# Automation of both sample preparation and analysis of a cell migration assay

Sarah E. Burroughs[1], Keren I. Hulkower[2], Simone Schicktanz[1], Jennifer Fronczak[2], Karin Boettcher[1], Timothy E. Cloutier[1]          [1]PerkinElmer, Inc.  [2]Platypus Technologies, LLC
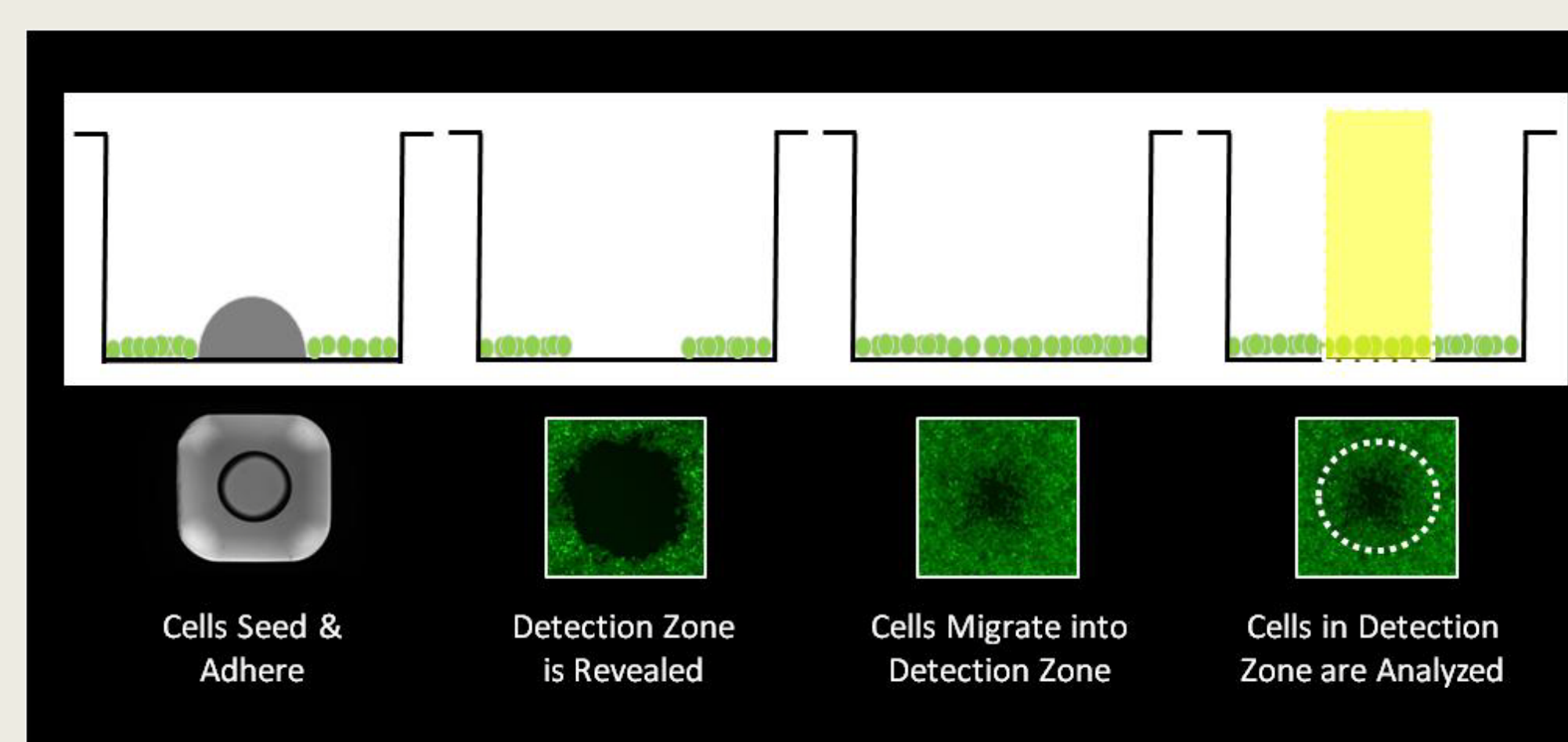
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## 1 Introduction

Cell migration is a critical event in the process of angiogenesis. Aberrant cell migration can contribute to metastatic cancer and other diseases. Understanding the signaling pathways involved in regulating migration is crucial for discovering new targets to develop drugs that inhibit migration and angiogenesis. Because cell migration is a phenotypic or whole-cell event, it can be challenging to conduct and quantitatively analyze such cell-based assays in a consistent and efficient manner. Automation of such assays allows researchers to analyze more conditions than would be feasible with manual methods. With advances in migration plate technology and automated liquid handling, it is now possible to increase one's capacity to run these important assays. However, liquid handling automation parameters are critical and must be optimized so as not to interfere with the biology or its analysis. Here we demonstrate a successful automation of the sample preparation of Platypus Technologies' Oris™ Pro Cell Migration Assay on a PerkinElmer JANUS® Automated Workstation. Analysis of cell migration using the Operetta® High Content Imaging System and the EnSpire® Multimode Plate Reader shows the assay to be a robust and reproducible quantification of cell motility.

### Key Technologies Used:
- Platypus Technologies Oris™ Pro Cell Migration Plate
- PerkinElmer JANUS Automated Workstation
- Operetta High Content Imaging System
- EnSpire Multimode Plate Reader



Cells Seed & Adhere | Detection Zone is Revealed | Cells Migrate into Detection Zone | Cells in Detection Zone are Analyzed

Graphical Depiction of the Oris™ Pro Cell Migration Assay Platform. The Oris Pro Plate uses a biocompatible gel (BCG) and is thus amenable to automation.

JANUS Automated Workstation with:
- Varispan™ Arm for independent pipetting of 1-8 channels and sample transfer from tubes to plates
- VersaTip® adapters for use of both fixed and disposable tips in a single protocol

Operetta High Content Imaging System

EnSpire Multimode Plate Reader

## 2 Materials & Methods

### Materials
- Oris™ Pro Migration Assay Collagen I Coated 96-well Plates (Cat. No. PROCMACC1); Platypus Technologies
- HT-1080 cells (Cat. No. CCL-121); ATCC
- sodium pyruvate solution, HBSS; Mediatech, Inc.
- Cytochalasin D (cyto-D), U0126 ethanolate, blebbistatin, phalloidin-TRITC, DMSO (>99.9%), ethanol (200 proof), Brilliant Blue G, formaldehyde 37% solution; Sigma Chemical Company
- Cell Dissociation Buffer, Hoechst 33258 (10mg/mL solution), fetal bovine serum (heat-inactivated), Hank's balanced salt solution (HBSS, +CaCl$_2$, +MgCl$_2$); Invitrogen Corp.
- EMEM, Cat. No. 06-174G; Trypan Blue 0.4% solution; Lonza
- JANUS Varispan 8 tip mini/standard deck- plate tiles, tip chute, Test tube rack (12mm tubes), flush/wash, WinPrep 4.7
- JANUS VariSpan disposable tips: 1mL, 200µL (conductive)

**Experimental Workflow:**

**Day 1**
1. Seed HT-1080 cells (30,000 cells/well) into Oris™ Pro 96-well plate (manual); incubate for 45-60 min
2. Prepare compounds in complete media (JANUS)
3. Replace seeding media with media containing compounds or controls (JANUS)
4. Incubate for 18-24 h

**Day 2**
1. Exchange media for HBSS$^{++}$ (JANUS)
2. Fix & stain cells (manual)
3. High Content Imaging & Analysis (Operetta) OR Absorbance measurement (EnSpire)

### Automated Method Development

A JANUS Workstation with Varispan Arm was used to prepare the compounds dose the cell plates, and perform media exchanges.
The JANUS VariSpan deck was outfitted with:
- disposable tips (both 1mL & 200µL)
- test tube rack for holding stocks & performing serial dilutions
- plate support tile for the Oris™ Pro Migration plate
- 4-well trough for media, a flush/wash station, & tip chute.
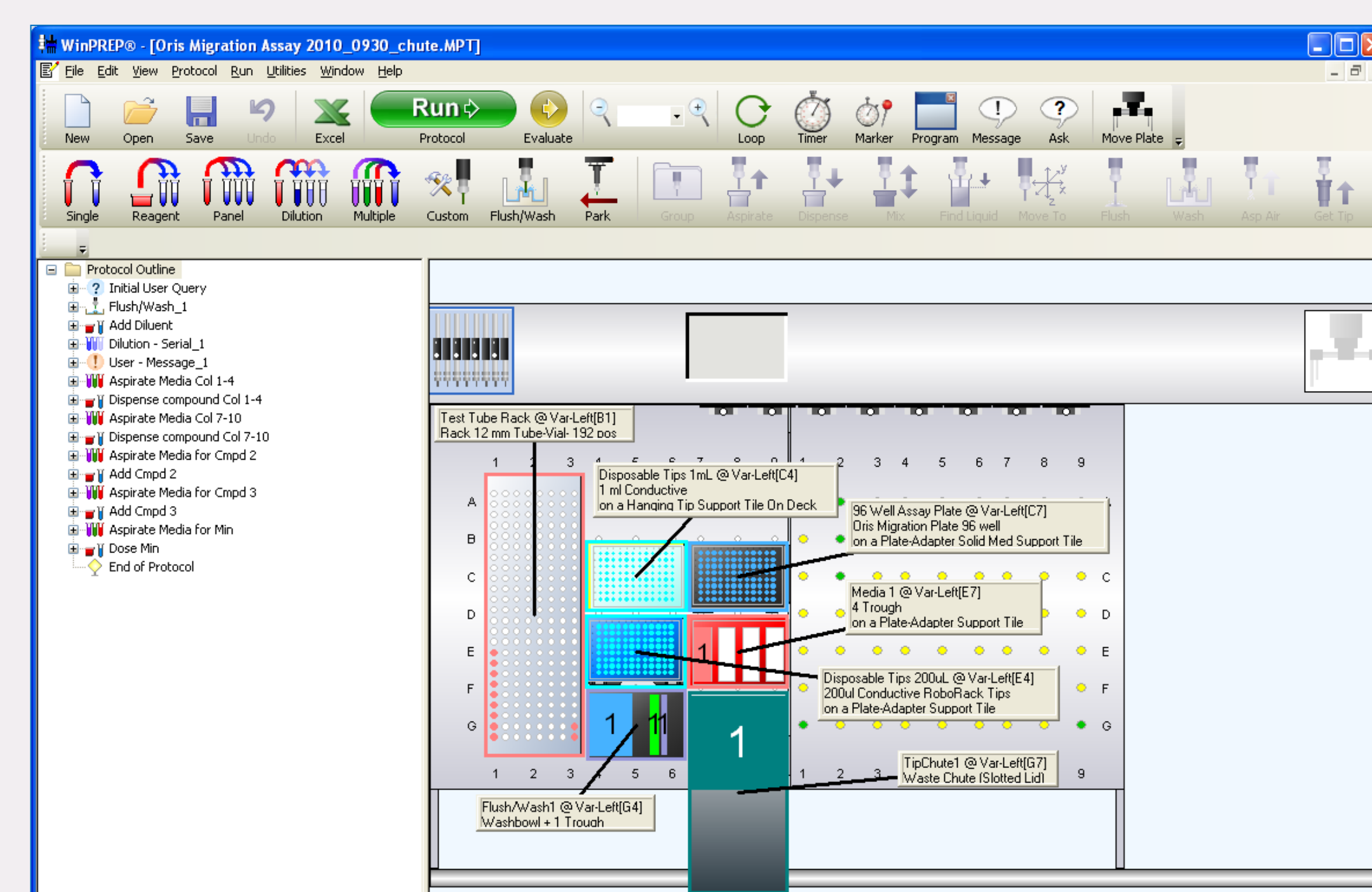


The WinWREP® software interface for JANUS, showing the protocol outline and deck layout for the automated cell migration assay.

Liquid handling parameters were optimized to avoid contact with the cell layer while still delivering liquid in an efficient manner. A combination of settings defined in the performance file & the plate definition file specify the height & speed of aspirate & dispense steps.

| Parameter | Optimized Value |
|---|---|
| Aspirate Height | 1.5 mm from well bottom |
| Aspirate Speed | 10 µL/sec |
| Dispense Height | 2.0 mm from well bottom |
| Dispense Speed | 10 µL/sec |
| XY Position | 9 o'clock |

## Materials & Methods, cont.

### High-Content Analysis (HCA)
Cells were fixed with 3.7% formaldehyde in PBS, permeabilized with 0.1% Triton X-100, and stained with Hoechst 33258 (1:10,000 in PBS-Tx) for 30 minutes.
The fluorescently stained plates were imaged and analyzed on an Operetta High Content Imaging System equipped with Harmony® software. The CellMigration-1 algorithm was used to identify and calculate the area of the region not covered by cells. The feature "Open Area" was used as the measurement for the HCA results.

**Image Analysis sequence: CellMigration-1**



Input Image: Hoechst | Cell Layer | Open Area
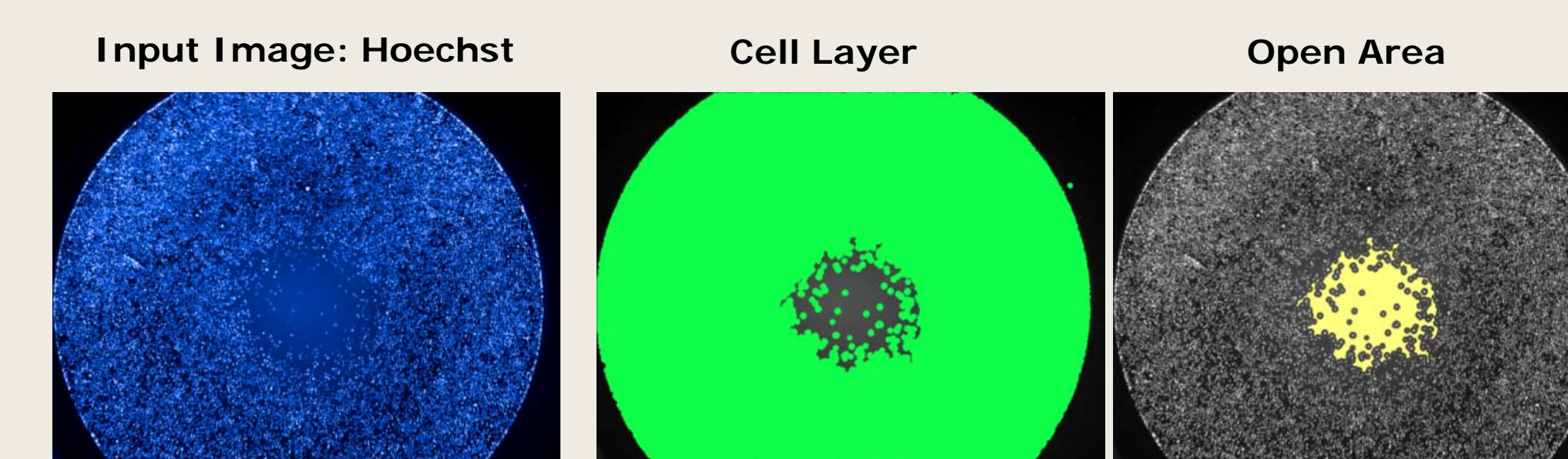
### Absorbance Measurement of Migration
Cells were fixed with 3.7% formaldehyde in PBS, washed with water, then stained with Coomassie reagent for 1 hour followed by several water washes.
The Coomassie-stained plates were read in an EnSpire multimode reader using a photometric 620nm filter. A special plate definition was created in which the scanned well diameter was reduced to 4.5 mm from the default 6.5 mm.
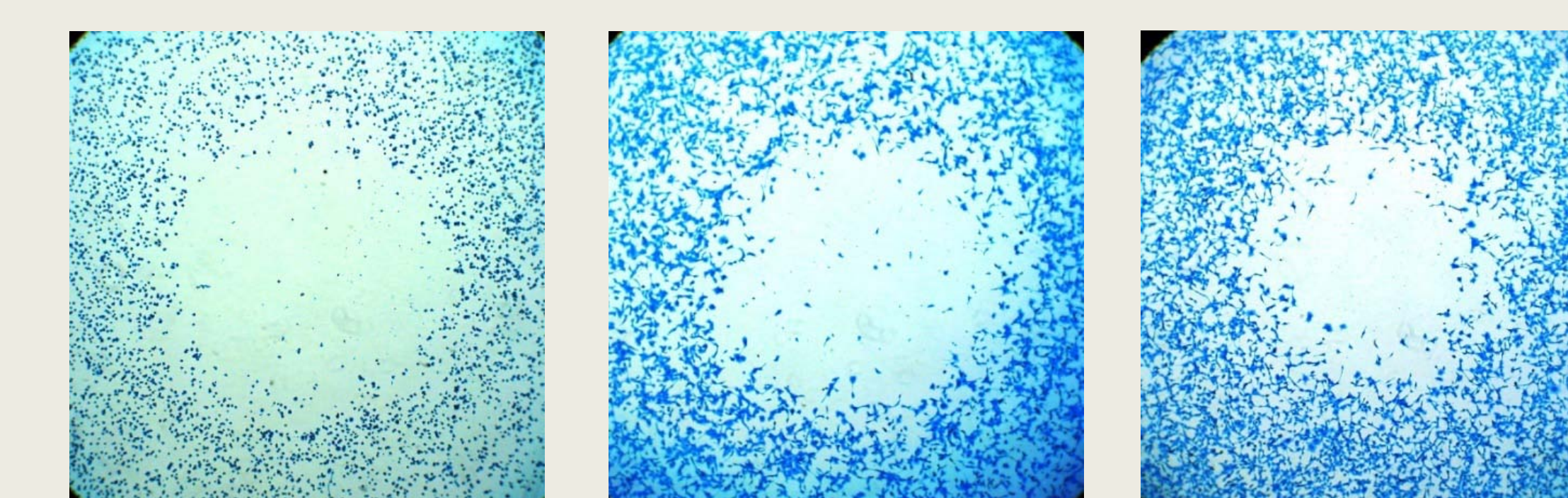


Photomicrographs of cells from an Oris™ Pro 96-well Cell Migration Assay stained with Coomassie blue. Left, 10 µM cyto-D; Center, 0.5 µM; right, 0.03 µM. Scale: the images represent the largest square that will fit into the well; photo edge ~4.6 mm, corner-to-corner distance ~6.5 mm.

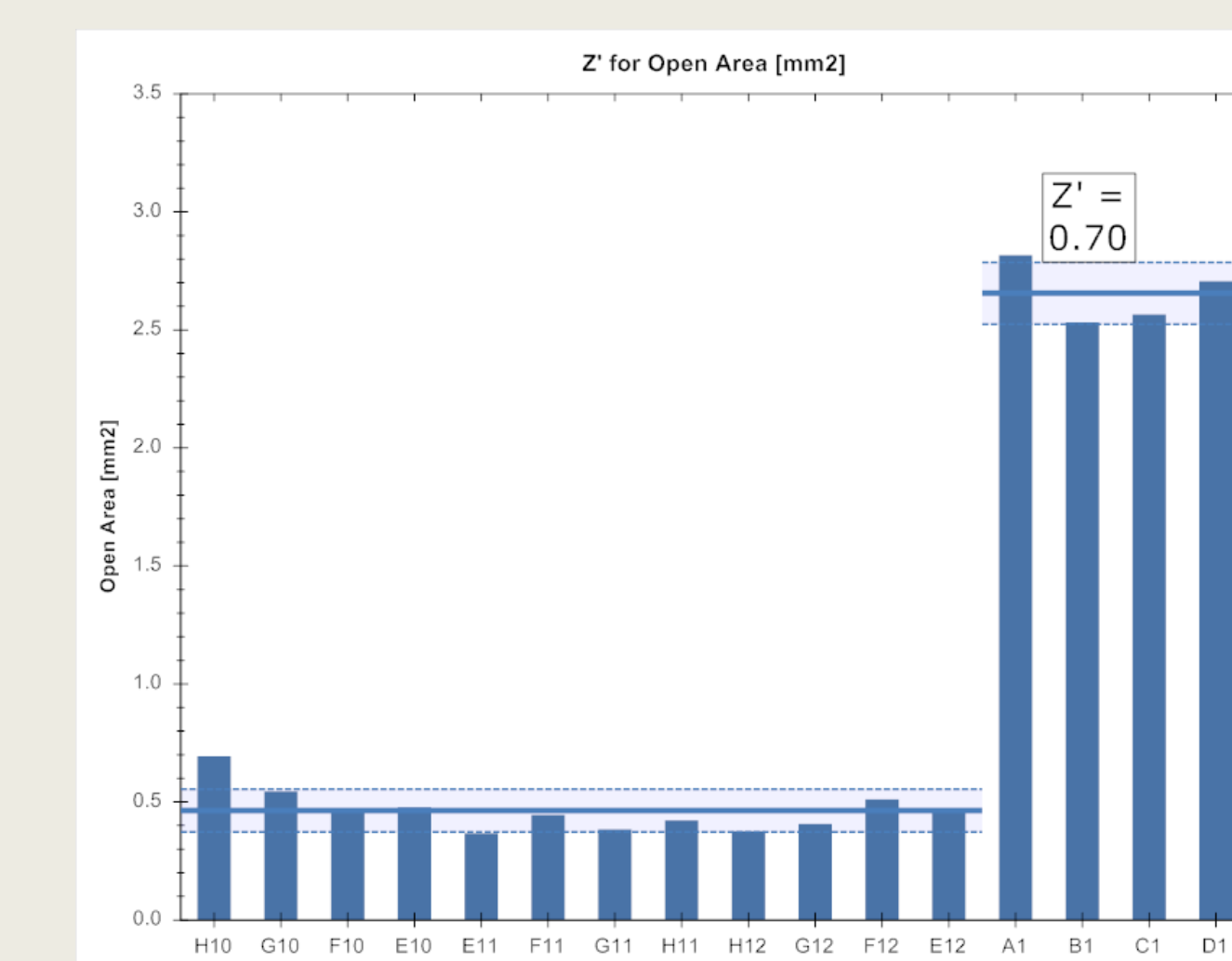## 3 Results

### Performance of the Automated Method
Liquid handling parameters were verified to avoid contact with the cell layer while still delivering liquid in an efficient manner. Colorimetric stained plates were visually checked and found to have no disturbance of the cell layer.
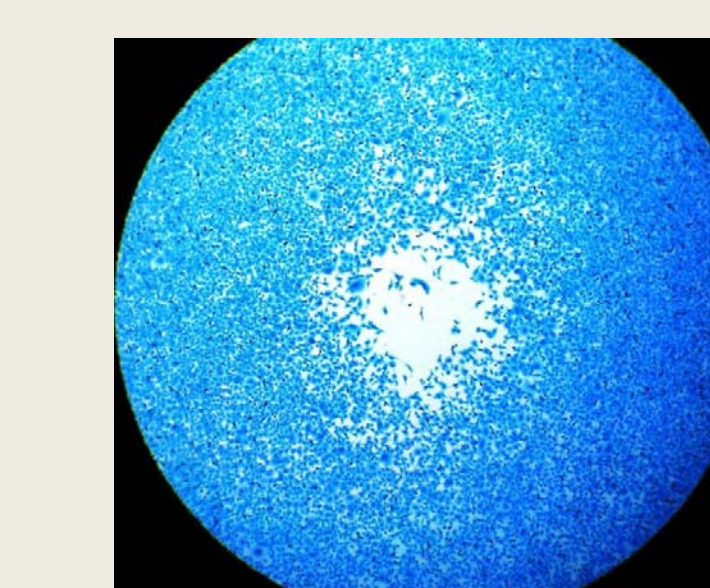


Color image of well after automated pipetting, migration for 18 hours, & manual Coomassie staining. Only the void from the BCG position is visible, and no evidence of perturbation in the 9 o'clock region of the well is observed.

High content analysis of a plate containing a number of negative control wells was used to assess assay robustness and well-to-well reproducibility. A Z' of 0.70 indicates a highly robust assay and is higher than reported values for manual plate preparation.



Analysis of robustness of the automated liquid handling/HCS method. Twelve negative control wells (0.1% DMSO) and 4 positive control wells (2 µM cyto-D) were used to calculate a Z' factor of 0.70.

## Results, cont.

### Quantitative Analysis of Inhibition by Cytochalasin D
Two-fold dilutions of cyto-D were prepared and added to the cell plates using the JANUS and processed as described. Samples imaged and analyzed with an Operetta instrument and Harmony software to determine the potency of cyto-D in this assay format.
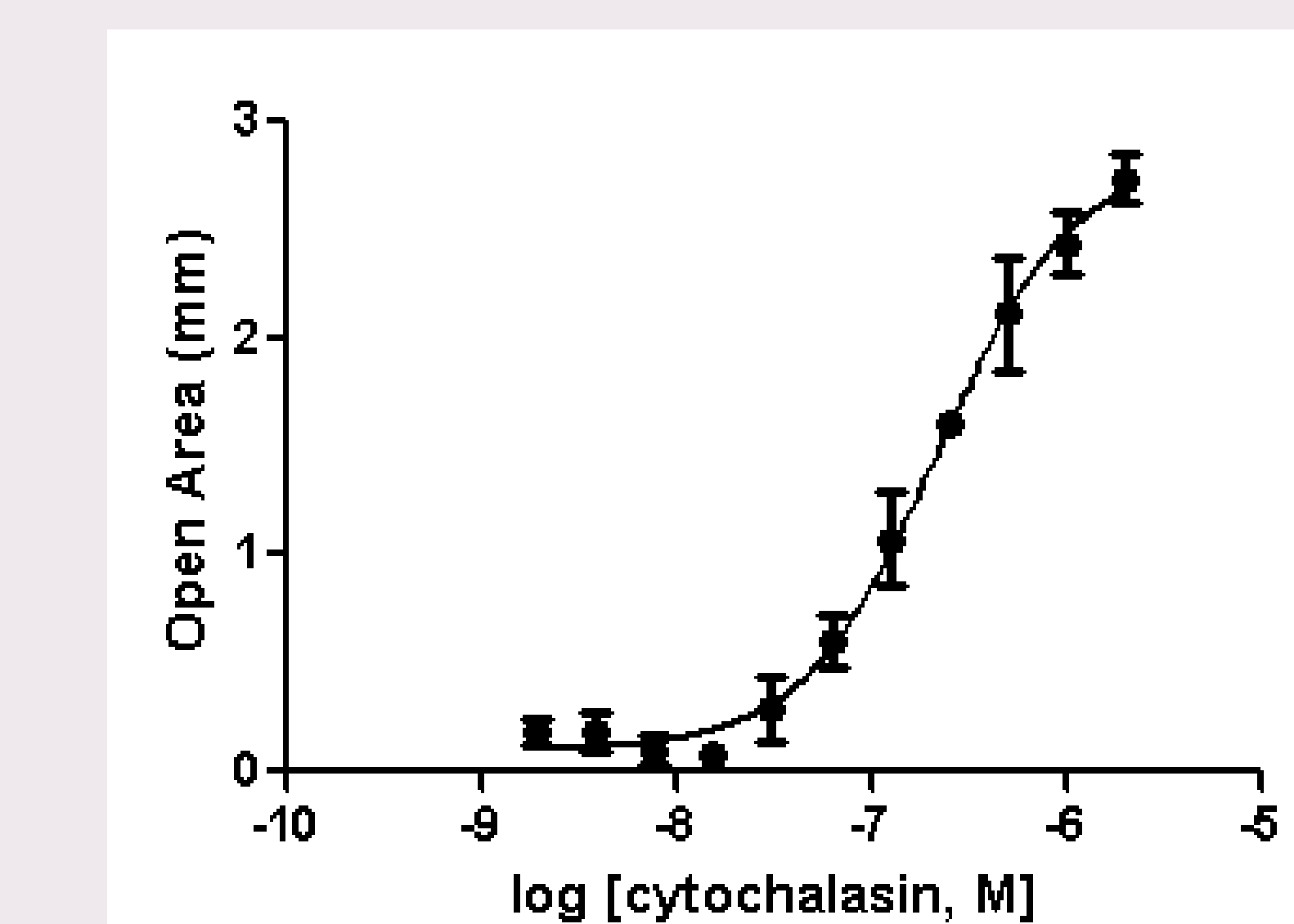


Dose-response of cyto-D on HT-1080 cells as measured by high-content analysis.
IC$_{50}$ = 215 nM

### Inhibition by other compounds
Additional compounds tested in the Automated Cell Migration Assay included U0126, a MEK inhibitor, & blebbistatin, an inhibitor of non-muscle myosin II which has had mixed results in cell migration assays. Percent Activity for these compounds was calculated as:

$$((Signal - Background)/Window) * 100$$

Where DMSO control is the background and window is the difference between the values for cyto-D and DMSO control.



Blebbistatin 23% | U0126 7% | DMSO Control | Cytochalasin D **
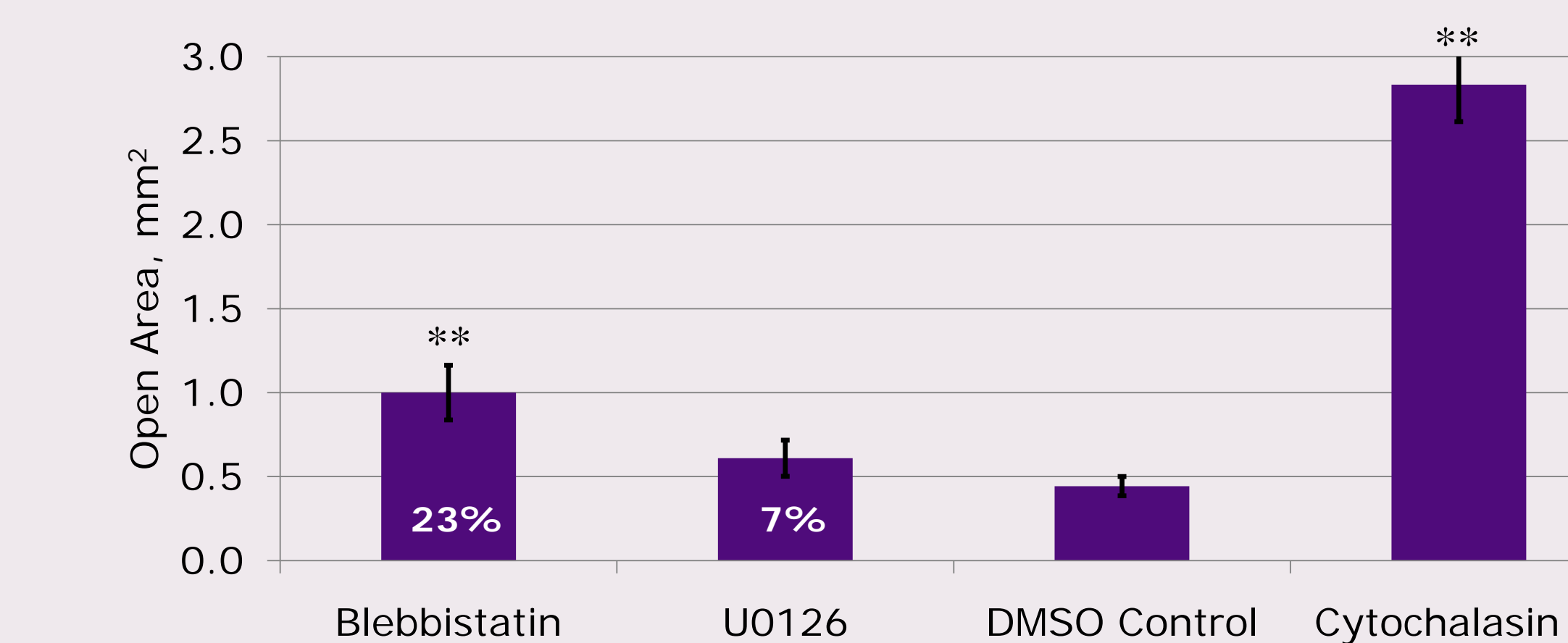
Automated Cell Migration Assay with High Content Analysis: 50 µM blebbistatin, 100 µM U0128, 0.1% DMSO (negative control), 2 µM cytochalasin D (positive control). Error bars represent std dev; n = 12 except cyto-D where n = 4. **, p-value <0.001

## 4 Summary/Conclusions

- An automated JANUS method for preparing samples in the Oris™ Pro Cell Migration Assay was developed and shown to perform robustly.
  - High-content analysis of the positive control (cyto-D) gave a Z' of 0.70.
  - Statistically significant inhibition of cell migration by blebbistatin was detected with high-content analysis.
- JANUS automated sample preparation:
  - Improves assay quality by reducing well-to-well variability
  - Reduces hands-on time for sample preparation
- Operetta imaging platform with Harmony high content analysis provides a robust and specific measure of cellular migration or inhibition thereof.
- Platypus Technologies' Oris™ Pro plates combined with PerkinElmer JANUS automated liquid handling and Operetta high-content analysis platforms make cell migration inhibitor screening feasible.