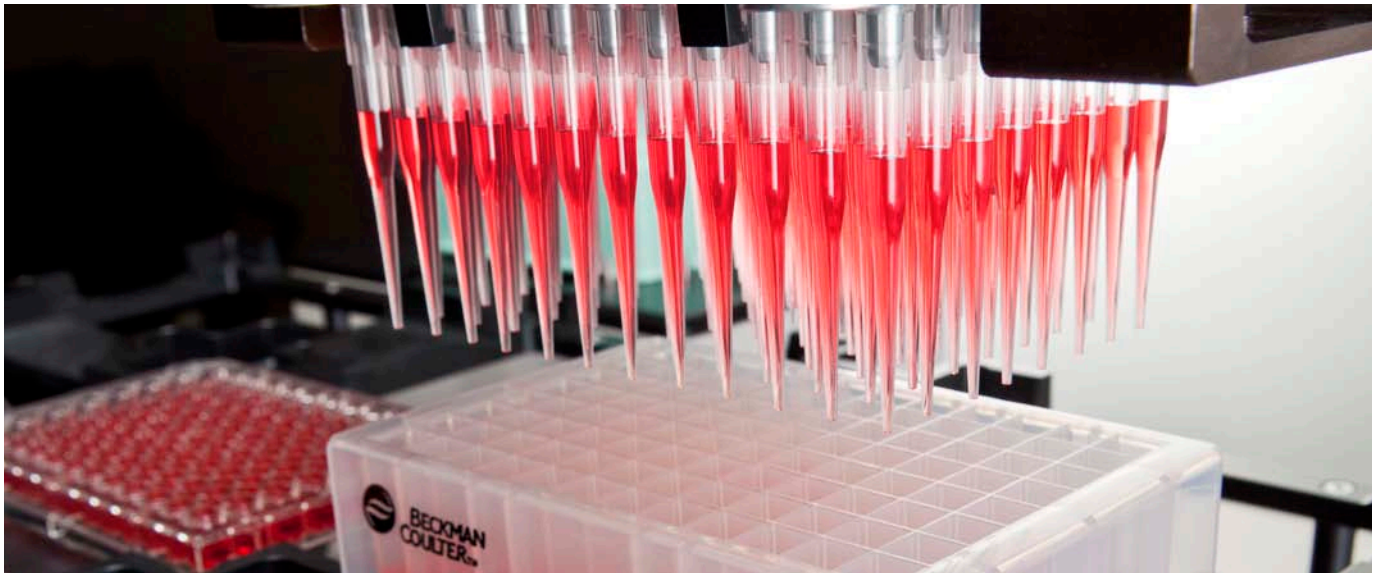
Automated Sample Preparation for Cell-Based Assays



Abstract

Cell-based assays are indispensable for drug discovery and necessary for exploring complex cell signaling pathways. Automating the sample preparation for these assays can improve consistency of results between users by ensuring the same treatment for all samples. Automation can also accelerate research by increasing sample throughput and/or reducing the active time required by scientists to process samples. Here we demonstrate the automation of sample preparation and analysis workflows for a variety of common cellular assays.

Introduction

Cellular analysis is at the heart of numerous lines of biological inquiry. Cell-based assays are used to interrogate the signaling pathways involved in a wide array of cellular functions—from growth and differentiation to metabolism and death. New techniques to ask and answer cellular questions are constantly being developed, and the preparation and analysis of samples is therefore always changing. As these techniques are accepted as the new standard, the need for higher sample throughput arises. One way of managing the time and effort associated with this increased throughput is through the use of automation.

Sample preparation for cell-based assays typically varies with the instrument used for analysis and whether the cells are grown as adherent or suspension cultures. Another consideration is whether cell sterility needs to be maintained or the cells are being prepared for an end-point assay. While flow cytometry is highly amenable to single-cell analysis of suspension cultures, the sample preparation frequently requires numerous interventions for centrifugation and reagent addition and removal steps. While adherent cells can be trypsinized to form suspensions for flow cytometry analysis, high content imaging can be used to acquire per-cell or even subcellular information directly from the adherent cultures. This method also typically requires numerous interventions for reagent exchange. Finally, both adherent and suspension cells may be analyzed with multimode plate readers. These assays could involve detecting cellular fluorescence at the well level, adding luminescent reagents for cell titer approximations, or sampling culture media for ELISAs.

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By automating the repetitive liquid handling steps that are often required for these assays, researchers are able to maximize their contributions by spending less time on repetitive tasks and more time on experimental design and data analysis. Automating cellular assay sample prep also reduces the variability that is seen when multiple users prepare samples through slightly different means. Descriptions follow for a variety of automated solutions for sample preparation of cell-based assays.

Automation Solutions

Biomek Workstations can be used to automate the liquid handling steps involved in the sample preparation of a wide variety of cellular assays and throughputs. If required, sterility can be maintained through the use of sterile pipette tips and HEPA-filtered custom enclosures or, in the case of the Biomek 4000 Workstation, by placing the unit in a standard laminar flow hood. For workflows that require more than just liquid transfers (i.e., incubations, centrifugation steps), Biomek Workstations can be integrated with numerous devices. Higher throughput and reduced user intervention is also enabled by integrating the final analyzer(s) to the instrument. The open platform of the Biomek Workstation allows these integrations to be highly flexible and useful for a wide variety of workflows. Figure 1a shows a system designed for maintaining and monitoring the viability of monolayer and 3D cultures; while Figure 1b illustrates a larger system that can automate a complete phenotypic screening of medium- to large-scale compound and siRNA libraries.

As higher throughput becomes possible with integrated automation, the complexity of the assay logistics and scheduling will increase. SAMI EX Workstation software controls the movement of multiple plates through the integrated system to optimize the workflow. It can also utilize assay data to drive liquid handling steps, such as "cherry-picking" wells with readings above a certain threshold. SAMI Process Management software is used to schedule instrument usage for assays that may span days or weeks (i.e., repeated visualization of cells over time), as well as to interleave overlapping experiments as necessary.

Demonstrations

Immunoassays

To demonstrate the utility of automating plate-based immunoassays such as the IP-One HTRF Assay, a Biomek NX^{P} Workstation was used to plate CHO cells overexpressing muscarinic m1 receptor in 384-well plates and treat them with various concentrations of carbachol and acetylcholine. HTRF reagents were added to detect inositol phosphate 1 (IP1) and after an on-deck heated incubation, plates were read on an integrated SpectraMax Paradigm. Figure 2 shows the resulting dose-response curves and the consistency of results across columns of a plate. Again, by integrating a Biomek Workstation with an analyzer, one can automate entire workflows with excellent reproducibility.

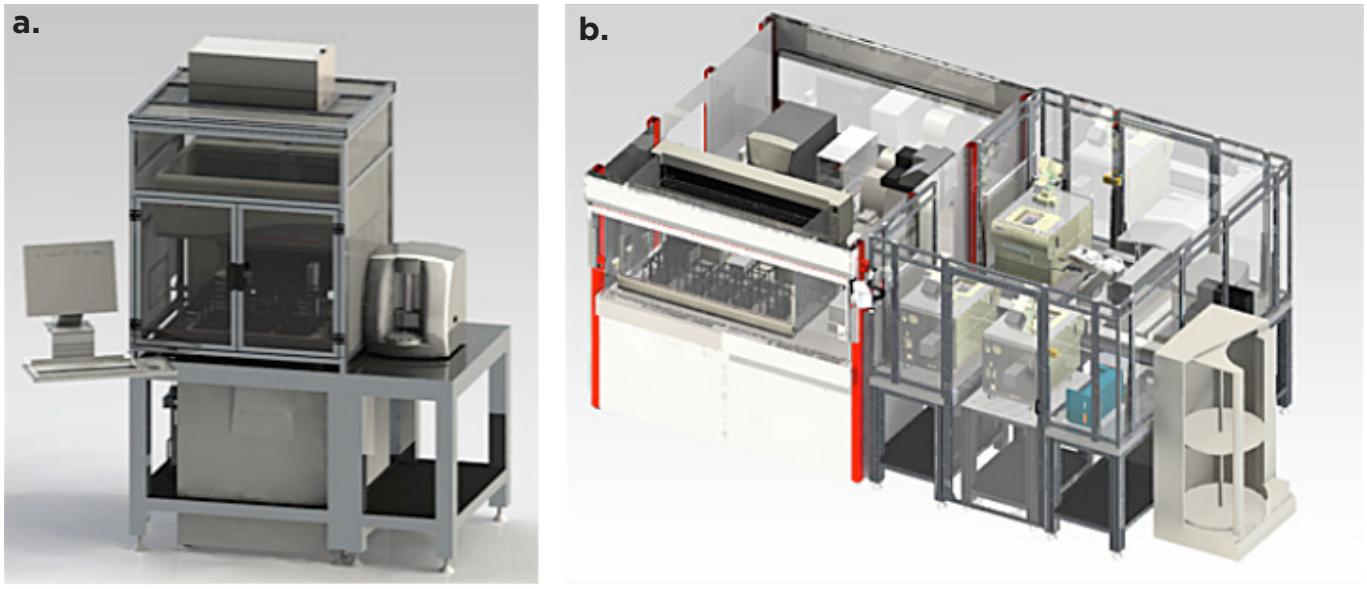
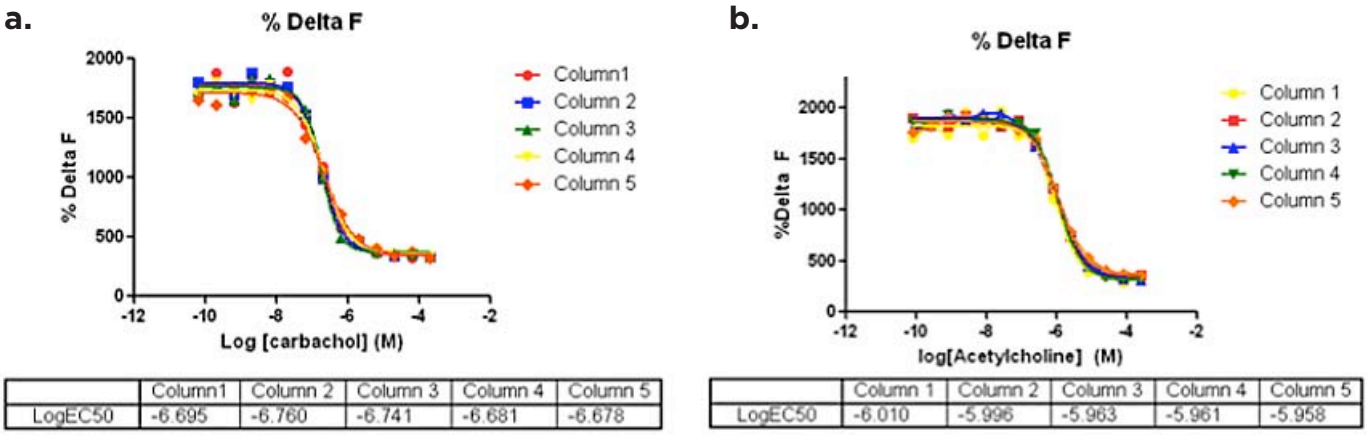


Fig. 1. Renderings of two integrated systems that are designed for two- and three-dimensional cell maintenance **(a)** and large-scale phenotypic screens **(b)**. Both systems include Biomek liquid handlers with filtered enclosures (top portion removed on **b**. for visualization) along with integrated storage components and analyzers. The first system **(a)** features a Beckman Coulter Vi-CELL XR Cell Viability Analyzer while the larger system **(b)** features a Beckman Coulter flow cytometer, as well as a multimode plate reader and multiple high content imagers from Molecular Devices. These systems provide sterile cell handling while automating complete cellular workflows.



	Column1	Column 2	Column 3	Column 4	Column 5
LogEC50	-6.695	-6.760	-6.741	-6.681	-6.678

	Column 1	Column 2	Column 3	Column 4	Column 5
LogEC50	-6.010	-5.996	-5.963	-5.961	-5.958

Fig. 2. Dose response curves of CHO-M1 cells overexpressing muscarinic m1 receptor to carbachol **(a)** and acetylcholine **(b)** in the Cisbio IP-One assay. Each curve is a replicate column normalized to wells with no agonist. Delta F is used for the comparison of data generated on different days and reflects the signal to background of the assay [(sample ratio—no agonist ratio)/no agonist ratio]. The tight replicates were reflected in a coefficient of variation for EC_{50} of 0.5% for carbachol and 0.3% for acetylcholine.

Cell Signaling

We have also utilized the Biomek NX^{P} Workstation to automate the stimulation and eventual fixation, permeabilization, and staining of leukocytes for flow cytometric analysis. Preparation of flow cytometry samples is assisted by the integration of a centrifuge to the liquid handler, as this allows automation of the entire workflow without repeated user intervention for cell pelleting steps. Whole blood was stimulated with either lipopolysaccharide (LPS) or phorbol-12-myristate-13 acetate (PMA), and CD14^{+} monocytes were stained for the phosphorylated forms of p38 MAP kinase and ERK. Cells were run on an FC500 flow cytometer and data was analyzed with Kaluza software. Figure 3 shows that LPS induced detectable phosphorylation of both p38 MAPK and ERK, while only phosphorylated ERK was detected in PMA-stimulated cells.

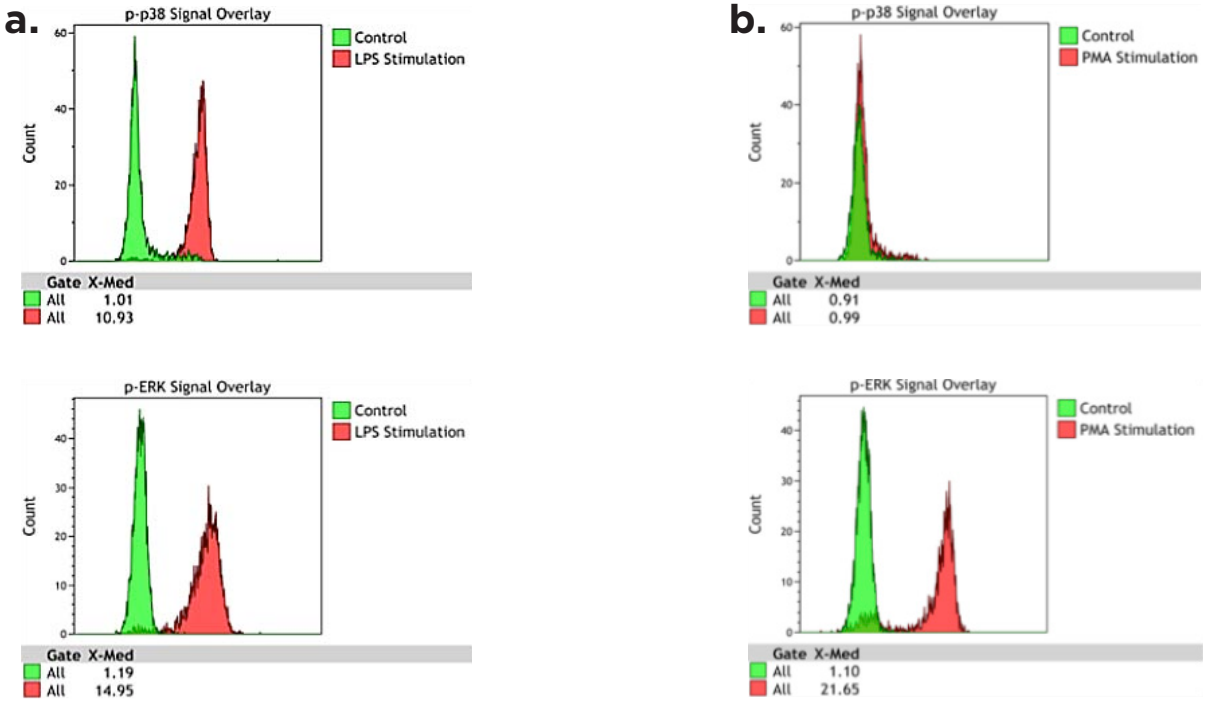


Fig. 3. Histograms indicating the phospohorylation state of CD14^{+} T-cells stimulated with lipopolysaccharide **(a)** and phorbol-12-myristate-13 acetate **(b)** (red peaks) as compared to unstimulated cells (green peaks). Cells were stained for phospho-specific forms of p38 MAPK and ERK. LPS induced phosphorylation of both proteins, as indicated by the shift in cell populations, while PMA only induced phosphorylation of ERK.

Cell Imaging

A final demonstration of sample preparation for cell-based assays utilizes the Biomek 4000 Workstation to prepare plates for high-content imaging. This system utilizes a user interface to enter reagents and incubation times to automate a wide variety of cell staining protocols. Figure 4 shows images of murine embryonic stem cells, co-cultured with feeder cells that have been stained for stem cell markers Nanog and Sox2, and analyzed with an ImageXpress Micro imager. As stated previously, the automation of the numerous pipetting steps involved in this sample preparation greatly alleviates the time spent at the bench, and the low coefficient of variation indicates a robust automated staining protocol.

Conclusion

Here we have illustrated a small sample of the cell-based assays that have been successfully automated on Biomek Workstations. These solutions include both stand-alone liquid handlers and integrated systems capable of automating entire sample preparation and analysis workflows. Automating cellular assays can reduce time at the bench and increase sample throughput while also reducing user-to-user variability. These benefits can help accelerate the cellular discoveries that will enhance our understanding of basic biology and lead to the identification of novel disease treatments.

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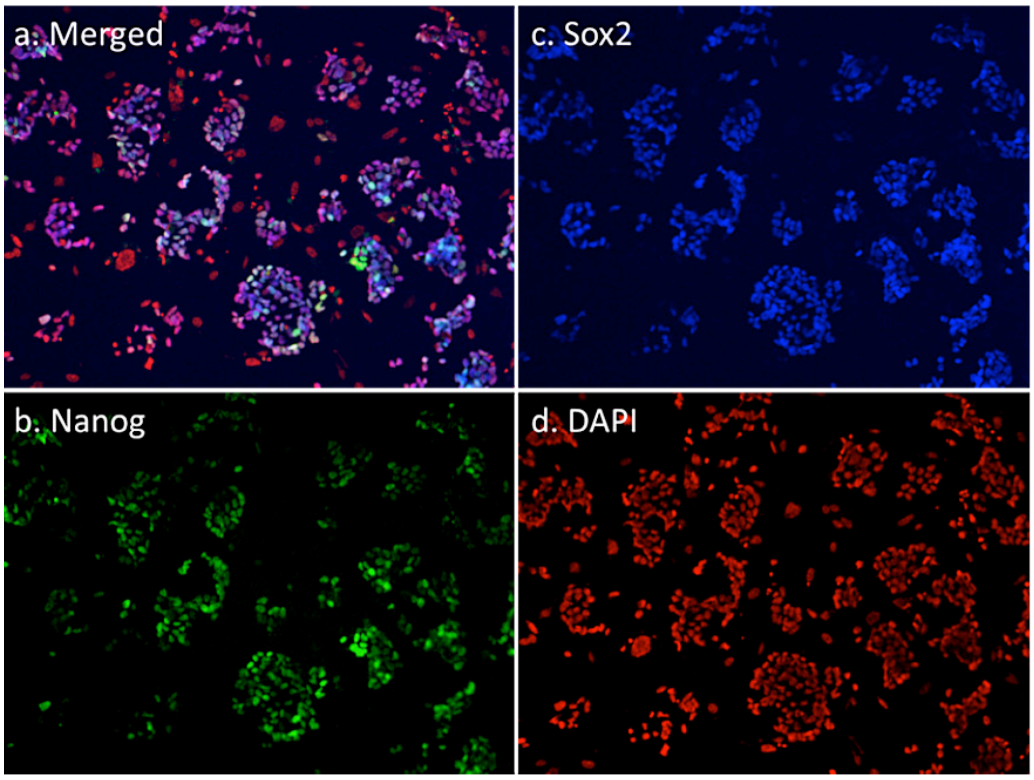

Fig. 4. Mixtures of murine embryonic stem cells and feeder cells were treated with PerFix-nc (Beckman Coulter) reagents and stained for Nanog (**b**., green) and Sox2 (**c**., blue) and with DAPI (**d**., red) to mark the locations of nuclei. Comparable percentages of positively stained cells were seen across 24 samples for Nanog (57.1%) and Sox2 (63.2%), with coefficients of variation of 6.6% and 7.6% respectively.

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