Simplified, user-friendly, automated workflow for phenotypic profiling based on the Cell Painting assay

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Introduction

Multiparametric high-content screening approaches, such as the Cell Painting assay, are increasingly being used in many applications ranging from drug discovery programs to functional genomics screening. The Cell Painting assay uses up to six fluorescent dyes to label and visualize a variety of organelles at the single-cell level. Morphological features extracted from the assay give unique cellular "signatures" that provide an overview of the cell. In addition, insights into the mechanism of action may be gained by comparing the phenotypic profiles of novel compounds with those reference compounds.

Cell Painting assays are typically carried out at scale with multiple assay plates. The workflows can be time and labor intensive, taking several days to complete a screen. Here, we developed a complete automated workflow for the Cell Painting assay. Lenti-X 293T cells were treated with colchicine for 4 hours or 24 hours, with untreated cells as controls. An automated liquid handler was used to fix and stain the cells, which were then imaged on a high-content imager equipped with a laser light source. Images were analyzed and their measurements uploaded to StratoMineR™, a web-based tool for further data analysis.[1] Three distinct clusters were observed, each representing a single colchicine treatment condition. Together, the data presented here highlights how the use of an automated workflow combining Biomek liquid handling with the the ImageXpress® Confocal HT.ai High-Content Imaging System can be used for morphological profiling, with the added benefits of reduced hands-on time and user handling errors, with increased assay throughput.

Methods

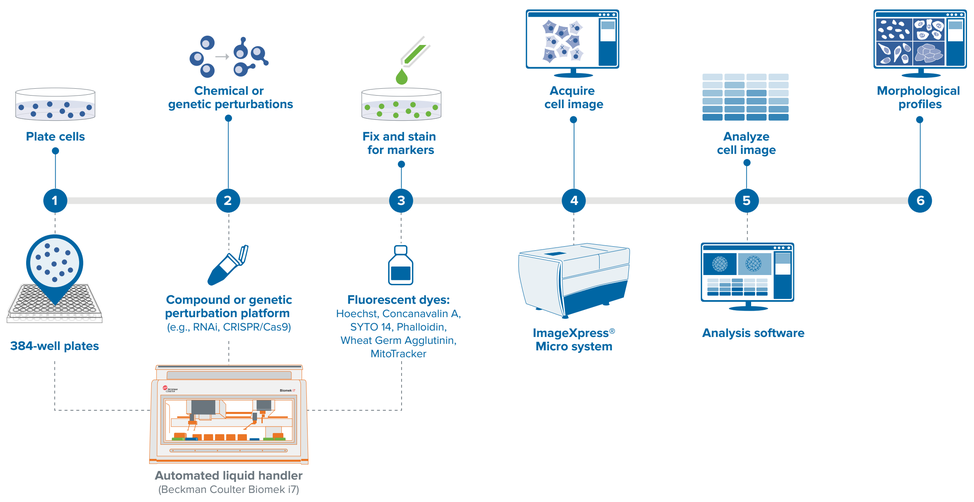


Figure 1. Cell Painting workflow. Simplified protocol for automated Cell Painting liquid handling, image acquisition, and data analysis. Cells were plated, treated, fixed and stained using a Biomek i7 Hybrid automated workstation (Beckman). Images were acquired using the ImageXpress HT.ai high content imager (Molecular Devices). Images were analyzed in the IN Carta image analysis software (Molecular Devices) and the data analyzed using StratoMineR (Core Life Analytics).

Cell culture

Lenti-X293T cells (Takara) were maintained at 5% CO_2 and 37°C in growth medium, which was composed of DMEM supplemented with 10% FBS and 1% 100X antibiotic/ antimycotic (Gibco). Cells were plated at 10,000 cells per well (100 µL total volume) using a Biomek i7 Hybrid workstation equipped with HEPA filtration unit.

Automated cell plate preparation

In general, the experiments performed here followed the protocol described in Bray et al.[2] For the automated liquid handling method, all aspiration and wash steps were performed using a 96-well Multichannel pod equipped with a 1200 µL head, and all reagent addition steps were performed with the Span-8 pod. All automated liquid handling steps were performed using a pipetting template that aspirated and dispensed at 5 µL/sec at a position >70% away from the center of the plate well. This ensured that cell monolayers on the bottom of the wells were undisturbed during the cell painting procedure. Paraformaldehyde solution was made fresh the day of the assay by dilution of a 16% stock solution to 6.8% PFA in ultra-pure water.

Image acquisition and analysis

Images were acquired using the ImageXpress Confocal HT.ai High-Content Imaging System (Molecular Devices) using the 20X Plan Apo objective, confocal pinhole size = 60 µm. The following filters were used (ex/em): DAPI 405/452, FITC 467.5/520, YFP 520/562, TRITC 555/598, TexasRed 555/624 and Cy5 638/692. Images were acquired in order of decreasing fluorophore excitation wavelength to reduce cross-talk. Four field of views were imaged per well. A small z-stack of 3 images were acquired with best focus projection option used to account for plate flatness issues which may compromise image focus. Image analysis was carried out with IN Carta® Image Analysis Software. Data was analyzed in StratoMineR™ (Core Life Analytics).

For a more detailed description of the protocol, please refer to the application note at www.moleculardevices.com.

Results

Six-fold reduction in hands-on time using automation for Cell Painting

Cell painting has been gaining popularity within the screening community as it can provide information-rich results following a variety of cellular manipulations. The plate preparation workflow for Cell Painting assays involves labor intensive liquid handling steps that can become impractical to perform manually, especially as the number of plates being screened increases.[2] The use of automation that includes liquid handlers could help to streamline these processes, saving valuable user time and increasing assay throughput.

		Manual		Biomek	
	Step	Incubation Time (Min)	Hands-on Time (Min)	Total (Min)	Hands-on Time (Min)	Total (Min)
1	Reagent Prep & Setup	—	5	5	10	10
2	Remove Media & PFA Fix	20	10	30	0	25
3	Wash Out PFA & Add Triton	10	10	20	0	20
4	Wash Out Triton & Add Stain	30	15	45	0	45
5	Wash Out Stain	—	20	20	0	15
	Total	60 min	**60 min**	120 min	**10 min**	115 min

Table 1. Timing of manual vs automated Cell Painting. A single 96-well plate was processed by hand using a 12-well multichannel or manual pipette and with the Biomek i7 Hybrid workstation. For a single plate, both workflows took approximately 2 hours to complete (120 manual vs 115 min Biomek), and 60 minutes of the total time was incubations at ambient temperature in the dark. For the Biomek method, the only hands-on time required was 5 to 10 minutes at the beginning of the method for deck setup.

Automated liquid handling for Cell Painting

In order to qualitatively evaluate this newly developed automated method, we stained HEK293 cells with fluorescent stains typically used in the Cell Painting assay (Figure 2).[1] The robust fluorescent signal observed in each channel shows that a) the staining step was successful and b) the automated liquid handling was gentle enough to keep cells adhered to the plate throughout the assay.

Results

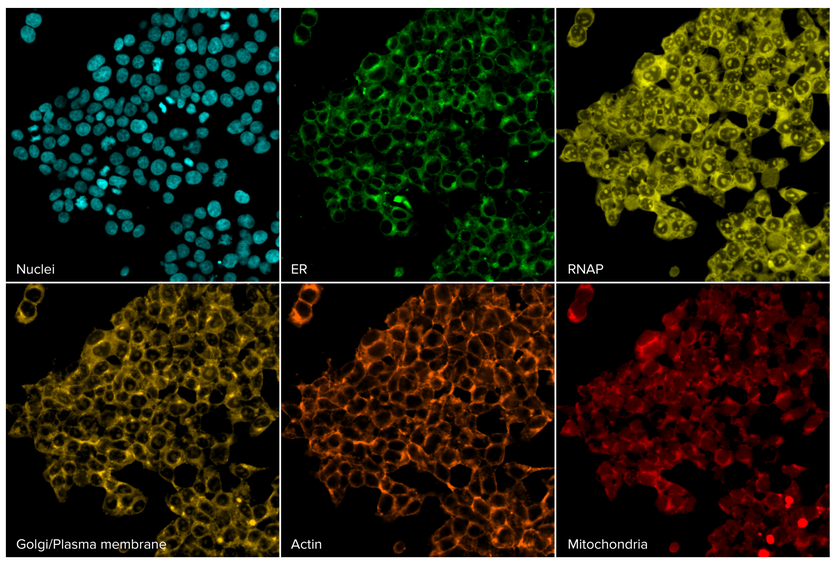


Figure 2. Example images acquired using an automated Cell Painting assay workflow. HEK293 cells were stained using a Biomek liquid handler and imaged in six fluorescent channels.

As Cell Painting is often used as a screening technique, each well will often contain a different cellular manipulation, such as a different drug-like small molecule. In order to simulate this assay setup, colchicine was selected as a test compound (Figure 3).

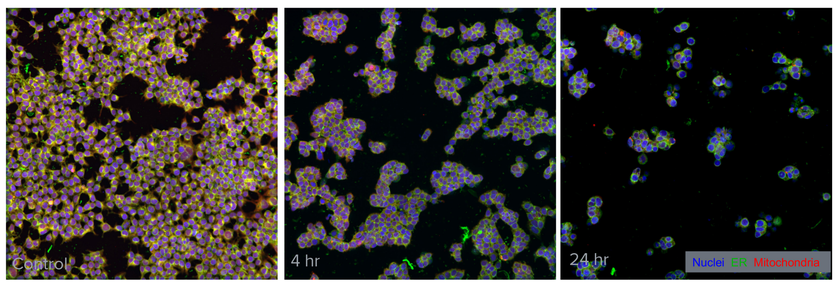


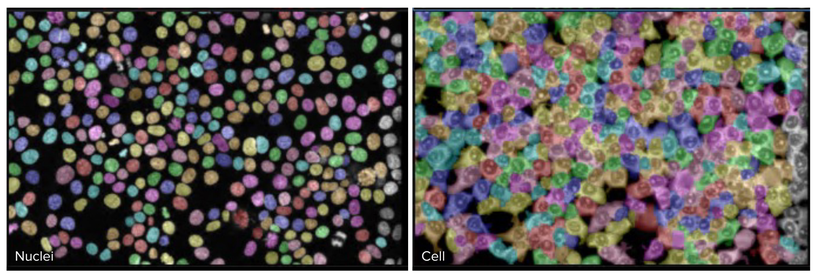
Figure 3. Colchicine treated HEK293 cells are phenotypically different from untreated control cells. Wells with colchicine contain significantly fewer cells after 24hr (646±37) compared to the control wells (4355±264) p<0.001.

Results

Automated image analysis with deep-learning improves nuclei segmentation

The image analysis routine can be adjusted to achieve robust detection of cells and organelles in IN Carta. Deep-learning semantic segmentation module (SINAP) may be used to improve detection of challenging features. Here, a pre-trained deep-learning model is used for the detection of nuclei (Figure 4).

A



B

#	Object ID	Row	Column	FOV	Nuclei Area wv6	Nuclei Form Fa..	Nuclei Elongati..	Nuclei Compa..	Nuclei Chord R..	Nuclei Gyratio..	Nuclei Displac..	Nuclei Diamet..	Nuc
302	301	F	2	4	111.32	0.82	0.81	1.15	0.54	4.52	0.90	15.07	
303	302	F	2	4	269.32	0.59	0.90	1.13	0.74	6.95	0.27	22.74	
304	303	F	2	4	115.99	0.93	0.99	1.00	0.92	4.30	0.86	12.60	
305	304	F	2	4	126.03	0.84	0.68	1.11	0.57	4.72	1.07	15.89	
306	305	F	2	4	113.07	0.91	0.89	1.01	0.88	4.27	0.51	12.80	

Figure 4. Image analysis was carried out in IN Carta. SINAP, a deep learning-based segmentation module was used segment individual nuclei. A) Example images from 2 channels with their corresponding segmentation mask overlaid. Objects touching the edge of the field of view were excluded. B) Measurements were linked to each segmented object allowing for easy overview of the analysis as well as for identifying outliers.

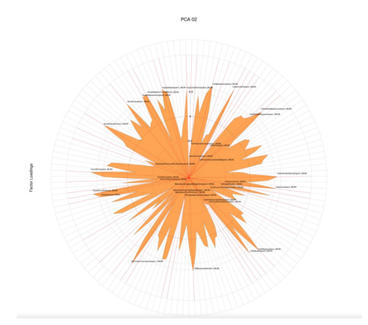
Data analysis workflow

The sheer volume of data generated from these experiments require powerful software and computational tools to extract meaningful interpretations. To circumvent the need of setting up in-house computational infrastructure, we used StratoMineR, a cloud-based tool for data analysis.

Principle component analysis was used to reduce the 487 measurements into 7 principal components. Three distinct clusters were observed, and each cluster was representative of a single colchicine treatment condition (Figure 5).

A

B



C

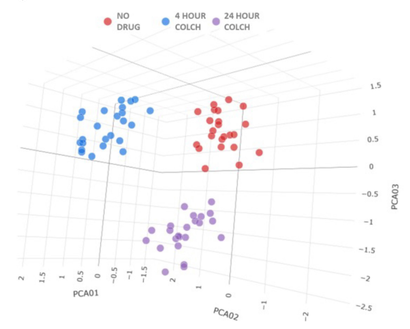


Figure 5. StratoMineR for data analysis. A) StratoMineR is a web-based platform which guides users through a typical workflow for analysis of high content multi-parametric data. B) Principal component analysis (generalized weighted least squares) was used for data reduction. Feature contributions to PCA2 is shown as a polar plot. C) 3D scatter plot shows interactions between data points in relation to 3 different PCAs. Note the 3 separate clusters correspond to each treatment condition.

Conclusions

- We designed and applied an automated approach the Cell Painting assay that includes cell seeding, fixing, and staining. Up to six times reduction in hands-on time can be achieved using a liquid handler.
- The automation protocol is easily scalable to handle multiple plates and 384 wells assays.
- Deep learning-based segmentation tools can be used to obtain robust image segmentation.
- Web-based data analytic tools such as StratoMineR allow for non-expert users to analyze and interpret complex data using guided workflows.

References

1. Omta WA, et.al., *Assay Drug Dev Technol.* 2016 Oct;14(8):439-452.
2. Bray MA et.al., *Nat Protoc.* 2016 Sep;11(9):1757-74.

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