FLUXION

# High content assays under flow using the BioFlux system

## Overview

The BioFlux, coupled to compatible High Content Imaging systems, is the only setup enabling cell imaging during compound addition for recording fast cellular responses

Traditional FLIPR cellular assays with a fast response time, like calcium flux assays, can now be performed on HCA instruments; this gives the ability to discern individual cell responses from the averaged population response.

Shear flow assays like cell adhesion, platelet aggregation, and mechanotransduction can be read out on any HCA system


**Figure 1: BioFlux and BF Quattro** The BioFlux System for live cell assays under controlled shear flow. The Quattro modules enables running 4 flow plates simultaneously.

## Introduction

High throughput studies for live-cell drug discovery seek to quantify cell signaling events important for directing cell migration, gene expression, morphological changes, and programmed cell death. Well plate assays offer throughput and convenience but, in many instances, do not take into account the true physiological shear flow conditions present in vivo. This is especially true in vascular biology applications where the presence of shear flow may influence compound effectiveness and the stability or functionality of molecular interactions involved in cell adhesion.

The BioFlux™ system (Figure 1) was developed to provide maximum physiological relevance for live cell assays. It consists of arrays of microfluidic flow cells within a standard well plate allowing multiplexing of flow-based assays. These microwell plates run live-cell assays under controlled shear flow conditions and are compatible with bottom-reading imaging modalities like inverted microscopy and high content analysis (HCA) readers.

We demonstrate three assays, both kinetic and endpoint, using the Operetta (Perkin Elmer), a commercially available high content screener located in drug screening labs around the world. All cell culture, treatment, fixation and staining was carried out taking advantage of flow in the BioFlux microfluidic channels. Endpoint readout of BioFlux consumables is compatible with any commercial HCA reader that works with standard well plates, including the Cellomics ArrayScan, GE IN Cell Analyzer, Perkin Elmer Opera and Operetta, and Molecular Devices ImageXpress.

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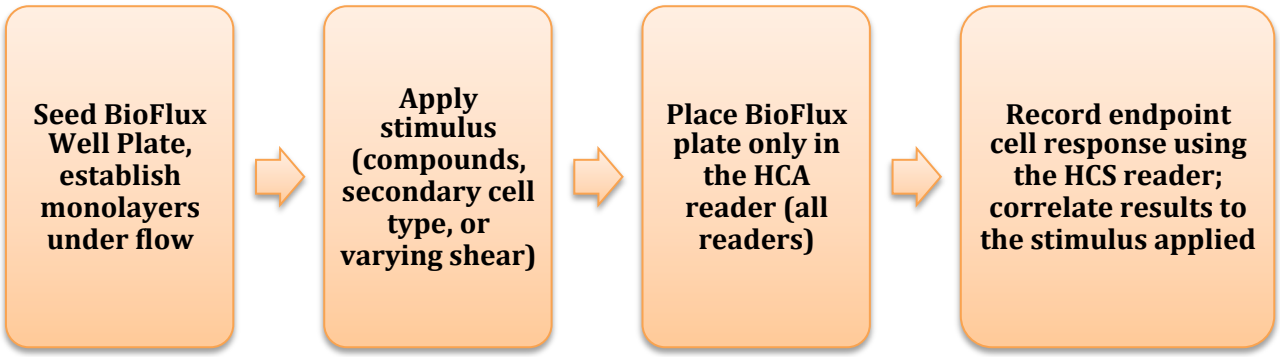


**Figure 2: Endpoint HCS Assay Workflow** This workflow was used to condition cells under shear flow, fix a desired endpoint, image and analyze

## NfκB Translocation

NfκB is an inflammatory master transcription factor that translocates from the cytoplasm to the nucleus after stimulation. Most cell based assays in the past looked at NfκB in static well plates. Here, we demonstrate that shear flow activates cells and causes NfκB translocation, independently of the classic inflammatory mediators.

CHO cell monolayers were grown on BioFlux plates coated with fibronectin (100 µg/mL ) overnight. Cells were subjected to 1 dyn/cm2 of shear flow for 10, 30, 60 and 120 minutes. Following stimulation, cells were fixed in 2% paraformaldehyde (Thermo Scientific), permeabilized in 0.1% Triton (Thermo Scientific), blocked with 5% BSA (Sigma), stained with anti- NfκB (Cell Signaling Technology), phallodin-555 (Sigma) and DAPI (Invitrogen). Images were taken using a Zeiss Z1 Axio Observer. The Operetta was used to quantitate translocation. Cells exposed to 60 minutes of shear flow exhibited the highest level of NfκB translocation (Figure 3 B). This is consistent with previous reports of maximum NfκB translocation at 60 minutes (Noursadeghi et. al., 2008).

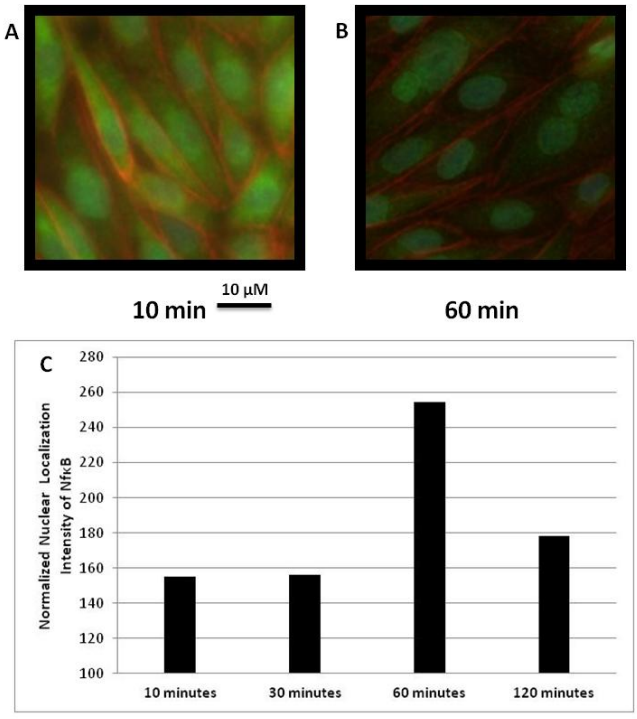


**Figure 3: NfκB translocation in response to controlled shear stress** Cells were treated with 1 dyn/cm2 and strained for nucleus (blue), NfκB (green) and actin (red). Representative images are taken 10 minutes (A) and 60 minutes (B). Operetta software was used to calculate the translocation (C)

## Cellular Alignment

Assays in standard microwell plates do not allow for the dynamic conditions cells experience in vivo. In the case of working with endothelial cells, researchers have gravitated towards flow chambers to mimic the shear force found in vessels. Endothelial cells exposed to shear flow experience cytoskeletal realignment, changes in transcription and translation signaling and epigenetic modifications. The BioFlux system operates an array of flow chambers that permit the multiplexing of flow based experiments. In this feasibility experiment, cytoskeletal alignment is used as a phenotypic indication that the correct mechanotransduction pathways are activated.

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Endothelial cells (SVEC mouse cell line) were seeded on a BioFlux 200 plate coated with fibronectin (100 µg/mL) overnight. Cells were exposed to static conditions (Figure 4A) or 20 dyn/cm2 (Figure 4B) using the BioFlux system for 5 hours. Cells were fixed, permeabilized and stained with phallodin-rhodamine (red), CellMask (green), and DAPI (blue). BioFlux plates were imaged and analyzed using the Operetta system. 400 cells were selected from the main population based on width/length ratio parameter (<0.35). Data are expressed as mean ± SEM, students two tailed t-test, ***p<0.001, **p<0.01 (Figures 4C and 4D). Data and images provided by Prof. Shoumo Bhattacharya and Dr. Aymen Ali Zen at the Wellcome Trust Center for Genetics in Oxford.

## Zinc Loading

Ion loading in cells is critical for signaling events. Classical calcium loading has multiple implications in homeostasis and disease including the "Calcium Hypothesis." (Kristián and Siesjö, 1998) Recent evidence has shown zinc to be critical for innate and adaptive immunity (Shankar and Prasad, 1998). BioFlux allows for rapid liquid handling of compound addition, critical for fast acting ion loading assays. These types of cell-based assays are typically handled using FLIPR instruments( Molecular Devices, Sunnyvale, CA), where the fluorescence of a large area inside the well is being recorded from (Franz et al, 2014). The advantage of the BioFlux approach is the combination of liquid handling and the response of each individual cell can be characterized along with the population response, enabling measurement of heterogeneous responses from different cell subpopulations. Figure 7 demonstrates the point of imaging heterogeneous cell populations after rapid compound addition.

A cellular monolayer of HeLa cells were plated on BioFlux 24 well dual flow plates coated with fibronectin (100µg/mL). Cells were treated with FluoZin-2 (Invitrogen) from one inlet and subsequently treated with zinc sulfate from outlet 2. Cells were imaged in the FITC channel (Figure 6A) and zinc loading was quantitated using Operetta's analysis software (Figure 6B).

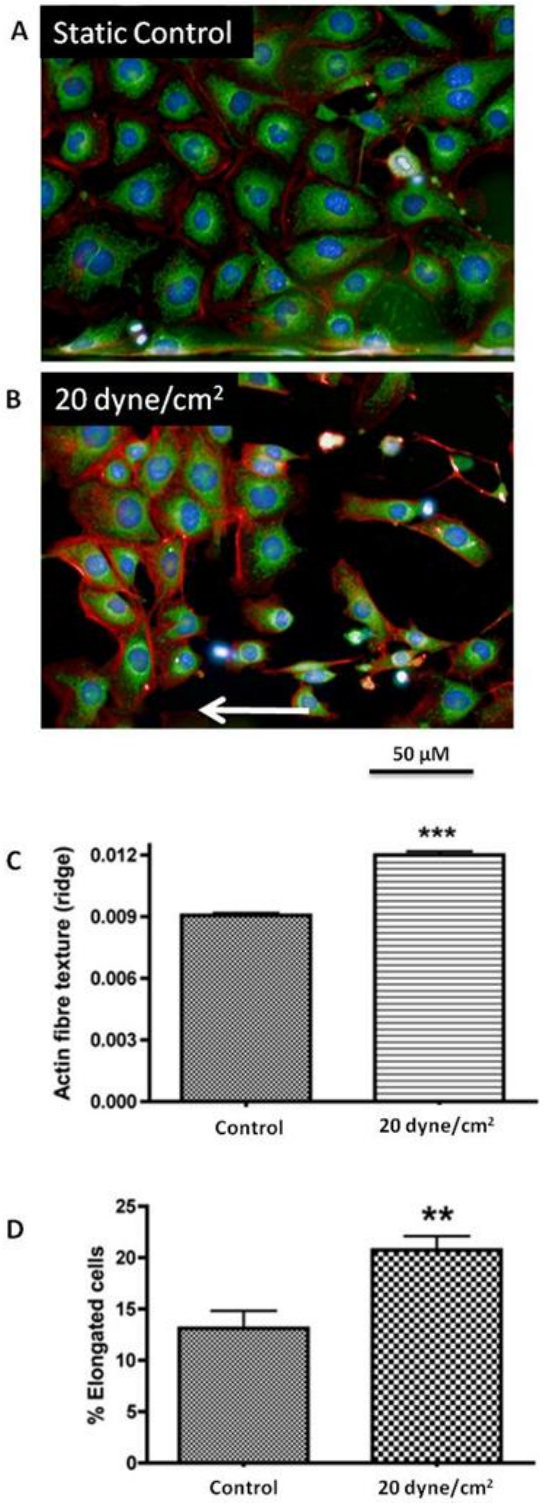


**Figure 4: Endothelial cell alignment** SVEC cells were treated with 20 dyn/cm2 or no flow for 5 hours and stained for nucleus (blue), NfκB (green) and actin (red). Representative images of the cells grown in static (A) and flow(B) environments. Operetta analysis software was used to determine the actin fiber texture (C) and the % elongated cells (D)

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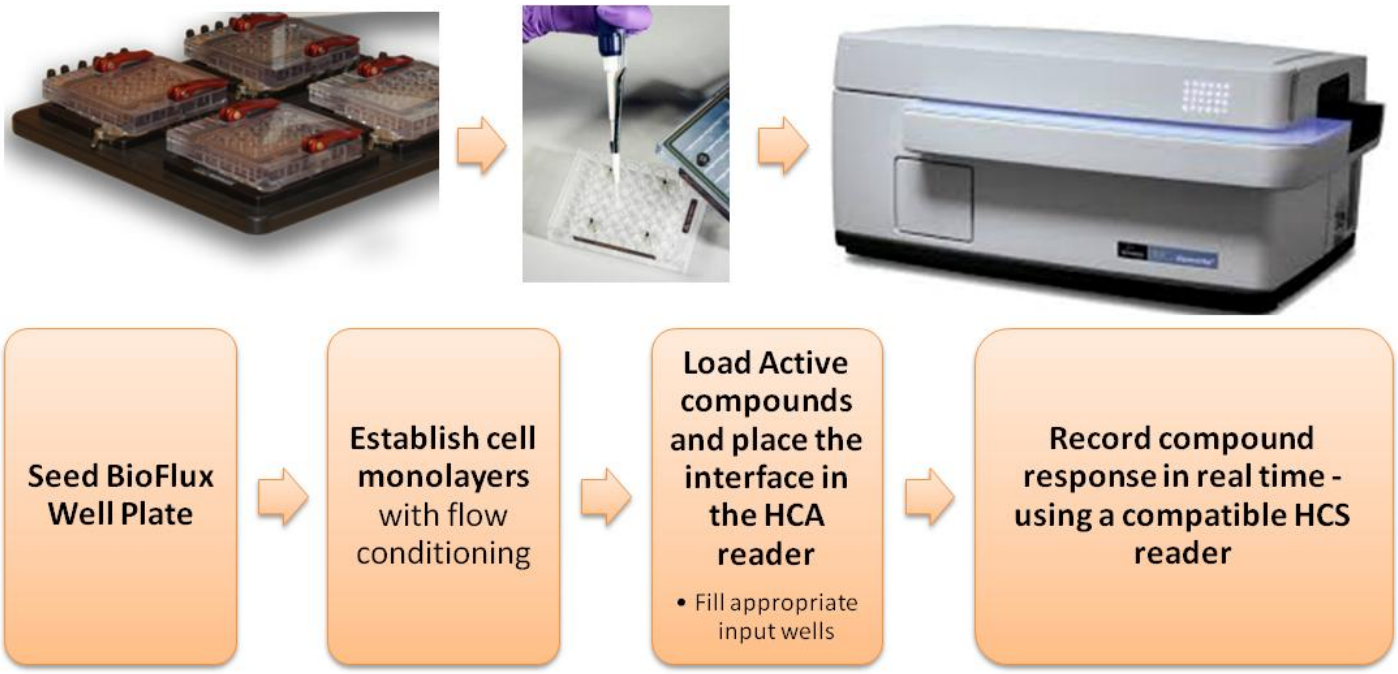


**Figure 5: Kinetic HCS Assay Workflow** using the BioFlux as a liquid handling device for rapid compound addition while imaging

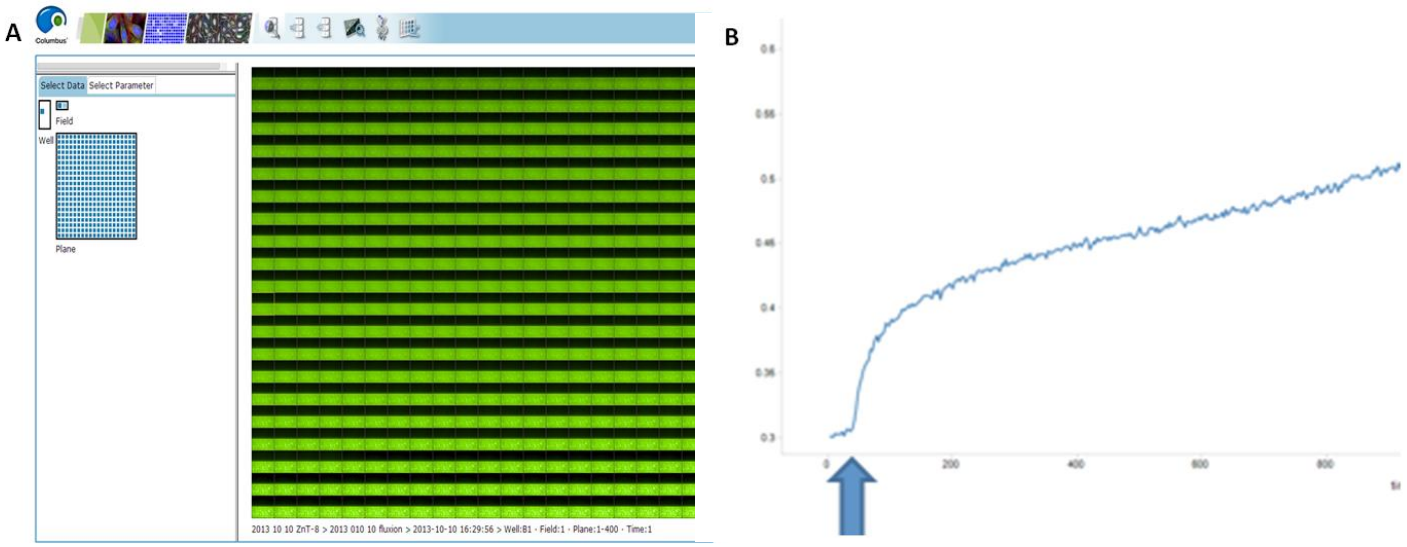


**Figure 6: Zinc ion loading in cells (kinetic assay)** Operetta screenshot of image stacks during the time course of adding zinc sulfate (A). Zinc loading was quantitated over time showing a 50% increase over 60 seconds (B)

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## Summary

We demonstrated the ability to use a commercially available high content analysis system with the BioFlux microfluidic well plate format. The Operetta (Perkin Elmer) was used to measure NfκB translocation, cellular alignment and zinc ion loading in the BioFlux plates. This capability holds particular importance for cells which exist under shear stress, such as vascular endothelial cells. Assays previously performed in microwell plates are well suited for the BioFlux microfluidic format.

The combination of the physiological relevance from the BioFlux microfluidics and high content imaging create a number of novel options for high content analysis, including:

- Real-time monitoring of cellular response during rapid compound delivery (BioFlux plate and interface mounted in the Operetta).
- The ability to run cellular assays with a fast response time, such as calcium flux assays, can now be performed on HCA instruments; this gives the ability to discern individual cell responses.
- Shear-mediated assays, such as cell adhesion, platelet aggregation, and mechanotransduction can be read out on any HCA system as a convenient endpoint assay after cell conditioning on the BioFlux system.

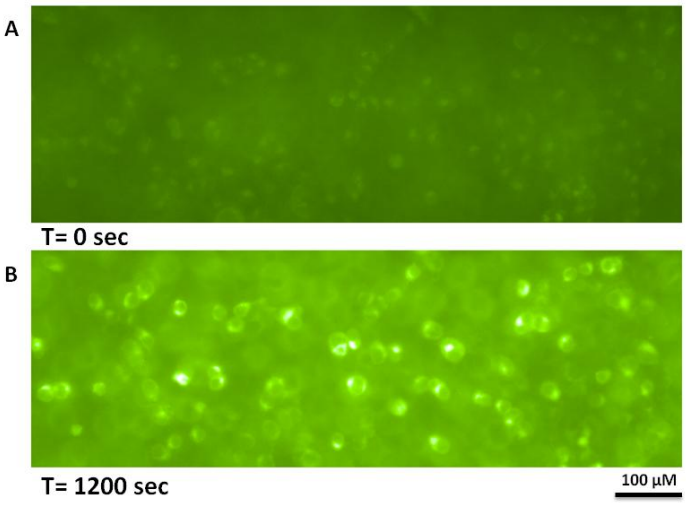


**Figure 7: Zinc loading – detecting heterogeneous responses** Zinc loading is excited in the green channel at time point 0 (A) and at 1200 seconds (B). Typical cell-based assays average fluorescence from an entire field, such as FLIPR like instruments when observations during compound addition are needed, thereby sacrificing the single cell resolution afforded by microscopy

## References

Franz MC, Simonin A, Graeter S, Hediger MA, and Kovacs G. Development of the First Fluorescence Screening Assay for the SLC39A2 Zinc Transporter. Journal of Biomolecular Screening. 2014 Mar 20. [Epub ahead of print].

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Noursadeghi M, Tsang J, Haustein T, Miller RF, Chain BM, and Katz DR. Quantitative imaging assay for NF-kappaB nuclear translocation in primary human macrophages. Journal of Immunological Methods. 2008 Jan 1;329(1-2):194-200.

Shankar AH and Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. The American Journal of Clinical Nutrition. 1998 Aug;68(2 Suppl):447S-463S.

## Support

For assistance with this application note, please contact Fluxion Biosciences through one of these methods:

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