

Eliminating Edge Effect in 96-well Plates by Controlling Thermal Conditions during Cell Plating

Poster
#5965

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ABSTRACT

The traditional approach to avoiding the variability in 96-well plate edge wells has been to avoid using them. This results in a 37% loss in usable assay space. Approaches to reducing this Edge Effect include reducing plate evaporation; however, we have preliminary data that plating cells at a constant 37°C reduces edge effect. Here, we extend these findings with studies of well-specific cell settling patterns associated with plate-wide thermal changes after plating. Our null hypothesis is that intra-well thermal currents as plates warm to 37°C after room temperature (RT) plating do not disrupt random cell deposition during settling. A549 human lung carcinoma cells were plated in traditional uncontrolled RT conditions or in an Xvivo System under completely controllable conditions. In this closed chamber, everything (chamber floor, atmosphere, the pipettor, tips, fluids, and plate) was the same temp as the incubator, where cells settle in the wells after plating. We used the PHI HoloMonitor M4 microscope to record time-lapse images of cells settling and adhering to the well floor. Crystal violet staining was used to assess cell settling patterns. We had previously found that in plates filled at RT, the cells in edge wells both experienced the earliest temp swings after cell plating, the most cells rolling, and the longest paths of directional cell rolling while settling. In contrast, cells plated at 37°C did not have thermal differences and the cells there settled randomly. Here, we present findings that cells settled in well-specific patterns on the well bottom due to thermal gradients and that we can alter or eliminate those patterns by changing the thermal conditions. When thermal changes were inverted, by plating cells at 37°C and letting them settle at room temperature, we were able to alter these patterns. We concluded that thermal changes drive these cell accumulation patterns and that constant temperature control during cell plating can reduce or eliminate edge effect. Full-time control of cell conditions could have a tremendous impact on cell-based drug discovery and pre-clinical drug testing, reducing assay time and materials, as well as improving assay reproducibility.

BACKGROUND

- The Edge Effect has been known since 96-well plates gained popularity in the 1980s
- Researchers often avoid using the edge wells, losing 37% of the space in a 96-well plate
- Moat plates and pre-incubation at RT have been used to try to eliminate edge effect
- We previously reported that plating cells with all materials at 37°C reduced edge effect

OBJECTIVES

To add evidence to the causal relationship between temperature changes and non-random cell settling in the well of 96-well plates:

- Reverse the temperature shifts that cells in 96-well plates are exposed to when plated in a traditional RT biological safety cabinet (BSC), plating cells at 37°C and incubating them at RT
- Record cell motion with the HoloMonitor M4 microscope during cell settling

REFERENCES

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EXPERIMENTAL DESIGN

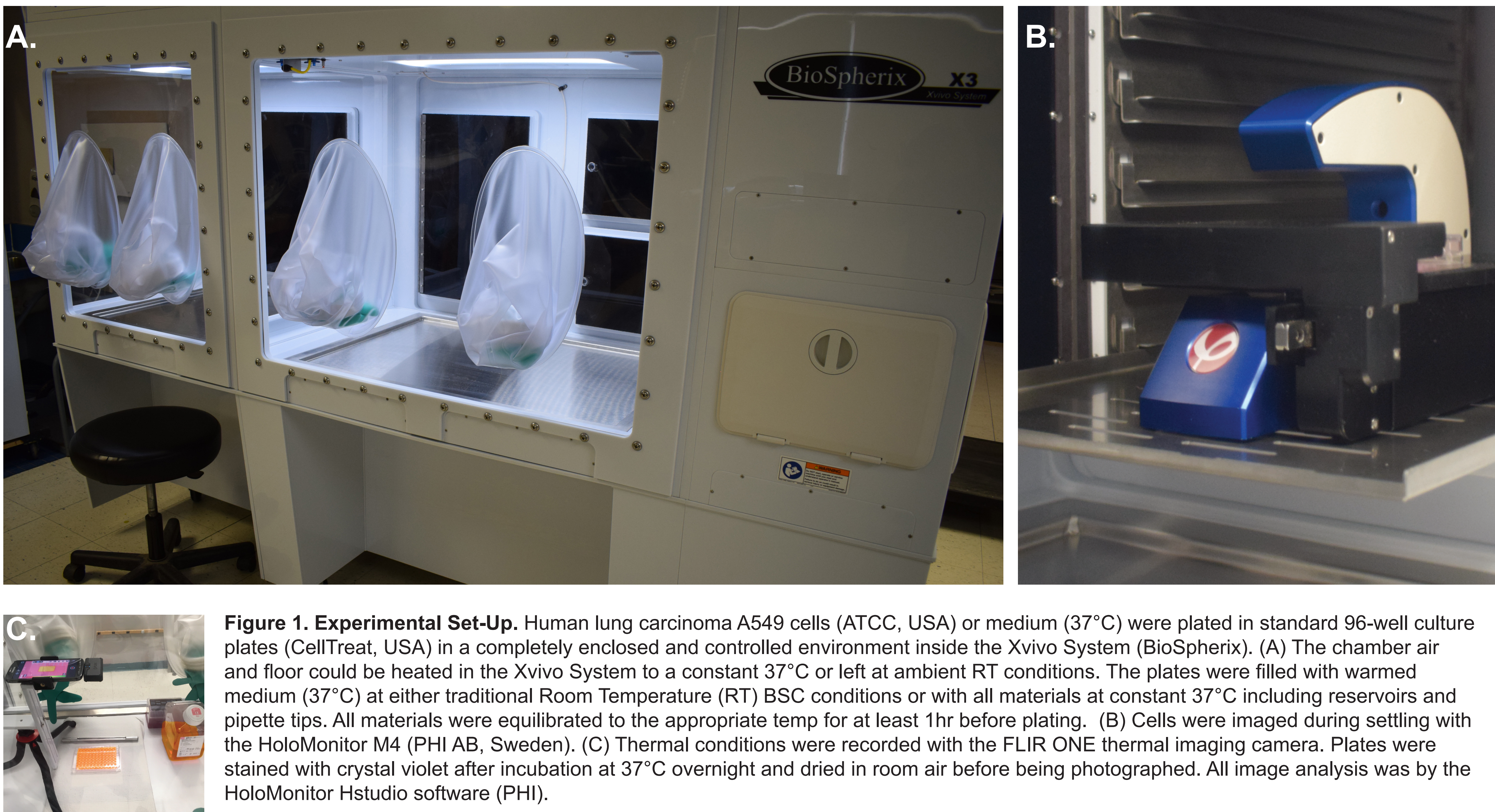


Figure 1. Experimental Set-Up. Human lung carcinoma A549 cells (ATCC, USA) or medium (37°C) were plated in standard 96-well culture plates (CellTreat, USA) in a completely enclosed and controlled environment inside the Xvivo System (BioSpherix). (A) The chamber air and floor could be heated in the Xvivo System to a constant 37°C or left at ambient RT conditions. The plates were filled with warmed medium (37°C) at either traditional Room Temperature (RT) BSC conditions or with all materials at constant 37°C including reservoirs and pipette tips. All materials were equilibrated to the appropriate temp for at least 1hr before plating. (B) Cells were imaged during settling with the HoloMonitor M4 (PHI AB, Sweden). (C) Thermal conditions were recorded with the FLIR ONE thermal imaging camera. Plates were stained with crystal violet after incubation at 37°C overnight and dried in room air before being photographed. All image analysis was by the HoloMonitor Hstudio software (PHI).

BACKGROUND DATA

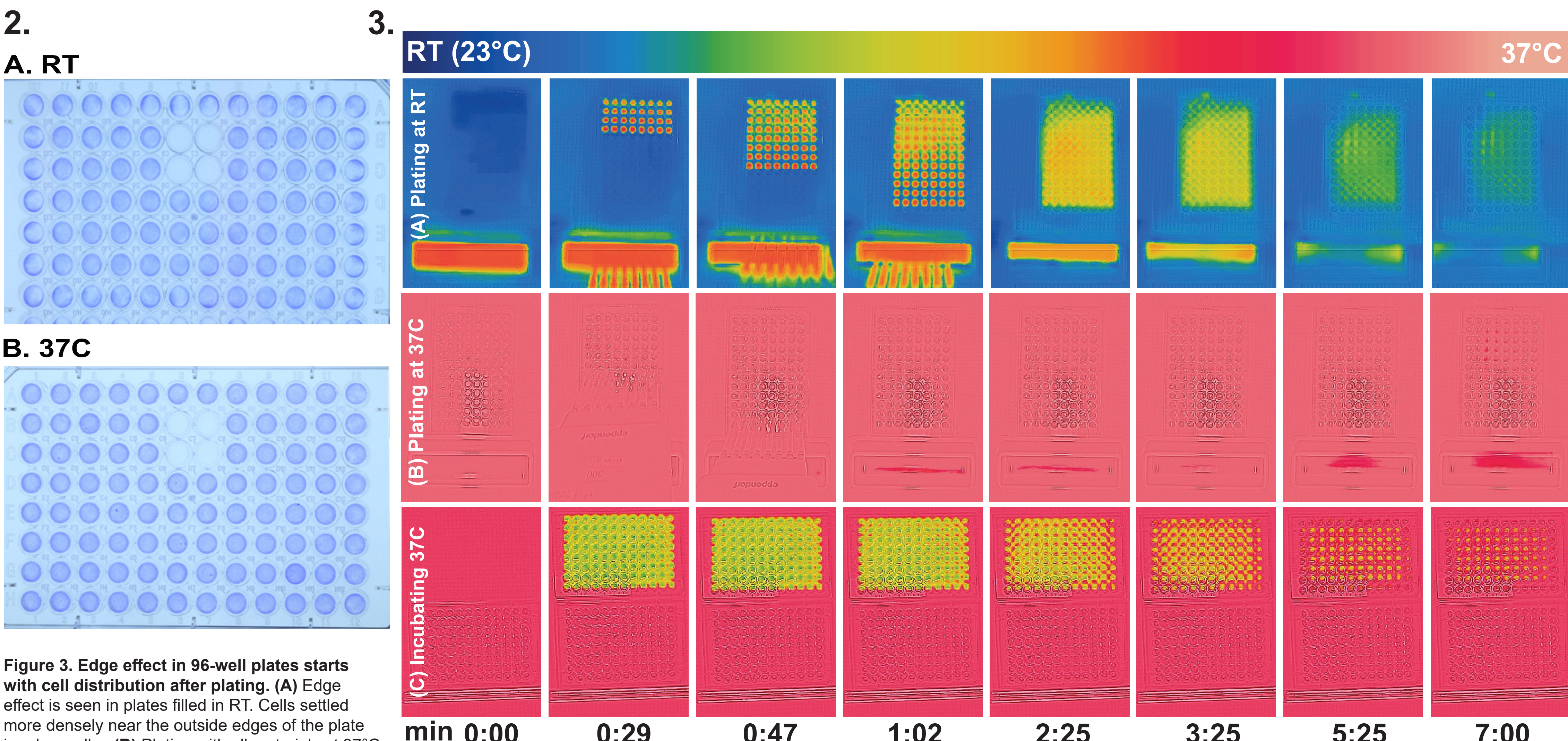


Figure 3. Edge effect in 96-well plates starts with cell distribution after plating. (A) Edge effect is seen in plates filled in RT. Cells settled more densely near the outside edges of the plate in edge wells. (B) Plating with all materials at 37°C in a chamber with a controlled temperature floor and atmosphere was able to reduce edge effect, resulting in a more random cell settling pattern. Four wells in each plate were filled with medium only.

Figure 2. Demonstration of thermal changes in 96-well plates during plating and cell settling. (A) Cell plating under room temp BSC conditions. Temperature changes were recorded using the FLIR One thermal imaging camera during manual cell plating. Cell culture medium was pre-warmed to 37°C as if in a waterbath. Pipettor, tips, plate and reservoir were at ambient room temperatures (~23°C). Medium in each well began cooling immediately before the plate could even be filled. Often cell plating takes more than 5 min, so the plate is near room temp before placing in the incubator. (B) Plating with all materials at 37°C in a chamber with a heated floor and atmosphere allows the medium to stay at temperature in the wells during plating. (C) When placed in the incubator next to a plate that was filled at 37°C, the room temp-filled plate warms from the edges inward during cell settling.

RESULTS

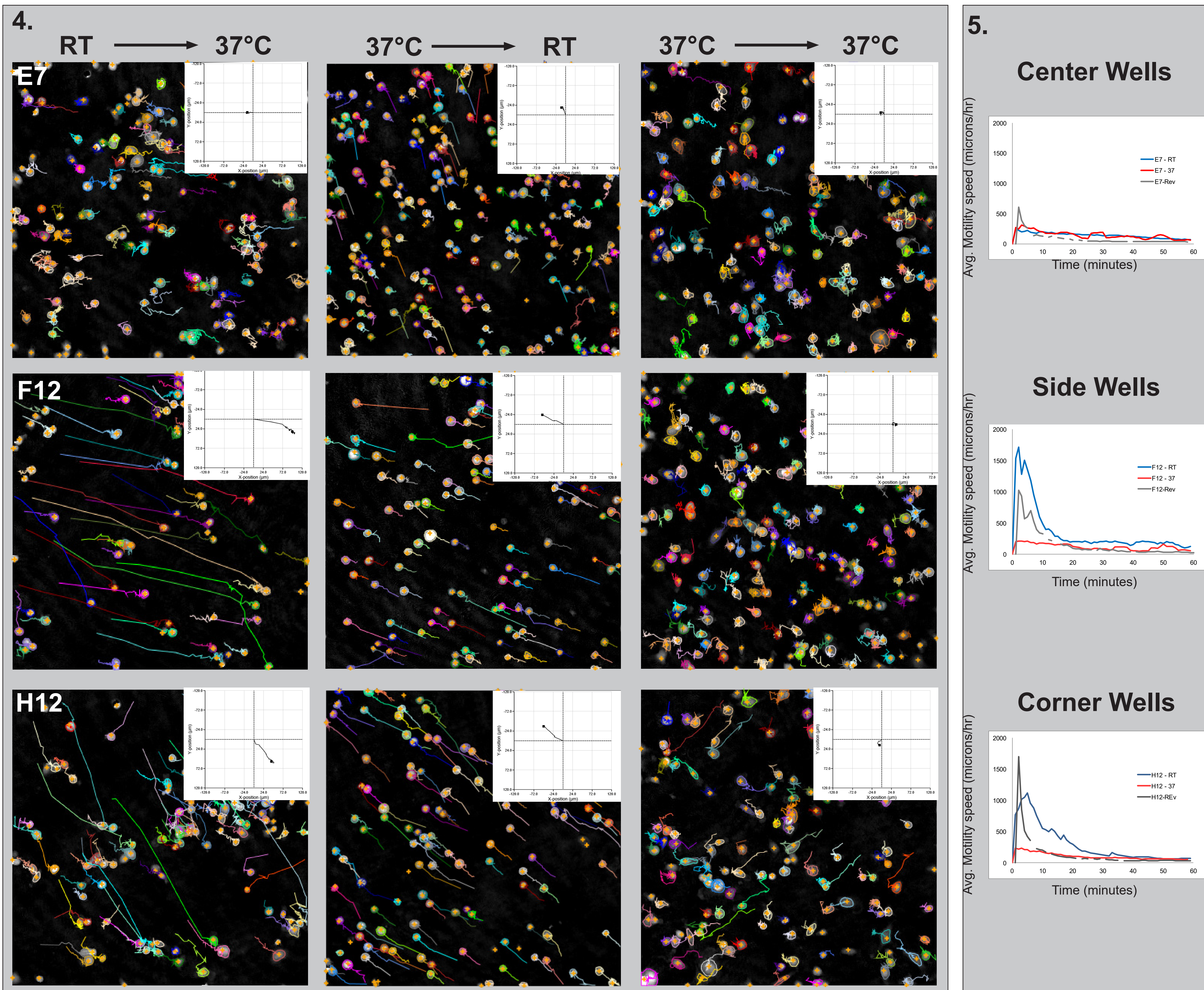


Figure 4. Cell Tracking shows strong directional motion in cells after they settle to the well bottom consistent with cells being rolled across the well bottom by convection currents. Images from center (well E7), right side (well F12) and corner wells of a 96-well plate (H12) were analyzed with Hstudio software for cell migration (duplicate wells, n=3 experiments). Cells were plated in RT or at 37°C and moved to the HoloMonitor M4 in a chamber set to either RT or 37°C. Unlike in center wells, cells in edge and corner wells moved toward the edge of the plate while plates warmed and moved toward the center of the plate while plates cooled. Combined ave. motility (inset) for each well showed a strong directionality when plates changed temp whereas plates in constant temp moved randomly with an average motility near the origin.

Figure 5. Less cell motion with constant temperature during cell plating and settling. Image analysis with the HoloMonitor Hstudio software showed that cells moved less after settling when plating and incubating both occurred in a controlled chamber set to 37°C. There was more average motion in edge and corner wells when plates experienced temperature shifts during cell settling.

Figure 6. Inversion of Edge Effect with Inversion of Temperatures. A549 cells plated at 37°C and incubated at RT show accumulations of cells on the inside edges of the edge wells. This is the opposite of the classical edge effect and lends evidence to the thermal model of edge effect. Cells were stained with crystal violet after an overnight incubation (n=3).

Figure 7. Thermal model of Edge Effect. In the first 30 min in the plate, cell settling is influenced in a way that correlates with temperature changes in the plate. As the plate warms from room temp in the incubator, the cells are still settling. Cells are rolled across the well bottom toward the warming side of the well. This is consistent with convection currents driving non-random cell settling and contributes to different cell distributions between edge and center wells. Cells piled in the well edges may not get even exposure to agents being

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CONCLUSIONS

Temperature changes during cell settling are associated with cells moving in non-random cell settling patterns, adding to variation between center and edge wells in the first 30 minutes in the plate.
Control cell plating thermal conditions to reduce edge effect.