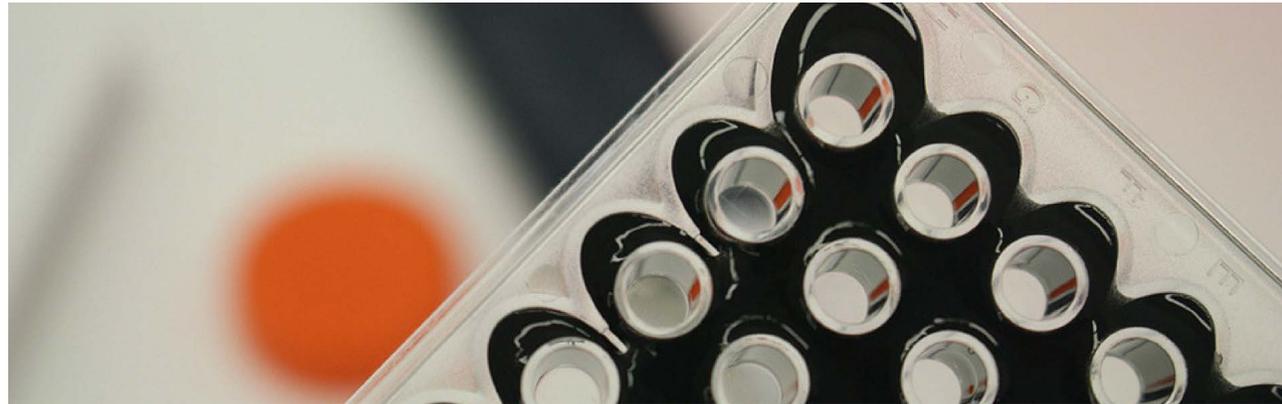


Vale High Performance (VHP) Plates

Patented Anti Edge Effect and Ultra-Miniaturization Technologies



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The Edge Effect problem

Microplates are essential tools for the assay biologist, whether performing biochemical or cell-based assays.

Biochemical and cell-based assays commonly share the issue of edge effect.

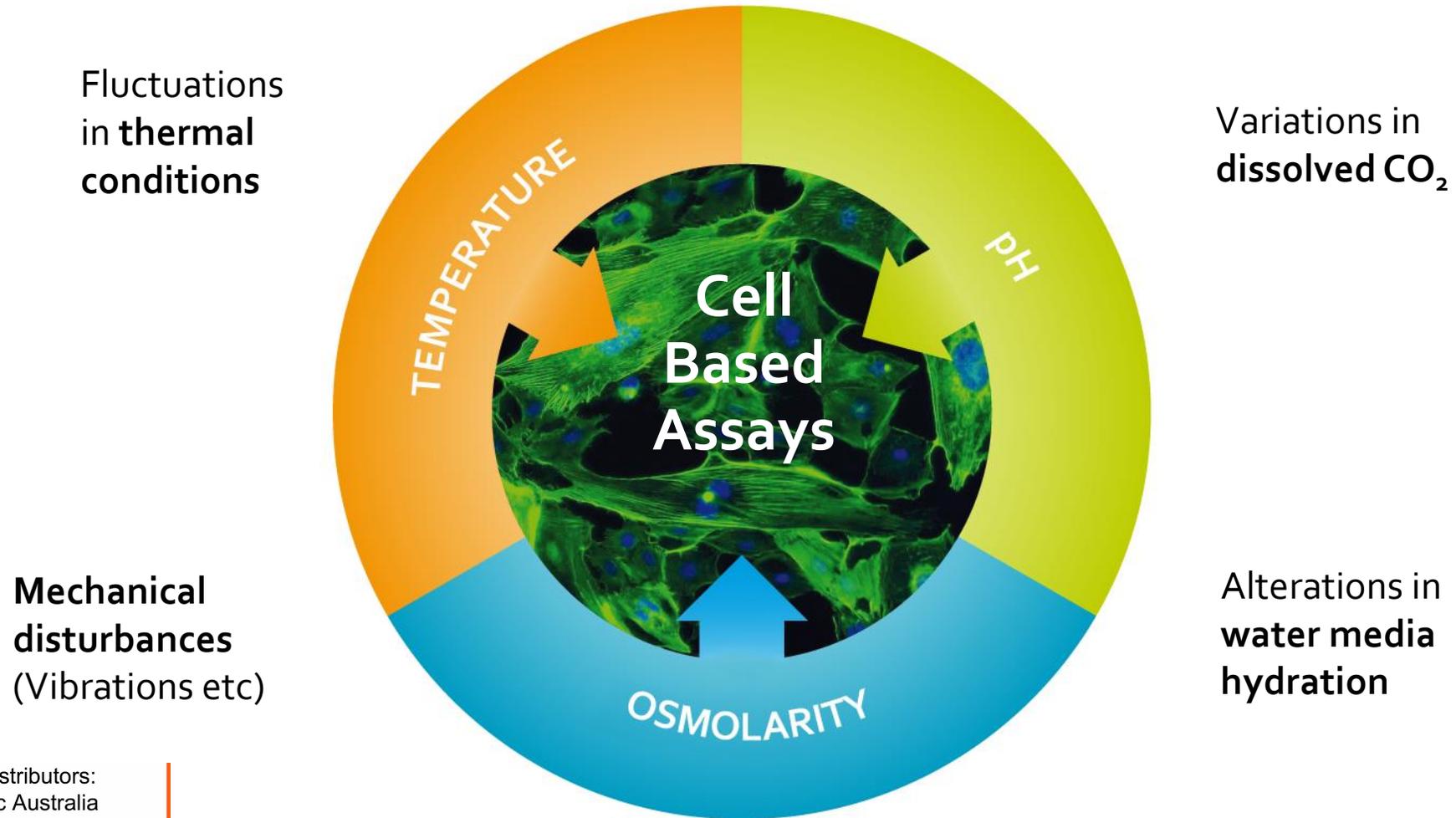
The primary cause for the “edge effect” phenomenon is the fluctuation of the environmental conditions.

All chemical, biological, physiological and physical processes are influenced by environmental elements such as temperature, pH and chemical composition.

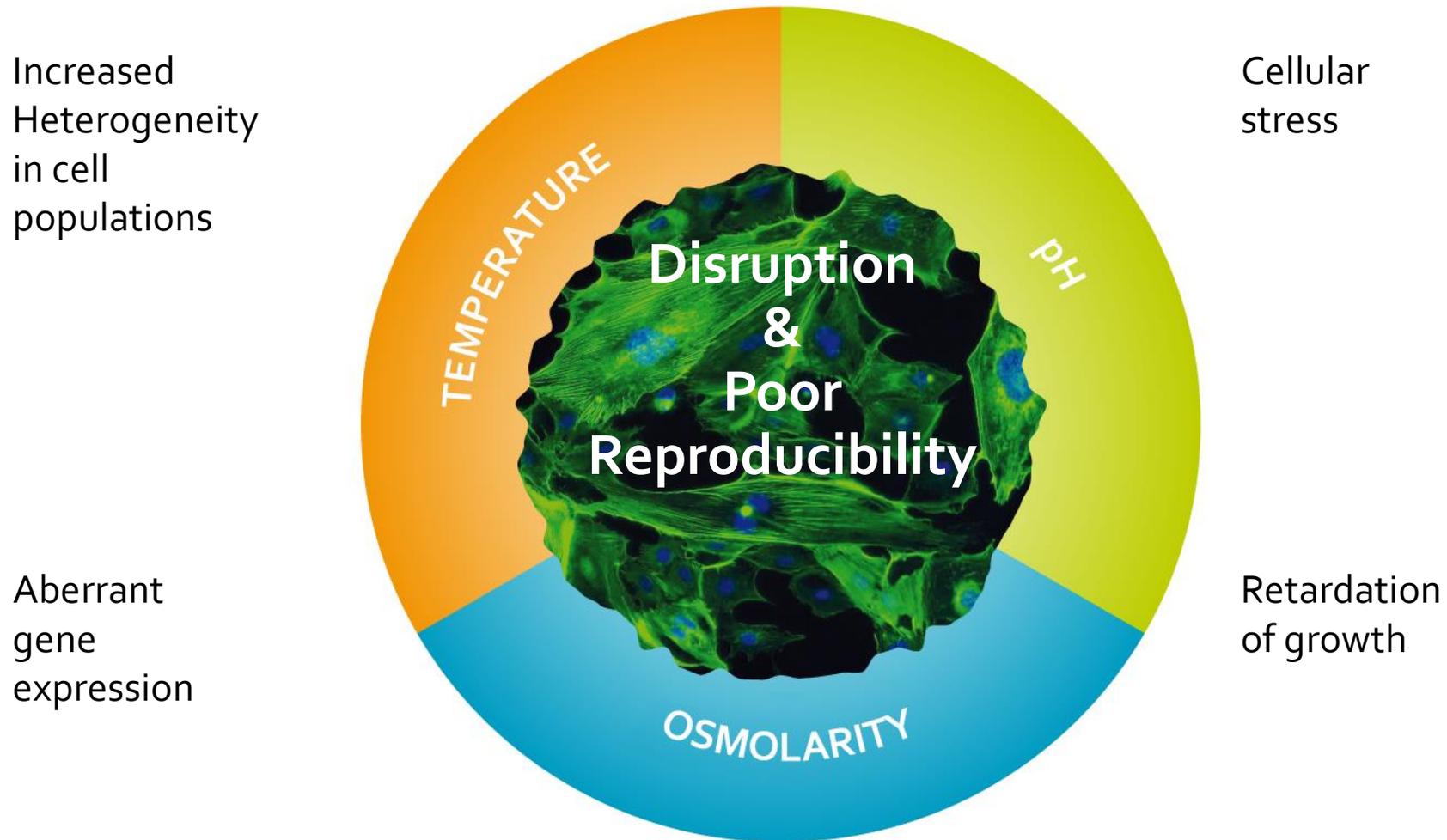
Uncontrolled changes in any of these factors can exert unwanted physical, chemical and/or biological effects on the experimental material leading to poor reproducibility or disruption of a given scientific and/or manufacturing process (figs 1a and b).

The “edge effect” is a discrepancy between the inner and outer wells (local environment), where each well has its own unique environment. Edge effects are commonly seen in high density formats as well volumes decrease as density increases.

The Environmental Factors that Lead to Edge Effect



The Resultant Biological Consequences of Edge Effect



Our solution

An Environmental Gel Buffering Technology Incorporated into the Microplate, that reduces environmental fluctuations by:

- (i) Absorbing heat and releasing via passive thermal gradients
- (ii) Absorbing and releasing environmental and respiratory gasses via passive concentration gradients
- (iii) Preventing Evaporation by maintaining high vapour pressure of water vapour in the microplate.

How our technology works

Vale High Performance Microplates incorporate a patented on-board environmental buffering system that protects the microplate environment from fluctuations in temperature, atmospheric gasses (such as CO₂) and moisture loss by evaporation

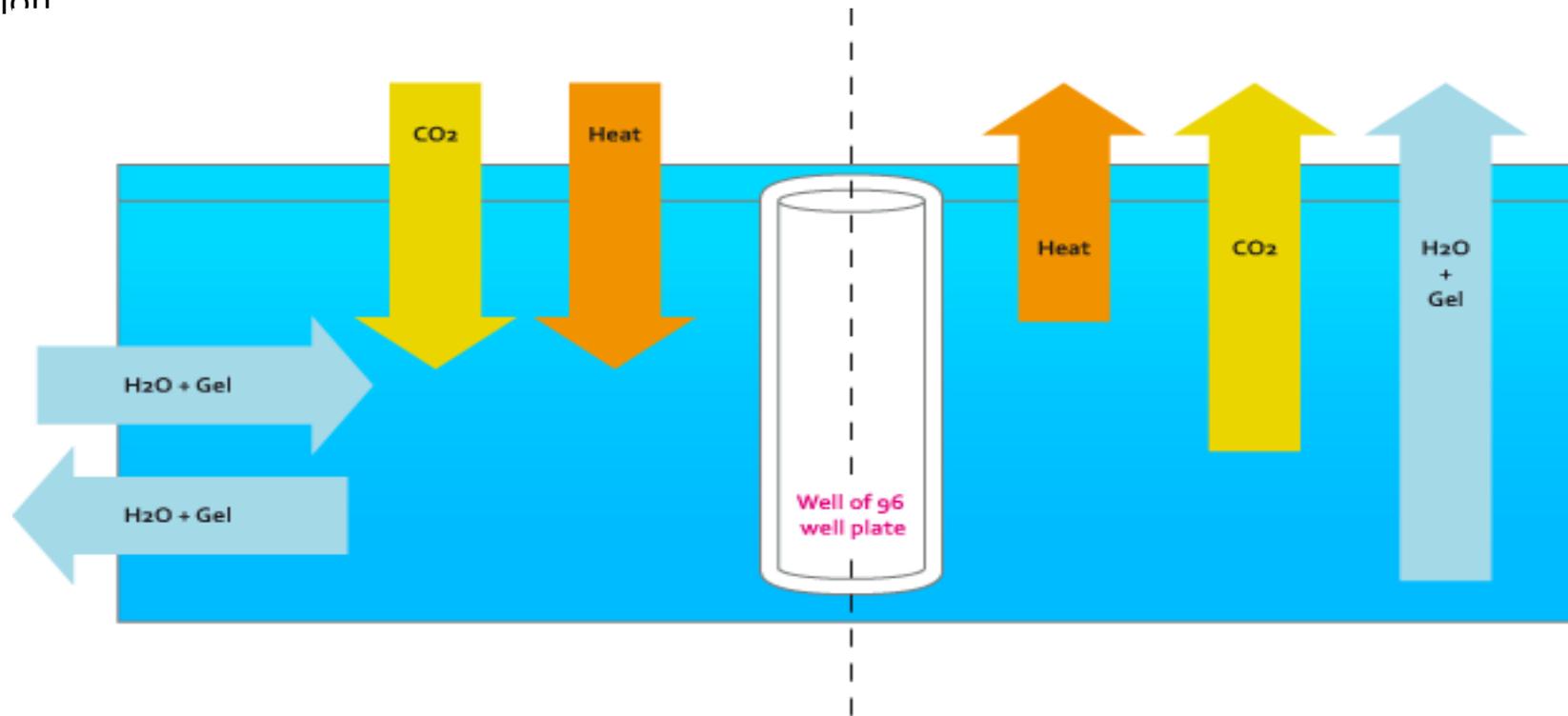


Figure. Showing the mechanism by which the microplate environment is maintained by patented buffering gel technology. When placed in the incubator the buffering gel absorbs heat, CO₂ and the vapor pressure of water reaches equilibrium with that of the incubator atmosphere. When the incubator door is opened, or the plate is removed from the controlled environment, the Buffering Gel releases heat, water and CO₂ down their respective gradients counteracting any changes in the microplate environment.

Cross section Diagram of Multi well bioreactor plate with gel, lid & base

Lid of Microplate

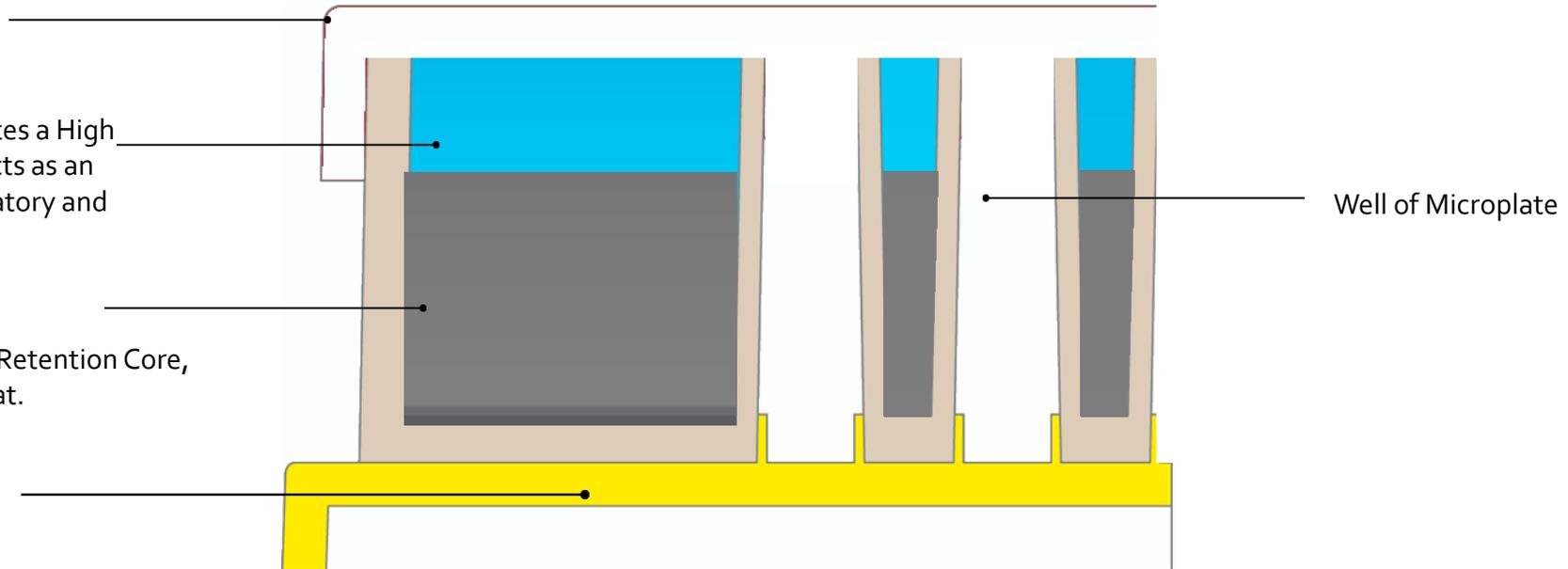
(i) Clear Gel Layer:

This Sacrificial Layer Generates a High Water Vapor Pressure and Acts as an Exchange Surface for Respiratory and Environmental Gasses.

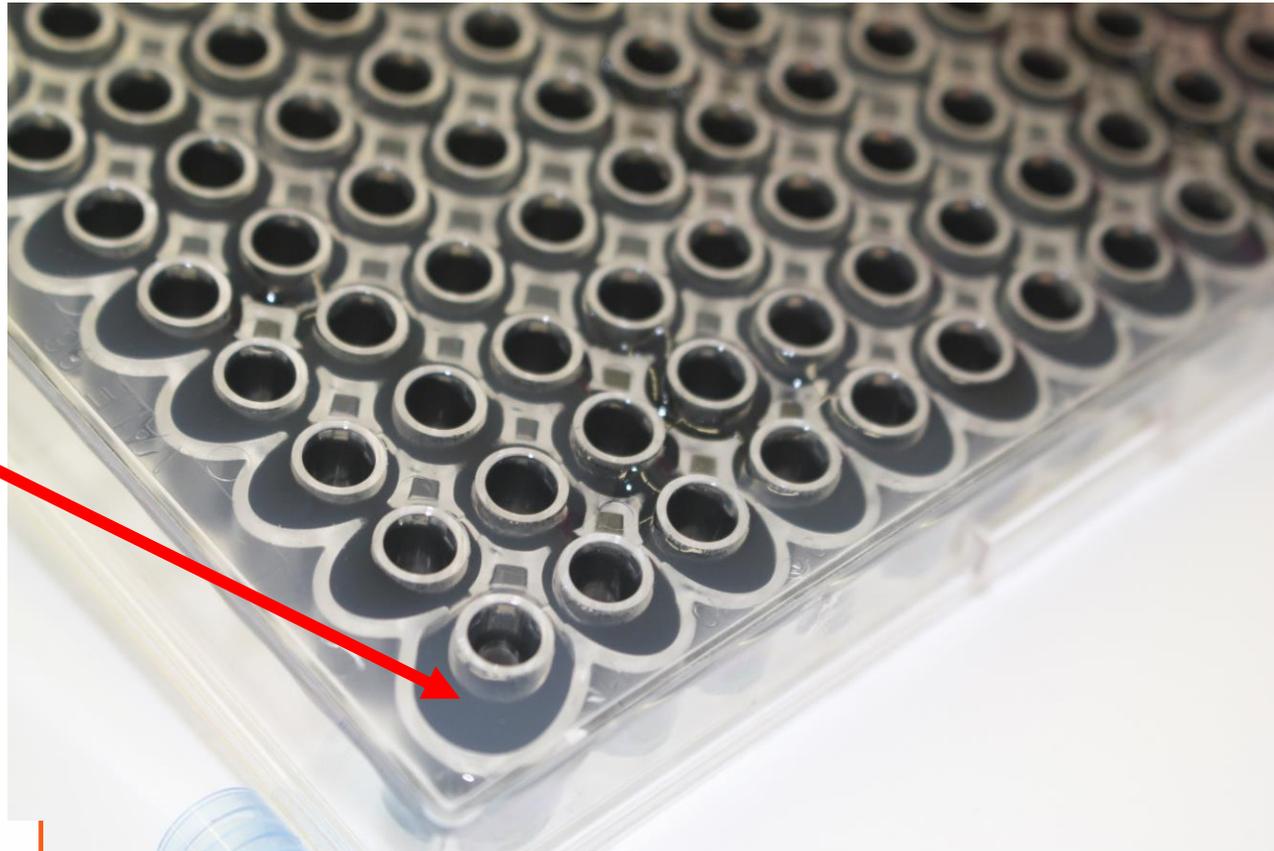
(ii) Dark Gel Layer.

This Layer acts as a Thermal Retention Core, Absorbing and Releasing Heat.

Micro Plate Base

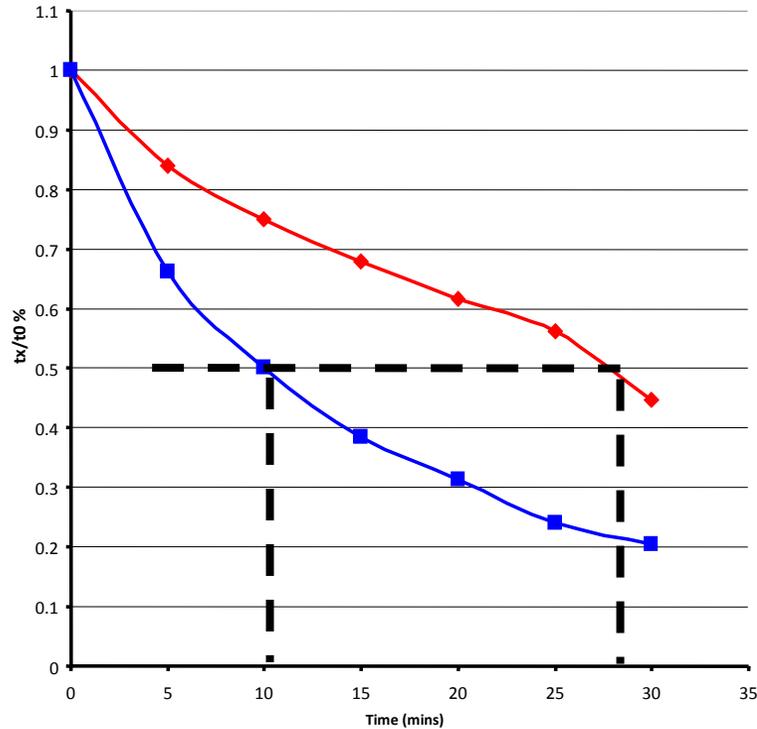


Outer Well Cavity Volumes Fine Tuned To Ensure Even Heating and Cooling Across the entire Plate



Outer cavity/gel receptacle

Thermal Buffering



Cooling rate reduced

Thermal retention ~ 3 X Improved

SBS format plate

Comparison of cooling between normal and Gel Buffered Plate

| | CV (%) | |
|------|----------------|-----------------|
| Time | Non-Bioreactor | Gel- Bioreactor |
| T0 | 0.00 | 0.00 |
| T5 | 7.59 | 6.38 |
| T10 | 13.84 | 6.24 |
| T15 | 18.92 | 8.44 |
| T20 | 22.87 | 10.90 |

Figure. Showing the coefficient of variation of cooling between designated wells in microplates containing Bio-reactor Gel Vs non Bioreactor Gel.

Anti Evaporation

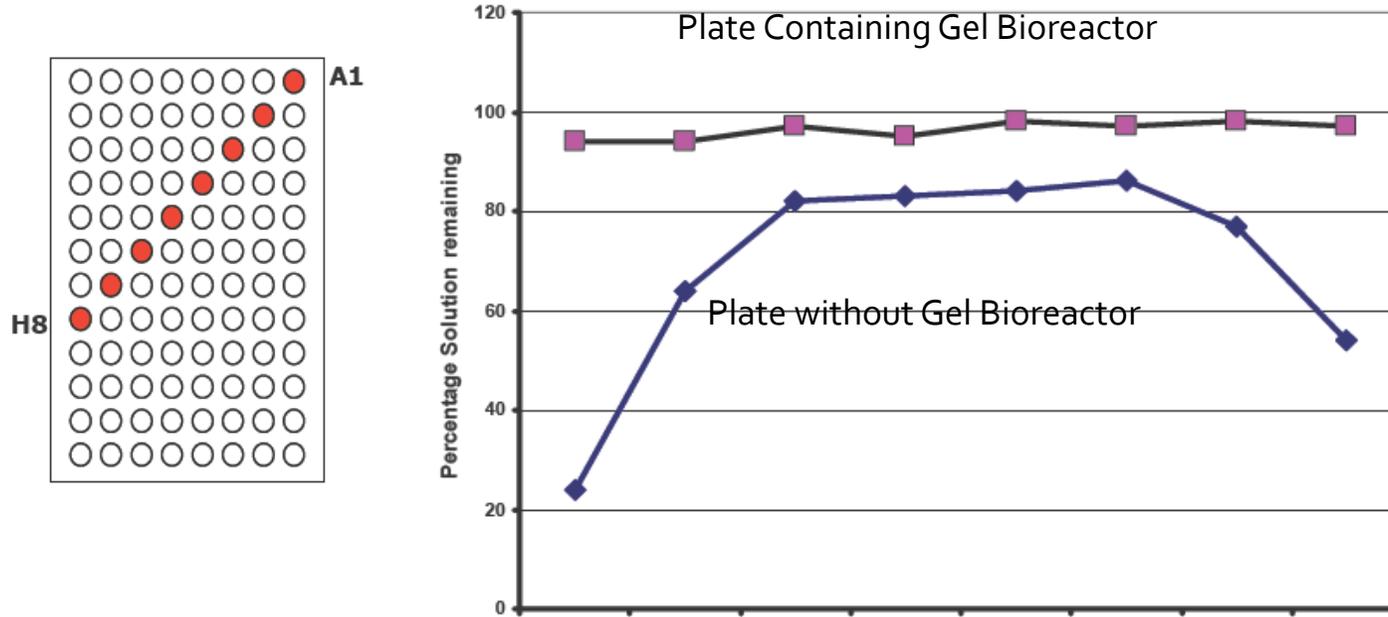


Figure. Showing experiment to assess the anti-evaporative effects of buffering gel Wells of 96 well plates were filled with 140 ul serum free culture medium and then maintained in a standard drying oven set at 50°C for 48 hours. (Data expressed as percentage volume remaining after incubation time.)

(See plate diagram to identify wells used in all experiments).

Improved Cell Growth

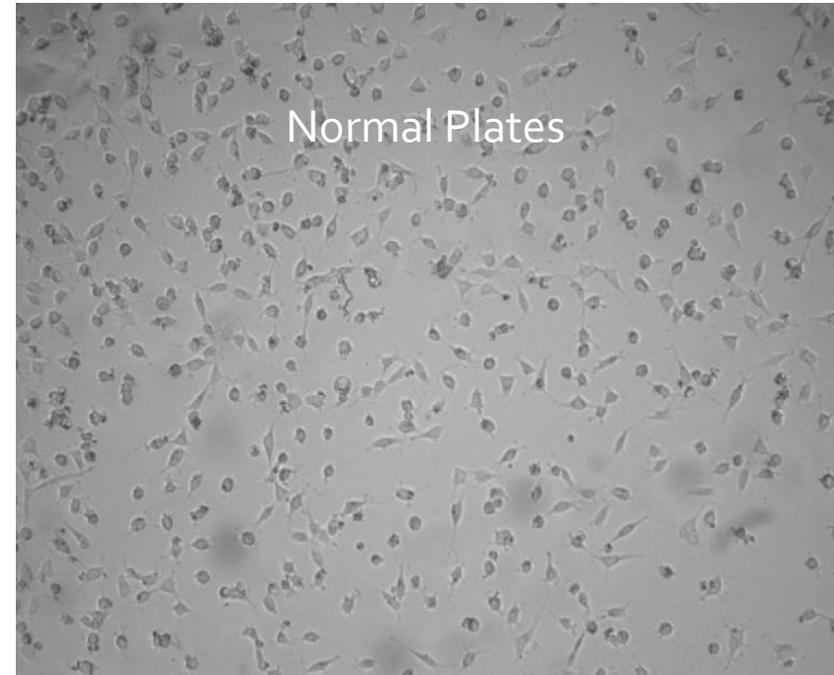
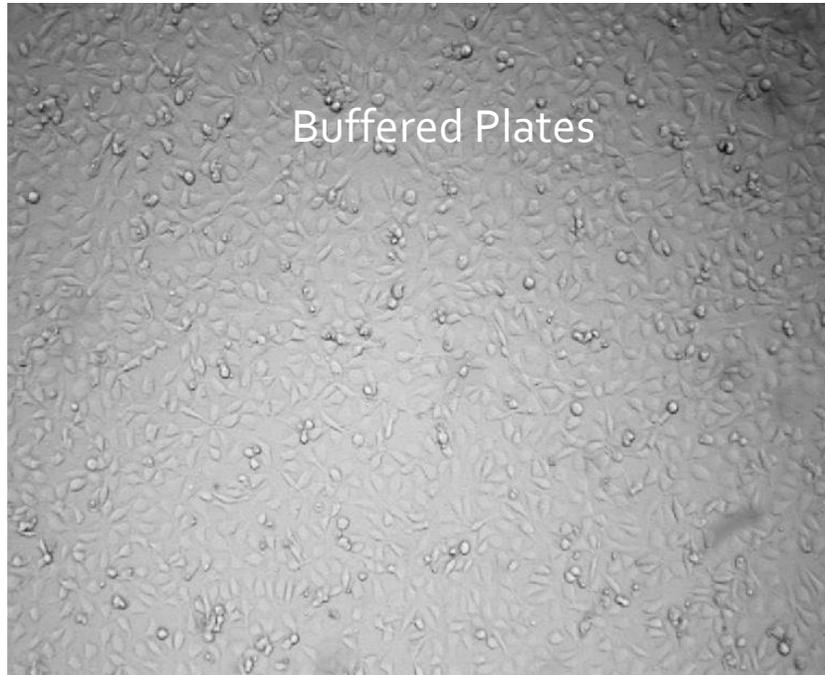
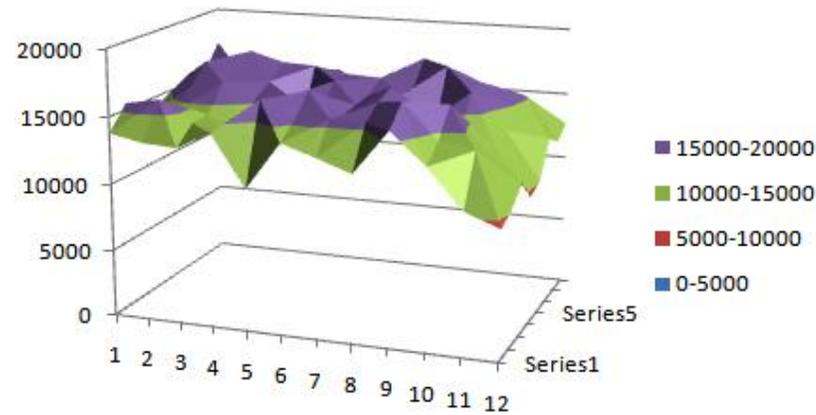


Figure. Showing a comparison of cells seeded into microplates in the presence and absence of gel buffering technology. Wells of buffered and normal 96 well plates were seeded at (equally) low density with cells of immortalised cell line prior to maintenance in a standard tissue culture incubator (set at 37C, 95% air/ 5% CO₂) for 48 hours. Both buffered and normal plates were placed in middle of incubator side by side.

Improved reproducibility of Cell Growth

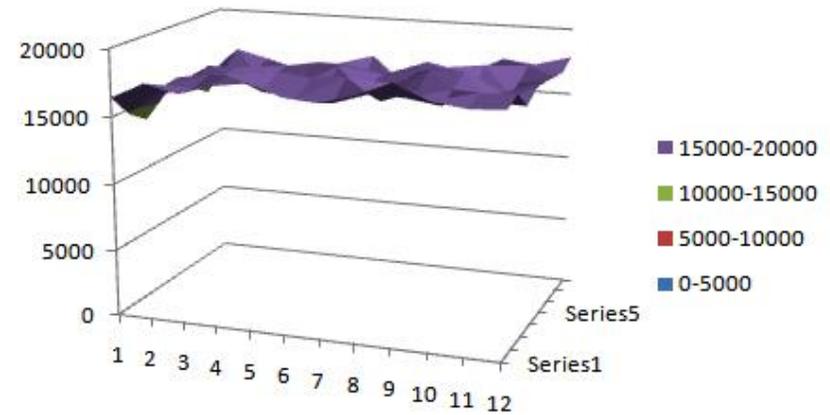
Non-Buffered 96 well plate



14710 (1871)

CV= **12.7%**

Buffered 96 well Plate



16805 (1029)

CV= **6.12%**

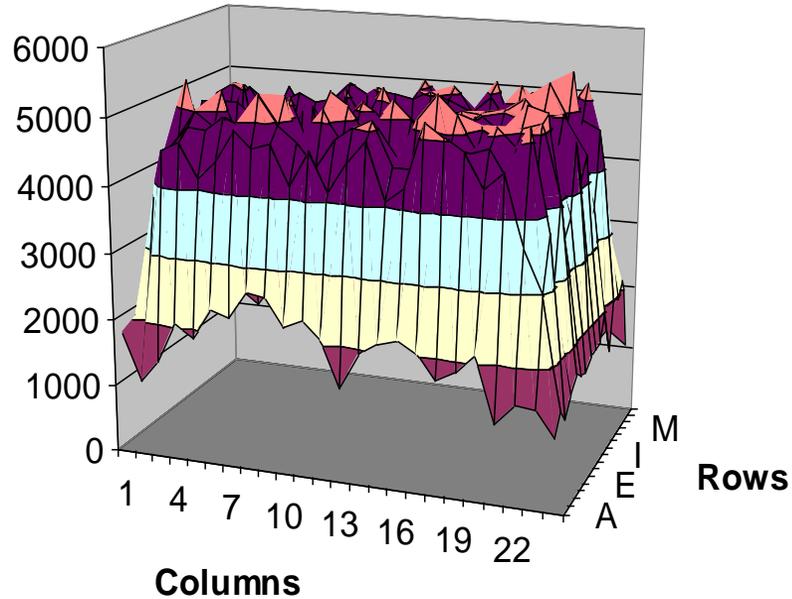
DRASTIC REDUCTION OF
EDGE EFFECTS

FIGURE Showing the consequences of edge effects on cell growth in STD 96 well plates (a) Vs 96 well plates modified with an environmental Gel Buffering System (b) It will be noted that environmentally buffered plates results in a lower coefficient of variation (cv cell number). A549 cells were incubated under standard tissue culture conditions (37°C 5% CO₂ >95% Relative humidity) for 7 days, cells were then stained with the nuclear dye Hoechst and then counted using High Content Analysis

Improved reproducibility of Cell Growth

Cell Number 384

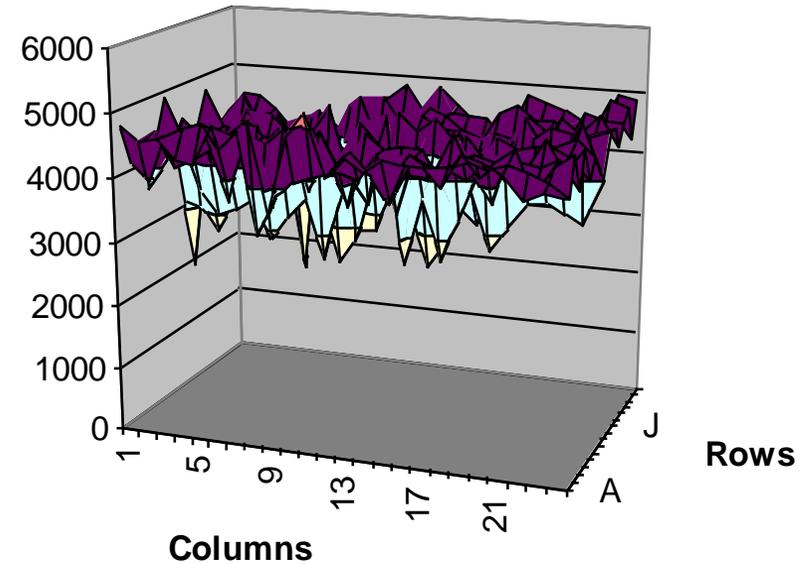
Non-Buffered 384 well plate



CV = **31.7%**

DRASTIC REDUCTION OF
EDGE EFFECTS

Buffered 384 well Plate



CV = **13.4%**

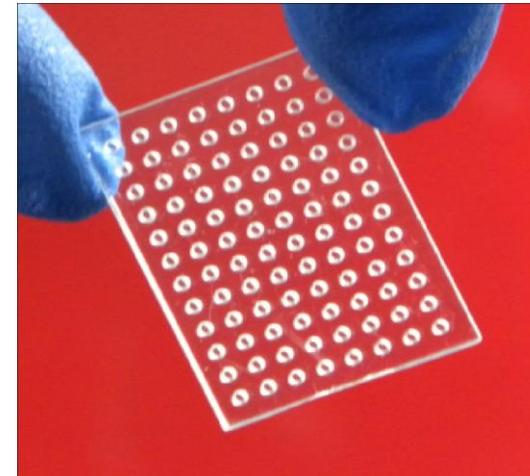
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Miniaturised plate formats

The 192 Nanoslide



Prototype 96



Ultra miniaturization using Vale Nanoslide technology

One of the major limitations of performing large-scale High Content Analysis (HCA) screens is reagent cost.

As well as the obvious financial advantages of reducing assay volumes, we have also identified other key benefits to this approach, namely:

- (i) Higher throughput
- (ii) Suited for the use of valuable cells such as primary cells
- (iii) Reduced storage and research space
- (iv) Improved mixing of reagents.

Despite the availability of a large range of low volume dispensing technologies and ultra-high density micro-plates (1536 and 3072) attempts to perform assays reproducibly even at the micro liter volume ranges has in many cases has been difficult to achieve. Often this is a direct consequence of performing experiments at low volume and the consequential reduced capacity of these experimental systems to buffer against environmental fluctuations such as changes in temperature, relative humidity (resulting in changes in osmolality due to moisture loss through evaporation) and local changes in the partial pressures of atmospheric gasses such as CO₂ (resulting in changes in pH). This poor environmental stability in turn can lead to edge effects in micro-plates resulting in reduced experimental reproducibility.

To address these issues, we have developed a new technology that permits the experimenter to perform cell-based assays in volumes as low as 10 nl.

To compensate for the issues surrounding the environmental stability we have utilized our novel environmental buffering technology.

As already mentioned bioreactor technology that retains heat and CO₂, prevents evaporation, hence reducing environmental fluctuations and hence reducing edge effects and evaporation of ultralow volumes of aqueous liquids. Depending on the needs of the experimenter, we have demonstrated that ultralow experiments can be performed in our VHP 96, 384 and Nano slide technologies.

The 192 VHP Nanoslides with gel buffering

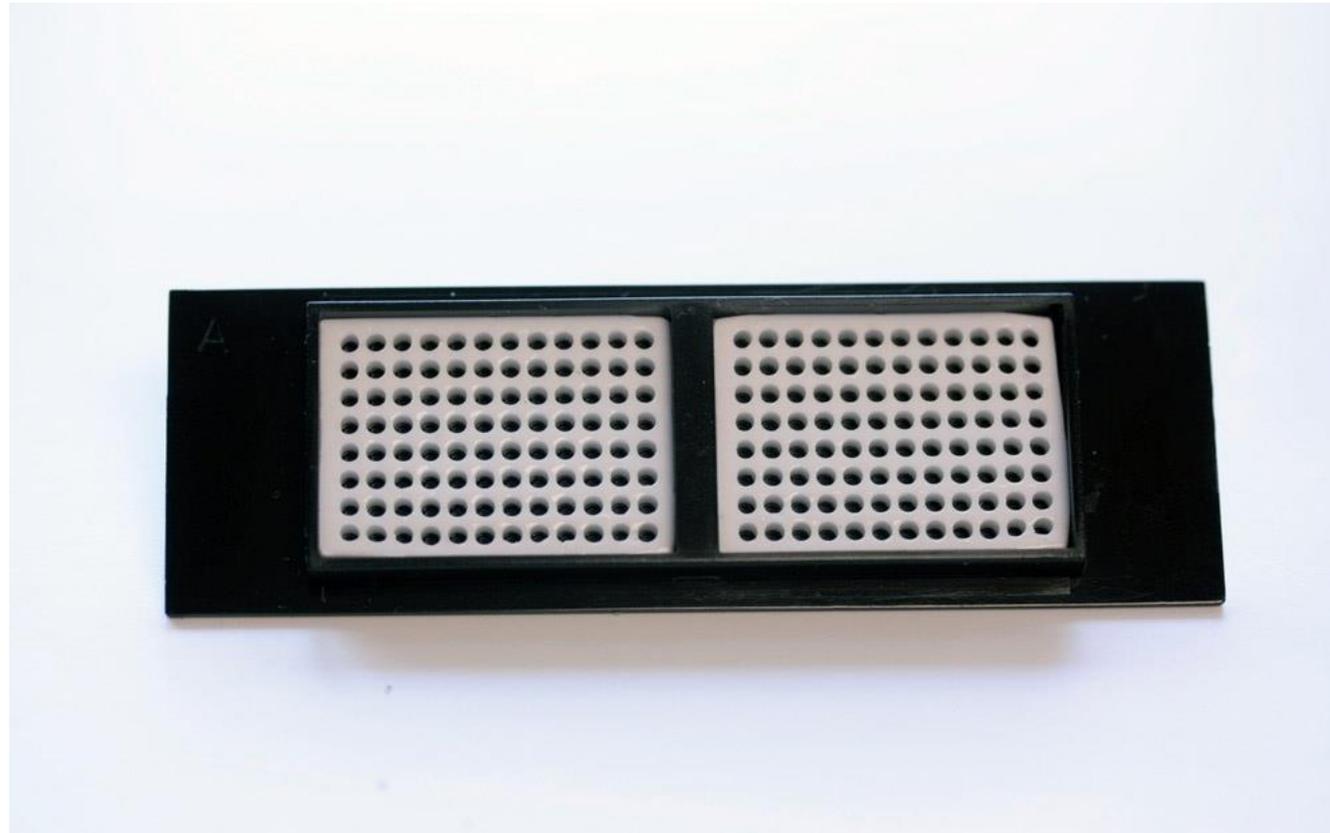
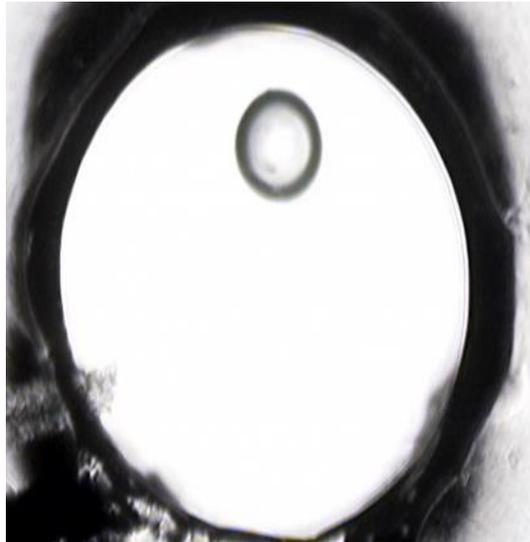


Figure. Showing specialised VHP Nano slide device with environmental buffering technology surrounding the wells (white). This array technology has been mounted into a standard microscope slide foot print.

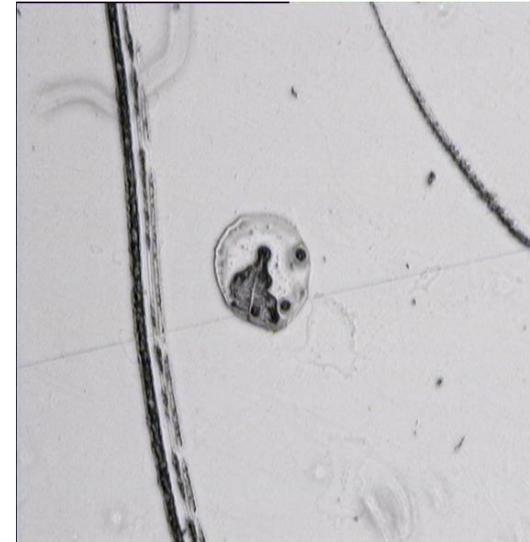
The excellent environmental buffering properties of Gel Buffered Plates allows miniaturisation of cell based assays down to nanoliter volumes.

Buffered



10 nl droplet at 15 minutes

Unbuffered



10 nl droplet evaporated in less than 60 seconds

Maintenance of ultra low volume droplets

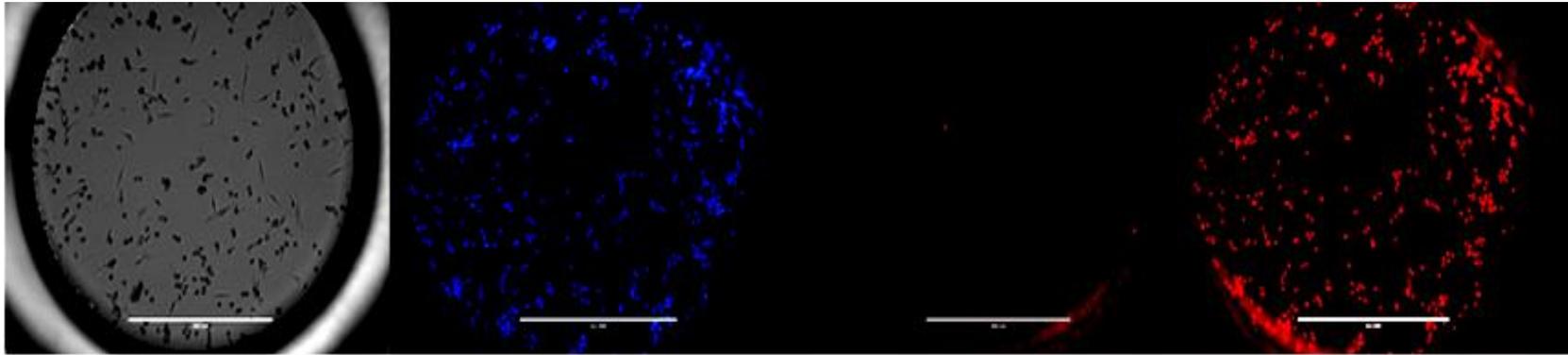
Subsequent experiments demonstrated maintenance of ultra low volumes of Aqueous Solution for several days.



Image of cells Grown
in buffered 192 nano
slide. (Volume 100nl)

Miniaturised 1000X
As compared to 96-
well plate assays
normally performed
at 100ul

No significant loss of Viability seen in cell maintained in 100 nl for 24 hours



Bright Field

Hoechst

PI

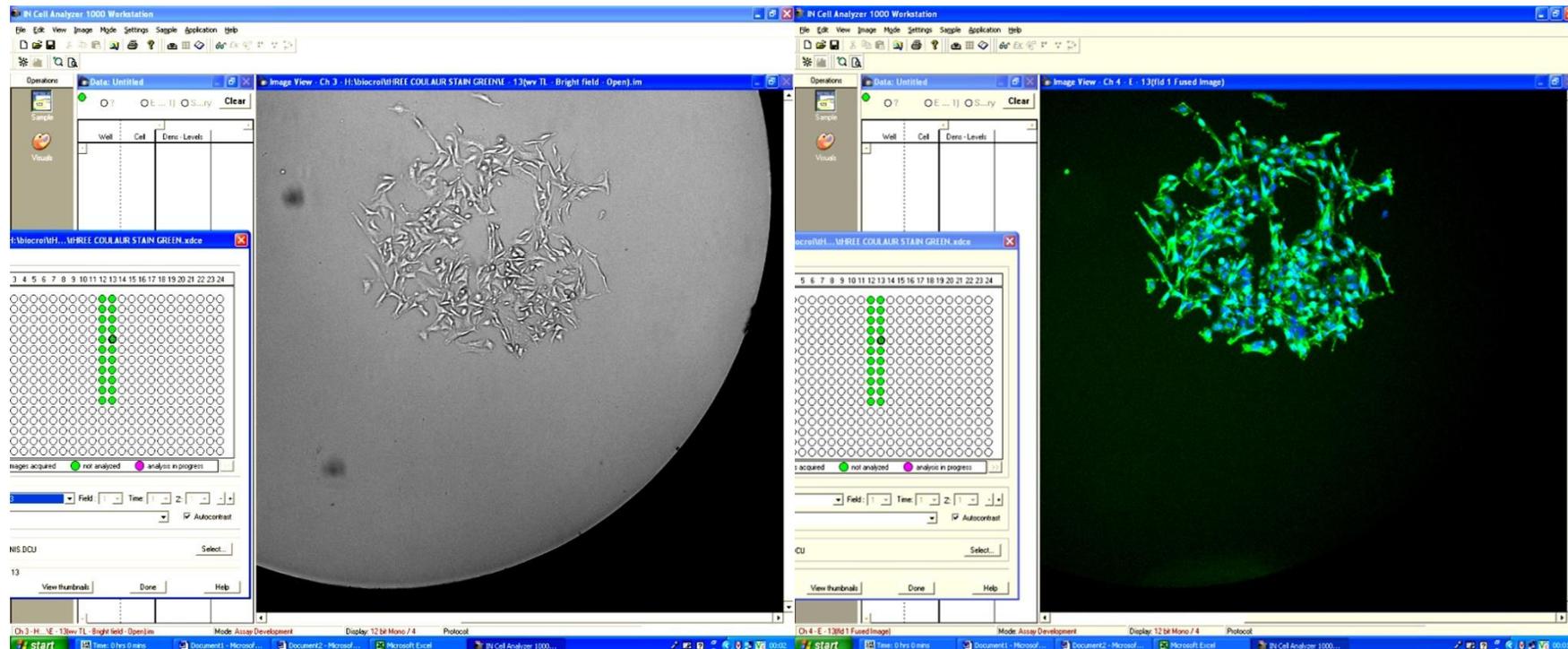
PI + Triton X 100

Images of the same cellular sample maintained in 100nl culture media for a period of 24 hours.

L to R:

- Panels 1 and 2 show bright field and fluorescently labelled cells and nuclei (respectively).
- Panel 3 shows cells stained with dead cell marker propidium iodide
- Panel 4 shows same cellular sample treated with the permeabilising agent Triton X 100 which was used as an additional control step for total cell counting.

High Content Image of cells incubated in **100nl** in a 384-well and Gel Buffered plate



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