experts in the micro-robotic cell injection procedure to review the advantages of the systems. In fact, some of the experiments can done on experts in the field obtain their feedback, from practitioner and trainer points of view. Having a group of experts who had prior training on the procedure to go through this VR-based training can provide useful feedback on the comparison and applicability of the techniques to real procedures.
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