Microfluidic Cell Culture System for Live Cell Imaging

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ABSTRACT

We describe a novel micro-fabricated live cell containment device (cellTRAY™) designed specifically for imaging live cells. The device enables the precise containment of cells in an optical glass substrate, the size of a standard 1"x3" microscope slide, comprising a micro-array of thousands of wells for isolating cells (or small groups of cells) for experimentation and imaging. Cells can be observed before and after replication allowing the researcher to monitor the response of cells to other stimuli. The cellTRAY also contains microfluidic channels that allow life support and reagents to be administered to the various wells. Nanopoint has two cellTRAY designs that differ in their ability to actively or passively flow fluid through their microfluidic channels. The active microfluidic cellTRAY has a syringe pump design, which allows users to regulate fluid flow. The cellTRAY is the only active microfluidic device that is mechanically open, but fluidically closed.

Keywords: microfluidic, array, cells

1 INTRODUCTION

Current methods of cell analysis involve living cells cultured in Petri dishes, 96, 384 and 1536 well plates and on microscope slides. The costs of running many experiments in well plates is high due to the amount of cell culture media, the number of cells required and the amount of reagent used. There is a trend moving towards lab-on-a-chip devices, which significantly reduces experimental costs and shrinks the physical footprint of current lab equipment. Nanopoint has developed a high-density cell containment device termed "cellTRAY", that significantly reduces the costs associated with running cellular experiments. Nanopoint is developing new tools for the biomedical market that are closer to the 'lab-on-a-chip' concept. These tools will greatly reduce the cost of running experiments while increasing the capability of cellular experimentation. The combination of the cellTRAY with an optical microscopy platform paves the way for a revolutionary high-resolution imaging system for bio-medical applications.

2 CELLTRAY

2.1 CT-1000

The cellTRAY allows biologists to hold multiple cells in an ordered array. Using a proprietary aperture mask template and an ion etching process, a rectangular array of 'wells' (or depressions) are produced that confine these cells. Figure 1 shows Nanopoint's passive cellTRAY device (termed CT-1000) sitting in a custom designed Petri dish.

Figure 1. Nanopoint's patent pending CT-1000 cellTRAY.

The cell array enables automated processing, simultaneous monitoring and analysis of a large matrix of cells. Cells can be observed before and after replication allowing the researcher to monitor the response of cells to other stimuli. Figure 2 is an image captured using a 10x objective and 100x magnification of a small section of wells contained on the CT-1000 device. Each square well is 200 µm in size.

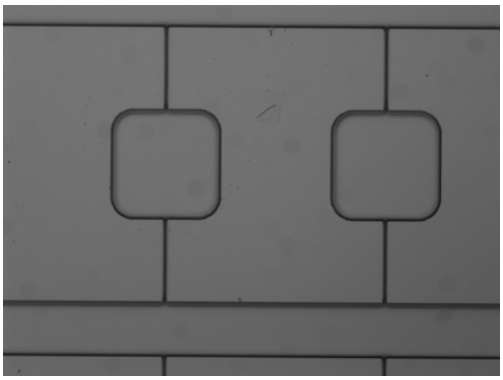


Figure 2. 200µm wells with micro-fluidic channels.

The passive cellTRAY is defined by eight regions (or sections) etched into a glass substrate and each one of these is individually addressable. The wells on the passive cellTRAY are either 200µm or 300µm in diameter and are 20µm deep. There are large "feeder" channels on either side of the eight regions that are 800µm wide with smaller microfluidic conduits leading to each of the wells. The total fluid volume required for the 200µm sized wells is 110 nanoliters and 143nL for the 300µm wells. The passive cellTRAY can be loaded with cells using a standard pipette. Cells are re-suspended in a small volume of cell culture media and dropped along the length of each region. Cells settle to the bottom of the fluid volume and into the wells. Excess cells left on top can be washed off once the cells in wells become adherent to the glass. The cellTRAY is also compatible with an automated spotter, which can more easily address each individual well. For the 200µm well size the cellTRAY contains 1104 wells and for the 300µm size there are 640 wells contained on one cellTRAY.

Nanopoint's proprietary data acquisition software, cellTRAY Manager, is used to calibrate the cellTRAY with an automatic scanning stage mounted to either an inverted or upright microscope (of various brands). The cellTRAY Manager software allows the user to capture an image at every well or designate regions of the cellTRAY to be scanned. The images are automatically stored in a folder that assigns a name matching the region that was scanned. The CT-1000 can be removed at any point and after returning it to the stage the software allows you to do an auto-stage calibration that quickly restores the previous settings.

2.2 CT-2000

Building on the CT-1000 concept, Nanopoint has brought out an active microfluidic device, the CT-2000. The CT-2000 has a glass substrate with etched wells, similar to the CT-1000, but it now has a silicon cover that is anodically bonded to the glass. There are etched through holes in the silicon that match the wells in the glass below.

Figure 3. Nanopoint's patent pending CT-2000 cellTRAY.

The result of this is the CT-2000 is considered a fluidically closed but mechanically open cellTRAY. Due to the opaque nature of silicon the CT-2000 cellTRAY can only be imaged with an inverted microscope. The cellTRAY has 14 regions on it with well sizes ranging from 50µm, 100µm, 200µm and 300µm along the length of the cellTRAY. There are 4 regions of the 50µm size wells, providing a total of 5040 wells. There are 4 regions of the 100µm size wells and 3 regions each of the 200µm and 300µm size wells. The total number of wells on this cellTRAY is 7614. The fluid volumes required for each well on this device are on the picoliter scale, with a total cellTRAY volume of 55.7µL.

A manifold attaches to the CT-2000 that addresses each of the 14 inflow and outflow ports that can be seen along the top and bottom edges of the CT-2000 in figure 3. The cellTRAY sits on a custom made platform that attaches to a commercial scanning stage (Prior Scientific), shown in figure 4. The manifold sits over the cellTRAY while leaving the 14 regions visible from the top. A window is used to enclose the air volume above the cellTRAY for environmental stability.

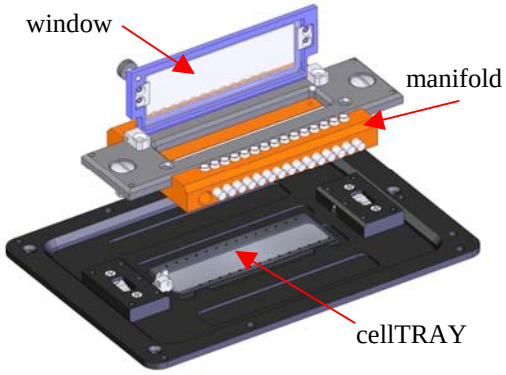


Figure 4. CT-2000 manifold and custom platform.

Figure 5 shows the enclosed CT-2000 cellTRAY as it fits on the microscope platform. The 14 white cylindrical points shown in figures 4 and 5 are the input and output ports connecting relay lines from a microfluidic control box to the cellTRAY.

Figure 5. CT-2000 containment apparatus.

An extra inlet port is included in the manifold, which will be used later for a CO_2 gas line. Fluid is regulated by syringe pumps that lead to and from a fluid reservoir and waste bottle. Each of the 14 regions on the CT-2000 can be individually addressed, allowing different reagents to be administered at each port. The syringe pumps are capable of a variety of flow rates, programmable by the user, with a flow resolution of 20nL/sec. The volume of fluid in a single syringe will cover a four hour experiment, although the volume is automatically replaced from the reservoir, extending the experimental time to overnight.

Figure 6 shows an illustration of the control box in which two fluid bottles can be seen as well as four syringe pumps. Two of the pumps are split to address seven regions each on the cellTRAY (dispense pumps), and two pumps are used to withdraw fluid, again addressing seven regions each, so the total 14 regions on the CT-2000 are utilized.

Figure 6. The CT-2000 microfluidic control box.

Environmental controls will be incorporated into the fluidic box later this year and will include temperature and humidity.

3 SOFTWARE

Nanopoint has developed software (termed cellTRAY Manager) that is used to navigate the cellTRAY by interfacing to a commercially available motorized stage (Prior Scientific). The software allows the researcher to easily and quickly navigate to any of the 7,614 wells on the CT-2000 and the 1104 or 640 wells on the two different CT-1000 cellTRAYs. This includes the ability to move to any x,y and z point in user defined step sizes, plus automated stage scanning along a predefined grid. The user can automatically acquire images and save those images for presentation and analysis by third party software products such as MetaMorph, ImageJ and CellProfiler.

Some of the supported applications include, counting live/dead cells in cultures, measuring different aspects of cellular self-destruct processes (apoptosis), evaluating cell growth cycles, assessing cell protein markers, screening individual cells for genetically tagged fluorescent proteins (GFP, YFP) and performing multiple RNAi (siRNA) experiments and optically monitoring the effects of gene silencing in live cells in real-time. Figure 7 shows the graphical user interface (GUI) for the cellTRAY Manager software. A CCD camera is also controlled through the software allowing researchers to change exposure times and gain.

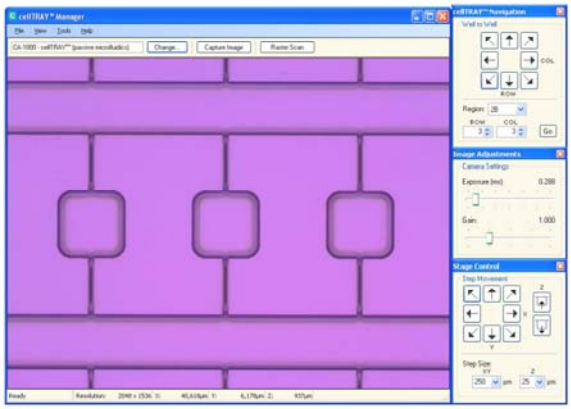


Figure 7. GUI window for cellTRAY Manager Software.

4 CELL EXPERIMENTATION

4.1 Cell Loading

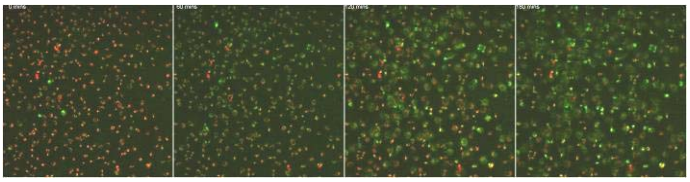
An Olympus IX51 inverted microscope with a motorized stage (Optiscan from Prior Scientific) was used with a 10x and a 40x LWD objective. Cells are loaded manually into the wells of the cellTRAY as described in section 2.1. The design of the CT-2000, which includes the bonded cover with wells left open, mean cells cannot inadvertently settle in the microfluidic channels. However, any cells outside the wells on the CT-1000, which does not have the bonded cover, are ignored by the cellTRAY Manager software which only scans and captures images of cells in wells. Figure 8 shows HEK-293T cells that were loaded into the CT-2000, the well size is 300 µm.

Figure 8. 300 µm size well in the CT-2000 containing HEK-293T cells.

4.2 Cell Viability

To more accurately determine the life expectancy of cells being cultured in the CT-1000 cellTRAY, the mitochondrial potential dye, JC-1 (Invitrogen) was used. JC-1 is a cationic dye that is used as an indicator of mitochondrial potential in cells. In cells with intact mitochondria, JC-1 will accumulate in the mitochondria and exhibit a fluorescence emission shift from green (~525 nm) to red (~600 nm). Mitochondrial depolarization, which usually occurs in dying or apoptotic cells, causes a decrease in the red/green fluorescence intensity ratio. Thus, although most cells will have mitochondria that fluoresce both red and green, healthy cells will exhibit a higher ratio of red/green fluorescence intensity, and dying or dead cells a lower ratio of this intensity.

For these experiments, HT1080 cells were cultured and plated onto CT-1000 cellTRAYs. The HT1080 cells were monitored over several hours and images were collected every two minutes to obtain color fluorescence. Figure 9 shows a four picture montage in which (a) is the start time and the red fluorescence spots indicate many live cells, (b) is an image taken after 60 minutes, (c) after 120 minutes, and (d) after 180 minutes. The progression to an increase in green fluorescence over red shows cells have been compromised and are dead or dying. The cell viability tests showed that the cellTRAY does not seem to compromise cells contained in the wells any sooner than is expected if cells are left in a Petri dish with no cell culture media over several hours. To increase cell lifetime and reduce fluid evaporation a cover slip can also be used with the CT-1000 cellTRAY.



(a) (b) (c) (d)

Figure 9. CT-1000 cellTRAY with HT1080 cells treated with JC-1. (a) 0 mins, (b) 60 mins, (c) 120 mins, and (d) 180 mins.

5 CONCLUSION

The cellTRAY is a micro-scale live cell containment device that creates new standards of precision and levels of efficiency for the study of individual or small groups of isolated live cells. Cells can easily be loaded into the wells of the cellTRAYs and show no signs of adverse reaction to their environment. Because the cell loading procedure is outside the pump and valve system automation is a viable technology to work with the cellTRAY. The cellTRAY allows researchers to work with picoliter volumes and a much smaller number of cells, which significantly reduces cost and is ideal for rare or expensive cell lines.