Basic Protocol for the SciFlow™1000 System
Create Fluorescein Standard Curve
Calibration for Flow Tracking
Drug Exposure & Monolayer Culture

System Description:

The SciFlow™ 1000 Fluidic Culture System is a benchtop tool for *in vitro* use to mimic cell, organ, and living systems. SciFlow operates like a shallow river bed with a series of compartments for cell culture. The design allows for isolated and stagnant culture during cell seeding then delivers real-time fluid flow and compartment-to-compartment signaling over time. The entire system is contained within a 96-well formatted culture plate that includes 8 repeatable channels. Each channel has the capacity to connect 1-to-10 cell culture wells in a linear array. As a benchtop tool, SciFlow is configured for cell and tissue assessments allowing easy access to all culture wells and media streams. Additionally, SciFlow is compatible with microplate readers, high content imaging platforms, and microscopes. Beneficially, no external pumps, or tubes, or controllers are required.

Protocol Focus: There are many applications of the SciFlow 1000, this protocol focuses on creating a fluorescein standard curve and subsequently estimating the dilution of test compounds across a SciFlow channel. Fluorescein is used as a tracer dye to extrapolate concentrations of compounds (concentration vs. time) across linked cell compartments as fluid moves across a channel.

Once the standard curve is generated, a line equation is calculated. This equation will allow back calculation of an absolute fluorescein concentration which is then correlated to the starting concentration of your drug or toxicant.

In order for this approach to be effective, users must first optimize gain settings for the highest concentration of fluorescein (recommended starting concentration 1 to 10 uM). The starting concentration will vary depending on instrument sensitivity and settings. Then, users must manually set the gain levels to be the same for each subsequent experiment.

Reagents and Supplies:

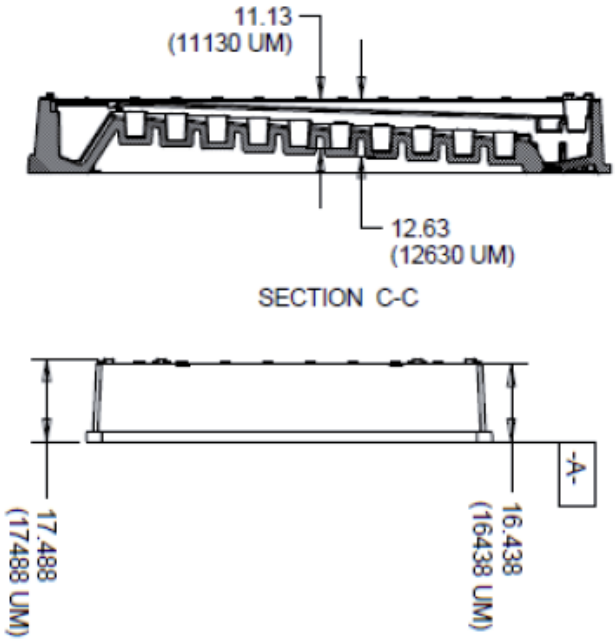
1. 100 uM Fluorescein: Make a stock solution by dissolving fluorescein sodium salt (Sigma F6377) in your base media without supplements.
2. Example: If your base media is DMEM, dissolve fluorescein in DMEM to 100 uM. Protect from light and maintain sterility. This stock solution can be stored at 4 degC wrapped tightly in foil.
    a. One SciFlow 1000 system.

3. Culture media. This is the media used to culture your cells. If you have different media for different cells, a standard curve calibration will need to be performed for each media.
4. CAUTION!! Check the height limitations on your plate reader to ensure the SciFlow plate will fit. Call your manufacturer with the height values. See Key Dimensions below for vertical height confirmation.

Key Dimensions for SciFlow plates

Dim-PD1000
SciKon Innovation, Inc. SciFlow™ plate dimensions for plate reader compatibility

SCIKON INNOVATION INC



Well 7 depth from top to well surface: 11.13mm
Well 7 height from bottom to well surface: 5.308mm
Well offset: 0.5mm each
Plate height with features: 17.488mm
Plate height without features: 16.438
Well diameter: 4.618mm
Well Area: 16.7mm^2

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Procedure:

1. Create a dilution curve from 1uM to .001uM Fluorescein in 2mLs dilutant. When creating dilutions, dilute into complete culture media (Base plus any serum or additives). Always protect from light as much as possible. Fluorescein rapidly quenches when left exposed to light.
2. Remove a sterile SciFlow™ System from packaging.
3. Load one concentration of dye along an entire row, 100ul per well with 500uL in the source well (well-1) and 350uL in the sink well (well-12). For example:

    a. Row A: 1uM
    b. Row B: 0.3uM
    c. Row C: 0.1uM
    d. Row D: 0.03uM
    e. Row E: 0.01uM
    f. Row F: 0.003uM
    g. Row G: 0.001uM
    h. Row H: Media only, no dye

4. Set fluorescent plate reader instrument to optimize gain based on 1uM Fluorescein. Read plate at excitation 485nm, emission 520nm
    a. Coefficient of variance across each row should be calculated from data. If the plate reader allows adjustment of z-height focal length, make z-height adjustments until CV is at lowest point. See Key Dimension specifications of the SciFlow plate to aid in creating appropriate z-height settings.
    b. **IMPORTANT**: Note the gain level that the instrument chooses for 1uM Fluorescein. This will be the gain level that must be used for all subsequent experiments
    c. **For some instruments, the gain is preset**. Call your manufacturer to clarify how to determine and set the gain appropriately

5. Plot data by averaging fluorescent units across rows against concentration of fluorescein.
6. Create a line equation that will be used to predict fluorescein concentrations based on fluorescent units collected at the gain level developed in step 5.
7. Record this line equation with the gain setting for future use
8. **With new stock reagents, changes in culture media, or from time to time, recalibrate the gain level by using a new plate and re-optimizing gain based on 1uM Fluorescein. Always store equation and gain levels together for accuracy.**

Fluorescein Standard Curve Example

Below is an example of creating a Fluorescein standard curve. In this example, a Tecan Infinite M1000 Pro was used to generate RFU. 100uL of each dilution was added to all the wells of a single row. The average and standard deviation was used to create a line equation for subsequent experiments.

Parameters

Mode	Fluorescence Top Reading	
Excitation Wavelength	485	nm
Emission Wavelength	525	nm
Excitation Bandwidth	5	nm
Emission Bandwidth	5	nm
Gain	88	Manual
Number of Flashes	10	
Flash Frequency	400	Hz
Integration Time	20	µs
Lag Time	0	µs
Settle Time	0	ms

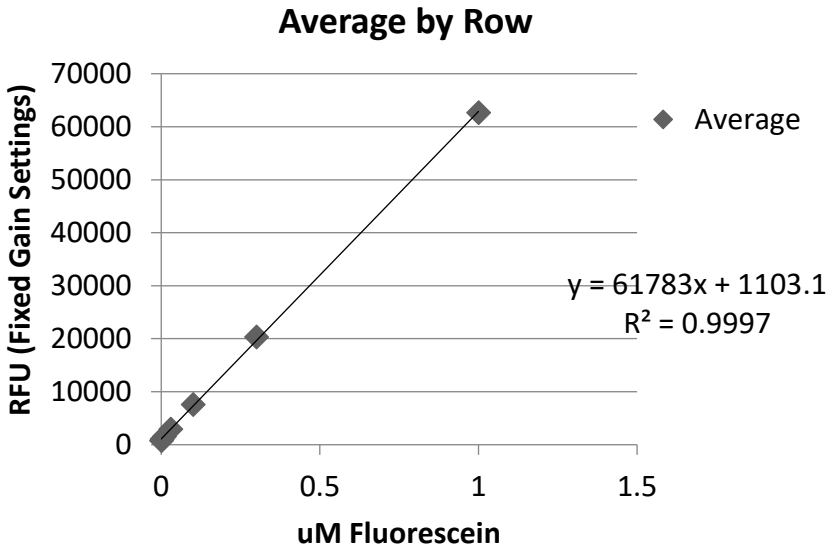
Raw Data

uM Fluorescein	2	3	4	5	6	7	8	9	10	11
1	61722	62521	63000	63000	62569	62916	62620	62820	62364	63000
0.3	18967	19849	20243	21107	20361	21105	20220	20112	20182	21087
0.1	6917	7468	7669	7535	7633	7673	7720	7696	8677	6787
0.03	2683	2952	2886	3044	3121	3008	2953	3021	2948	3049
0.01	1437	1580	1507	1417	1505	1548	1621	1493	1574	1628
0.003	1055	1063	1040	1056	1042	1017	1026	1057	980	1051
0	806	799	803	782	811	818	793	744	762	726

Summary Stats

uM Fluorescein	Average	Stdv	%CV
1	62653.2	399.2	0.6
0.3	20323.3	663.0	3.3
0.1	7577.5	509.8	6.7
0.03	2966.5	119.7	4.0
0.01	1531	71.9	4.7
0.003	1038.7	25.2	2.4
0	784.4	30.7	3.9

Graph and Line Equation



Tips and FAQ: This section outlines some very useful techniques for handling SciFlow 1000.

Removing Media (if required): SciFlow can be emptied by inversion and flicking into an appropriate waste container. Additionally, the entire row can be emptied via vacuum aspiration through the sink well.

Moving SciFlow 1000: SciFlow is a fluidic system and if the plate is tipped along the lengthy axis (x-axis), this can disrupt or modify both flow and any established gradients. Reasonable care should be taken when moving the plate to minimize unintended flow caused by tipping the plate.

Evaporation: Though the SciFlow 1000 does have a lid, evaporation can be observed, and for experiments over 7 days, a 10 – 20% larger volume can be added to the source well than is removed from the sink, to combat decreasing volumes in the source.

Sample Plate Map:

	Source	2	3	4	5	6	7	8	9	10	11	Sink
A	Fluorescein Tracer		Acellular Fluidic Tracer									
B	Compound 1 (triplicate rows)		Cellular Model (e.g. HepaRG cells @ 40,000 cells/well)									
C												
D												
E	Vehicle control (triplicate)		Cellular Model (e.g. HepaRG cells @ 40,000 cells/well)									
F												
G												
H	Fluorescein Tracer		Acellular Fluidic Tracer									

Example Cell Seeding Densities

Table of Example Cell Seeding Parameters: SciFlow culture well areas are ½ the size of traditional 96-well culture surface areas (0.167cm^2 or 16.7mm^2).

Cell Seeding Examples, 2D monolayers	Number of cells per SciFlow plate	Number of cells per well	How many culture wells	Seed Time	Initial confluence	Adjustment
Primary human hepatocytes with collagen coating	2.0 E6	27,500	72 (3 – 11)	Overnight	Confluent	By viewing
Primary rat hepatocytes with collagen coating	1.0 E6	14000	72 (3 – 11)	Overnight	Confluent	By viewing
Primary mouse hepatocytes with collagen coating	6.5 E5	9000	72 (3 – 11)	Overnight	Confluent	By viewing
Primary duck hepatocytes with collagen coating	6.5 E5	9000	72 (3 – 11)	Overnight	Confluent	By viewing
Primary canine hepatocytes with collagen coating	1.0 E6	14000	72 (3 – 11)	Overnight	Confluent	By viewing
HepG2	2.2 E6	30000	72 (3 – 11)	Overnight	80%	By viewing
HepaRG	2.9 E6	40000	72 (3 – 11)	Overnight	80%	By viewing
HepaRG (no spin)	2.9 E6	40000	72 (3 – 11)	Overnight	80%	By viewing
Cell Line MCF7	7.2 E5	10000	72 (3 – 11)	Overnight	20%	By viewing