CELL-IQ® APPLICATION NOTE

Monitoring chemotaxis using CellDirector® microfluidic device

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INTRODUCTION

It is now clear that cells are constantly interacting with the extracellular matrix and their phenotypes change with the evolving microenvironments [1,2]. In chemotaxis studies, there is a need for building spatially and temporally controllable chemical gradients, as well as following cellular dynamics in real time and space. Microfluidic chemotaxis devices have the potential to perform these tasks and lay the foundation for theoretical modeling of mammalian cell chemotaxis [as reviewed in 3]. Gradientech's CellDirector® is a commercial microfluidic chemotaxis device for studying cell migration along a well-established and stable chemical concentration gradient either toward or away from the chemical stimulus.

Combining CellDirector® with Cell-IQ® provides a simple way to study biologically relevant chemotaxis automatically, label free and in real time. Cell-IQ® is an ideal platform for this type of study as it provides an optimised incubator for cell maintenance, a time-lapse microscope for image capture and built-in analysis software (Figure 1). Use of the z-stack and dynamic focusing functions ensures images are of high quality and in focus throughout the experiment. Cell-IQ® is compatible with a variety of commercial products for live cell studies, here we demonstrate the use of Gradientech's CellDirector® to study migration of prostrate adenocarcinoma cells, PC3 cells, in the presence/absence of FBS over a 16 hour incubation period. Data generated includes centred trajectories, cell migration distance and rose plots for all experimental conditions.

Figure 1. Cell-IQ® incubator loaded on the left plate holder with a CellDirector®2D (green) and the right plate holder with a CellDirector®3D (red).

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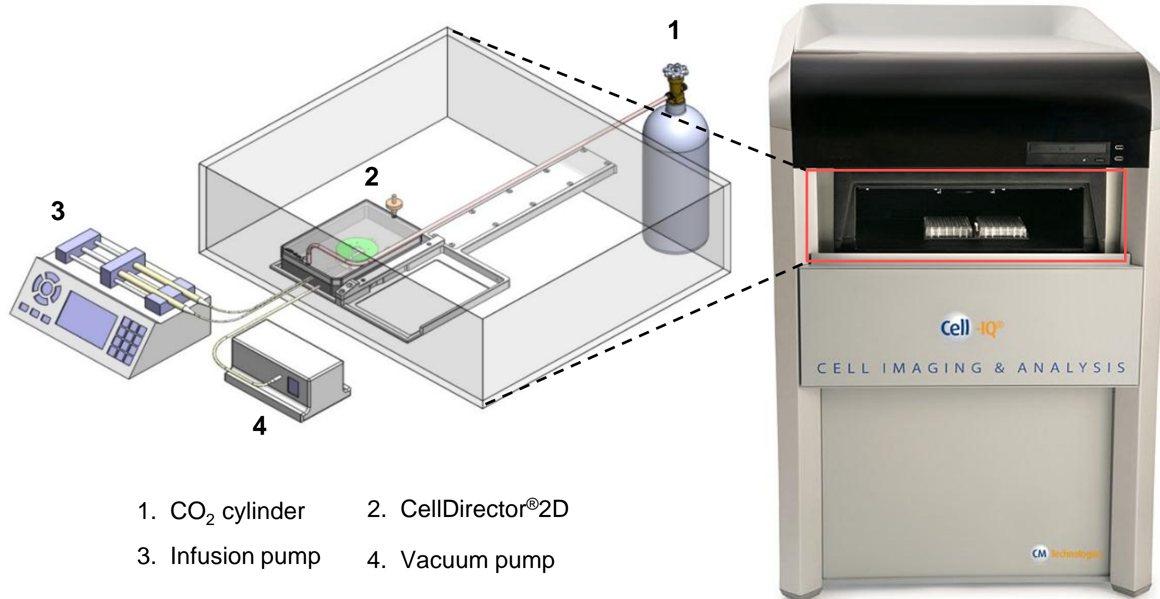


Figure 2. Schematic of Cell-IQ® and CellDirector® experimental set up.

MATERIALS & METHODS

Cell culture

PC3 cells were cultured in Ham's F12 medium (BioWhittaker®) supplemented with 10% FBS and 2 mM L-glutamine at 37°C with 5% CO_2. The media was replaced 16 hours prior to starting the chemotaxis assay with Ham's media containing only 0.5% FBS. Cells were harvested and diluted/suspended to a density of 1 x 10^6 cells/ml in medium containing 0.5% FBS and 200 U/ml penicillin-streptomycin.

Coating and seeding with cells

All reagents were stored at 37°C to equilibrate over night to avoid bubble formation. To the CellDirector® 200 µl of type I collagen (200 µg/ml in PBS) was added and incubated at 37°C to allow the collagen to set. After 1 hour 200 µl of PC3 cell suspension (1 x 10^6 cells/ml) was added to the CellDirector® and the assay incubated for a further 1 hour at 37°C to let the cells adhere. Two syringes were filled, one with 1 ml of media containing 0% FBS (control) and the second with 1ml media containing 10% FBS (chemoattractant), and placed in the incubator to equilibrate.

Connection and loading into Cell-IQ®

The CellDirector® was placed onto a holder which was loaded into plate position 1 inside the Cell-IQ incubator. The syringes and infusion tubes were connected to the CellDirector® as required and the vacuum outlet connected to the vacuum pump. The Cell-Secure™ lid was secured in place and connected to the CO_2 gas supply which was run at a flow of 35 ml/minute for the duration of the experiment (Figure 2).

Image capture and flow rate

Once in position, three different regions of interest (ROI) were defined and selected for imaging purposes, control of 0% FBS, gradient and control of 10% FBS (Figure 3). The Cell-IQ Imagen™ software was set to capture phase contrast images from all selected positions at 90 second intervals for 16 hours using a Z-stack of 19.2 µm and the dynamic focus feature enabled. Once the Imagen protocol was complete the syringe pump was started at a flow rate of 1 µl media/minute and the Imagen program started.

Quantitation using Cell-IQ Analyser™ software

The Cell-IQ Analyser™ software was used to follow the cells throughout the entire image series providing graphical output of their total trajectories. In addition to single cell trajectories, combined trajectories of all cells in the image, their velocities and direction of movement was also determined (Figure 4). This data is presented using standard graphical tools such as centered trajectories, rose plots and distance charts (Figures 5 & 6). All parameters, including movement angles and persistence of movement, was generated for each cell and as an average for the whole population. The batch process facility was utilised to process all captured images automatically for the entire experiment.

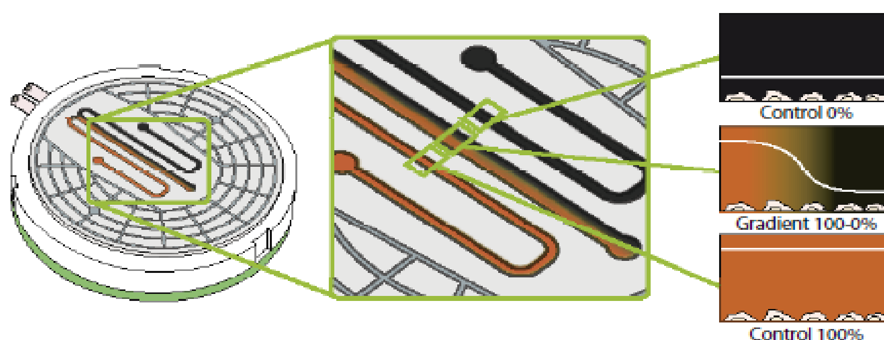


Figure 3. Schematic representation of Gradientech's CellDirector®2D.
Cell-IQ® was programmed to capture images from all three sample areas (control 0%, gradient and control 100%) at 90 second intervals.

RESULTS

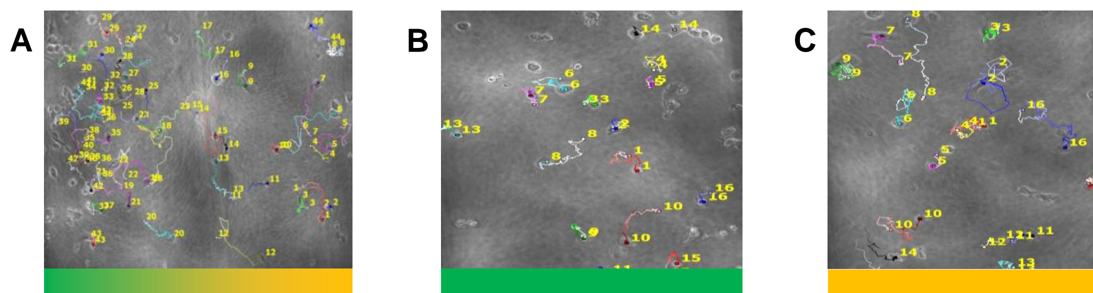


Figure 4. Trajectories of PC3 ells imaged within the flowing media and chemoattractant gradients created using Cell-IQ Analyser™ software. Images captured by Cell-IQ® were analysed for all three ROIs, A) gradient channel, B) 0% FBS channel and C) 10% FBS channel
■ 0% FBS, ■ 10% FBS.

Figure 5

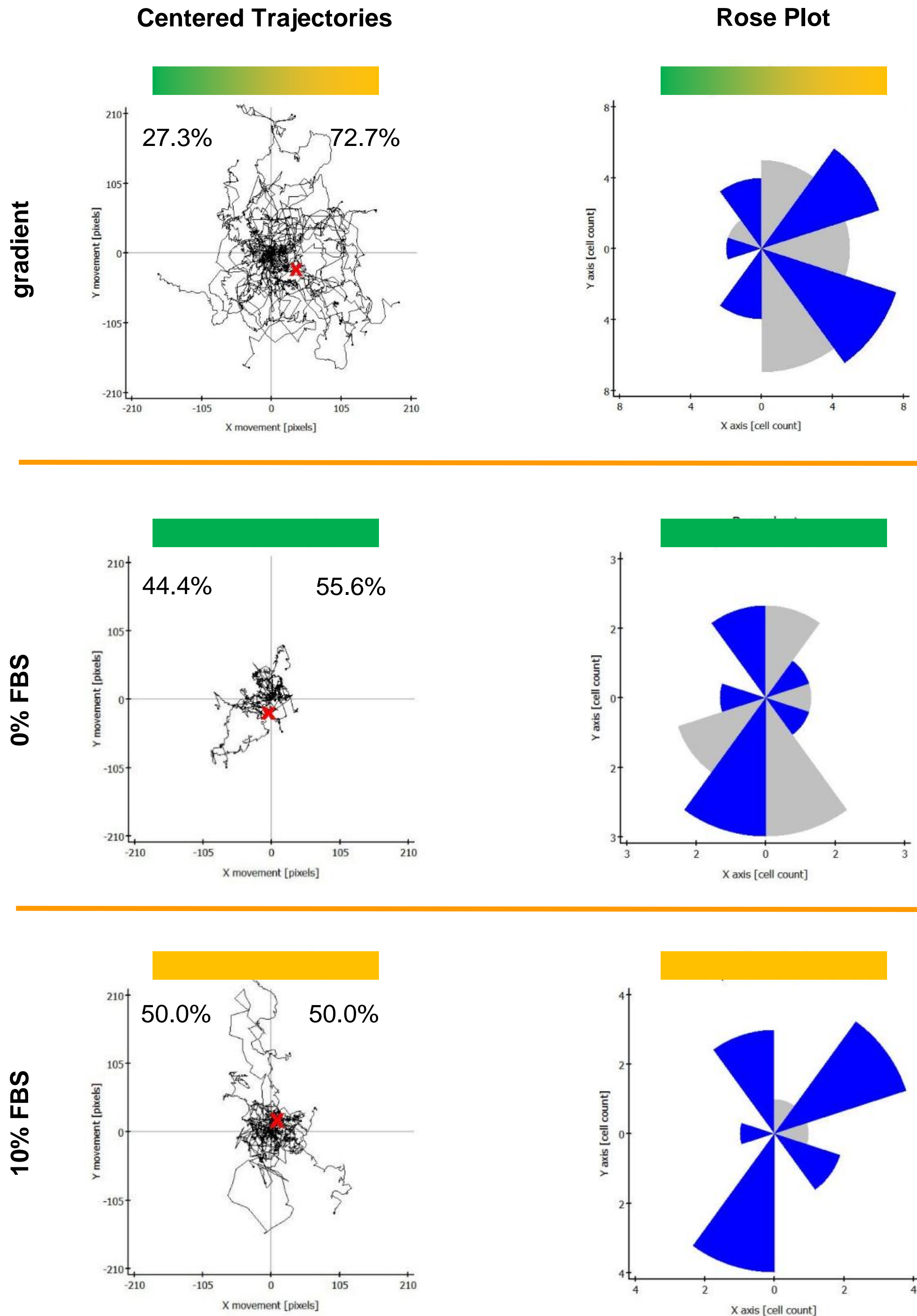
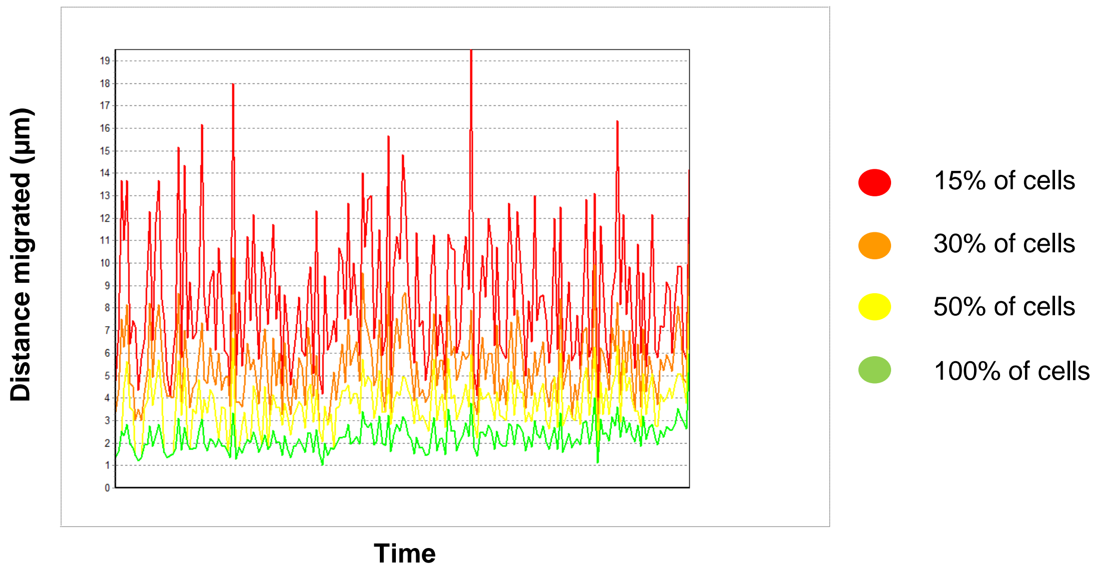


Figure 5. Data generated indicated that PC3 cells actively migrated toward the higher concentration of FBS. Centered trajectory graphs and Rose plots show that in the gradient channel, the majority of cells migrated from 0% FBS toward 10% FBS. In both 0% and 10% FBS control channels, the number of cells migrating in each direction were comparable. The Centre of Mass is indicated as a **X** on the graphs for each sample type. Cell movement was quantified from 3 to 8 hours of culture. ■ 0% FBS ■ 10% FBS.

Figure 6

A



B

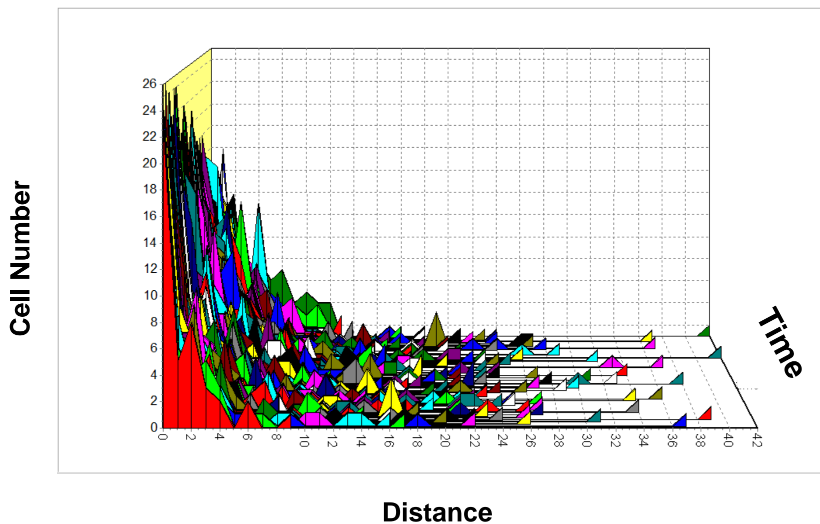


Figure 6. Cell-IQ Analyser™ graphical representation of the data in 2D (A) and 3D (B) plots for cell migration distances in the gradient channel. Graph A indicates the distance migrated in micrometers by the total cell population over time. Whilst Graph B provides the additonal parameter of cell number incorporated into the data set. Cell-IQ Analyser™ software identifies, counts and classifys every cell on the captured image, in addition, it also records the X,Y and Z coordinates for each and every cell making this form of graphical representation possible.

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CONCLUSIONS

Together Cell-IQ® and CellDirector® provide a powerful, label free, automated solution for the real time observation and quantification of cell chemotaxis in a microfluidic environment. As expected PC3 cells were seen to migrate with district directional movement along the concentration gradient from the 0% FBS medium toward the higher 10% FBS medium. There was no obvious directional movement of the cells cultured in 0% FBS, however cells cultured in 10% FBS did show an increase in movement i.e. increased chemokinesis.

KEY POINTS

- Cell-IQ® and CellDirector® provide a simple automated solution for the study of cell chemotaxis in real time
- Image capture and analysis was performed truly label-free so cell behavior was not compromised
- Using the two technologies combined significantly reduces cell, reagent, time and labor costs
- Cell-IQ Imagen™ Software ensures high quality images are captured from all selected positions and use of the dynamic focusing features maintains image focus at all times
- Cell-IQ Analyser™ Software automates analysis of captured images quantifying multiple parameters at one time for the entire experiment
- Graphical data is easily generated for trajectories, directionality and cell velocity as well as cell numbers and classification

REFERENCES

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