FLUXION

APPLICATION NOTE

Cellular Migration and Invasion Assays

Real-time, dynamic measurements of cellular behavior & controlled microenvironmental conditions

Relevant Research Areas: Oncology, Vascular Biology, Immunology, Ophthalmology, Stem Cell and Developmental Biology Research, Microbiology

Introduction

Cellular migration is an important biological process that occurs in both in health and disease as a part of wound healing, development, differentiation, inflammation, immune response, angiogenesis and metastasis. Chemical messengers are integral to most migration events and serve to direct cells to tissues or the vasculature via a process called chemotaxis. Chemotaxis is a dynamic event with numerous steps of cell communication and response.

Typically, cellular migration is studied using a two compartment device called a Boyden chamber or a transwell insert system. The data gathered using a Boyden chamber are narrowly-focused snapshots into complex biological phenomena. Events occurring before the endpoint are typically lost using the Boyden chamber or transwell approach. The processing of transwell inserts for data capture is considerably labor-intensive. Further, it is impossible to control environmental conditions within the well due to the static nature of the assay. In order to examine these processes in detail over time, it is necessary to observe cells *in situ*.

Figure 1: The BioFlux 200 System for live cell assays under controlled shear flow.

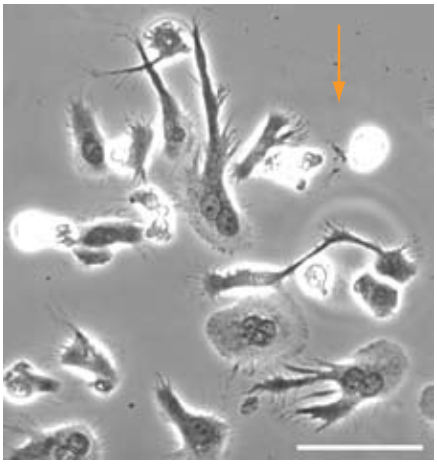


Figure 2: HI-60 cells migrating within BioFlux channels as viewed from beneath the well plate. Cell movement is in response to fMLP, a potent chemokine (direction is indicated with arrow). Scale bar is 50 µm.

BioFlux allows observation of chemotaxis and migration as a dynamic event

- Up to 16 conditions tested in parallel
- Flexible format (liquid or gel) enables a wider range of assays
- Better quality data with real-time measurements *in situ*
- Minimizes variability with a continuous chemotactic gradient
- Saves time by collecting data direct from the assay with no transfer steps
- Assays can be performed label-free

Representative Applications

The BioFlux system can be used for angiogenesis assays with endothelial cells, screening different types of tumor cells for invasive phenotypes, screening compounds to inhibit or otherwise affect invasion and or angiogenesis, or to study chemotaxis of circulating cells in response to stimuli or inhibition.

This application note will describe two model migration assays. The first assay is a chemotaxis study with neutrophilic HL-60 cells responding to stimulation with chemoattractants, fMLP and IL8. The second assay, using HUVEC, HT1080 and MCF7 cells, leverages parallel flow in the microfluidic channel to create an environment to study invasion.

Cellular Migration and Invasion Assays

Real-time, dynamic measurements of cellular behavior & controlled microenvironmental conditions

Mode	Cell Type	Description
Transmigration	Circulating Cells	Migration of cells through the endothelium to the underlying tissue at the site of insult
Angiogenesis	Endothelial Cells	Invasion via degration of the extracellular matrix
Extravasation	Abnormal Cells Cancer Cells	Transmigration of tumor cells from the vasculature to the tissue
Homing	Stem Cells	Migration and/or transmigration to tissue target
Invasion	Abnormal Cells Cancer Cells	Cell mobilization away from primary tumor
Wound Healing	Epithelial Endothelial	Migration and proliferation of cells to repair a wound

Table 1: Special cases of cellular migration or chemotaxis

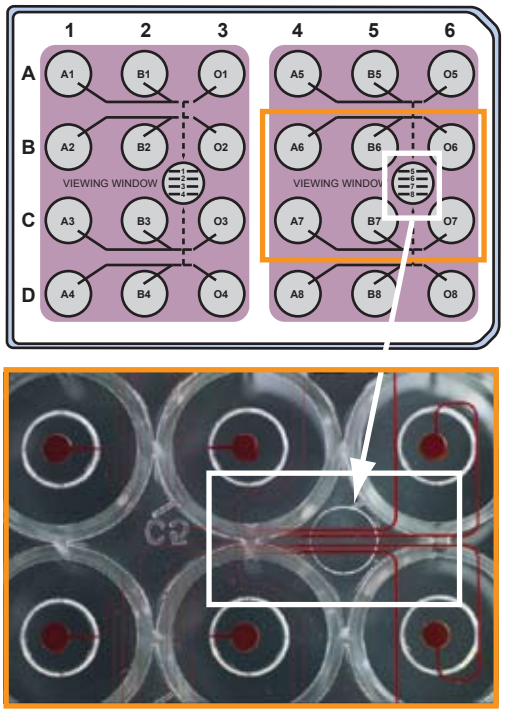


Figure 3: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Parallel Flow Principles of Operation

BioFlux can be used to create microenvironments within the channel

Creation of two microenvironments within a microfluidic channel is achieved using a two inlet channel and parallel flow streams (Figure 3). When flowed under laminar fluid dynamic conditions, fluids originating from inlet A and B travel in non-mixing streams from beginning to the end of the channel. Laminar flow conditions were demonstrated by flowing a fluorescent dye from one inlet and non-fluorescent buffer from the other while varying where pneumatic pressure was applied (Figure 4).

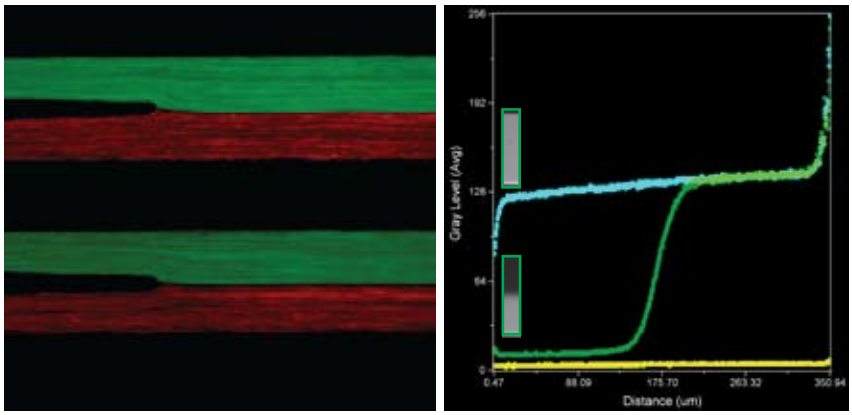


Figure 4: Microfluidic channels are shown (left) with different colored fluorescent beads flowing from separate inlets. Flow control provides the ability to fine tune microcompartments (right). No pressure on the inlet with dye (yellow trace) or the inlet with buffer (blue trace) resulted in a channel with no fluorescence or all fluorescence. Equal pressures applied to both inlets (green trace) resulted in equal-sized zones of dye and buffer.

Typical BioFlux experimental setup for each assay type

Chemotaxis Assay:

Channel	Cell Type	Condition	
		Plate 1 fMLP [nM]	Plate 2 IL-8 [nM]
1 control	n-HL-60	0	0
2 exp	n-HL-60	2.5	0.0625
3 exp	n-HL-60	25	0.312
4 exp	n-HL-60	125	0.625
5 exp	n-HL-60	250	1.25
6 exp	n-HL-60	2500	12.5
7 exp	n-HL-60	10000	6.25
8 control	HL-60	250	1.25

n-HL-60 are differentiated into a neutrophil-like cells using simulation with growth factors. HL-60 are fully undifferentiated with no neutrophilic capabilities. In this experiment the compounds were flowed from one inlet and buffer from the second inlet. Chemotaxis was followed using timelapse microscopy. Image capture was performed using the BioFlux 1000 imaging station. The duration of data collection was 45 minutes with an image captured every 15 seconds.

Angiogenesis & Invasion Assay:

Ch	Cell	Treatment	Cell	Treatment
	Angiogenesis		*Invasion*	
1	HUVECp2	Matrigel (MG)	HT1080	MG
2	HUVECp2	MG+bFGF(1)	HT1080	1 exp
3	HUVECp2	MG+bFGF(2)	HT1080	2 exp
4	HUVECp2	MG+VEGF(1)	HT1080	3 exp
5	HUVECp2	MG+VEGF(2)	MCF7	MG
6	HUVECp2	MG+Fum(1)	MCF7	1 exp
7	HUVECp2	MG+Fum(2)	MCF7	2 exp
8	HUVECp2	MG	MCF7	3 exp

Matrigel, with or without treatments added, is patterned into one side of the BioFlux channel; cells are added to the other. Timelapse data can be collected at any frequency for any duration desired. This technique is especially amenable to longer term experiments where long term culture is desired.

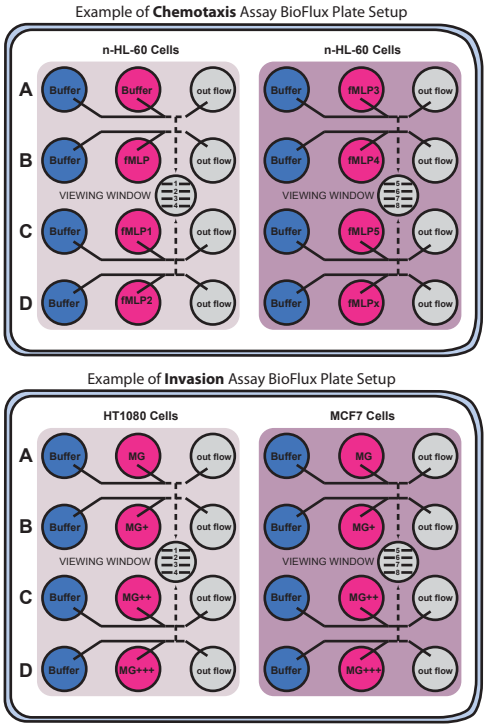


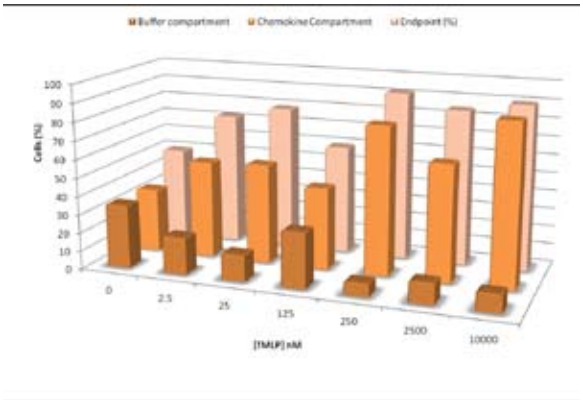
Figure 5: Plate setups for common parallel flow assays.

Results

1. Cellular migration in response to fMLP. At both the beginning and the endpoint of the experimental period, cells were counted in the channel and in the regions of the channel where buffer and chemokine were present during the active experiment and expressed both as a percentage of starting number of cells (Buffer compartment and Chemokine compartment) and because some cells are removed from the channel due to flow, as a percentage of total cells remaining at the endpoint (Endpoint %).

2. Angiogenesis: Invasion of Endothelial Cells into the Matrigel Matrix. After 36 hours cells WGA (green) was used to stain cells and gel for 15 min post-flow. Nuclei are stained as well with Hoescht 33342 (blue). Individual cells were observed invading the gel matrix under conditions of Matrigel alone, VEGF, bFGF. Sprouting and process formation, which are early steps of angiogenesis, were noted under these conditions. The addition of fumagilin, a angiogenesis inhibitor, abrogated the invasion and the cells multiplied outside of the gel.

1



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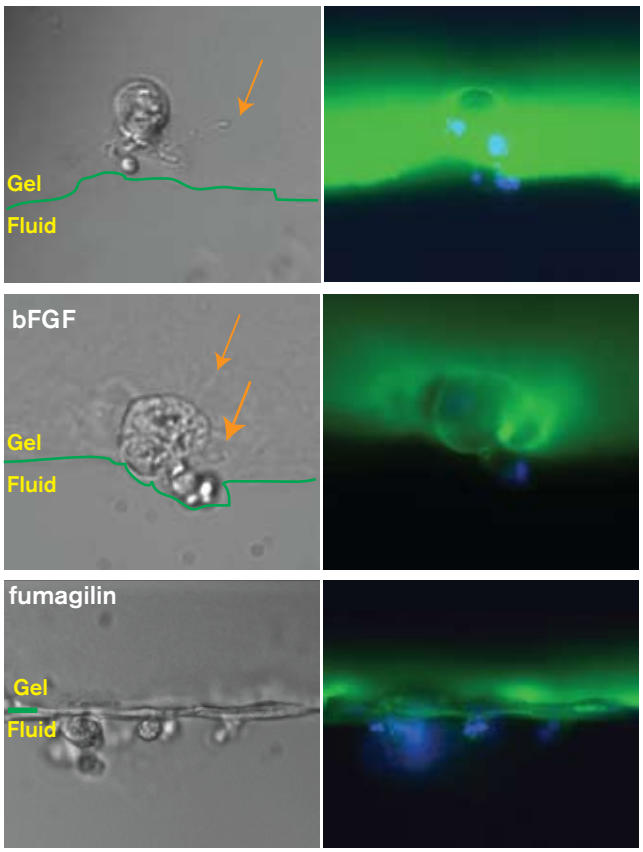
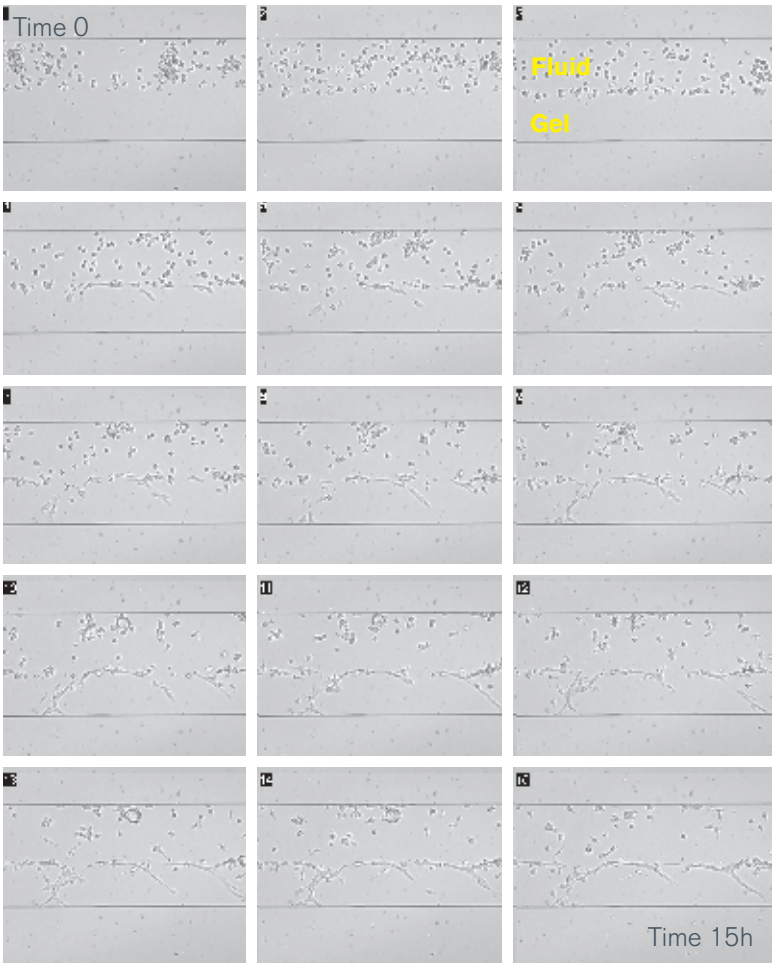
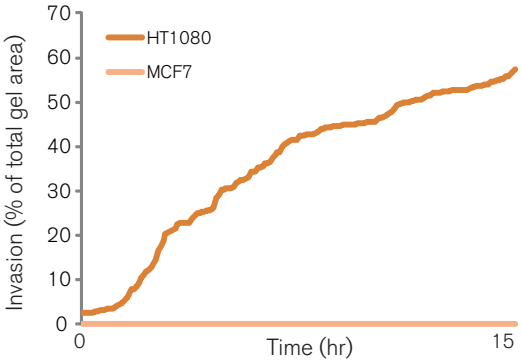


Results (continued)

3. Cancer cell invasion into the Matrigel Matrix with model invasive vs non-invasive cells. Invasive HT1080 cells began to migrate after 4 hours of perfusion into the matrix and continued invasion throughout the entire experiment. In contrast the MCF7 cells, were as expected completely non-invasive (not shown). Timelapse over 15 hours of either invasive (HT1080) or non-invasive cancer cells(MCF7) into Matrigel. Cell invasiveness expressed as pixel intensity over total gelled area of the channel as a function of time.

3





Data Processing for Cell Migration using BioFlux Montage

Data analyses are typically carried out using Multidimensional Motion Analysis module in BioFlux Montage (Fluxion) using template matching. This module enables the user to follow many aspects of cellular movement over time, including distance, velocity, direction, angle and many others (Figure 6 &7).

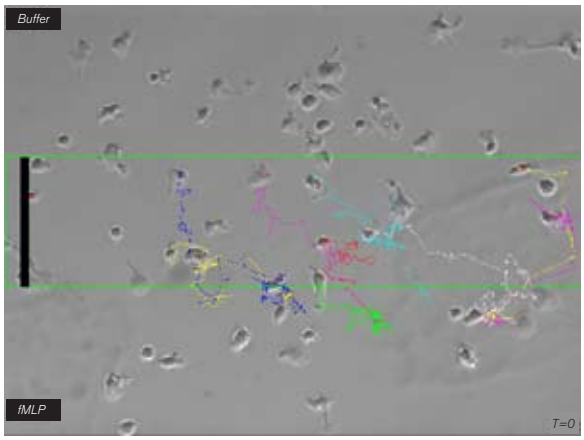


Figure 6: A representative microscopic field at the beginning of a timelapse data set used for migration analysis (time=0). The green box represents the midline search region where each cell is tracked through a total of 178 microscopic fields. The black box is 100µm in height. The tracks shown as colored lines indicate the paths of each cell during the timelapse.

Summary

- Imaging i*n situ* provides temporal insights into cellular migration.
- Consistent gradient within the microchannel provides experimental reliability and reproducibility
- A wide range of conditions can be queried in parallel
- Other relevant applications include:
  - >Wound healing
  - >Chemotaxis of microbes
  - >Chemotaxis on substrates
  - >Transmigration

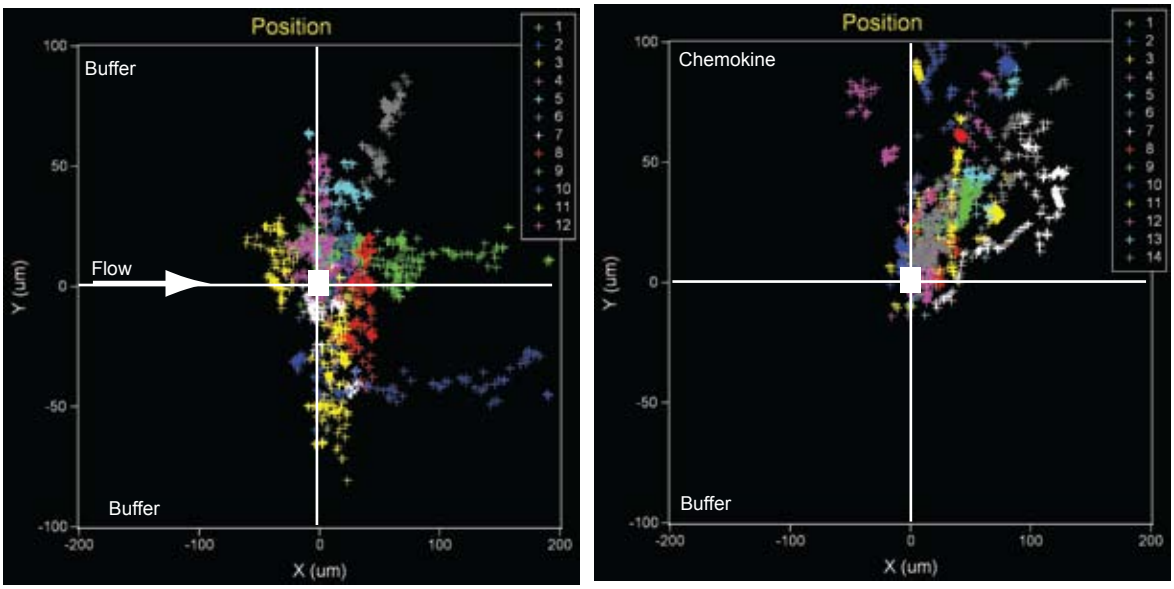


Figure 7: Typical analysis results: Directional motion analysis for timelapse migration experiments. Cell motion was tracked over the duration of the timelapse. X,Y spatial position with respect to the cell's original position (origin, white box) over time is shown for cells within the interrogation area, (left) Buffer only, (right) 125nM fMLP.

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