

BioFlux System for Cellular Interactions

Microfluidic flow system for live cell assays

Introduction to the BioFlux System

A vast number of biological processes involve cells interacting with other cells or molecules under shear flow conditions. Examples include blood clotting, tissue repair, immune and inflammatory response, bacterial infections, and cancer progression. There are two main approaches to studying these phenomena in drug discovery and cellular biology laboratories: static well plates and laminar flow chambers. Well plates can offer higher throughput, but their lack of flow control typically lowers the physiological relevance of the assay. Laminar flow chambers provide shear flow, but current systems suffer from limited throughput, long setup times, poor dynamic range of flow, large reagent consumption, and often unreliable results (i.e. leaks, bubble formation, etc.).

The BioFlux System is a versatile platform for conducting cellular interaction assays which overcomes the limitations of static well plates and conventional laminar flow chambers. BioFlux Plates form the core of the system, and provide a convenient well-plate format for running parallel experiments. Each system also includes a benchtop controller, well-plate interface, heating stage, and simple to use automation software. The principal advantages provided by the platform lie in the experimental control, throughput, and reliability. Shear force, flow rates, temperature, and compound addition each can be independently controlled and automated via the software. Up to 24 independent experiments can be run on a single BioFlux Plate, each of which is single-use and maintains an SBS-standard format. Other benefits include low reagent consumption, quick setup times, presterilized plates, and integrated image acquisition. BioFlux has been used for numerous applications in cellular biology and drug discovery, some of which are described here (for complete list of Technical and Application notes, please see www.fluxionbio.com).

BioFlux Principles of Operation

Well Plate Microfluidics

Fluxion uses a proprietary approach to creating microfluidic channels (70µm tall) into its devices. The process begins with photolithographic etching of a silicon wafer. This creates a template from which microfluidic devices can be molded from (see Fig 1).

The BioFlux System leverages the advantages of microfluidics to create a network of laminar flow cells integrated into standard well plates. Each BioFlux Plate utilizes an SBS-standard well plate to ensure compatibility with common microscope stages, liquid handlers, and plate readers. In the manufacturing process, a network of microfluidic channels becomes integrated to the bottom of the plate. These channels connect the wells together into groupings of independent experiments. Reagents are added to the wells of the plate using conventional means (i.e. pipette, syringe, liquid handler). Pressures can then be applied to each individual well using the automation software, which applies the pressure from the Controller through to the Interface and into the well. As that pressure is applied, it drives the reagent in that well into the corresponding channel underneath the plate. After the reagent goes through the channels, it ends up in a separate well on the plate, i.e. an 'output' well (see Fig 2).

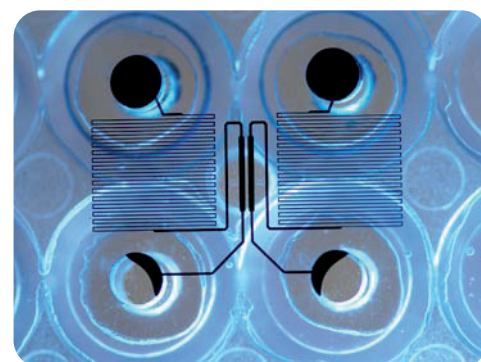


Figure 1: A Fluxion microfluidic device coupled to an SBS-standard 48-well plate (viewed from the bottom). Each fluidic channel has a unique input (top wells) and an output well (bottom wells).

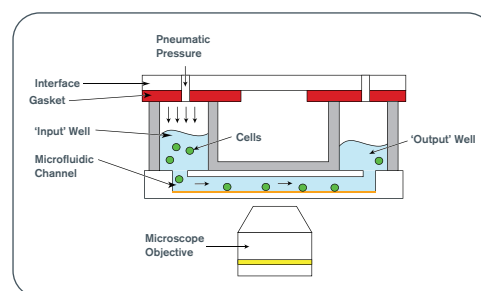


Figure 2: Schematic depiction of Fluxion's Well Plate Microfluidics. Fluid reagents are loaded into wells on the plate, and pressures are applied via the Interface to move them throughout the channels. The channels run on the bottom of the plate from well to well.

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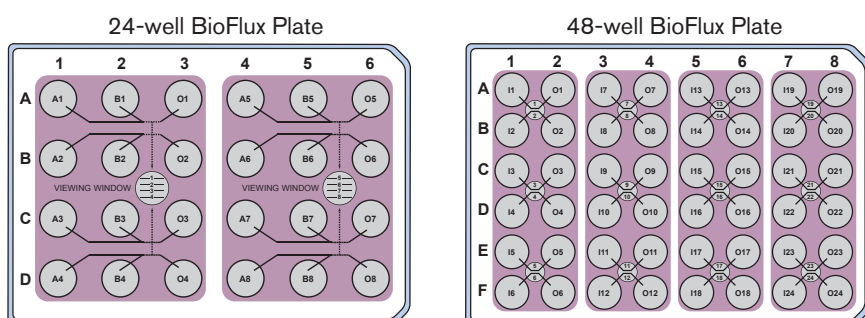


Figure 3: Representative fluidic layouts of a 24-well and 48-well BioFlux Plate. 24-well Plates have 8 experimental channels per plate, each with 2 inputs ('A/B') and one output ('O'). 48-well plates have 24 channels each with an input ('I') and output ('O').

In this manner, it is possible to set up networks of microfluidic channels that run between wells of the plate. In the BioFlux System, one experimental channel typically consists of an 'input' well and an 'output' well. This is the case with the 48-well BioFlux Plate, which contains 24 experimental channels (i.e. each with an input and an output well). In some assays, it is desirable to have multiple input wells for compound addition or other reagents that need to be added during the course of an experiment. This is the case with the 24-well BioFlux Plate, which offers two input wells and one output well per experimental channel. As such, the 24-well plate provides 8 experimental channels (see Fig 3).

Materials of Construction

The BioFlux Plate upper well structures are made from clear polystyrene. The microfluidic structures are cast from a material called poly dimethyl siloxane (PDMS), which is similar in consistency to elastomeric silicone. This material forms the sides and roof of the microfluidic channels. A standard 180 μ m cover slip glass is used to close off the channels on the bottom. As cells, reagents, etc. are deposited onto the bottom of the channels, they attach to the glass surface. Similarly, when images are being acquired, they are observed through the cover glass and are thus compatible with bright field, fluorescence, and confocal microscopy (see Fig 4).

BioFlux Controller, Interface, and Heater

The BioFlux System is designed to automate the experimental workflow on the BioFlux Plates. The BioFlux Controller contains an air compressor and electropneumatic regulators to deliver precisely controlled pressure outputs. That pressure is delivered via a series of valves inside the Controller and tubing connections coming from the Controller. The tubing connections connect to the Interface, which attaches to the top of the BioFlux plate. The Interface serves as a pressure distribution manifold, and can deliver precise pressures to one or more wells at a time as specified by the user in the software. The BioFlux Heating Stage is a thin transparent heater used to maintain temperature of the BioFlux Plate experimental channels. The BioFlux Plate and Interface can be placed directly on the Heating Stage while experiments are running (see Fig 5).

BioFlux Software

The BioFlux Software serves as the primary user interface, and controls the operation of the experimental channels. The software runs on Windows PC or notebook computers, and connects to the Controller via the supplied USB cable. In the software, the user can control which channels are active, set a precise shear flow, and select flow direction and source. Commonly used routines can be saved and applied to a run table, which automates a series of experiments on a given plate. Image acquisition and analysis can be automated as well with the optional camera and imaging software.

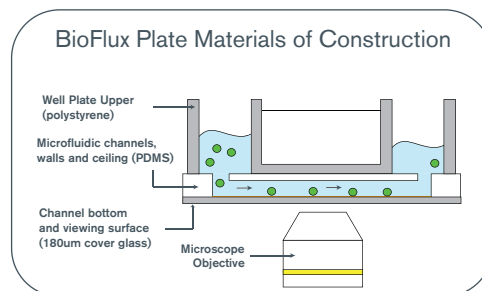


Figure 4: BioFlux Plates utilize SBS-standard polystyrene well plates. The fluidic channels are cast from PDMS to create the tops and sides. The bottoms of the channels are closed off by a 180 μ m cover slip.

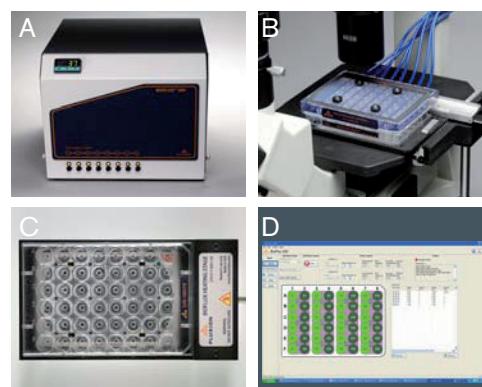


Figure 5: Bio Flux System components: A) BioFlux Controller – contains a compressor and electropneumatic regulators to control pressure output. B) Interface – serves as a pressure distribution manifold to the BioFlux Plates. C) Heating Stage – transparent indium tin oxide heating stage to control temperature during experiments. D) Software – automates many of the common, repetitive tasks needed during setup and experiment.

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Image Acquisition and Analysis

Most endpoints for data collected on the BioFlux System inherently involve taking images of the microfluidic channels. BioFlux Plates have a standard SBS footprint and fit on conventional inverted microscope stages (flat or with a well-plate tray). The cover glass bottoms make it possible to acquire high resolution images using brightfield, phase, fluorescence, and confocal microscopy.

Typical Workflow of a BioFlux Experiment

The BioFlux System enables a wide variety of cellular interaction assays, including bacterial biofilms, cellular adhesion, live cell imaging, stem cell applications, and more (see Applications Section). Even though these assays can vary greatly in their reagents used and experimental objectives, they typically follow similar procedures on the BioFlux platform. Some of the common steps are described below:

Coating:

It may be desirable to start with a particular coating on the microfluidic channels. This coating could be an extracellular matrix molecule for cell culturing into monolayers, an adhesion factor of interest in a cellular adhesion assay, or other functionalized coatings of interest. The coating substrate can be introduced into one or more of the wells, and advanced through the channel simply by applying pressure from the Controller. Once the coating has gone through the entire channel, the plate can be stored at the appropriate environmental conditions (i.e. room temperature, 37°C 5% CO₂, etc.) until ready for use.

Priming:

Prior to use, the channels of the BioFlux Plates are typically primed with fluid. This step helps prevent air bubbles from entering the channels on subsequent loading steps. The priming fluid can be selected as appropriate for the experiment (e.g. cell growth media).

Cell Seeding or Inoculation:

Many experiments on the BioFlux System will call for an initial seeding or inoculation step. In the case of mammalian cell experiments, a monolayer of cells can be grown on the bottom of the channel. For microbial experiments, bacteria or other microbes can be seeded on the bottom surface which can culture into biofilms. These cellular layers can then be stored at appropriate environmental conditions on the supplied Stage Heater or in an incubator (see Fig 6).

Running Experiments:

The BioFlux System enables a wide range of cellular assays under shear flow. As such, the experimental protocols will vary depending on which features of the system are being utilized. One of the key features available is controlled shear flow. Using an intuitive software interface, the user can program a constant shear flow or create automated protocols for changes in shear rate over time, for example as steps or gradient ramps. Each protocol defines the duration, shear value, and direction of flow over as many intervals as desired. Protocols can then be applied to individual channels on the BioFlux Plate being used, and both the protocols and the channel assignments can be saved into a library for easy reproducibility of experiments. The 24-well BioFlux Plates have the ability to switch between two inputs. These can be used, for example, to switch on the fly between cell growth media and a compound of interest.

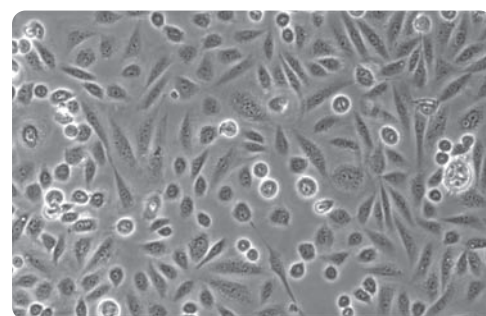


Figure 6: A Bio Flux Plate microfluidic channels with an adherent monolayer of CHO cells (20X phase objective). Each channel is 350µm wide and 70µm tall.

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Data Acquisition and Analysis:

Images of the experimental channels can be acquired at any time during an experiment. An inverted microscope is required to capture images from the BioFlux Plates. BioFlux Plates can be used with most inverted microscopes. BioFlux Systems can be configured with a range of cameras suitable for brightfield and fluorescence applications. The BioFlux Software automates user-defined imaging routines, which include single images or time-lapse sequences. The software can also be configured for analysis routines for common cellular assays, including cellular adhesion studies. Third party cameras and their associated imaging software may also be used to acquire images, which can then be imported into the BioFlux Software for image analysis.

Performance Benefits

Higher throughput and parallelism	Compared to conventional flow cell technology, BioFlux offers a significant gain in overall throughput. Up to 24 independent experiments can be run simultaneously on a single plate.
Wider dynamic range of laminar flow	BioFlux Plates have channels which are 1/10 the size of conventional flow cells, which means the flow remains laminar over a much wider range. Shear flows from 0-20 dynes/cm ² are supported.
Integrated Compound Addition	The BioFlux 24-well Plate offers dual inputs for automated compound addition. This feature enables monitoring of the experiment before and after compound introduction, while keeping other conditions constant such as temperature and shear flow.
Integrated temperature control	The vast majority of cell biology experiments need to maintain a specific temperature. The BioFlux Heating Stage provides this control while still maintaining the ability to acquire images.

Workflow Benefits

Faster setup times	BioFlux eliminates the need to change tubing, gaskets, and other cumbersome accessories. Everything is ready to go out of the box, and starting a new experiment is as simple as pulling out a new BioFlux Plate.
Sterility assurance	BioFlux Plates are supplied sterile which eliminates the autoclaving and rinsing steps often required with conventional flow cells. All of the fluidics are contained entirely within the plate, which is safer for both the user and the experiment.
Intuitive software automation and image acquisition	BioFlux Software makes it easy to setup, run, and process data for complex experiments. Protocols for flow conditions and image acquisition are easily entered and saved for future use. Complete control is provided for shear flow, direction, compound addition, and duration.
Lower reagent volumes and fewer cells required	Fluxion's unique microfluidic technology enables experimental channels 1/10th the size of conventional flow cells. This translates into reagent volumes two orders of magnitude less than what is typically required. This means fewer cells used and less volume of costly reagents.

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Applications

The BioFlux System enables a wide variety of applications in cellular biology and drug discovery. A few of these applications are described below which illustrate some of the BioFlux capabilities.

1) Biofilm Assays

Microbial biofilms assays are commonly performed throughout microbiology labs and drug discovery labs. Biofilms are communities of surface attached microbes, and have clinical relevance in hospital infections, short and long term medical implant infections, cystic fibrosis related lung infections, and others. A typical biofilm experiment involves inoculation of the channels with a microbial species, followed by the culturing of these cells into a biofilm. Representative experiments can range from: testing different mutants simultaneously to see which ones are most prone to biofilm formation, adding different compounds automatically to measure effects of biofilm prevention or eradication, or following genetic or morphological changes to the biofilm over time in response to varying shear flow (see Fig 7A).

2) Cellular Adhesion Studies

Numerous biological processes are mediated by various types of cellular adhesion. These include blood clotting, autoimmune responses, and lymphatic circulation. To simulate these phenomena in the BioFlux System, users typically coat the channels with either cells or adhesion molecules. The cells or adhesion molecules deposited in the channels emulate the physiological conditions of interest, such as airway epithelial cells, platelets, selectins, or integrins. After the channels are conditioned, circulating cells (i.e. leukocytes, red blood cells, Jurkats, etc.) can be introduced at a specified shear flow. Adhesion can be monitored and automatically measured in software as a rolling velocity or static adhesion calculation. Compounds can be introduced to assess their ability to modulate cell adhesion. Shear flow can be altered systematically (i.e. as a shear ramp up protocol) to quantify adhesion strength (See Fig 7B/C).

3) Live Cell Imaging Under Shear Flow

Live cell imaging has emerged as a preferred method for cell biology research and drug screening. While commercial systems exist for high content screening, it is difficult or impossible to run these assays under continual shear flow. Applications such as neuronal development, stem cell research, and vascular physiology can all benefit from being run under flow conditions. In these cases, monolayers of cells can be cultured in the BioFlux channels with shear flow being applied during or after monolayer formation. Possible assay endpoints can include: rates and directions of axonal growth, fluorescence staining for protein expression, calcium flux, and morphological characteristics of stem cell differentiation. Many imaging modalities are supported including brightfield, phase, fluorescence, and confocal microscopy (see Fig 7D).

Conclusion

Fluxion Biosciences has developed the BioFlux System for simple and reliable cellular biology experiments under controlled shear flow. The platform provides a simple to use software interface and controlled, reliable experiments using the BioFlux multi-well microfluidic plates. Many common cell based assays can be performed with higher throughput, easier setup, and more precise control over experimental conditions.

For more information, please visit www.fluxionbio.com or send an email to info@fluxionbio.com

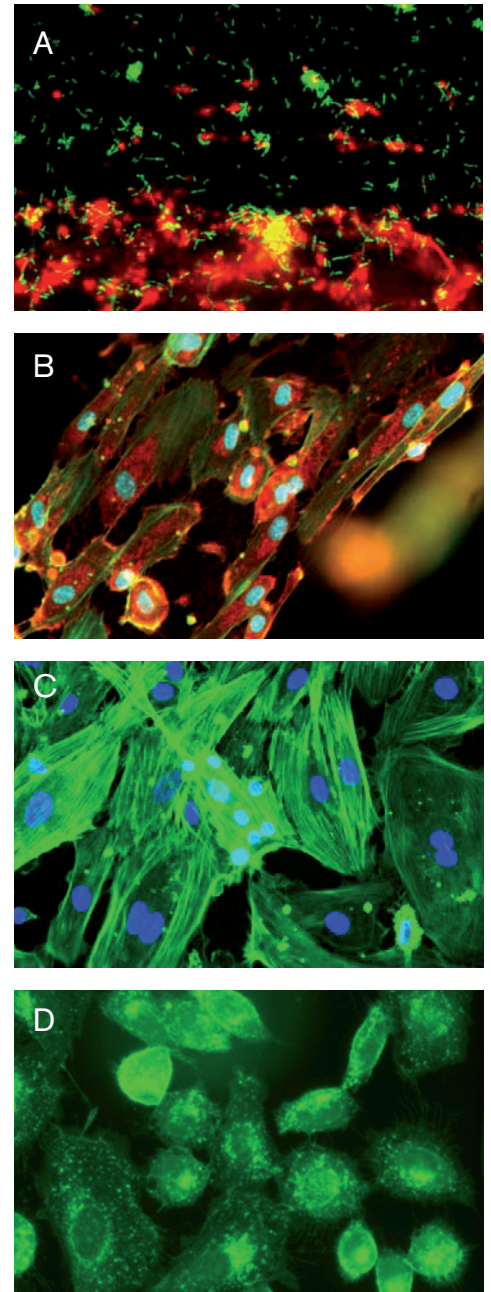


Figure 7: Representative Applications.

- A) *Pseudomonas* biofilm grown under shear flow on the BioFlux System and stained with the BacLite Kit (Invitrogen). (20X objective)
- B) An endothelial cell monolayer stained with F-actin. (20X objective)
- C) An adhesion assay showing Jurkat cells adhered to a HUVEC monolayer. (20X objective)
- D) CHO cells grown on a CellTak-coated channel for 16 hours under shear flow. (20X objective)

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Appendix I: Specifications

BioFlux Plates

Available configurations:	24-well Plate (8 independent experimental channels, two inputs per channel) 48-well Plate (24 independent experimental channels, one input per channel)
Channel cross section:	350 μ m wide X 70 μ m tall
Channel materials:	polystyrene (well plate), PDMS (channel sides and roof), 180 μ m cover slip glass (channel bottoms and imaging path)
Shear Flow:	0.5 – 20 dyne/cm ²
Uninterrupted Run Time:	Up to 24hrs at 1 dyn/cm ² (wells can be refilled at any time)

BioFlux Controller

Dimensions:	12" wide, 13" deep, 9" tall (30cm X 33cm X 23cm)
Power Supply:	5A 12V DC (included)
Connections:	USB cable (included)

BioFlux Software and PC Requirements

Operating System:	Windows 2000, XP
Hardware Requirements:	CD drive, USB 2.0 connection (for Controller), USB or FireWire connection (for camera)



