Bringing Physiological
Flow To Live Cell Assays

FLUXION

BIOFLUX™

Automated instrumentation for cellular experiments under shear flow

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BioFlux combines the throughput and convenience of a well plate with the physiological relevance of controlled shear flow.

Live cell assays are often performed in well plates to test multiple conditions and compounds in parallel. But many biological questions require more than simply adding cells into a dish. The BioFlux system provides the ability to emulate physiological shear flow in an *in vitro* model, while still maintaining a convenient well plate format. This opens up a multitude of experiments which could never be done in static well plates.

The power of flow

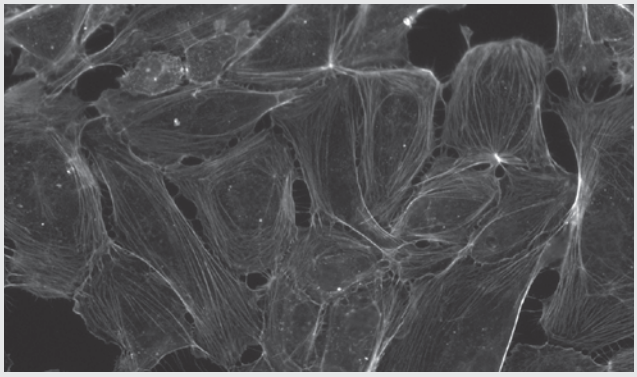
Many physiological processes take place under flow conditions. Blood flows through the vasculature. Cancer cells circulate throughout the body under flow. Plaque forms on teeth under the presence of saliva flow. Numerous studies have shown the presence of flow has a profound impact on cellular physiology. Yet many of these processes are still studied *in vitro* without the presence of flow. The BioFlux System gives you the ability to introduce flow to your research and drug discovery experiments and emulate *in vivo* conditions.

Shear flow is a critical physiological factor in many areas of biology and drug discovery:

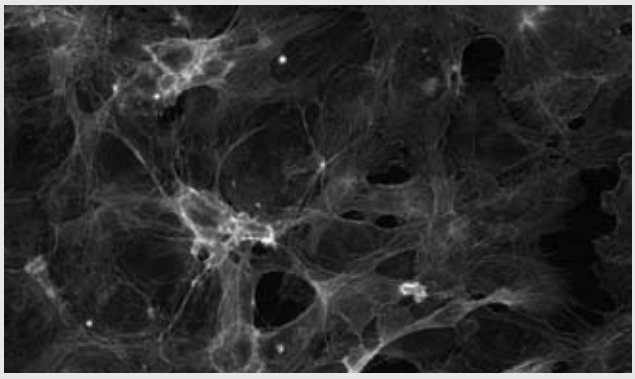
- Vascular biology
- Platelets and cardiovascular disorders
- Oncology
- Stem cells and developmental biology
- Biofilms

Vascular biology

With Shear



Without Shear

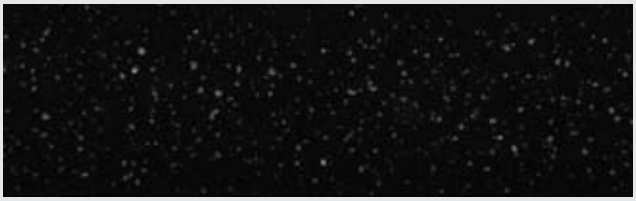


Shear stress activates mechano-sensors in endothelial cells, a critical factor in both health and disease

Platelets and cardiovascular disorders

High Shear

Low Shear



Shear flow governs the nature of platelet-receptor interactions and influences how platelets behave in the vasculature.

"Given the critical role of shear in regulating platelet adhesion and thrombus growth, these findings may have potential pathophysiological significance."

Jackson, et al.
Arterioscler. Thromb. Vasc. Biol.26(2006);663-669

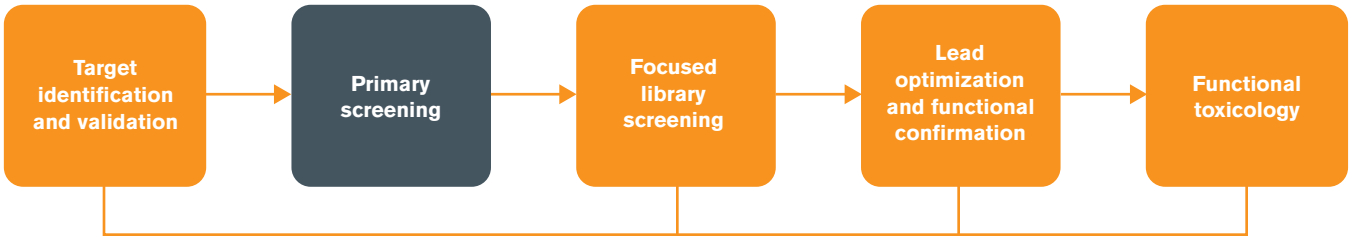
In life science research…

Shear flow plays an important role in creating physiologically-relevant models to answer fundamental questions in cellular biology.

- Structure function relations
- Mutant and knockout screening
- Functional cell interactions
- Morphology and cell cycle analysis

In drug discovery and development…

BioFlux helps ensure that only the most promising compounds will be passed through to clinical trials, saving tremendous time and expense versus conventional screening methods.



BioFlux Application Areas

BioFlux brings the power of flow to your research and drug discovery applications…

Now you don't have to choose between high throughput well plate assays and highly-functional flow cell experiments. BioFlux offers the physiological relevance of laminar flow chambers with the throughput and convenience of standard well plates.

Features	Benefits
High throughput, up to 96 samples run in parallel	Reduces time to publication Accelerates drug discovery and development
Controlled, physiological shear flow up to 200 dyne/cm^2	Bridges the gap between *in vitro* and *in vivo* experiments Helps optimize lead compounds Helps eliminate compounds before going to costly clinical trials
Advanced automation of experiments and data analysis	Reduces workflow bottlenecks
Well plate simplicity – standard format, pre-sterilized, no tubing to change	Ensures quick setup and experimental reproducibility/repeatability

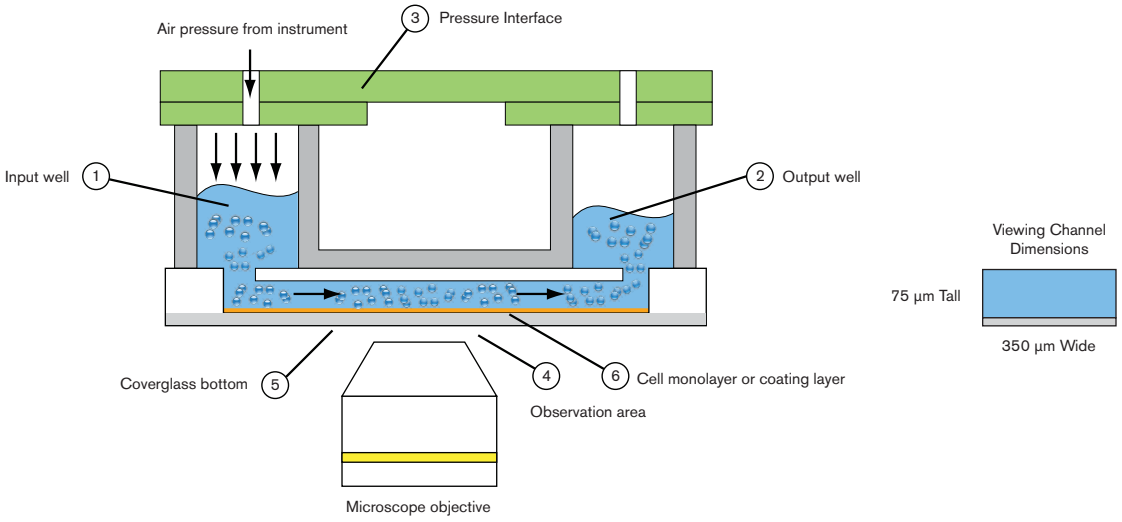
How we do it

Well Plate Microfluidics: Providing the best of both worlds

BioFlux utilizes the innovative Well Plate Microfluidic™ technology to embed micron-scale fluidic channels on the bottom of a standard well plate. By controlling the flow across the experimental channel, you can simulate a wide range of physiological conditions. Using the well plate format ensures that the experimental setup and image acquisition fit right in to your existing workflow.

View from underneath the well plate.

Principles of Operation



BioFlux Plates contain an array of microfluidic flow channels arranged on a well plate format. Each flow channel connects to an input well (1) and an output well (2) on the plate. To begin an experiment, cells and other reagents are loaded into designated input wells using a standard pipettor or automated liquid handling robot. The BioFlux Pressure Interface (3) couples to the top of the well plate and applies a controlled pneumatic pressure from the control instrument to the top of the wells which drives the fluid through the channels at a user-defined flow rate. Reagents in the channel flow across an observation area (4) which is situated between the wells. High resolution imaging data is acquired at this location. The bottom of the channels are comprised of 180µm coverslip glass (5) which make them compatible with brightfield, phase, fluorescence and confocal microscopy. After clearing the observation area, the fluidic reagents collect in the output well.

The flow channels can be coated with proteins, adhesion molecules and cellular monolayers (6) to conduct a wide range of cell-cell and cell-ligand interaction assays. The flow profile in BioFlux Plates is extremely uniform and laminar. This ensures that each experiment is reliable, meaningful and reproducible. The micron-sized channel dimensions reduce the required reagent volumes to as little as 20µL per well.

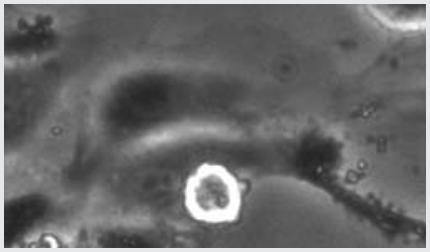
One system… many applications

Oncology

Understanding the molecular and cellular mechanisms of cancer progression, from primary malignancy to metastatic disease, is the key to development of successful treatments. Cancer cells travel through the vasculature under flow and interact with cells in the endothelium. BioFlux provides a physiologically-relevant in vitro model for studying these interactions under controlled shear flow conditions.

Representative applications:

- Cell adhesion and rolling
- Transmigration
- Migration and invasion
- Chemotaxis



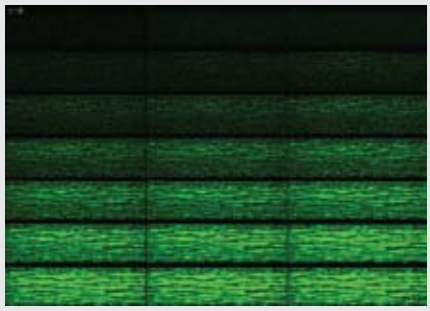
A colon carcinoma cell (HT29) arrested on an endothelial cell under 0.8 dyne/cm^2 shear flow. Up to 24 simultaneous transmigration assays can be run in parallel under physiological shear flow conditions.

Platelet Function

Platelet aggregation, or thrombosis, occurs in response to vascular injury where the extracellular matrix below the endothelium has been exposed. It can be triggered in the presence of shear flow and is dependent on many biochemical interactions present in whole blood. The BioFlux system enables simulation of vasculature shear rates up to 200 dyne/cm^2 (5000 s^{-1}) using whole blood, platelet-rich plasma or other cells of interest. Experiments can be run on purified matrix proteins (von Willebrand factor, collagen, etc.) or endothelial cell monolayers. The system uses as little as 20µL of reagents per experiment.

Representative applications:

- Platelet aggregation and adhesion
- Thrombosis
- Atherosclerosis models



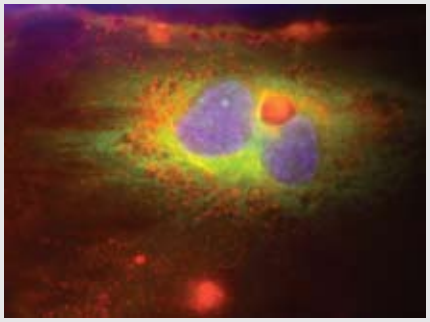
Thrombosis on vonWillebrand factor under high shear - 10 minute timelapse (going from right to left then down) with calcein AM labeled whole blood. Anti-thrombotic compounds can be screened and evaluated for dose-response using human cells and whole blood under physiological shear, a model which is impossible to replicate in a conventional well plate.

Vascular Biology

The endothelium is a dynamic mediator of many physiological phenomena and the crossroads for immune system function, cancer metastasis and atherosclerosis among others. Endothelial cells have been shown to alter their morphology and gene expression in the presence of the shear flow they routinely see in the vasculature. As such, the relevance of endothelial cell culture and downstream assays increases significantly with the presence of flow.

Representative applications:

- Specialized cell culture
- Cell adhesion
- Atherosclerosis
- Mechanical loading
- Thrombosis
- Migration and invasion



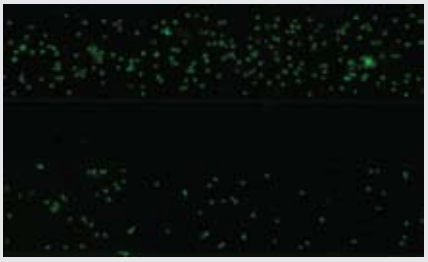
A primary peripheral blood monocyte (red) attached to endothelial cells. Post-fixation and permeabilization, cells were stained with anti-tubulin (green), wheat germ agglutinin (red) and Hoescht 33342 (blue). The BioFlux system enables a variety of assays which span the entire adhesion cascade from initial rolling adhesion to transmigration.

Immunology

Proper immune function depends on the interconnected relationships of many diverse cell types. When improperly functioning, these cell-cell interactions can lead to allergy, asthma, autoimmune and infectious disease. Immune surveillance in large part occurs within the vasculature in the presence of constant flow, where cell-cell and cell-ligand interactions are influenced by in situ forces. BioFlux provides the ability to control the timing of these interactions as well as the shear rate.

Representative applications:

- Transmigration
- Migration
- Cell adhesion and rolling
- Wound healing



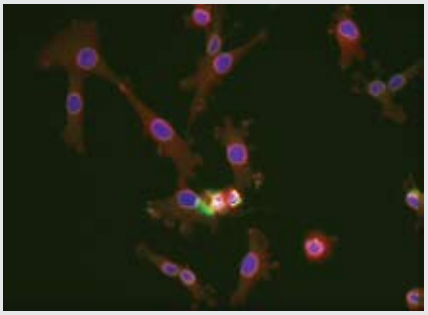
T-cells (Jurkat) attached to VCAM-1 under flow. Top channel is a no-treatment control and bottom channel is anti-VLA4 treatment. Parallel screens of up to 96 assays at a time can be run to evaluate compounds which modulate adhesion. These screens provide quantitative data for compound efficacy, dose-response or target validation.

Stem Cells

Stem cell research has the potential to produce novel treatments for previously incurable diseases and injuries. The application of controlled shear flow to undifferentiated embryonic stem cells promotes enhanced expansion of cell lines. Shear stress can be used as a stimulus for differentiation especially for cell types that naturally respond to physiological shear such as endothelial cells. Differentiation of cells into specific cell types and subsequent production of biomaterials is also facilitated by mechanical forces such as shear.

Representative applications:

- Controlled differentiation
- Growth conditions
- Mechanical loading
- Bioproduction



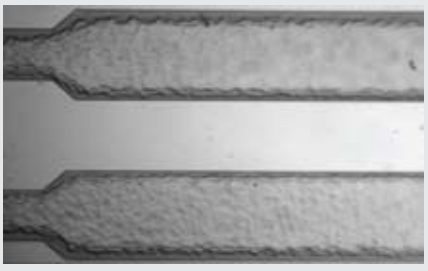
Mesenchymal stem cells cultured under continual shear flow for 48 hours in the presence of VEGF. Treatment with shear induces differentiation into endothelial cells (green stain). This image here shows the early signs of differentiation.

Microbiology

Bacteria and fungi organized into biofilms are highly refractory to known antibiotics and biocides. In order to develop new solutions to combat biofilms, screening technologies must be designed to grow biofilms under conditions that represent an *in vivo* or *in situ* community while maintaining the ability to add and observe the effects of compounds. BioFlux offers a comprehensive solution for running both fundamental biology assays as well as anti-microbial compound screens.

Representative applications:

- Biofilm growth
- Mutant screens
- Antibacterial or antifungal screening
- Host-pathogen interactions
- Adhesion strength



Pseudomonas aeruginosa biofilms grown under uniform shear flow in the BioFlux microchannels.

A system that meets YOUR needs

The BioFlux System was designed with a keen understanding that every lab has its own unique needs. That's why the BioFlux can be configured in a variety of ways to meet your specific requirements for automation, analysis and budget.

BioFlux 200 System

Requires an inverted microscope capable of holding SBS-standard well plates and PC computer.

- Perfect for getting started with flow experiments
- Integrates into an existing inverted microscope
- Upgradable to BioFlux 1000

The BioFlux 200 System comes with everything you need to start running physiologically-relevant assays right on your own inverted microscope. It is ideally suited for research labs, core centers and biotech/pharma research. The system comes with the following components:

- BioFlux Controller
- Pressure Interface (24-well or 48-well)
- Vapor Trap
- Heating Plate
- BioFlux 200 Software
- CCD Camera (optional)

BioFlux 1000 System

- Ideal for high use, core centers, drug screening, and large studies
- Fully-integrated system comes with complete automated microscope
- Enables high throughput acquisition, timelapse imaging, unattended overnight operation

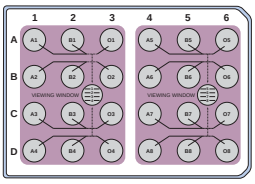
The BioFlux 1000 System is a fully-integrated high content screening system designed for BioFlux applications. It features a research-grade inverted microscope from Nikon and a suite of customized automated microscopy components. It enables rapid image acquisition from BioFlux Plates an d synchronization with flow-based protocols. BioFlux 1000 utilizes the BioFlux Montage software which provides a comprehensive image analysis toolkit based on the industry-leading MetaMorph application. BioFlux 1000 can be configured in a variety of packages to meet your particular application needs.

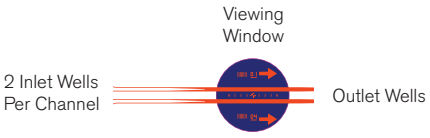
BioFlux Plate Configurations

BioFlux Plates are consumable devices featuring Well Plate Microfluidic™ technology. There are different plate designs to accommodate a wide range of applications.

24-well Plate, 20 dyn/cm²

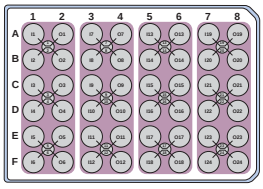
This plate features 8 experimental channels, each with two input wells. It provides the ability to dynamically (<500msec) change between two input sources. The secondary input can be used to add compound, change media type, introduce a second cell type or exchange wash buffer. Ideally suited for overnight experiments, stem cell applications, drug screening, and high content analysis.

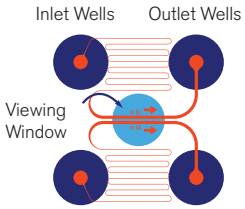




48-well Plate, 20 dyne/cm²

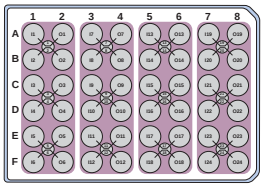
This plate features 24 experimental channels, each with an input and output well. The higher number of experimental channels makes it ideally suited for testing larger sets of conditions, such as compound screening or genetic variants.

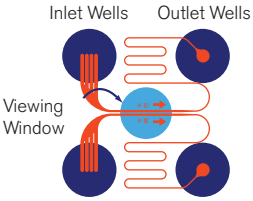




48-well Plate, High Shear, 200 dyne/cm²

This plate features 24 experimental channels, each with an input and output well. The channels have been designed to reach a maximum shear stress of 200 dyne/cm² making them suited for platelet adhesion and aggregation assays, as well as other high shear applications.





	24-well Plate, 20dyn/cm²	48-well Plate, 20 dyne/cm²	48-well Plate, 200dyn/cm²
Number of experiments per plate	8	24	24
Number of input channels to each experiment	2	1	1
Maximum shear stress (shear rate)*	20 dyn/cm² (2000s⁻¹)	20 dyn/cm² (2000s⁻¹)	200 dyn/cm² (20,000s⁻¹)

*Shear rates are listed for water at room temperature

Intuitive control, advanced data analysis

BioFlux 200 Software

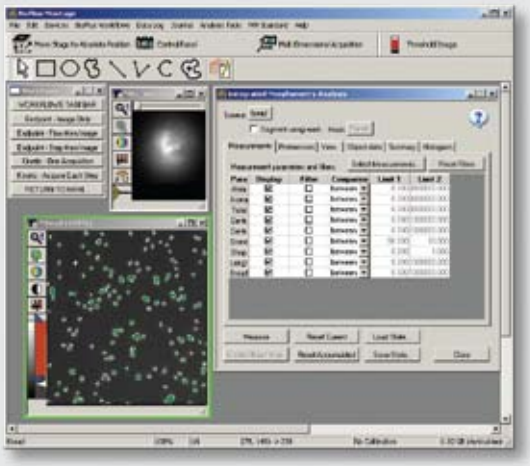
The BioFlux 200 Software provides a simple to use interface for controlling the BioFlux 200 system. Once your experiment is designed, you can easily create and run advanced scripts in the AutoRun Mode. This allows you to dynamically change flow rates, flow directions, compound additions and timings without having to adjust the instrument. Images can be acquired directly in to the s oftware using one of the integrated CCD cameras. Several image analysis modules are included for commonly used routines.

BioFlux 200 Modules:

- Manual Mode
- Protocol Mode
- Image Acquisition (supported cameras)
- Data Analysis
  - Fluorescence intensity
  - Percent adhesion
  - Rolling velocity/particle tracking
  - Image stacking

BioFlux Montage – POWERED BY METAMORPH®

The BioFlux Montage Software is a complete solution for running automated live cell experiments and data processing. This package is provided with the BioFlux 1000 System and serves a single-point control for the entire system. BioFlux Montage was devel oped in conjunction with MDS / Molecular Devices and features the industry-leading capabilities of MetaMorph. All the necessary drivers are included to control the BioFlux 1000 automated stage, CCD camera, Z-drive, automated fluorescence and BioFlux Controller. Data collection is automated with innovative modules which couple directly to the BioFlux flow control. The Montage package offers sophisticated image analysis capabilities featuring the legendary MetaMorph image analysis toolkit, as well as customized modules designed specifically for analyzing BioFlux applications.



BioFlux Montage Modules:

- BioFlux Flow Control
- Multi-dimensional timelapse imaging
- Automated scanning of BioFlux plates
- Sample Reload Adjustment
- Data Analysis
  - Morphology analysis
  - Fluorescence intensity
  - Cell/object counting
  - Percent adhesion
  - Multi-wavelength cell scoring
  - Co-localization
  - 3D Motion Tracking
  - And more…

Bioflux Montage Process Only

The BioFlux Montage image analysis toolkit is available as a standalone application. This is an ideal option for BioFlux 200 users who need additional analysis capabilities. For BioFlux 1000 users, this provides an option to have multiple computers configured for offline analysis of images acquired from the primary hardware workstation.

BioFlux Specifications

BioFlux 200 System

BioFlux Controller:
Shear flow range: 0.5-200 dyne/cm²
Temperature Control: ambient to 50°C
Dimensions: 12"W x 13"L x 9"H (30cm x 33cm" x 22cm)
Air filter: 0.2µm in-line filter

BioFlux 200 Software:
Operating modules: Manual, AutoRun, Image Acquisition, Image Analysis
Operating System: Windows 2000 or XP
Computer Requirements: 1GB RAM, 1GB HD, USB 2.0 Connection

Inverted Microscope:
Available, but not included in base system
Requires inverted microscope with moveable well plate holder

BioFlux 200 System

BioFlux 1000 System

BioFlux Controller:
Shear flow range: 0.5-200 dyne/cm²
Temperature Control: ambient to 50°C
Dimensions: 12"W x 13"L x 9"H (30cm x 33cm" x 22cm)
Air filter: 0.2µm in-line filter

BioFlux Montage Software:
Drivers: BioFlux Controller, CCD camera, stage, focus, filter wheel changers
Modules: BioFlux Control, multidimensional acquisition, digital auto focus, image analysis

Inverted Microscope:
Brightfield Illumination: 12V 100W
Objectives: Nikon, 4-40X (others available)

Anti-Vibration Table:
Size: 30" wide by 24" deep by 4.5" tall
Pressure hookup: 80psi air supply (house air or tank recommended)

CCD Camera:
Imaging array: 1392 x 1040
Pixel size: 6.45µm
Digital output: 12-bit @ 20MHz
Cooling: 25°C below ambient
Frame rate: 10fps at full resolution (165fps max with BIN/ROI)

Automated Stage and Z-Drive:
XY Travel: 4" x 3" (114 x 76mm)
Repeatability: <1µm
Resolution: 0.01µm
Sample holder: customized interface for BioFlux plates
Focusing: automated Z-drive and digital autofocus

Automated Fluorescence:
Lamp: 200W metal halide
Filters: 6-position integrated filter wheel, 55msec switch time
FITC, TRITC, DAPI filters included (others available)
Automated brightfield shutter

BioFlux 1000 System

Automated stage

Automated focus system

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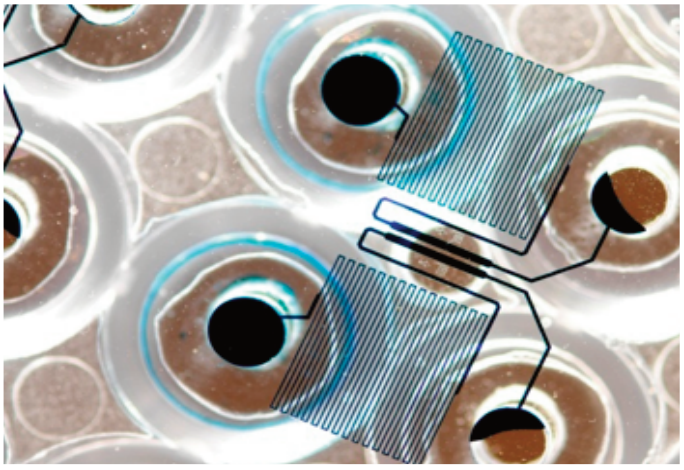
L'innovation à la mesure de vos recherches



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