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# The cell injury device: A high-throughput platform for traumatic brain injury research

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## HIGHLIGHTS

- Novel, automated system delivers controlled scratch-induced trauma to brain cells.
- High-throughput imaging/analysis provide quantitative measurement of cell migration.
- Scratch-area variation (3.9–8.4%) is improved over manual methods (10.7–19.6%).
- Drug screening: enlarged gap area of migration/proliferation inhibitor UO126 at 72 h.
- Effective, yet inexpensive, method for quantifying the injury response of cells.

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## ABSTRACT

A novel, automated system for delivering controlled scratch-induced trauma to brain cells cultured in multi-well plates was created and characterized. The system is equipped with high-throughput imaging and analysis capabilities, enabling quantitative measurements of cell migration. The scratch-area coefficient of variation of the device was between 3.9% and 8.4%, a significant improvement over traditional manual methods, which provided a scratch-area coefficient of variation of between 10.7% and 19.6%. The device's inexpensive imaging and analysis capabilities were comparable to a well-known system, the Discovery-1 (Molecular Devices), with no significant difference found between the two. When used for drug screening, the gap area of Neuro2a cells after 72 h was significantly larger in samples containing UO126 (20 μM), averaging 0.89 $mm^2 \pm 0.21$ $mm^2$; compared with an average vehicle control gap area of 0.42 $mm^2 \pm 0.1$ $mm^2$. A gradient response could also be detected among samples with increasing UO126 concentrations (0–20 μM), due to decreased migration and/or proliferation of cells into the gap over the time period.

Our device provides an inexpensive method for delivering a standardized, closely controlled pressure/scratch to brain cells cultured in multi-well plates. The system provides more consistent patterns of scratch-induced trauma to cultured cells when compared to traditional methods. This device is an effective platform for quantifying the injury response of cells, and has applications in testing the effectiveness of drugs on cell migration and proliferation which might potentially treat traumatic brain injury.



## 1. Introduction

An estimated 10 million people are affected by Traumatic Brain Injury (TBI) annually, which is becoming one of the major causes of death and disability in the world (Gean and Fischbein, 2010). Currently, no treatment for TBI has proven successful in humans. To develop better drug therapies for TBI there is a need for closely controlled trauma delivery to cultured human brain cells such as astrocytes, neurons, pericytes and meningeal fibroblasts.

### 1.1. Cell migration after traumatic brain injury

Shortly after a brain trauma, meningeal fibroblasts, pericytes and astrocyte cells infiltrate injured regions of the brain. These cells are involved in fibrotic and glial scar formation and are believed to provide immediate mechanical, chemical and electrical

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stability to the surrounding neurons and synaptic connections. The scar tissue remains long-term, physically obstructing the regeneration of neurons and synapses (Silver and Miller, 2004; Kawano et al., 2012) and impeding axon regeneration and remyelination (Fawcett and Asher, 1999), which are necessary for the recovery of cognitive function. Inhibiting fibrotic scar formation shortly after a traumatic brain injury could allow for an improved recovery in the long term (Stichel and Müller, 1998; Stichel et al., 1999a,b) because of increased neuro-, axonal-, and synapto-genesis. Limiting the migration/influx of cells involved in scar formation at the injury site could improve the overall recovery in TBI. Therefore great interest remains in testing migration inhibitors using high throughput in vitro testing to identify compounds with therapeutic potential (Parmentier-Batteur et al., 2011).

### 1.2. Scratch wound assay

Although no completely automated systems exist for the high throughput screening of migration inhibitors, there are well-known protocols for evaluating the migration of cells after a trauma event. The most popular method is the Scratch Wound Assay described by Cory (2011), Hulkower and Herber (2011), Liang et al. (2007), Lim et al. (2007), Wu and Schwartz (1998), Seniuk et al. (1994), and Yu et al. (1993). In this procedure, confluent monolayer cell cultures are scratched with a filtered pipette tip to create an area which is cell-free, thus allowing for the directional migratory response of the remaining cells to be studied within this area. Although the physiological relevance of the wound mechanism of this in vitro model is imperfect, previous literature suggests cell injury may be present and even similar to that seen in TBI. Cells along the border of the injury site often show altered behavior (Lackler et al., 2000) due to being moved, or mechanical damage including membrane rupture, and severing of cell processes. In addition, they are exposed to chemical cues from adjacent dead and dying cells. The migration of cells into the scratch, or Gap Area, can be imaged and analyzed to provide *quantitative* measurements of cell migration over time. Common quantitative methods utilize intensity-thresholding and other post-processing image analysis techniques (Lind et al., 2006). Although the resulting metrics are approximate, having numerical values of the rate of cell migration allows migration experiments to be analyzed quantitatively, and conclusions to be drawn with a known confidence level. For high throughput screening, such quantitative measurements are essential.

The primary disadvantages of the manual scratch wound assay are the variabilities between scratch size, shape, location, and spacing (Hulkower and Herber, 2011). The manual procedure is susceptible to human error and provides a relatively slow throughput. Additionally, the cells are subject to variable (and unknown) forces during this process which might affect cell behavior and migration. An automated system could provide a standardized scratch, delivered with a known and controllable force. The improved repeatability and accuracy of an automated device would enable better detection capabilities for scratch wound assays.

### 1.3. Scratch devices

Although the majority of published studies perform scratch assays manually (using a pipette tip) there are few devices that exist to facilitate this process. These range from simple 'comb-like' devices to expensive microscope-based automated systems. Peira (Belgium) makes two varieties of manually driven comb-like devices which contain multiple tips to induce a number of scratches with each stroke. While economical, these manually driven devices do not include automated imaging functions for cell migration analysis and the force of the scratch cannot be controlled accurately.

A commercial system which encompasses scratch generation, imaging, and analysis is the LEAP system by Cyntellect (San Diego, CA). This instrument allows for laser induced scratch generation, fluorescent live-cell imaging at high magnification, and fully integrated image processing and analysis. This system was originally designed for "cell culture purification" in which individual cells can be identified, selected, and then removed from the culture by laser ablation. Essen Bioscience (Ann Arbor, MI) makes a live cell imaging system, Incucyte, which can be used for cell migration experiments. However, the scratch generation for this device is performed manually with a hand-tool. 'In-house' devices, such as those used by Yue et al. (2010) and Simpson et al. (2008), have also been developed. To our knowledge no devices are available for automated scratch migration assays which combine scratch generation, imaging, and analysis together in one high-throughput low-cost system.

In this paper we evaluate the advantages of an automated system for delivering a closely controlled pressure/scratch to brain cells cultured in multi-well plates: the Cell Injury Device (CID). Our system is also equipped with high-throughput imaging and analysis capabilities, providing quantitative measurements of cell migration. We quantify the repeatability and effectiveness of our CID system, and compare its performance with scratch assays performed manually with a hand-held scratch tool.

## 2. Materials and methods

### 2.1. Cell injury device (CID)

The Cell Injury Device consists of a four-axis positioning system that manipulates interchangeable plastic indenter tips for the delivery of a scratch to monolayer cell cultures grown in standard tissue culture plates (see Fig. 1(A and B)). The device was custom made by the Biomimetics Lab at the Auckland Bioengineering Institute (Auckland, New Zealand) and can be replicated to order. A control box panel allows the user to select the desired function (scratch, impact, image acquire), along with the appropriate program and setting (number of rows or wells, sequence), and then the device performs functions automatically in a high-throughput manner (see Fig. 1(C and D). The Arduino microprocessor (Italy), programmed in C++, executes functions in a state-machine fashion (e.g. if current condition/state is *this*, machine performs *that)*. A Loadstar Sensors load cell (Fremont, CA) provides real-time force feedback, allowing delivery of a repeatable scratch/injury independent of plate geometry/dimensional inaccuracies. The positioning resolution of each Velmex linear axis (Bloomfield, NY) is 5 μm. Positioning repeatability is limited by micro-switch resolution (<1 mm), located at the ends of travel, which is used to find the zero (or home) position. The range of motion is 250 mm × 150 mm × 100 mm ($L \times W \times H$). The range of applied force is 0.2–10 N; the force transducer accuracy is 0.015%. The maximum stage velocity is 40 mm/s, allowing a 96 well plate to be completed in 7 min. A 2.0 MP Logitech digital camera (Newark, CA) allows for detailed image acquisition, and the USB computer interface saves images automatically according to well location. The open-source software contains a macro for image analysis batch processing which quantifies the Gap Area of the scratch, providing a measurement for migration and/or proliferation.

#### 2.1.1. Cell culture

Mouse neuroblastoma cells (Neuro2A's: ATCC, Rockville, MD; No. CCL 131) were grown in Nunc 96 well plates in MEM-alpha/10%FBS/1%PenStrep/1%SodiumPyruvate at 37 °C/5% $CO_2$. At confluence, the cultures were scratched by the device in a sterility hood and then fixed with 4% PFA for 15 min. Total time to scratch a 96-well plate was approximately 7 min, therefore no extra