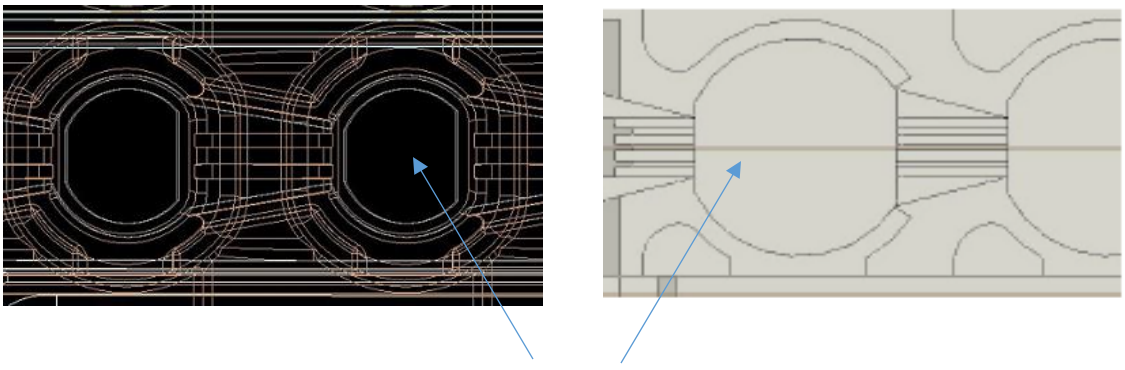
Basic Protocol for the SciFlow™1000 System
Collagen Coating of Well Surfaces
Pre-Coat of a Thin Protein Layer
Monolayer Culture

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 collagen coating of well surfaces prior to plating monolayer type cells. Adjustments for specific coatings should be explored by users. This protocol will focus on every well surface being coated with collagen type-1, separate wells can contain distinct surface coats.

Overview: Culture areas within SciFlow 1000 are analogous to a ½ area 96-well plate (0.167 cm^2 per well). As purchased, all culture wells are tissue culture treated.



Surface images of linear aligned culture wells (partial circles)

Protocol:

1. To achieve a 10 ug/cm^2 surface collagen coating.
    a. Add 1.67 ug of collagen to each well. Use a 30 ul volume per well to avoid surface tension variations and climbing of liquid up culture walls.

Example: To prepare Rat Tail Collagen Type-1

1. Dilute collagen type-1 (Sigma C3867 or equivalent) to 0.056 mg/mL using base media without serum (e.g. DMEM or Williams E) in 0.1N Acetic acid.
2. Keep the solution cold on wet ice.
3. Add 30 ul of prepared diluted collagen solution to each well.
4. Spread evenly by rocking the plate in a figure-eight pattern.
    a. For best results, set plate on top of a paper towel on top of a bed of wet ice prior to applying collagen.
5. Place in humidified 37degC incubator $\geq$ 1 hour providing enough time for protein adhesion.
6. Remove excess fluid.
7. Rinse plate twice with base cell culture media or PBS prior to plating cells.

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