Automated Liquid Handling for Cell Staining and Imaging

Abstract

High content imaging is a powerful way to acquire per-cell data for adherent cell cultures. As the throughput increases for this method, the preparation of samples for analysis quickly becomes burdensome and time consuming with increased opportunities for error. Here we describe a variety of automated solutions to reduce the time, effort, and complexity associated with sample preparation workflows for cell imaging.

High Content Imaging

High content imaging can provide some of the highest data granularity of any cellular assay and this has driven its increasing use in cellular applications. While "well-level" details can be sufficient for initial experiments or screens, imaging can provide per-cell data of a mixed population sample. High content imaging can even move beyond the single-cell level and provide data such as cell shape (e.g. myotube formation or neuronal migration) or subcellular localization (e.g. nuclear/cytomplasmic translocation or vesicle trafficking). This data can provide information about cellular changes and signaling pathways that would either require surrogate readouts or be missed entirely by other technologies.

While imaging can be used with suspension cells, the technology is better adapted to use with adherent cells due to the single plane of focus. Using adherent cells also makes the liquid handling steps more amenable to automation, as no centrifugation steps should be required. However, one must be careful not to disturb the cellular layer during sample preparation. The workflow for cellular imaging frequently involves culturing and treating cells, followed by fixation and staining procedures. Automating the liquid handling for any or all of these steps on a Biomek Workstation can significantly reduce the active time needed for sample preparation as well as eliminate the user-to-user variability present in any experiment.

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Automated Liquid Handling

The family of Biomek Workstations provide the flexibility required to automate a host of applications and throughputs. For high content imaging sample preparation, differing staining reagents can be added to different wells by either a single channel tool on the Biomek 4000 or by a Span-8 pipettor on the Biomek NX^{P} or FX^{P}. In addition, the 96- or 384-well multichannel heads available on the Biomek NX^{P} or FX^{P} can facilitate reagent exchanges across multiple plates for higher throughputs. Staining reagents can be chilled on the deck of the instrument to help ensure stability and reduce evaporation.The Biomek software enables a high level of pipetting control, such as following the liquid level as reagents are added or removed or aspirating around the circumference of a well, as ways of minimizing disruption to the cellular monolayer. The software also ensures that all data is tracked across plates and samples so no information is lost during liquid handling steps.

These same tools can also be used to automate the upstream experiments prior to cell staining, such as cell maintenance (media exchange, passaging, plating), nucleic acid transfection, and compound addition. These previous steps can be performed in a sterile fashion through the use of sterile Biomek tips and custom HEPA-filtered enclosures or through the placement of the Biomek 4000 in a standard laminar flow hood. Here we provide a number of illustrations of how the Biomek Workstations can be used to automate the cell staining frequently associated with high content cellular imaging.

Automated Cell Staining

Biomek 4000 Workstation for Cell Staining: Figure 1a shows the deck layout of a Biomek 4000 Workstation specifically configured for cell staining. This solution is designed for lower throughput applications and includes a user interface that allows the user to select wells for staining and input the number of reagents and their volumes, as well as incubation times (Figure 1b). Following these entries, the required reagent volumes are

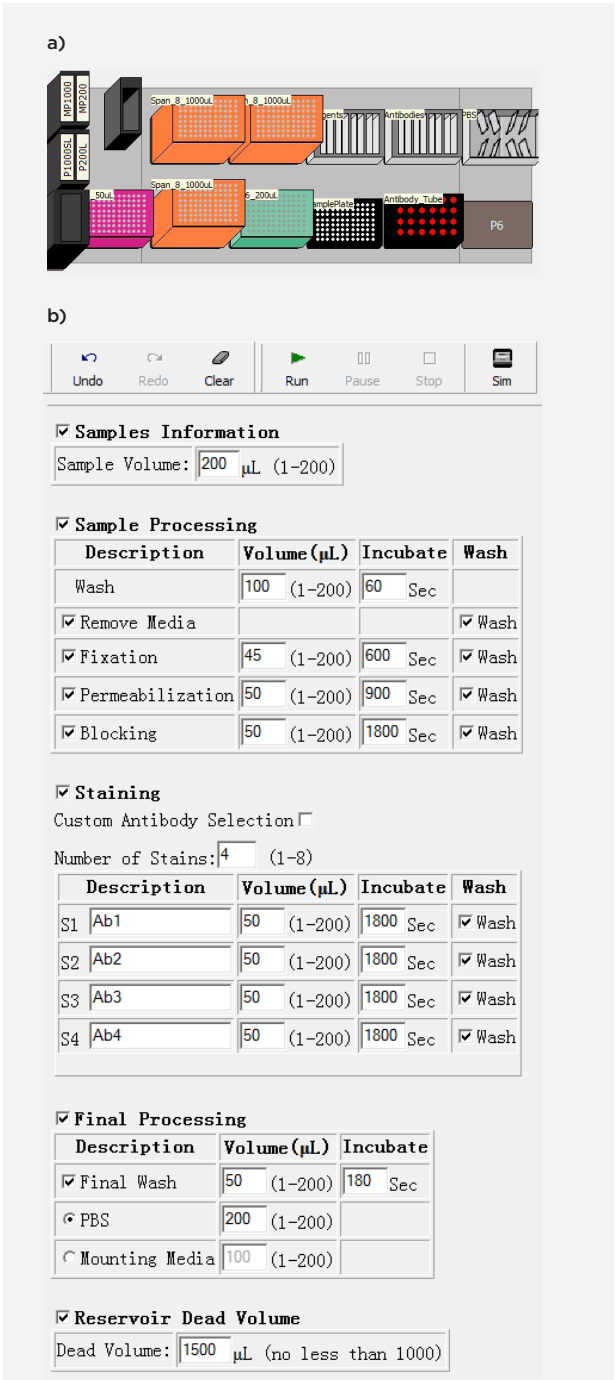

Fig. 1. Cell staining on the Biomek 4000 Workstation. **a)** Software image of the standard deck layout for a cell staining application on the Biomek 4000 Workstation. **b)** Screen capture of the software interface that guides users through a rapid and flexible setup for a cell staining experiment. Subsequent screens display the volumes of reagents needed (based on sample numbers) and their locations on the deck.

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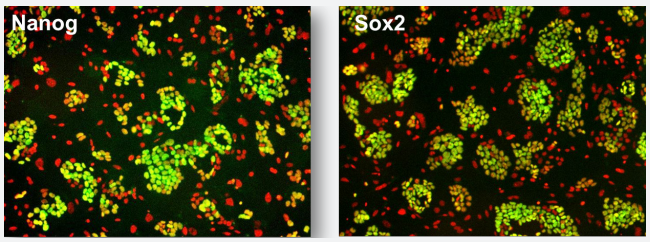
a)



b)

Antibody Staining	Positive Yield	Standard Deviation	Coefficient of Variation
Anti-Nanog	50.1%	2.6%	5.3%
Anti-Sox2	55.5%	3.4%	6.2%

Fig. 2. Automated staining of stem cells using the Biomek 4000 Workstation. **a)** Images of cocultures of murine embryonic stem cells and irradiated murine embryonic fibroblasts stained for Nanog (left, green) and Sox2 (right, green). Nuceli were stained with DAPI (red). **b)** Results of staining across 24 wells for each condition. Consistent staining is indicated by the low coefficient of variations (-6%).

calculated and displayed and the user is guided through setting up the deck. While this application can still be customized to accommodate specific needs, the established method and user interface serve to dramatically reduce the time required for multiple users to run this process in the laboratory.

As a proof of principle, we utilized this Biomek 4000 configuration to stain co-cultures of murine embryonic stem cells and irradiated murine embryonic fibroblast cells with anti-Nanog and anti-Sox2 antibodies (Figure 2a). Cell nuclei were stained with DAPI and images were acquired using an ImageXpress Micro high content imager. The percentages of positively stained stem cells were calculated using Metamorph software. Automated staining of 24 wells with each antibody showed high reproducibility, as distinguished by coefficient of variations below 7% (Figure 2b). These results indicate that, in addition to the reduced active time achieved by automating the liquid handling steps of the sample preparation, the consistency of staining across samples leads to robust experimental data.

Higher Throughput Solutions: As researchers move towards using imaging as a primary screening tool, a sample preparation system with a higher throughput capability becomes necessary. One example of such a system is the Biomek FX^P Workstation. The high level of flexibility in this instrument is illustrated in Figure 3a, which shows two alternative deck setups for staining partial plates using the Span-8 pipettors or full plates using the multichannel head. This optimized staining method uses a Microsoft® Excel® worklist to select the antibodies added to each well and then uses this information to calculate the required reagent volumes. The Biomek FX^P workstation also has the ability to switch between 96- and 384-well heads for staining of higher density plates.

Figure 3b shows a mosaic of high content images covering two wells of murine embryonic stem cells that were differentiated under different conditions. The Biomek FX^P was used to stain cells for α-actinin to identify the conditions that promoted cardiomyocyte formation (right) as compared to control differentiation conditions (left). This methodology can be used to screen for a variety of phenotypes and the Biomek FX^P can assist with the preparation for a broad spectrum of assays over a wide array of throughputs.

Integrated Workflows: An even greater level of process automation can be achieved through the integration of devices to the Biomek Workstations. Beckman Coulter has parternered with numerous companies to integrate instuments that enable high content imaging workflows. By integrating a device that tilts the plate, one can remove media from the edge of the well to avoid disturbing poorly adherent cells during processing. Other

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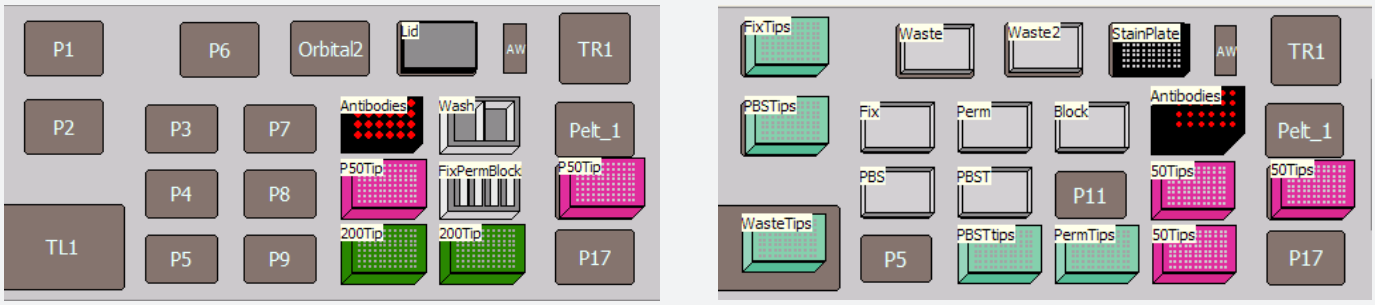

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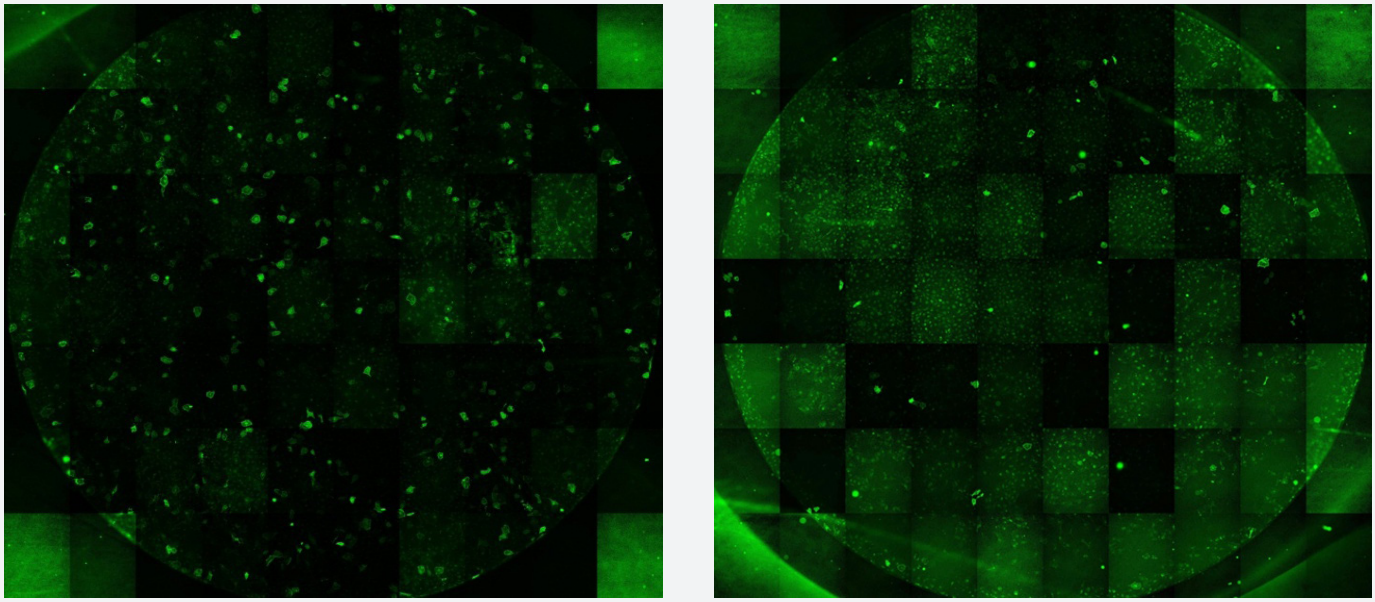

Fig. 3. Higher throughput automated cell staining. **a)** Biomek FX^P deck layouts for staining of partial plates (left) or full plates (right). Partial plates can be stained using Span-8 pipettors while full plates can be rapidly stained with the 96- or 384-channel head. **b)** Mosaic of high content images following automated staining of differentiated murine embryonic stem cells for α-actinin as a marker of cardiomyoctes. Optimized conditions (right) show greater numbers of cardiomyocytes than control conditions (left).

integrations include storage units that hold additional tips and plates necessary for high-throughput applications. Throughput can also be improved by integrating instruments that can accelerate sample processing, such as plate washers. The complete workflow can then be automated by integrating the high content imager to the Biomek Workstation so processed plates can immediately be analyzed without user intervention. Figure 4a shows a SCARA robotic arm transporting a 96-well plate to the ImageXpress Micro for analysis. These integrations can also enable large screening systems in which imaging is just one component of the workflow (Figure 4b).

Integrating devices to automate a greater portion of an imaging application has advantages beyond increasing throughput or reducing the active time required for a given assay. By reducing the number of user interventions, automation helps to reduce the opportunity for human error such as disordering tubes or inverting plate orientations. Data integrity is also improved as any liquid transfer or assay data generated

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a)

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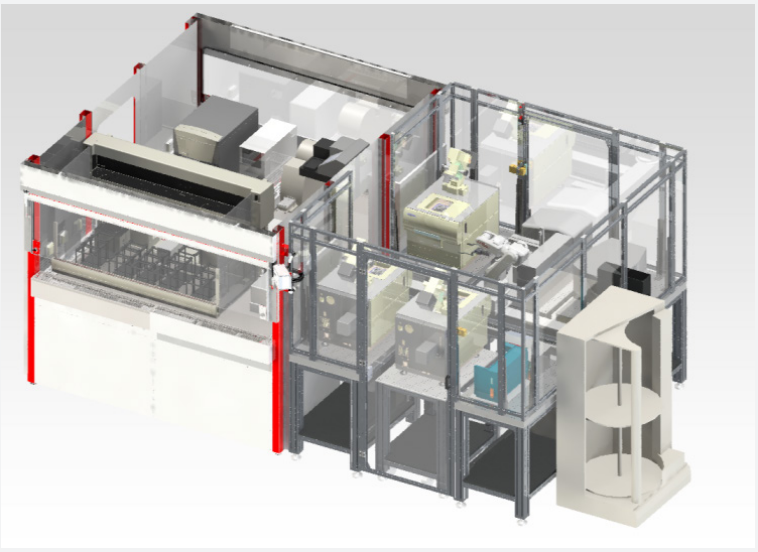

Fig. 4. Integrations facilitate automation of complete high content imaging workflows. **a)** Image showing the automated delivery of a processed plate to an ImageXpress Micro high content imager. **b)** Large integrated system that utilizes the Biomek FX^{P} for processing plates and multiple ImageXpress Micro imagers for phenotypic screens.

throughout the workflow is tied back to the original samples without the possibility of these mistakes. The ability of Biomek Workstations to integrate with high content imagers and other devices can increase the throughput and data integrity of cellular imaging assays by reducing the number of user interventions in a workflow.

Conclusion

As high content imaging moves into greater use for screening experiments, automation becomes a necessity to process the higher volume of samples. While the adherent nature of the cells being interrogated is ideal for liquid handling instruments, there still must be sufficient pipetting control to ensure that no cells are lost or disturbed during processing. In addition, the software must be able to accommodate numerous staining reagents and conditions while tracking data throughout the process. The staining reproducibility and flexible user interfaces shown here demonstrate that the Biomek Workstations deliver these abilities. In addition, the Biomek's open platform provides the flexibility to customize the instrument for various workflows. For cell staining applications, this customization can include the integration of high content imagers, incubators, or other instrumentation to accelerate sample preparation. These properties make the Biomek Workstations ideal solutions for automated liquid handling for high content imaging of any throughput or complexity.

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