Application Note

Cell Analysis

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Analysis of Cell Retention Following Rigorous Automated Cell Fixation and Staining

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Abstract

Automated liquid handling procedures can greatly enhance microplate processing throughput for a variety of assay formats. Retention of the appropriate cell density in the microplate wells following these procedures can be critical to the performance of downstream processes and analysis of cell-based assays.

Introduction

The use of microplates allows for increased throughput of cell-based assays in combination with instrumentation to automate processes such as media exchanges, cell washing, fixation, and reagent addition. By using a combination of fluorescent reporter proteins and dyes spanning the visible and near IR light spectrum multiparametric analysis can be accomplished on a single microplate well. Many procedures require a number of liquid handling steps including cell washing and reagent dispensing. Optimization of automated cell washing and dispensing parameters can reduce variability and insure retention of the desired cell density. This application note describes the use of automated image capture to visualize cell retention and monolayer integrity across the microplate well surface.

Materials and methods

Materials were sourced and prepared as previously described.[1] Cell fixation and staining was performed as previously described.[1] Prepared plates were kept at room temperature and protected from light prior to imaging. Cells were imaged using an Agilent BioTek Cytation 5 cell imaging multimode reader configured with DAPI, GFP, and Texas Red light cubes. Images were captured using a single, green channel prior to automated cell washing, fixation, and staining. Post-washing, fixation, and staining images were captured using three channels: blue, green, and red.

Results and discussion

Automated image capture allows a series of images to be captured and reconstructed to render a single image depicting the entire well of a microplate. Capture of single color images in the green channel provides visualization of the monolayer prior to automated fixation and staining. The 3 × 2 monochrome image depicting GFP shows evenly dispersed cells creating a cell monolayer of ~ 40 to 50% confluency (Figure 1A).

Following automated cell fixation and staining using the Agilent BioTek EL406 automated washer dispenser, the wells can be imaged using the green, red, and blue channels for analysis (Figure 1B). An entire well can be imaged as a montage and stitched to determine the integrity of the cell monolayer.

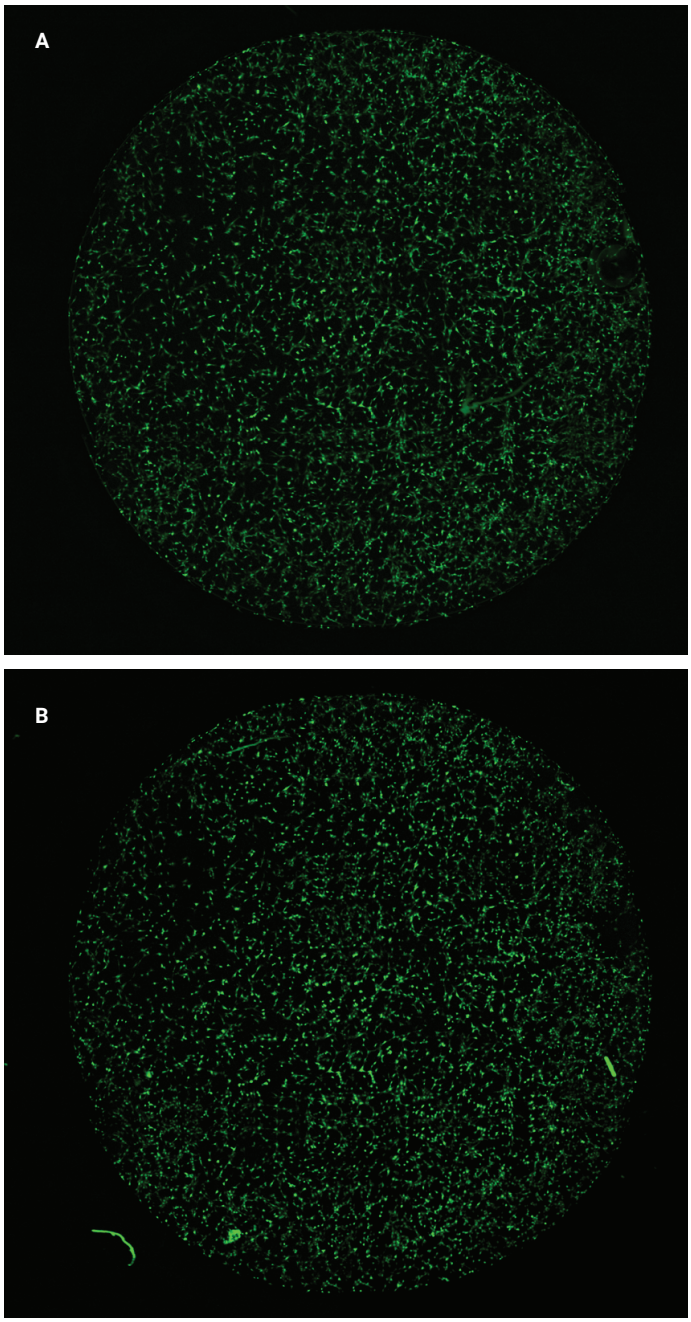

Figure 1. Stitched montage of a 3 × 2 matrix of captured images. HEK293 cells expressing GFP were seeded in a 96-well microplate for automated cell fixation and staining. The cell monolayer was imaged as a montage using a 2.5x objective to capture the entire well surface. Stitched images of GFP expressing HEK293 cells (A) pre- and (B) postautomated fixation and staining for visualization of integrity of the cell monolayer.

A comparison of whole-well stitched montages captured in the green channel, detecting constitutively expressed GFP in HEK293 cells, shows excellent cell retention following rigorous automated cell washing, fixation, and staining (Figure 3). The entire well can also be investigated as an overlay of all captured channels overlayed and stitched into a single image (Figure 2).

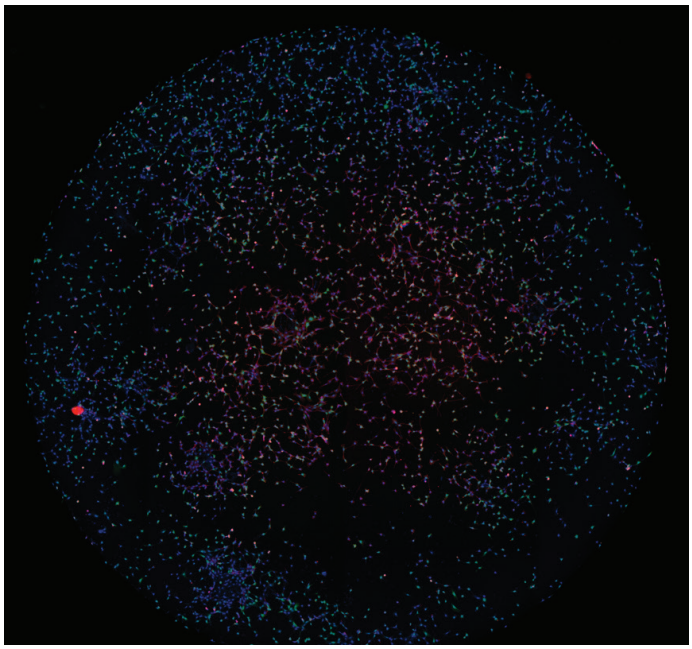

Figure 2. Stitched montage of a 3 × 2 matrix of captured images. HEK293 cells expressing GFP were seeded in a 96-well microplate and subjected to automated cell fixation three channels (blue, red, and green) that were automatically stitched and overlaid for analysis and staining.

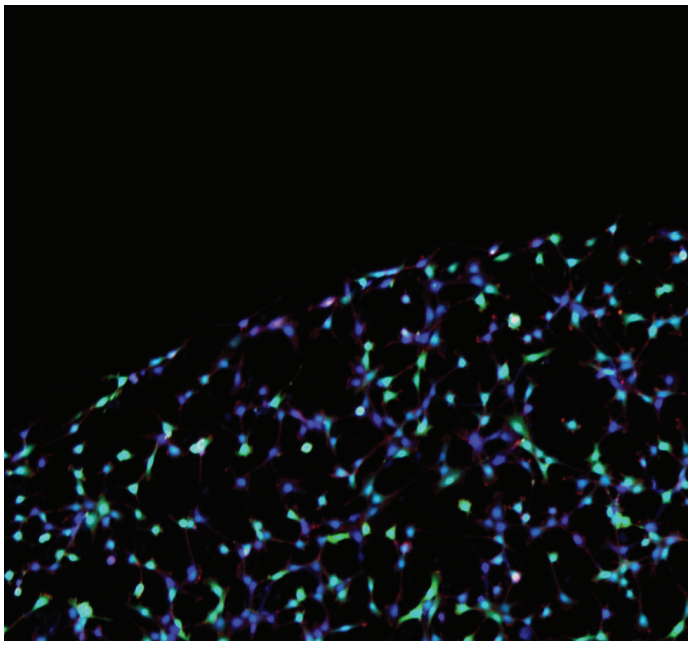

Figure 3. HEK293 cells expressing GFP. Actin was stained with Texas red-phalloidin and nuclear DNA was identified with DAPI. Image represents the overlay of separately captured red, blue, and green fluorescent signals using a 4x objective.

Conclusion

The use of automated liquid processes to improve cellular workflow can dramatically reduce variability, labor, and improve results. The use of microplates for cell-based assays can further improve throughput and help reduce costs associated with reagent use. Optimization of automated liquid processes such as routine cell washing and reagent additions required for cell fixation and staining help improve assay repeatability. The ability to perform automated imaging of the entire well both before and after automated liquid processes can provide valuable information regarding the uniformity of seeding density, the robustness of cell washing and retention and integrity of the cell monolayer.

Reference

1. Held. P. *Agilent Technologies application note,* **2005** Automated Tissue Culture Cell Fixation and Staining in Microplates (agilent.com)

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