Application Note

Traceable 3D Fluid Mixing and Rapid 3D Media Uniformity in the SciFlow™ 1000 Fluidic Culture System as Detected by BMG LABTECH's CLARIOstar High-Performance Reader

SCIKON INNOVATION INC

BMG LABTECH

Rapid Mixing Establishes 3D Uniformity in the SciFlow™ 1000 Fluidic System using CLARIOstar

3 Dimension Mixing
Nutrient Uniformity
Repeatable Exposures
Variable "Z" Adaptable
Easy / Quick High Quality

Introduction and Quick Summary

SciFlow 1000 is a flexible fluidic 96-well system that is designed to mimic *in vivo* like conditions, enabling the evaluation of a compound's effects in a human biology relevant fashion. The SciFlow System operates like a shallow river bed with a series of linked 3D compartments for cell culture and monitoring of downstream cell signaling. The design allows for isolated and static culture during cell seeding then delivers real-time 3D fluid flow and compartment-to-compartment signaling over time. The entire system is contained within a 96-well format that includes 8 identical channels, each consisting of a linear array of 1-to-9 cell culture wells. All wells are easily accessible for media sampling, media changes, performing assays and cell visualization.

The SciFlow 1000 System is an SBS formatted device compatible with plate-readers, imaging systems, and automated liquid handling systems. In order to enable efficient flow of fluids, SciFlow 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 (i.e. z-height changes). This allows for cascading flow of fluids across the system without the need for external pumps or connected tubes (Figures 1 & 2). The BMG LABTECH's CLARIOstar High Performance Microplate Reader provides the ability to dynamically track a bolus dose of infusion compound and the ensuing 3D fluid mixing in downstream cell culture wells. This is critical as drug exposures enter and exit, in a temporal fashion, each well of the SciFlow System.

This application note demonstrates high-performance microplate reading in 3D culture wells involving a 4 mm vertical range. This was achieved by fluorescent characterization of fluid mixing within cell compartments of the SciFlow System. By pairing SciFlow and the CLARIOstar Reader, we demonstrated the ability to dispense, visualize, and monitor the time course of 3D fluid transport to quantify the uniformity of mixing kinetics using the fluorescence of a Fluorescein salt in culture media.

Materials Used

SciFlow™ 1000 System (SciKon catalog # AA-1-50)
Culture media: DMEM (Gibco Cat # 31053) supplemented with 10% FBS (Sigma # F2442)
Fluorescein salt (Sigma Cat # F6377)

Instrument: BMG LABTECH's CLARIOstar High-performance Microplate Reader with Revolutionary LVF Monochromator Technology

SciFlow ™ 1000 System Features

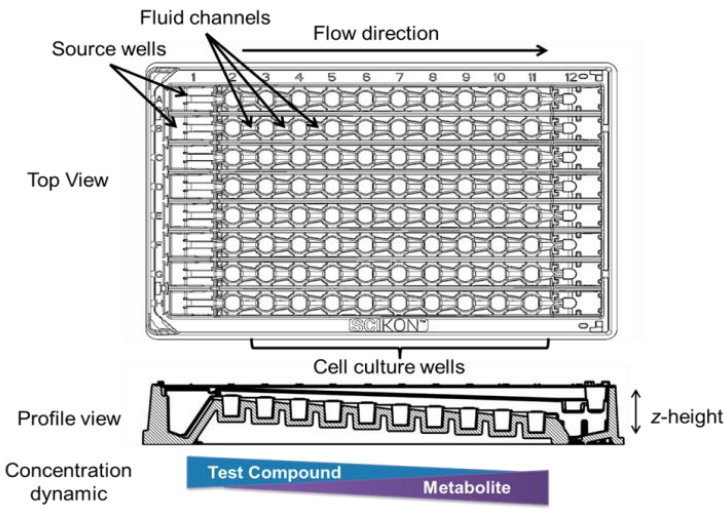

Figure 1. Top view and profile view of SciFlow™ 1000 System illustrating fluidics and multiwell major features. Note the difference in z-height of each well in profile view. Functionally, by applying a test chemical (e.g. fluorescein tracer) to the source well (well 1), serial exposure into subsequent 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.

Table 1	Z- Height Positions in the SciFlow System; well-7 culture surface as reference								
Column	3	4	5	6	7	8	9	10	11
microns	+ 2000	+ 1500	+ 1000	+ 500	0	- 500	- 1000	- 1500	- 2000

Methods

Optimizing z-height focus using the BMG LABTECH's CLARIOstar

Background: z-height characteristics of SciFlow comprise two attributes: 1) The flat culture surfaces have distinct z-height locations for each SciFlow column; see Table-1. In this table, the well-7 culture surface is shown as the mid-system or zero-reference point for comparing other surface locations. 2) The maximum fluid volume of each culture well is 100 µL which equates to an in-well z-height of 4 mm. This in-well z-height is the emphasis of this application note (Figure 2).

Calibration: To determine microplate reader accuracy of in-well z-heights, 100 µL of 1 µM fluorescein solution was added into well-3 of SciFlow, then fluorescein signal was auto analyzed by CLARIOstar software to determine the location (z-height) of the optimum signal (maximal signal).

Experiment: Figure 2 outlines the experimental design of 3D fluid mixing using a fluorescein tracer that temporally enters well-3, blends with culture media over time, then exits as a gradient solution into subsequent culture wells (e.g. wells 4 - 11). Briefly, the SciFlow System was primed with culture media using 100 µl/well (wells 2-11). Then, 400uL of culture media was applied to the source well (well-1). The system was incubated for 30 minutes to allow for connection of the fluidic channels between wells (i.e. fluid priming). Once fully connected, 100uL of 1uM fluorescein (fluid tracer) was added into the source well (Rows A & B). The fluorescein tracer was monitored (well-3 and well-7) on the CLARIOstar microplate reader using three z-height levels. These levels correlate with the optimum (max) signal height, and 1mm above and 1mm below the optimum signal height.

Microplate Reader Analysis Workflow

Process	Details
• Prepare Fluid in SciFlow™ 1000	*prime without flow*
• Activate fluid flow in SciFlow™ 1000	*when ready to begin*
• Initiate fluorescein treatment	*apply in source well*
• Create temporal flow gradients	*directional flow cascades*
• Track fluorescein over time	*3 z-levels per culture well*
• Acquire images at time points	*real-time exposures*
• Analyze outcomes in real-time	*3D mixing kinetics*

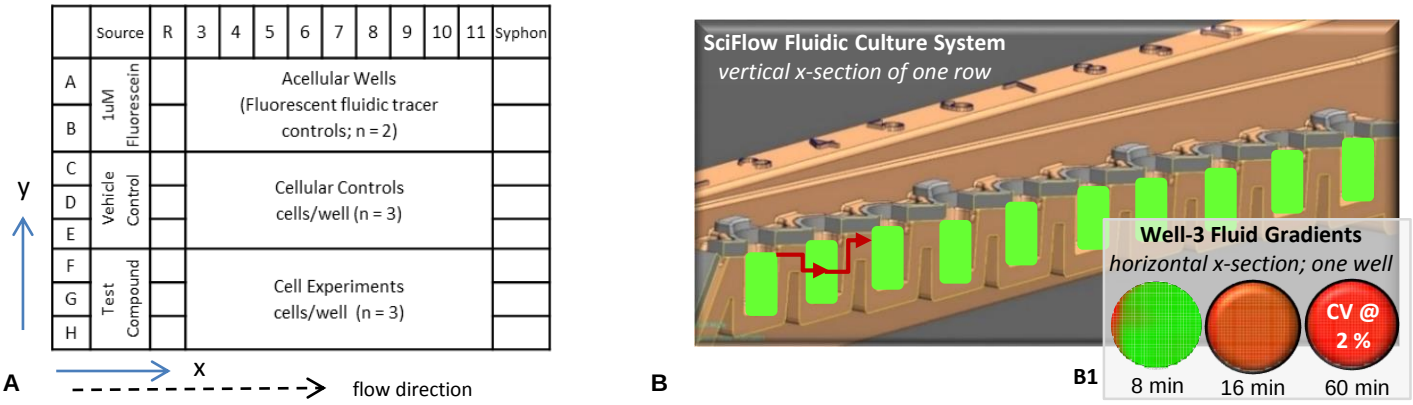

Figure 2. **A)** Example of SciFlow System experimental layout with fluid tracers, control cells, and cells with test compound. **B)** A vertical x-section of SciFlow culture wells along a connected row (wells 1-12); green is volume of culture media in culture wells (2-11). **B1)** A horizontal 30x30 grid circle at one optimal z-height, signals at 3 different times. Grids capture fluorescein signal movement (red) as tracers enter and mix with culture media (green).

Temporal Gradients at One z-Height; Captured by CLARIOstar

The SciFlow 1000 System is configured similarly to a 96-well culture format (1/2 area well design). Flow of fluid enters and exits from the top of each well (Figure 2B red arrow) providing opportunities to track compounds that permeate into, blend with, mix, and exit cell culture wells that already contain media (Figure 2B1). In this study we utilized the CLARIOstar Wellscan feature and inlaid a 2D horizontal grid, 30x30, to monitor fluorescein movement over time; Figure 2B1. By 60 minutes in well-3, the CV across the grid was 2% indicating nearly complete distribution.
Outcome: Analysis was used to monitor real-time 2D gradients of temporal movement of fluorescein. Fluorescein can be considered as a surrogate exposure of toxicant, drug, or compound within SciFlow's fluid transport.

Mixing at Three z-Heights; Fluid Uniformity in SciFlow Wells

The CLARIOstar Microplate Reader is a high-performance system with monochromators, spectrometer, and filters. CLARIOstar is equipped with revolutionary LVF technology for versatility, sensitivity, and flexibility and making it ideal for assay development. CLARIOstar has the capability to quickly and efficiently analyze real-time 3D fluid mixing kinetics inside culture wells. The power to correlate three separate horizontal Wellscans (3 z-heights) in one acquisition routine equates to 2700 traceable data points, 30x30x3, in 100 uL of fluid (at one time point). Outcome: Analysis makes it practical to trace 3D fluid flow and to quantify mixing data over time (Figure 3). If one considers fluorescein as the surrogate exposure of toxicant, drug, or compound, then concentration exposures are quantifiable.

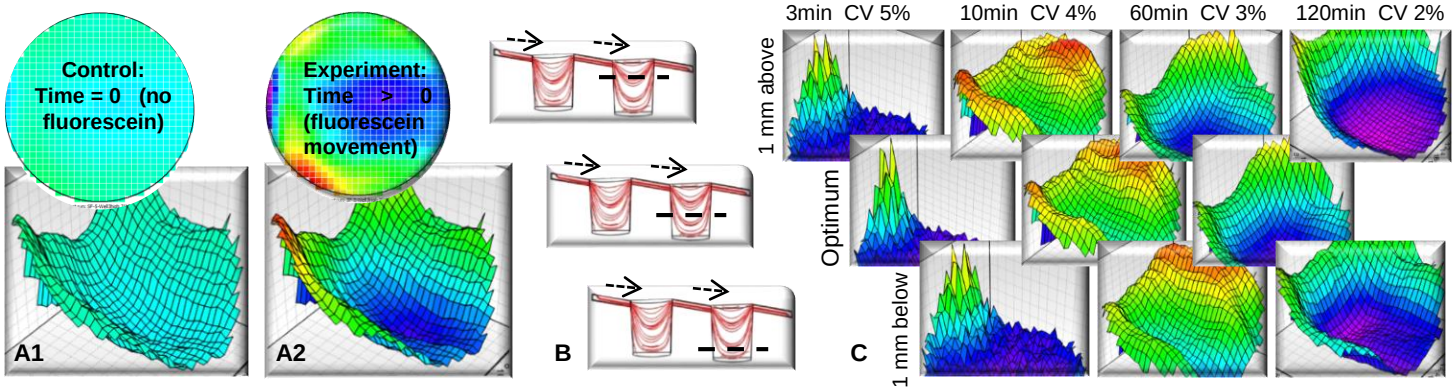

Figure 3. Wellscans of SciFlow well 3; 30x30 grids. **A1)** Control Background: media only. **A2)** Example: permeation of fluorescein into culture media (left to right). **B)** Flow Paths: fluid vector lines show flow direction through culture wells. Horizontal dash-lines annotate z-height locations within culture wells (1mm above, optimum, 1mm below). **C)** Experiment: z-height relationships and fluorescein movement dose-response times. Data used to evaluate 3D mixing kinetics. The coefficient of variance (CV) is between the three z-heights in well 3 (a vertical CV; mixing).

Fluid Equivalence *for* Horizontal Gradients and Vertical Mixing

Fluorescein cascades or temporal exposures in the SciFlow Culture System.

Fluorescein Dose Well	Culture Well Analyzed	Fluorescein Enter Well Time	Horizontal 2D Gradient, Δ-time till CV < 5%	Vertical 3D Mixing, Δ-time till CV < 5%
1	3	< 1 min	< 60 min	< 60 min
1	7	< 75 min	< 75 min	< 75 min

Conclusion: Track Real-Time Fluid Flow and 3D Nutrient Mixing. Quantify Compound Exposure Kinetics vs. Time.

- The SciFlow™ 1000 Fluidics Culture System is x, y, and z format compatible with the BMG CLARIOstar High-performance reader. Reproducible signal acquisition was achieved across greater than 4 mm of z-height change in the SciFlow System.
- The CLARIOstar Reader can effectively interrogate SciFlow's real-time fluid flow using two methods: 1) via horizontal 2D gradient exposures in a 900 sample segmented grid, and 2) by means of compound 3D mixing via "image mapping" of three meshed vertical grids with 2700 signals attained by fluorescent activity.

Rationale: Merge viable cells and tissues in connected fluidic chambers to create mini-organ systems. Evaluate chemicals, drugs, and compounds in benchtop human systems.

Ordering Information:

Phone: 919-354-1083
Email: Info@scikoninnovation.com Web: www.scikoninnovation.com
Web Product Page: http://scikoninnovation.com/shop/category/96-well-waterfall-culture-plate/

Product Number	Description
AA-1-50	SciFlow™ 1000 (Pk of 5)

SCIKON INNOVATION INC

Enabling Human Physiology on the Benchtop™