



Real-time, label-free monitoring of cellular invasion and migration with the xCELLigence system

For researchers performing invasion and migration assays, the CIM-Plate 16 used with the xCELLigence RTCA DP Instrument from Roche Applied Science offers a means to monitor cellular responses in real time, without exogenous labels, through impedance-based technology. The CIM-Plate 16 provides a kinetic cell-response profile throughout an experiment, detailing the onset and rate of invasion and migration. This information can help researchers understand processes that current endpoint experiments are unable to address.

Principles of invasion and migration for cellular analysis

The study of cell invasion and migration is of great importance to enable a better understanding of underlying biological and molecular mechanisms. The new CIM-Plate 16 device from Roche Applied Science detects real-time invasion and migration of cells without the use of labels.

Cell invasion is the intrusion on and destruction of adjacent tissues, particularly with respect to cancer cells. Cell migration is the movement of cells from one area to another, generally in response to chemical signal, and is important in diverse physiological and pathological processes including embryonic development, cell differentiation, wound healing, immune response, inflammation and, notably, cancer metastasis.

Conventional methodologies for migration detection are endpoint and label-based assays. One of the more commonly used methods is the Boyden chamber (also called trans-well migration assay), whereby cells placed in inserts are allowed to invade and migrate through an artificial microporous membrane into lower chambers containing a given chemoattractant.

There are drawbacks to this approach. The migrated cells (typically attached on the reverse side of the membrane) must be stained with chemical dyes or labeled with fluorescent molecules. In most cases, the membrane is removed from the insert, and then the stained or labeled cells are counted manually using a microscope or evaluated using a spectrometer. This labor-intensive process limits throughput and, as a result of labeling the cells, may alter gene expression profiles and lead to intra-assay variability because of differences in labeling efficiency. Additionally, cell counting by means of microscopy may not be accurate, often resulting in inconsistent and irreproducible migration data.

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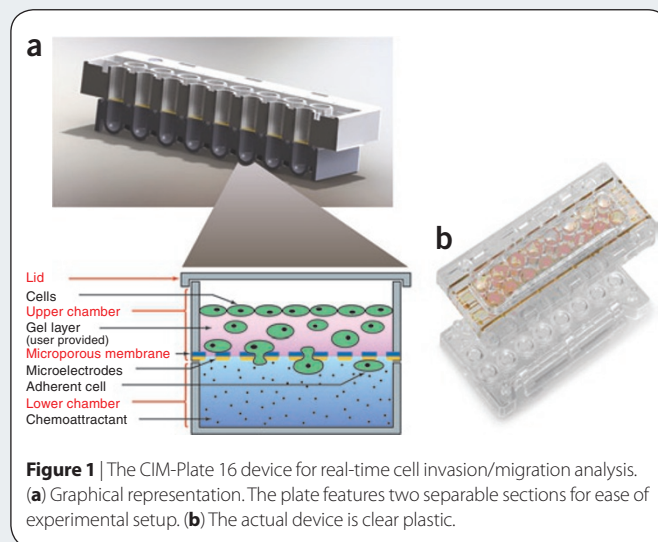
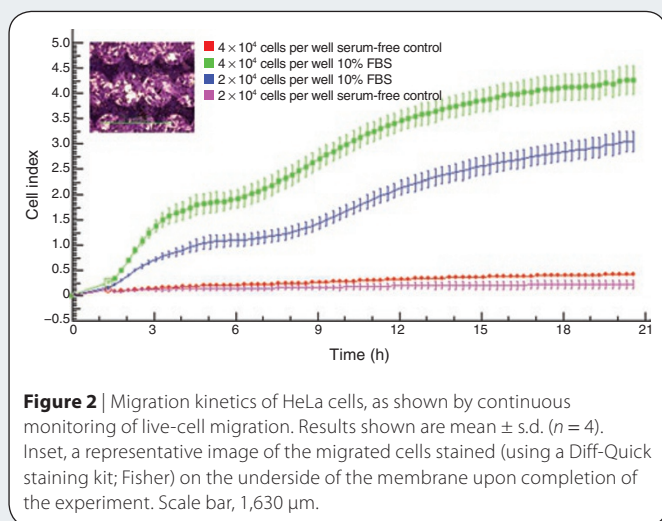


Figure 1 | The CIM-Plate 16 device for real-time cell invasion/migration analysis. **(a)** Graphical representation. The plate features two separable sections for ease of experimental setup. **(b)** The actual device is clear plastic.

CIM-Plate 16 detects cell invasion/migration in real time

In contrast to the methods mentioned above, the Roche xCELLigence Real-Time Cell Analyzer (RTCA) DP instrument provides kinetic information about cell migration by dynamically recording the entire cell migration and invasion process in real time without labeling cells, considerably improving invasion and migration assay quality. The RTCA DP instrument uses the CIM (cellular invasion/migration)-Plate 16, which features microelectronic sensors integrated onto the underside of the microporous polyethylene terephthalate (PET) membrane of a Boyden-like chamber (**Fig. 1**). As cells migrate from the upper chamber through the membrane into the bottom chamber in response to chemoattractant, they contact and adhere to the electronic sensors on the underside of the membrane, resulting in an increase in impedance¹. The impedance increase correlates to increasing numbers of migrated cells on the underside of the membrane, and cell-index values reflecting impedance changes are

APPLICATION NOTES



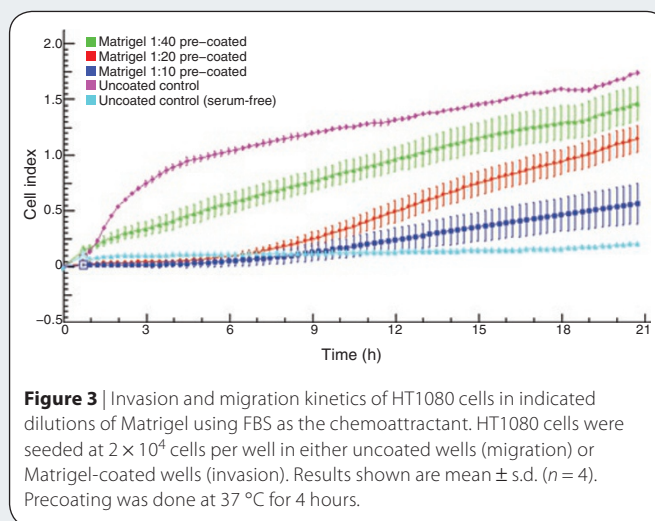
automatically and continuously recorded by the RTCA DP instrument (Figs. 2 and 3). Therefore, cell migration activity can be monitored via the cell-index profile.

Experimental design

The following is a basic migration assay protocol used on the RTCA DP instrument, with fetal bovine serum (FBS) as chemoattractant. The protocol is optimized for two cancer cell lines: a human fibrosarcoma cell line, HT1080, and a human cervical cancer cell line, HeLa. Assay conditions may require further optimization if different types of cell lines or chemoattractants are used. (i) Cells should be grown in serum-free medium for 4–24 hours. (ii) For invasion experiments, the top chamber of the CIM-Plate 16 is coated with Matrigel (BD) before adding medium (with or without chemoattractant) to the bottom chamber. (iii) The CIM-Plate 16 is assembled by placing the top chamber onto the bottom chamber and snapping the two together. (iv) Serum-free medium is placed in the top chamber to hydrate and preincubate the membrane for 1 hour in the CO_2 incubator at 37 $^\circ\text{C}$ before obtaining a background measurement. (v) Cells are lightly trypsinized, pelleted and resuspended at the indicated cell densities in serum-free medium. (vi) Once the CIM-Plate 16 has equilibrated, it is placed in the RTCA DP station and the background cell-index values are measured. (vii) The CIM-Plate 16 is then removed from the RTCA DP station and the cells are added to the top chamber at the desired density. (viii) The CIM-Plate 16 is placed in the RTCA DP station and migration is monitored every 2 minutes for several hours.

Migration of HeLa cells

We analyzed two densities of HeLa cells in the absence or presence of 10% FBS in the bottom chamber (Fig. 2). Cell migration kinetics were recorded by the RTCA DP instrument for approximately 18 hours. Migration kinetics were distinct and easily detected by automated real-time monitoring. The low and high seeding densities were quantitatively monitored and reflected by the cell-index values, and were correlated with a manual cell count determined at the end of the assay by fixing and staining the cells on the membrane of the top chamber.



Invasion and migration of HT1080 cells

We monitored migration and invasion kinetics—in the absence and presence of Matrigel, respectively—in response to 10% FBS (Fig. 3). In the absence of serum, we observed little to no migration. As indicated by the cell-index profiles, cell invasion and migration was affected in a dose-dependent manner by the concentration of Matrigel. The rate and onset of invasion were readily quantified by real-time monitoring while concurrently assessing migration. The delay of cell-index signal (Fig. 3) was due to the time required for the cells to invade through the Matrigel and was dependent on the concentration of Matrigel used.

Conclusion

The cancerous cells shown have varying migratory and invasive behaviors, specific to chemoattractant stimuli or matrigel concentration. The new CIM-Plate 16 for the RTCA DP instrument is a versatile device for the analysis of invasion and migration, and is at present the only platform capable of quantitatively measuring the invasive and migratory behavior of cells in real time without exogenous labels. This enables the identification of migratory and invasive time points, which will help increase throughput by shortening assay time, minimize the number of endpoint assays and help determine optimal time points for inhibitor studies. Moreover, analysis using the xCELLigence System reduces hands-on cell-manipulation steps, leading to more physiologically relevant results.

The CIM-Plate 16 will be available in the fall of 2009. More information about the xCELLigence System is available at <http://www.xcelligence.roche.com/>.

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- Solly, K., Wang, X., Xu, X., Strulovici, B. & Zheng, W. Application of real-time cell electronic sensing (RT-CES) technology to cell-based assays. *Assay Drug Dev. Technol.* **2**, 363–372 (2004).

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