MOLECULAR DEVICES | SCIKON INNOVATION INC

APPLICATION NOTE

High-throughput imaging of a unique continuous flow microfluidics plate

Introduction

The continued drive towards cell based assays that better mimic the *in vivo* environment has led to innovative cell culture systems such as the SciFlow™ 1000 Fluidic Culture System, which utilizes capillary channels to enable physiologically relevant fluid flow across cultured cells. In this application note, we present the ability to accurately image the wells of this novel culture system with the ImageXpress® Micro High-Content Imaging Systems to visualize and quantify cytotoxic exposure kinetics.

The SciFlow 1000 System (Figure 1) is an SBS formatted, 96-well device compatible with plate-readers, imaging systems, and automated liquid handling systems. In order to enable efficient flow of fluids, the system is designed with descending well-heights from left-to-right so the bottom of each subsequent well is 500 microns lower than the previous well in the row (i.e. z-height changes). This allows for cascading flow of fluids across the system without the need for external pumps or connected tubes.

However, this changing z-height across the plate presents a challenge for most imaging systems due to the inability to accurately determine a focal position in each well. This application note will show that the ImageXpress Micro Systems are capable of dynamically adapting focus position across the required 4000 µm vertical range to rapidly deliver clear, focused images for quantification.

Materials used

- SciFlow 1000 Fluidic Culture System (SciKon Cat. No. AA-1-50)
- HepaRG Cells (Triangle Research Labs Cat. No. NSHPRG)
- Culture media MH100, MHTAP, MHMET (Triangle Research Labs)
- CellTox™ Green (Promega Cat. No. G8731)
- Hoechst DNA Dye (Sigma Cat. No. 33342)
- Tamoxifen (Sigma Cat. No. T5648)
- Fluorescein salt (Sigma Cat. No. F6377)
- Instrument: ImageXpress Micro High-Content Imaging System (Molecular Devices)

Benefits

- **Utilize microfluidic channels to model fluid flow in organ and living tissue systems**
- **Acquire in-focus images across a range of well depths in a single 96-well format**
- **Monitor real-time fluid flow, dynamic cell culture responses, and compartment-to compartment signaling**

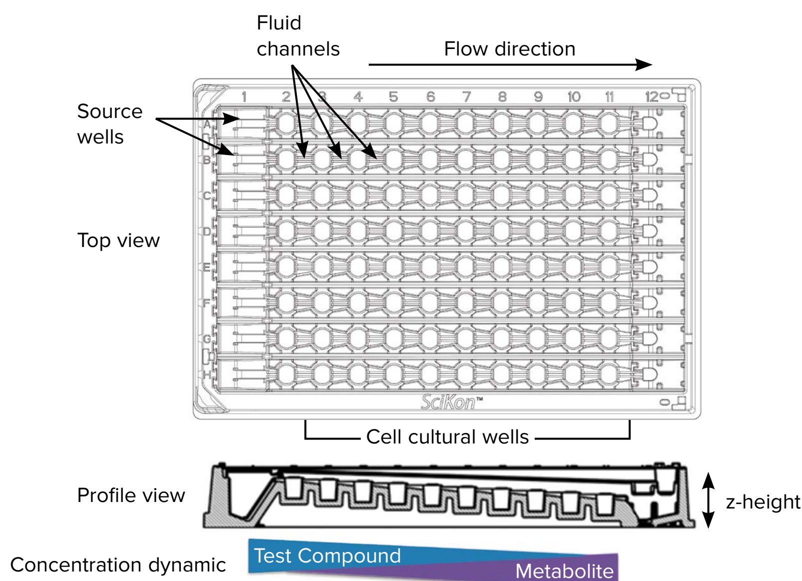

Figure 1. SciFlow 1000 System Features. Top and profile views of SciFlow illustrate fluidics and multiwell major features. Note the difference in z-height of each well in the profile view. By applying a test chemical to the source well (column 1), serial exposure into subsequent wells results in a gradient of decreasing chemical concentration and a concurrent increase in metabolites and cellular responses across the linear array of linked wells.

Method for optimizing focus position using the ImageXpress Micro system

To determine z-focus position in each well, HepaRG cells were plated in the SciFlow 1000 System then analyzed via nuclear DNA staining with Hoechst. The z-height adjustments for the system are listed in Table 1, with Column 7 being the mid-system or zero-reference point used for configuring laser autofocus during imaging.

Experiment

Our method workflow (Figure 3) outlines the experimental design (Figure 2) of Tamoxifen treatment of HepaRG cells. HepaRG cells were plated in 50 µL media. Following cell attachment, the media was exchanged with media containing 1:2000 dilution of the cytotoxicity indicator CellTox™Green and 0.5 µg/ml Hoechst (100 µL/well). Then, 400 µL of media containing CellTox Green was applied to the source wells. The plate was incubated for 30 minutes to activate fluid flow and allow for connection of the fluidic channels. Once fully connected, 100 µL of test compound, vehicle control, or 1 µM fluorescein (fluid tracer control) was added to the respective source well. To maintain fluid flow over multiple days, 100 µL was removed manually from wells in column 11, and fresh drug, vehicle, or fluorescein standard was added to the source well three times per day. This resulted in a complete turnover of media over four days while providing a constant infusion of reagent in each treatment group.

Automated Imaging with Z-height focal adjustments to accommodate variable well depths

Hoechst (all cells) and CellTox Green (dead cells) staining was monitored by imaging the entire plate at multiple time points. Plates were read prior to compound addition and at 12 subsequent time points over 4 days. Between imaging runs, environmental control was maintained by keeping the plates in an incubator. Images were analyzed with MetaXpress software using the Live Dead Application Module or Custom Application Module to quantitate cell viability.

The SciFlow 1000 System is configured similar to a 96-well culture format. SciFlow also incorporates z-height steps of 500 microns between neighboring columns to create a maximum z-height change of 4 mm between wells three and 11 (Table 1). Rapid imaging of the whole plate is advantageous as the SciFlow 1000 System has active fluid flow and real-time compartment-to-compartment (well-to-well) signaling as a result of temporal toxicant concentration gradients and parent-metabolite interactions.The ImageXpress Micro System was used in widefield mode and utilized laser autofocus (4X) and, optionally, image-based autofocus (20X) to accomodate for the large z-height changes across the SciFlow 1000 System. The ImageXpress Micro system imaged all 72 wells (1 field of view per well) in 90 seconds at 4X and 5 minutes at 20X. Imaging at 20X included use of image-based autofocus.

SciFlow 1000 System z-height adjustments									
Column	3	4	5	6	7	8	9	10	11
microns	+ 2000	+ 1500	+ 1000	+ 500	0	- 500	- 1000	- 1500	- 2000

Table 1. Z- Heights in the SciFlow 1000 System using ImageXpress Micro system.

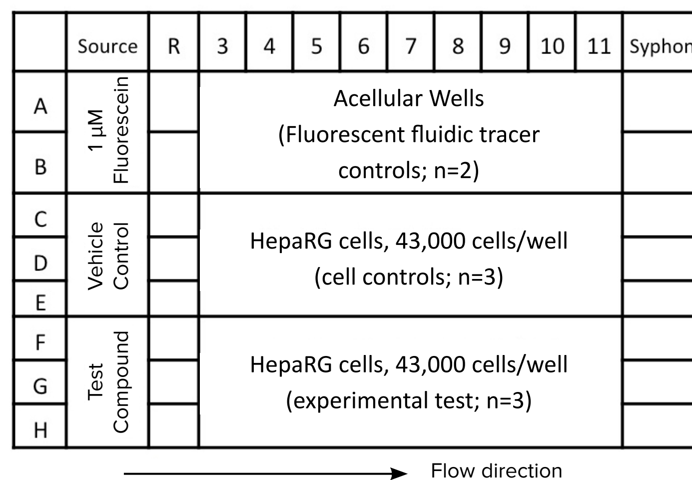

Figure 2. Example of a single SciFlow 1000 System with the experiment layout. Cell seeding and attachment (50 µL) is done in columns 3-11. Then, well volume is increased to 100 µL allowing fluid connection between wells. Lastly, the test compound is added to the source well in column 1.

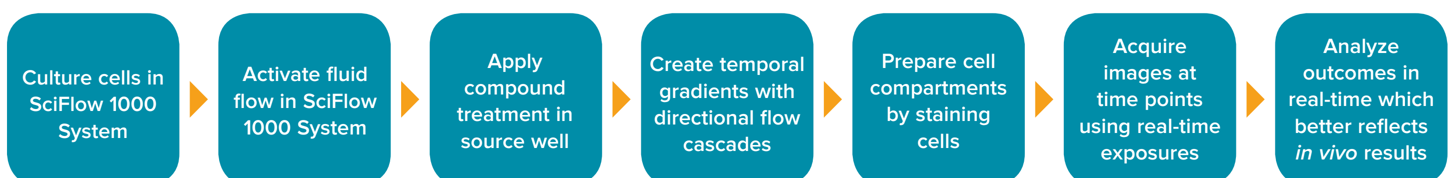

Figure 3. Method workflow.

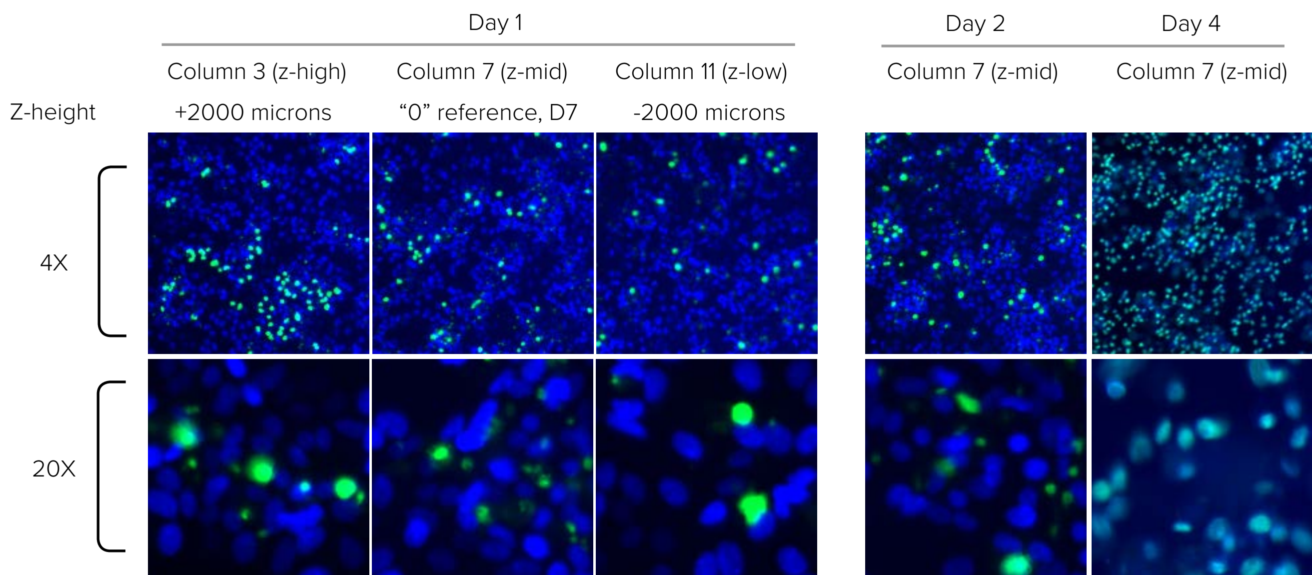

Figure 4. Images of cells at 4X and 20X. Blue = all cells, Green = dead cells. **(Left)** Day 1, concentration gradient across Row D (vehicle control) before initiation of tamoxifen flow. **(Right)** Day 2-4, real-time tamoxifen exposure kinetics (Row G) of concentration vs. time.

Summary

The future of high-content imaging is the capability to accommodate more complex and physiologically relevant culture formats. Imaging of non-standard formats provides flexibility in workflow while producing high-quality images for research needs. The SciFlow 1000 Fluidics Culture System is one such format, which can be used to monitor real-time fluid flow, direct cell culture responses, and dynamic compartment-to-compartment signaling of cellular responses. We are able to show that the ImageXpress Micro systems are compatible with variable z-height format plates such as the SciFlow 1000 System. Automated imaging was achieved across greater than 4mm of z-height change, rapidly generating high quality, reproducible data.

A Vehicle controls

Hours of exposure/experiment

	3	4	5	6	7	8	9	10	11
0.0	63.0%	62.1%	70.3%	65.1%	66.4%	70.5%	70.2%	74.2%	76.5%
0.5	61.0%	62.6%	67.6%	66.2%	64.4%	69.3%	66.7%	73.8%	73.6%
18.0	70.1%	72.5%	77.2%	75.2%	70.5%	79.3%	76.0%	80.2%	81.2%
20.4	68.4%	70.8%	77.8%	75.5%	72.0%	78.6%	77.4%	79.4%	80.4%
22.8	68.5%	71.5%	79.0%	76.3%	73.9%	80.7%	79.5%	80.9%	81.8%
24.9	68.3%	73.0%	79.1%	77.5%	74.1%	81.4%	80.2%	82.1%	82.8%
39.8	65.6%	68.1%	76.2%	75.3%	74.4%	81.8%	82.7%	83.1%	87.3%
44.4	64.9%	68.8%	74.8%	75.6%	75.7%	82.7%	82.9%	82.4%	87.7%
46.9	67.0%	68.4%	76.4%	76.0%	76.5%	84.1%	84.4%	83.7%	87.4%
49.5	67.9%	68.9%	74.6%	75.1%	76.4%	83.6%	83.8%	84.7%	88.4%
65.3	62.5%	63.2%	69.5%	70.5%	75.2%	82.5%	84.4%	85.5%	89.0%
68.0	61.7%	63.2%	69.9%	71.1%	76.3%	82.4%	84.6%	85.9%	89.7%
70.5	60.4%	61.1%	71.1%	72.5%	77.3%	83.1%	85.4%	86.4%	89.4%

B Tamoxifen

Hours of exposure/experiment

	3	4	5	6	7	8	9	10	11
0.0	64.3%	65.0%	57.4%	60.7%	66.8%	69.5%	69.3%	79.9%	75.5%
0.5	61.5%	62.6%	56.1%	59.9%	66.3%	67.4%	68.9%	77.7%	75.0%
18.0	73.2%	75.7%	66.5%	68.0%	75.8%	73.8%	74.1%	84.1%	78.6%
20.4	70.5%	72.2%	66.7%	68.0%	73.3%	74.5%	73.4%	82.5%	77.7%
22.8	71.7%	72.7%	68.8%	70.0%	77.1%	75.4%	75.1%	83.4%	79.1%
24.9	69.3%	73.3%	68.2%	71.9%	78.7%	76.3%	74.4%	82.4%	79.0%
39.8	6.0%	6.0%	27.6%	43.5%	51.4%	57.8%	74.3%	82.4%	82.6%
44.4	4.0%	2.6%	21.0%	40.2%	49.2%	56.3%	75.0%	83.5%	84.0%
46.9	2.9%	1.9%	17.2%	30.2%	44.8%	52.3%	72.2%	82.8%	83.5%
49.5	2.1%	1.3%	13.1%	22.4%	31.9%	40.7%	61.1%	76.7%	81.7%
65.3	2.6%	0.8%	0.4%	0.3%	0.3%	0.5%	1.1%	5.5%	21.7%
68.0	2.9%	1.0%	0.2%	0.2%	0.1%	0.3%	0.7%	3.8%	20.8%
70.5	4.2%	2.0%	0.4%	0.3%	0.2%	0.2%	0.4%	3.0%	18.7%

Figure 5. Tamoxifen cytotoxicity measurement. Table data show cell viability across SciFlow 1000 System rows. Table columns represent corresponding columns (3-11) in SciFlow 1000 System. Table rows are time points over which the experiment was measured. Values were generated from 4X images. **(A)** This table shows the percentage of viable vehicle control cells over time. The values are an average of C, D, and E rows. **(B)** This table shows cell viability for tamoxifen. The values are an average of F, G, and H rows.

For additional information on the SciFlow 1000 System, please contact:

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