

CELL MIGRATION

probing cell movement with smarter tools

Cell migration comprises the movement of cells from one location to another in response to events, biological signals or environmental cues. Cell migration plays a critical role in numerous normal biological processes and also in many diseases. Most recent commercial activity around cell migration has been directed towards developing tools that facilitate easy access/setup and add greater reproducibility to assays. Researchers wanting to investigate cell migration are now increasingly spoilt for choice. This is reflected not only in the variety of cell culture inserts, stoppers, specialised microplates, microfluidic devices and live cell compatible trackers that are now available to support cell migration, but also by the efforts of instrument manufacturers to make detection, reading, imaging and analysis of fluorescent and unlabelled cells easier in both end point and kinetic motility assays. Many new and innovative assays tools, some of which are reported in this article, not only facilitate the wider investigation of cell migration assays but also contribute to a better understanding of the process by which cells migrate. The latter is expected to play a pivotal role in the development of novel therapeutics strategies and to improving the treatment of a wide range of diseases.

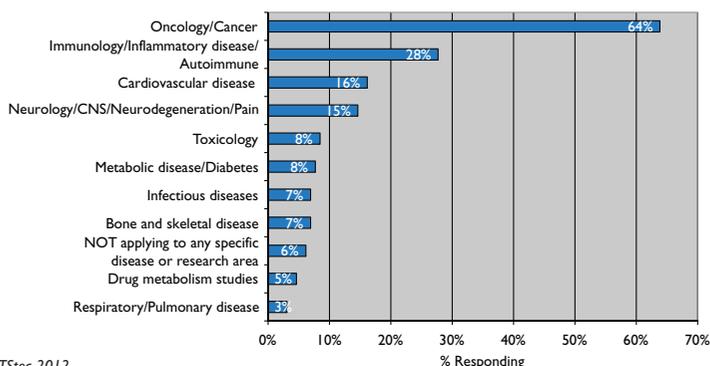
Comprising the movement of cells from one location to another in response to events, biological signals or environmental cues, cell migration plays a critical role in numerous normal biological processes and also in many diseases. For example, during an infection immune cells move in an orchestrated fashion towards the site of infection and during wound healing cells move to the site of injury in order to repair the damage. In pathology, erroneous or abnormal migratory signalling may induce the movement of the wrong cell type to the wrong place. This may have serious consequences, including mental retardation, car-

diovascular disease, arthritis, tumour formation and metastasis. A better understanding of the process by which cells migrate may lead to the development of novel therapeutic strategies, for example controlling invasive tumour cells, and is essential for improving the treatment of a wide range of diseases.

There are various types and definitions of cell migration. Migration in response to, either toward or away from a particular chemical signal, is usually referred to as chemotaxis. Cell invasion is related to, and encompasses, cell migration, except that cells move through the extracellular matrix

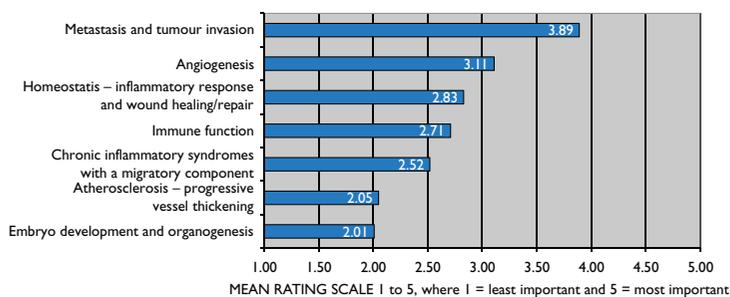
By Dr John Comley

Figure 1: Disease areas investigated/targeted with cell migration assays



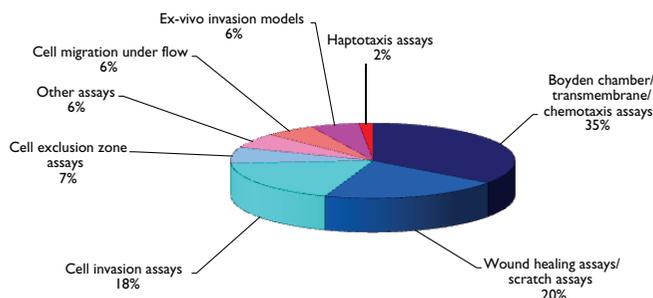
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Figure 2: Most important applications/reasons for investigating cell migration assays



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Figure 3: Breakdown of assay types used for cell migration



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into neighbouring tissues. Transmigration describes the migration of cells (usually leukocytes or tumour cells) through the vascular endothelium toward a chemoattractant. The list goes on, but the term ‘cell migration’ is broadly used and covers most of assay types involving cell movement including: cell migration, transmigration, cell motility, wound healing, chemotaxis, haptotaxis, cell invasion and cell exclusion.

Some of the classic and most commonly used methods for studying cell migration include the scratch wound healing and the Boyden Chamber assays. To perform a scratch assay, a wound is typically introduced in a cell monolayer on a coverslip or in a multiwell plate using a pointed object such as a pipette tip or syringe needle. The open gap is then inspected microscopically over time as the cells move in and fill the damaged area. In the Boyden Chamber assays a cell culture insert is nested into the well of cell culture plate. Cells are seeded in the top of the insert typically in serum-free media, while serum or similar chemoattractants may be placed in the well below. Migratory cells move through the pores toward the chemoattractant below and can be stained and visualised by microscopy/imaging or quantified using a plate reader. Invasive cells may be similarly measured by the placement of a coating of extracellular matrix proteins on top of the membrane. There are many variants of these assays and little standardisation in assay protocols.

Most commercial activity around cell migration assays has been directed towards developing tools and assays kits that facilitate easy access and add consistency, plus yield quantitative results and higher throughput, mainly through microplate compatibility. This is reflected in the variety of cell culture inserts, stoppers, specialised microplates, microfluidic devices, fluorescent probes or related consumables now available to support cell migration.

HTStec’s Cell Migration Assay Trends 2012 survey and market report¹ published in August 2012 sought to understand current use of cell migration, motility or invasion assays. The survey also examined the application requirements, market opportunities, metrics, costs, unmet needs and demand for such assays. We now report on some of the survey findings and discuss them with reference to new vendor developments in cell migration assays.

Disease areas targeted

The disease area most investigated/targeted with cell migration assays by the majority (64%) of survey respondents was oncology/cancer. This was followed by immunology/inflammatory disease/autoimmune (28% investigating); cardiovascular

Figure 4: Types of instruments applied to read cell migration assays

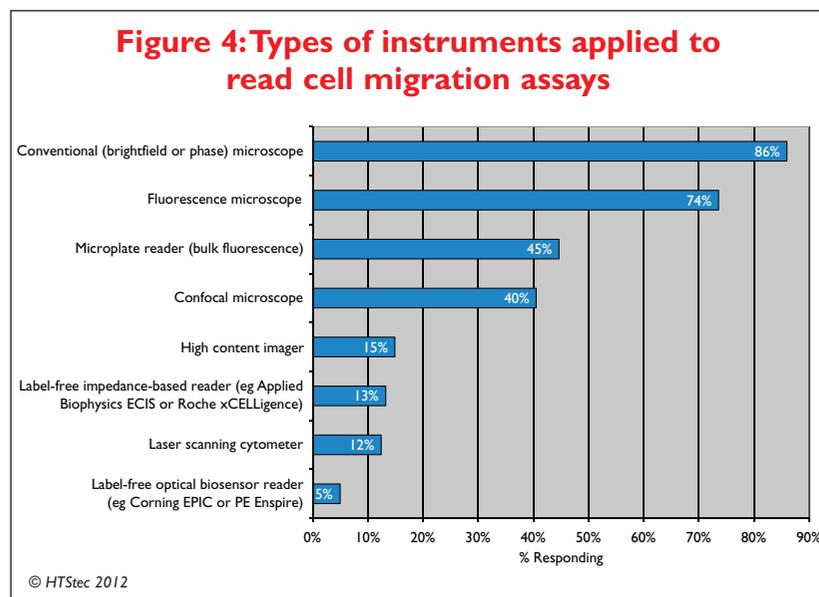


Figure 5: Cell types used in cell migration assays

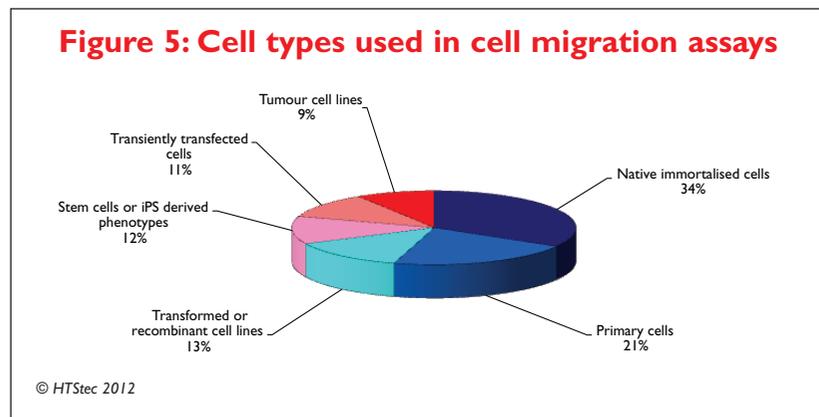
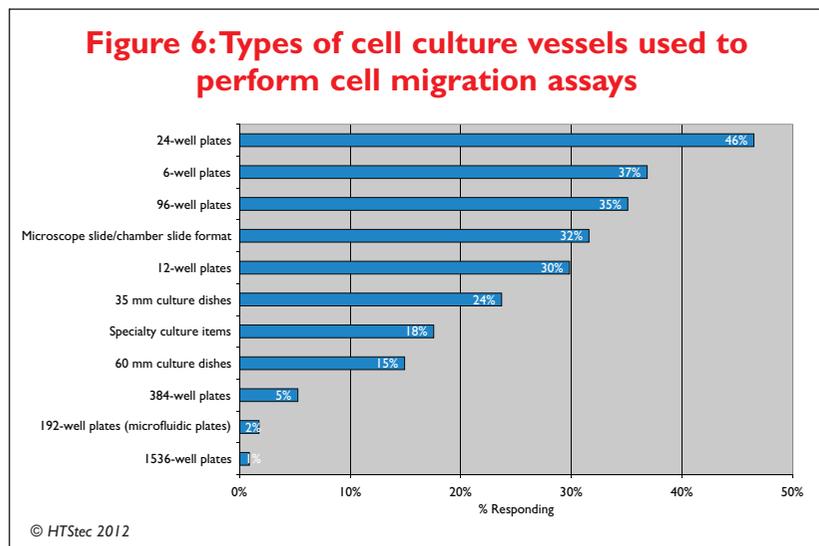


Figure 6: Types of cell culture vessels used to perform cell migration assays



disease (16% investigating); neurology neurodegeneration/pain (15% investigating); and then toxicology (8% investigating) (Figure 1).

Most important applications

Survey respondents rated metastasis and tumour invasion as the most important application/reason for investigating cell migration assays. This was followed by angiogenesis; homeostatis – inflammatory response and wound healing/repair; and then immune function. Least important was embryo development and organogenesis (Figure 2).

Most used assay types

The assay type most used by respondents today (2012) for cell migration assays was Boyden chamber/transmembrane/chemotaxis assays (35% using). This was followed by wound healing assays/scratch assays (20% using) and then cell invasion assays (18% using). All other assays types had 7% or less use (Figure 3).

Instruments used to read/analyse assays

The type of instruments survey respondents have most applied today (2012) to read/analyse cell migration assays were conventional (brightfield or phase) microscopes (86% applying). This was followed by fluorescence microscopes (74% applying) microplate readers (bulk fluorescence) (45% applying); and then confocal microscopes (40% applying). Other instruments (high content imagers, laser scanning cytometers and label-free readers) were applied to only a small proportion of cell migration assays today (<15%) (Figure 4).

Cell types used

The cell type most used by survey respondents today (2012) for cell migration assays was native immortalised cells (33% using). This was followed by primary cells (21% using) and then transformed or recombinant cell lines (13% using). Least used today was tumour cell lines (9% using) (Figure 5).

Types of cell culture vessels used

The type of cell culture vessel survey respondents have most applied today (2012) to cell migration assays were 24-well plates (46% applying). This was followed by 6-well plates (37% applying); 96-well plates (35% applying); microscope slides/chamber slide format (32% applying); and 12-well plates (30% applying) and then 35mm culture dishes (24% applying). All other vessel types were used by 18% or less of survey respondents for their cell migration assays (Figure 6).

Current use and future interest in cell migration assays kits

Survey respondents today make approximately equal use of home brew assays versus those made using a commercial kit or product to setup cell migration assays.

Survey respondents rated gradients that are not linear, well-established, or controlled as the major hurdle/limitation of existing cell migration assay products. This was closely followed by quantification of migratory cells based on endpoint measurement alone; requires many controls in order to generate robust and interpretable results; and real-time imaging of the cells while they are migrating is not possible. Rated least limiting was pre-labelling of cells with fluorescent dyes (Figure 7).

Capable of outputting parameters such as cell velocity, directionality and migration index was ranked by survey respondents as the most desired feature of a new cell migration assay product. It was closely followed by distinguishes between chemotaxis and random movement, and then simple one (or few) step protocol (Figure 8).

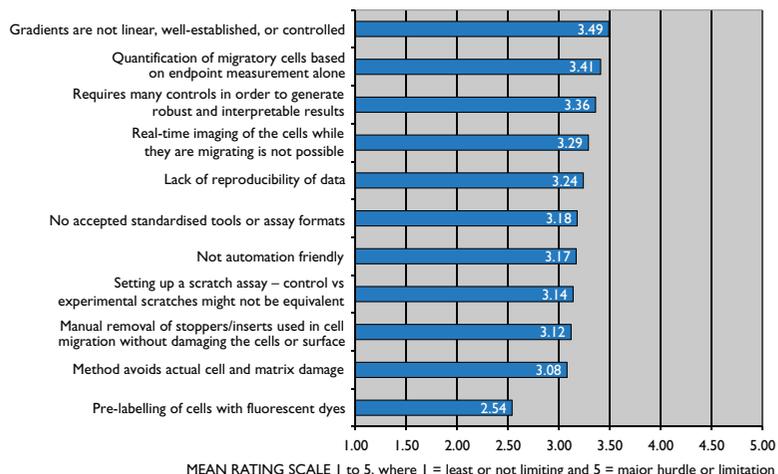
Survey respondents rated 3D cell culture environments as the aspect of cell migration and invasion assays they would most like to see supported by a broader/better range of new assays or kits. This was followed by co-culture of multiple cell types in motility assays; chemotaxis; and then *ex vivo* cell invasion models. Rated least needed were better haptotaxis assays (Figure 9).

Products that remove the variability from assays were rated by survey respondents as the most important factors for future cell migration-related research efforts. This was followed by the flexibility to address different cell types (pore sizes) and then the ability to handle diverse range of applications. Rated least important were solutions that enable turnkey approach to high content screening (Figure 10).

Latest developments in cell migration assays

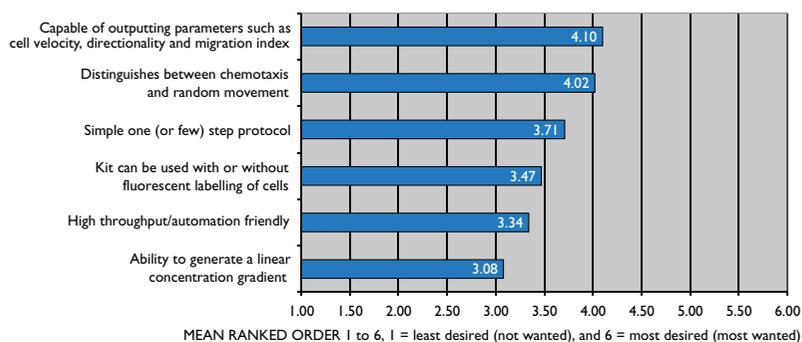
In vitro cell migration and invasion assays are frequently used as model systems for quantifying the directed movement of cells towards a chemoattractant stimulus, or to measure how a particular drug, antibody or extra cellular matrix (ECM) coating affects that movement. Analysis of this movement is often accomplished through the use of qualitative visual methods (eg, scratch or plug assays, transmembrane Boyden chambers) that are time-consuming and labour-intensive. To address these issues, an improved fluorescence blocking version of the Boyden chamber, the FluoroBlok™ insert

Figure 7: Main limitations of existing cell migration assay products



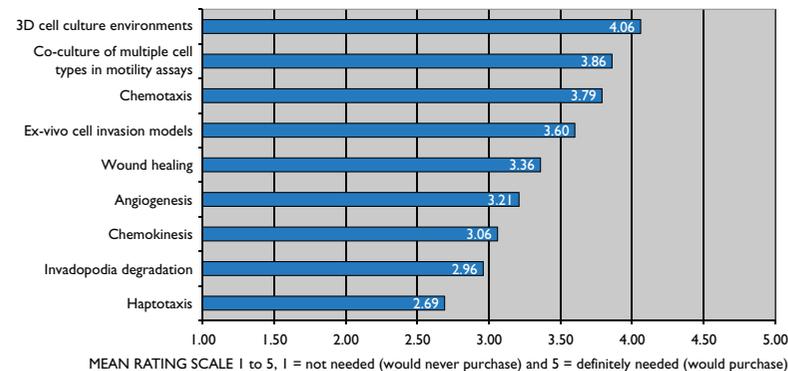
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Figure 8: Most desired features of a new cell migration product



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Figure 9: Aspects of cell migration assays in need of an improved product offering



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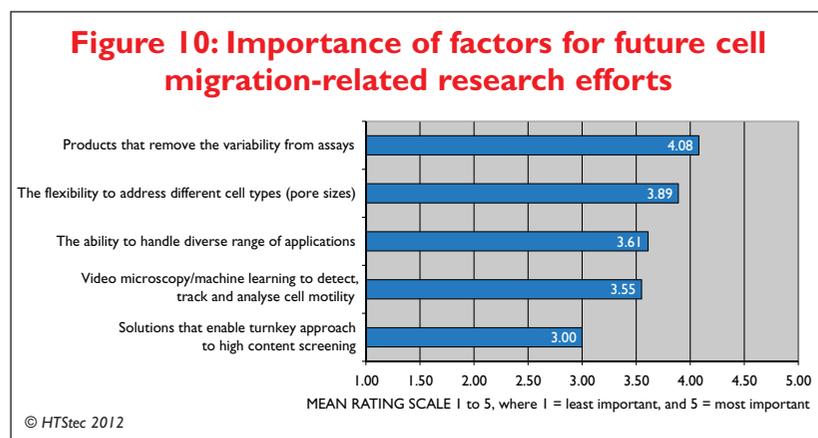


Figure 11: With the BD FluoroBlok insert system from Corning (various formats shown), cells migrating through a fluorescence-blocking microporous membrane can be detected using a bottom-reading fluorescent microplate reader

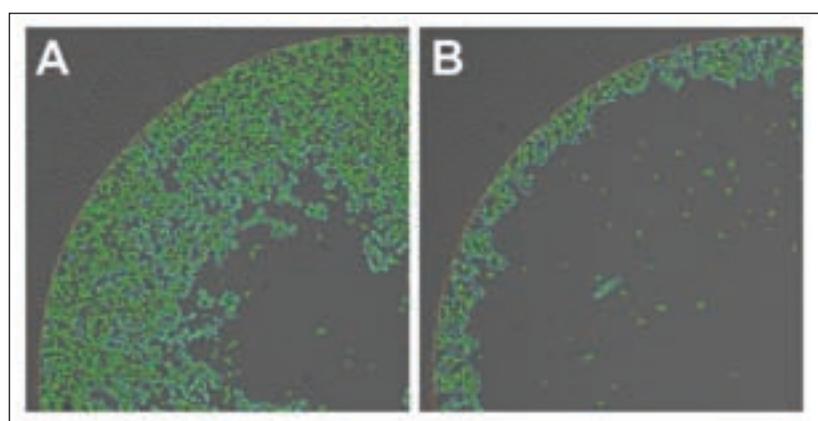


Figure 12: Cell migration analysed on Brooks Automation Celigo. HT-1080 cells can be visualised using the Celigo brightfield channel. The Celigo software reports cell counts and percentage wound healing (green and blue outlines respectively). Images were acquired and analysed 24 hours after removal of silicon inserts to allow for cells to migrate into the cleared area. In control wells (panel A), cell migration is observed in the cleared area while cells remain to the outer of the cleared zone in wells treated with cytochalasin D (panel B)

system, was developed by BD Biosciences. This system is now supplied exclusively by Corning (www.corning.com/discoverylabware). As cells migrate through the fluorescence blocking microporous membrane, they can be detected using a bottom-reading spectrofluorometer. Cells remaining in the upper chamber of the insert are shielded from this detection, allowing for cell quantification in a homogeneous assay system. FluoroBlok membrane blocks $\geq 98\%$ transmission of light from 360-700nm and $>50\%$ more light in the range of 360-490nm, enabling the use of the blue fluorescent Hoechst dyes and DAPI for direct cell enumeration and multiplexing. Functional applications of this technology have been demonstrated in several well-established assays: endpoint staining in a model tumour invasion system yielded a Z'-factor >0.5 ; real-time chemotaxis of monocytes demonstrated a peak signal between 25-35 minutes; and migration of human endothelial cells to VEGF was demonstrated with high reproducibility (CV $< 15\%$). FluoroBlok cell culture inserts are automation-friendly, and can be used for high-throughput cell migration studies yielding highly reproducible results (Figure 11).

Cell migration can easily be quantified using the Celigo Imaging Cytometer from Brooks Automation (www.brooks.com/lifescience). The Celigo is an easy to use multi-channel brightfield and fluorescence imager that allows full well image acquisition and processing of multi-well plates. Typically, cell migration assays such as 'scratch assays' are difficult to quantify, lack reproducibility and are challenging to scale up to levels appropriate for high-throughput screening. Moreover, standard protocols often require either the use of cytotoxic fluorescent stains or cell transfection with fluorescent proteins which are not adequate when using primary cells. To overcome these challenges, the Brooks Celigo is able to count cells and measure wound healing in label-free brightfield mode. Typically, experiments are carried out using plates with silicon inserts that prevent cells from adhering at the well centre. The area cleared from cells is then used to define the edge of the wound and the Celigo measures cells migrating into it. The fact that the Brooks Celigo does not require fixing cells permits plates to be imaged repeatedly over time while the Celigo software generates wound healing curves. Using the Celigo to transform a typical end-point fluorescent assay into a kinetic brightfield assay enables the simultaneous use of various drugs or cells types. The Celigo reports data for every time point, even if cell migration in

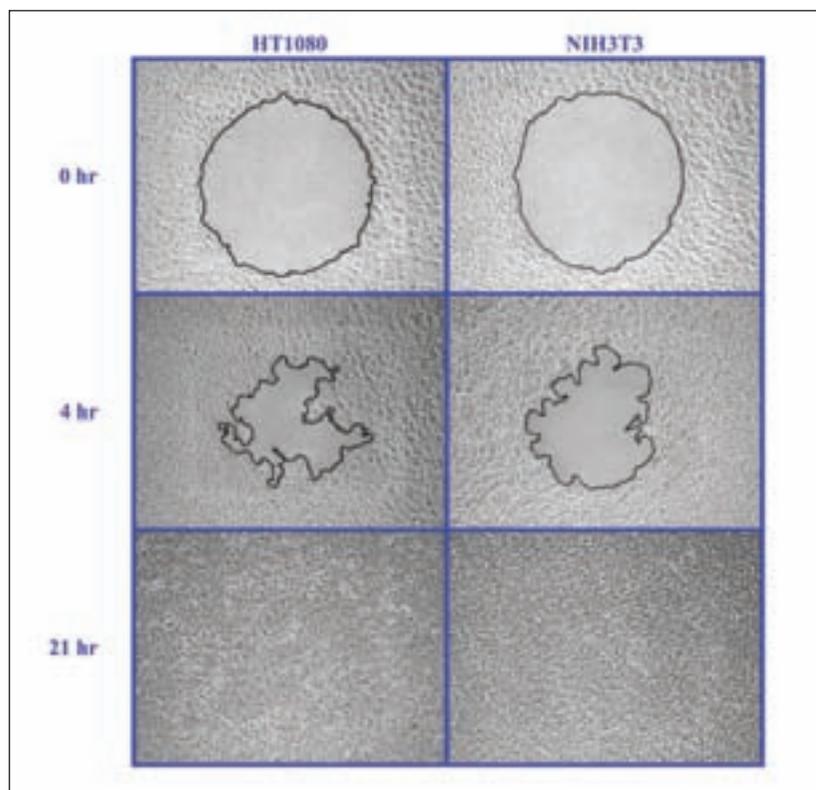


Figure 13: Time course of cell migration using the Cell Biolabs Radius™ 96-well cell migration assay kit and two different cell lines (HT1080 and NIH3T3)



Figure 14: Corning's range of Transwell permeable support inserts for cell migration assays

various wells is not synchronised and provides additional cell migration kinetic measurements compared to standard assays. Additionally, the integration of the Celigo into a robotic platform brings cell migration assays into high throughput applications and provides increased productivity to screening labs (Figure 12).

Various assays have been developed for testing cell migration, but not all formats are created equal. Currently there is no 'holy grail' migration assay which provides the perfect environment for all cell migration experiments. Therefore, choosing the right migration assay depends on a good understanding of your experimental goals. Traditional Boyden Chambers with a cell culture insert nested inside a microplate well allow the creation of a chemoattractant gradient, providing incentive for cells to move from one location to another. Such assays are ideal when testing the effects of a suspected molecule on migration rates. However, they are not amenable to time course migration studies, nor are they sensitive enough to measure small changes in migration rates. Recently developed Gap Closure assays, in which cells are seeded around a barrier (physical or chemical) and then migrate across the gap created by removal of the barrier, provide an environment to monitor migration rates in real time. They may also provide greater sensitivity in detecting smaller changes in migration rates, but they do not allow for a chemoattractant gradient. Cell Biolabs (www.cell-biolabs.com) is unique among cell migration assay manufacturers by providing kits in both formats. CytoSelect™ Boyden Chamber Assays are ideal for absolute quantitation and for true chemotaxis experiments where the goal is to measure the effect of a chemoattractant. The Radius™ Cell Migration Assays provide a two-dimensional gap closure format in 24-, 96- and 384-well plates which is optimal for relative quantitation between different cell types or between treated and untreated cells (Figure 13).

Cell migration – the movement of cells from one area to another in response to a chemical gradient – is central to a variety of functions such as wound repair, cell differentiation, embryonic development and the metastasis of tumours. To facilitate the study of these mechanisms, Corning Life Sciences (www.corning.com/lifesciences/surfaces/en/transwell.aspx) developed Transwell Permeable Support Inserts. Transwell inserts offer a simple *in vitro* approach to studying the movement of cells in response to a variety of attractants. Polycarbonate

inserts are available in 6-, 12-, and 24-well plates as well as automation friendly 96-well HTS versions. Several Polyester versions are also available; including the latest addition to the product line, PET, 6.5mm, 8µm inserts in 24-well plates. These inserts provide sufficient optical clarity for the visualisation of cell outlines in culture and a smooth surface for easy identification of stained cells post migration. Inserts can be coated with a variety of extracellular matrices (ECM) or basement membrane extracts (BME) for enhanced attachment as well as invasion assays. The PET inserts can also support more elaborate invasion assays requiring the establishment of a monolayer of endothelial cells on the permeable support prior to the addition of the transmigratory cell line. Similarly, cells that secrete a paracrine growth factor can be cultured in the tissue culture treated receiver wells of the permeable support system to act as the source of chemoattractant for a variety of migratory assays. In addition, to the standard multiple well insert offerings, Corning also offers custom 75mm PC inserts, available upon request (Figure 14).

EMD Millipore (www.millipore.com) has several new products that address outstanding issues in the field of cell migration and invasion. One new product, the QCM™ High Sensitivity Non-cross-linked Collagen Invasion Assay, extends the capabilities of EMD Millipore's existing cell invasion assays to permit more rapid detection of invasion through extracellular matrix by cells with lower invasive potential. Another key issue in cell migration research is that the biochemical events underpinning cell migration are incompletely understood, in part due to the lack of commercially available methods for scaling up and isolating sufficient migrating cells to perform detailed biochemical and proteomic analysis. EMD Millipore has developed the Cell Comb™ Scratch Assay to enable biochemical analysis of the popular scratch wounding assay, which is typically performed with a pipet tip. The Cell Comb™ Scratch Assay employs a novel comb-like device with narrowly spaced teeth that, when applied to a cell monolayer in a complementary rectangular plate (also included in the kit), creates high density, uniform scratches and results in a high ratio of migrating to stationary cells. Lysates from the scratched cells and unscratched controls can be analysed by mass spectrometry, Western blotting, coimmunoprecipitation and enzymatic assays. The high ratio of migrating to stationary cells allows for sensitive detection of biochemical changes occurring specifically in the migrating cell population (Figure 15).

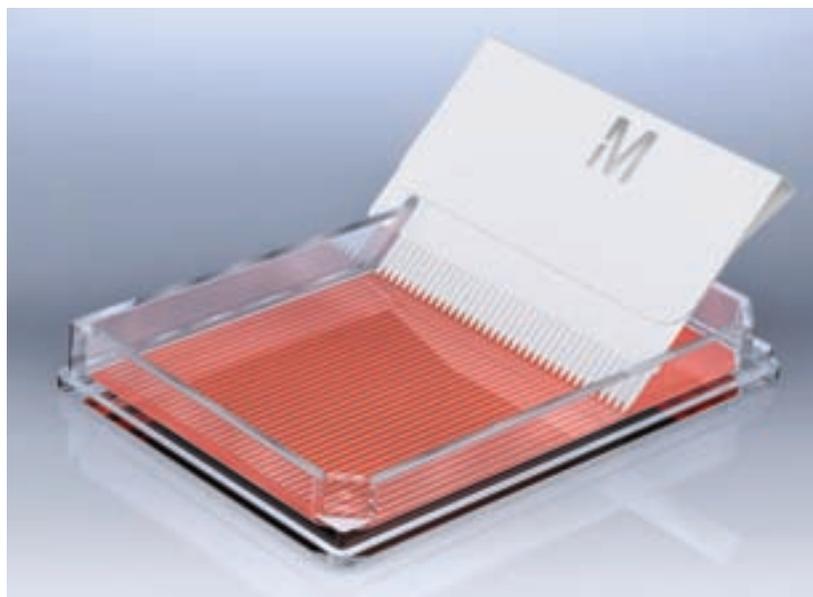


Figure 15: EMD Millipore's TCell Comb™ Scratch Assay employs a novel comb-like device with narrowly-spaced teeth that creates high density, uniform scratches and results in a high ratio of migrating to stationary cells

The BioFlux systems offer a fully integrated solution for running live cell assays under biologically relevant conditions. At the core of the system is Fluxion Biosciences (www.fluxionbio.com) Well Plate Microfluidics™, which incorporates microfluidic design into traditional microplate formats with



Figure 16: The BioFlux system from Fluxion Biosciences is able to create *in vivo* microenvironments that mimic the vasculature. By allowing user-controlled temperature, gas composition and shear flow rates in convenient microplate formats, the system is ideally suited for cell migration assays in drug screening labs



Figure 17: Greiner Bio One's ThinCert™ cell culture inserts provide high quality and easy-to-use tools for trans-membrane migration experiments

temperature and gas control. This design enables real-time, dynamic measurements of adhesion and migration in physiologically relevant microenvironments. This significantly simplifies complex assay workflows while providing high quality, high content imaging data for live cell assays. The BioFlux system can be used for screening different types of tumour 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. Transmigration of cells to underlying tissues and through the blood brain barrier from the vasculature is involved in many disease states, making transmigration an important assay for drug devel-

opment. While the blood-brain barrier is a difficult *in vivo* environment to mimic, the microfluidic design of the BioFlux system has allowed customers to study how cell adhesion molecules transmit extracellular cues into intracellular signals. As a result, the system has been used to show how cell adhesion molecules inhibit migration and invasion of brain tumour cells in a convenient microplate format. The use of live cell imaging with the BioFlux system has allowed our customers to quantify cell migration in an automated, straight-forward, and reproducible manner (Figure 16).

Porous membranes have proven as excellent tools for migration and invasion assays *in vitro*. Cells in the compartment above the membrane may be attracted by chemokines in the lower compartment, thereby changing their cytoskeletal architecture and migrating along the chemotactic gradient through the pores. With ThinCert™ cell culture inserts Greiner Bio One (www.gbo.com/bio-science) provides high quality and easy-to-use tools for trans-membrane migration experiments. The membrane of the ThinCert™ is made of polyethylene terephthalate (PET) – a material well known for its excellent biocompatibility and absence of adverse effects, such as unwanted macrophage activation. An innovative track etching process enables the manufacture of ThinCert™ inserts with well-defined capillary pores for highly reproducible migration assays. The product is optimised for easy accessibility of the lower compartment and all-over handling under aseptic conditions. For example, the insert has an excentric position in a well plate, thus opening a large pipetting window between insert and well plate. Furthermore, the insert arms are ergonomically designed and arranged in a way facilitating the handling of the

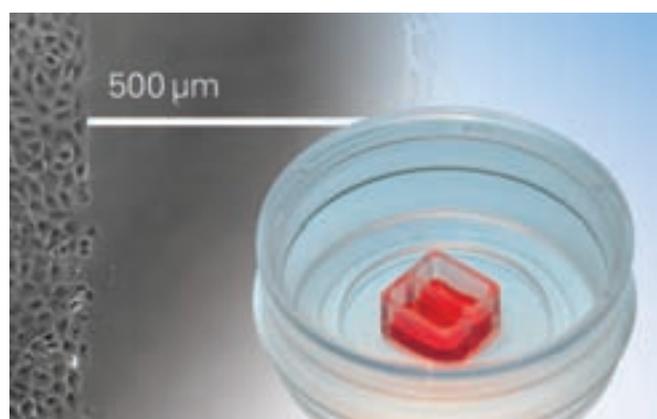


Figure 18: ibidi's μ-Slides Chemotaxis provide reliable and reproducible chemotaxis assays with adherent and non-adherent cells (left) ibidi's Culture-Inserts enable highly reproducible wound healing experiments due to defined cell free gap (right)

insert with forceps under aseptic conditions. ThinCert™ cell culture inserts are widely applied in tumour migration assays as well as studies on trans-endothelial immune cell migration. Furthermore, the inserts can be pre-coated with extracellular matrices, hence providing a meaningful tool for studying tumour cell invasion and the formation of metastases (Figure 17).

ibidi (www.ibidi.com) μ -Slides support chemotaxis experiments of adherent cells using μ -Slide Chemotaxis 2D and the directed migration of non-adherent cells embedded in a gel matrix using the new μ -Slide Chemotaxis 3D. In both types of μ -Slides, two large reservoirs are connected by a narrow observation area. The cells in the observation area become super-imposed by a linear and time stable gradient. This enables chemotaxis measurements in real-time using time lapse video microscopy, providing reproducible results with reliable and user-independent data. The web-based, automated image analysis tool 'WimTaxis' and the chemotaxis analysis software 'Chemotaxis and Migration Tool' complete ibidi's chemotaxis assays and provide exact data for critical parameters such as cell velocity, directionality and migration index. ibidi's wound healing and migration assays require only a few steps from sample preparation with the Culture-Insert to image analysis done using ibidi's automated image analysis platform 'WimScratch'. When placed on a cell culture surface, the Culture-Insert provides two cell culture reservoirs which are separated by a 500 μ m thick wall. The cells are cultured in both reservoirs. Removing the silicone insert from the surface results in two precisely defined cell patches, which are separated by a zone that is exactly the same width as the separation wall. The Culture-Insert provides reproducible results due to a defined cell-free gap, no leakage during cultivation and no material left behind after the insert's removal. After data acquisition on a microscope, the wound healing image analysis solution 'WimScratch' evaluates 2D cell migration (Figure 18).

Quantifying the extent of cell migration due to wound healing, cancer metastasis or compound inhibition provides valuable information for drug discovery and development efforts. However, performing such assays in a consistent, high-throughput manner can be challenging. The ImageXpress® Micro XL System, coupled with the MetaXpress® Image Acquisition and Analysis Software from Molecular Devices® (www.moleculardevices.com), offers a solution for fast and reliable high content

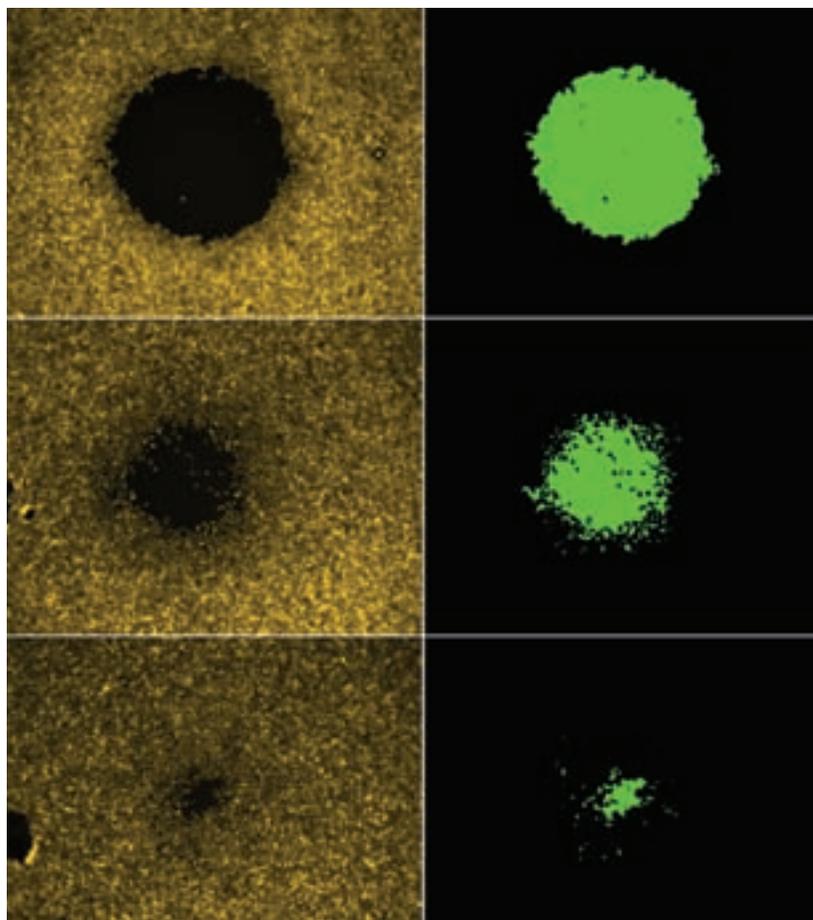


Figure 19: A simple custom module on Molecular Devices on ImageXpress® Micro XL System allows measurement of the Cell-free zone after cells have been allowed to migrate for 18 hours. Different surface treatments (top – tissue culture; middle – fibronectin; bottom – collagen I) dramatically affect the extent of migration

screening (HCS) of cell migration assays in a multi-well plate format. The ImageXpress Micro XL System is a flexible live cell imaging platform with a large field-of-view that is three times larger than standard HCS systems. This feature enables acquisition of fewer sites per well to capture the entire invasion zone. Researchers can take advantage of the system's environmental control chamber, brightfield microscope and fluidics capabilities to observe live cell kinetics spanning long time-courses using fluorescent or unlabelled cells. Precise stage and z-axis positioning allows for 3-D visualisation of invading cells. Quantification of cells into the zone of migration can be readily automated with the MetaXpress Software. The software offers pre-configured application modules such as Count Nuclei and Cell Proliferation, a flexible Custom Module Editor for creating sophisticated analysis and capabilities for analysing multi-planar invasion using more advanced software functions. Researchers can utilise

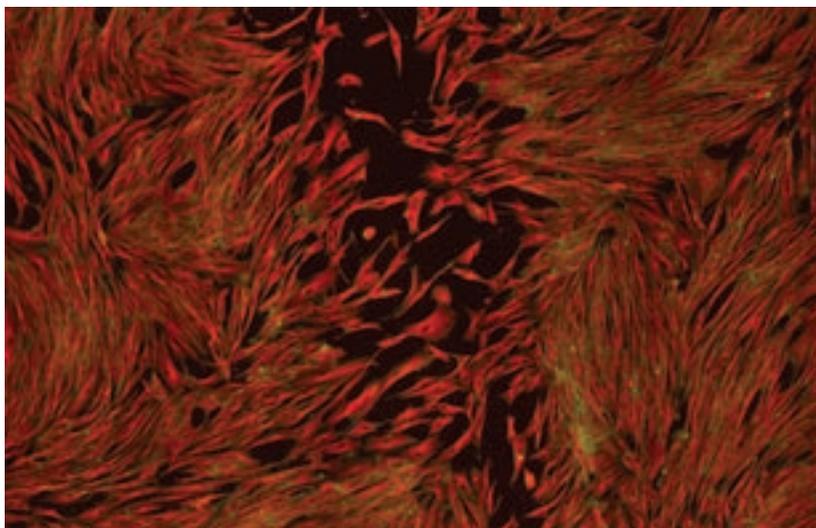


Figure 20: Gibco® HDFn cells were plated and allowed to grow to confluence. A scratch wound was made down the middle of the well and cells were incubated overnight to allow wound closure. Following fixation and permeabilisation, cells were labelled with an anti- α -tubulin IgG detected with an Alexa Fluor® 555 secondary antibody (red) and Alexa Fluor® 488 phalloidin (green) from Molecular Probes

these tools to measure 2-D area or 3-D volumetric changes in the detection zone as a function of varying compound concentration. Analysis can also run speedily on parallel processors to satisfy high-throughput needs (Figure 19).

Cell migration is critical for normal cellular processes such as embryogenesis, tissue growth and homeostasis, and wound healing; it is frequently deregulated in diseases such as cancer. Migration is guided by both chemical signals and physical

forces. For fixed end-point assays, the superbly bright and photostable Alexa Fluor® dyes are ideal indicators when conjugated to secondary antibodies or probes such as phalloidin (Figure 20). However, the greatest need is for kinetic live cell-compatible trackers and the selection from Molecular Probes (www.invitrogen.com) has greatly expanded in this area. Although each probes class is unique, all were specifically designed to meet demanding cell tracking requirements. They are cell permeant, stable, nontoxic, well retained during the experimental interval (from hours to days) and brightly fluorescent at physiological pH. Moreover, they are available in colours that span the spectrum, allowing combinations with other live-cell probes for simultaneous detection of other critical cell functions. Calcein, AM, has traditionally been used for cell tracking and viability assays. It loads easily, generating an extremely bright, but transient, fluorescence. CellTracker™ (thiol-reactive) and CellTrace™ (amine-reactive) probes afford a uniform, well-retained cellular fluorescence that enables multi-colour monitoring of individual cells for several generations. The Qtracker® Cell Labeling Kits contain a targeting peptide for selective cellular delivery and are distributed in vesicles throughout the cytoplasm. These Qdot®-based probes exhibit an intense, photostable fluorescence that can be observed using continuous illumination, without photobleaching or degradation. The fluorescence is maintained under changes in intracellular pH, temperature and metabolic activity. Qtracker® probes are passed to daughter cells through at least seven generations. Importantly, they are not transferred to adjacent cells in the population, making them useful for co-culture experiments. Both CellTracker™, CellTrace™ and Qtracker® probes are stable, bright and extremely well tolerated, with no discernible impact on cell viability, migration or gene expression.

Traditional methods for monitoring cell motility, such as ‘Scratch’ assays and Boyden Chambers, have limited researchers’ ability to obtain robust and informative data due to the high level of noise that is inherent to these assays. To overcome such limitations, Platypus Technologies (www.platypustech.com) developed the Oris™ and Oris™ Pro Cell Migration Assays to enable reproducible, facile and efficient measurement of cell motility. These assays avoid the need to scrape away cells which can damage cells and the surface. Quantification of cell movement with a multi-well plate reader or microscopy is achieved by pre- or post-migration staining of cells or fluorescent

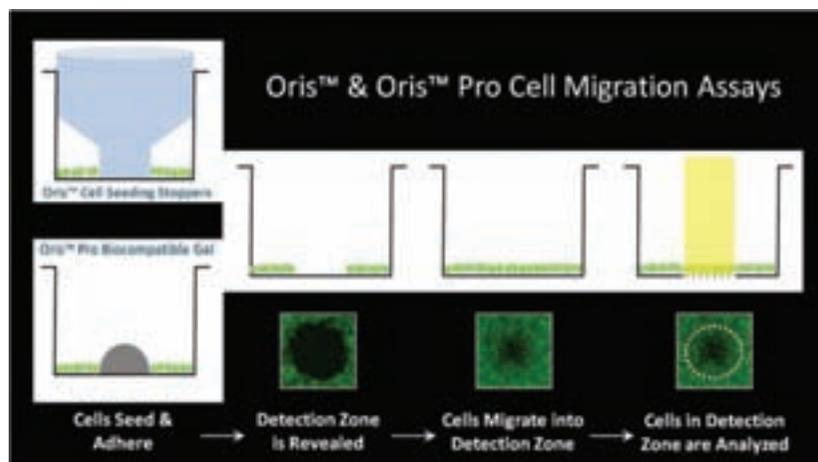


Figure 21: Schematic of Platypus Technologies’ Oris™ and Oris™ Pro Multi-well Migration Assays. Cells are seeded and allowed to adhere in an annular monolayer surrounding the Oris™ Cell Seeding Stoppers or Oris™ Pro Biocompatible Gel (BCG). Cells are allowed to migrate into the Detection Zone. Cells are imaged via confocal microscopy or High Content Imagers. Images are then analysed for cell migration as well as phenotypic changes

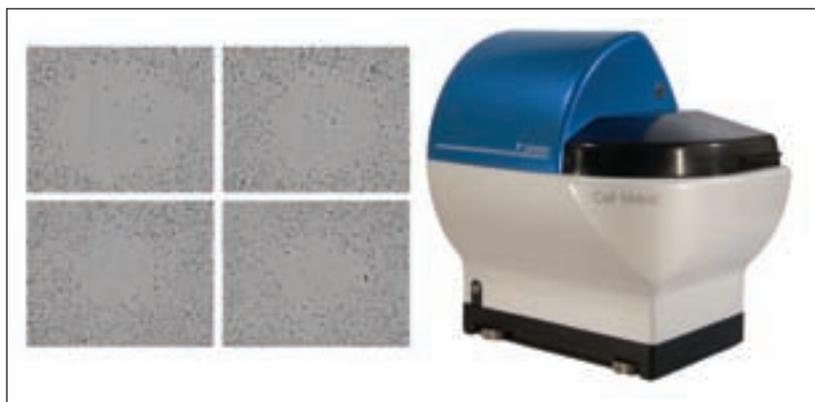


Figure 22: The Solentim Cell Metric supports high-throughput time course migration assays in micro-plate format

trackers. The Oris™ assays feature silicon-based stoppers that provide a temporary physical barrier to prevent cells from adhering to the centre of the wells and facilitate creation of an annular monolayer of cells with a cell-free, central exclusion zone into which cell movement can occur. Addition of an overlay of extracellular matrix enables monitoring of cell invasion, thus providing a viable alternative to Boyden Chambers and Transwell inserts. The 96- and 384-well-based Oris™ Pro assays utilise a non-toxic biocompatible gel to form cell-free zones. These assays facilitate drug discovery research by enabling the use of automated liquid handling equipment for cell seeding and allowing unrestricted access to cells throughout the experiment. This novel format increases the data gathered from each well by enabling monitoring of cell



Figure 23: Tecan's Infinite M200 PRO multimode microplate reader with Gas Control Module (GCM™) on top

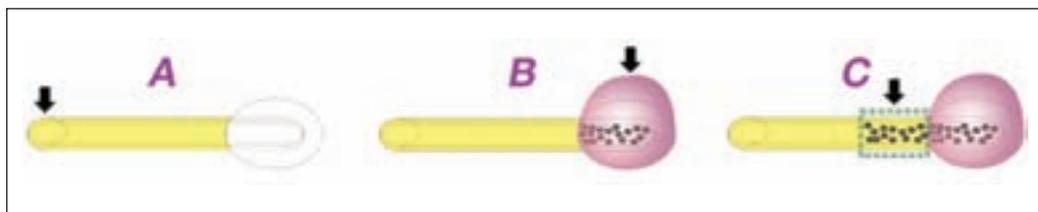
movement and cell phenotype changes. Images of cells in the detection zone can be captured and quantified in real-time using microscopes and HCS/HCI instruments (Figure 21).

The Cell Metric from Solentim (www.solentim.com) is a compact, bench-top instrument that supports high throughput cell migration and motility assays without the use of fluorescent labels. Brightfield microscope optics coupled with a high resolution camera and automated stage support a variety of 2D and 3D assays in 96 and 384-well microplate format, such as the Oris and Oris Pro Cell Migration Assay kits available from Platypus Technologies. The label-free approach is well matched to the kinetic nature of migration assays and ensures more physiologically relevant results as the cell function is unaffected. The system generates cell migration readouts by analysing the brightfield images to calculate changes in cell area coverage, thus enabling a simple, non-invasive measurement of cell migration progress. The Cell Metric is also an important quality control tool that can improve the consistency of assay results by verifying that the cell seeding density is correct before the first time point and by monitoring cell morphology and proliferation during the assay, helping to pinpoint cell culture problems unrelated to the assay treatment that would affect the assay results and might otherwise go unnoticed in a fluorescence-based readout. Fast readout times of between three and five minutes per plate (whole well) allow readings to be taken frequently so that changes in growth rate can be monitored closely. The system is available with an automated plate loader featuring 10-microplate capacity and environmental control, providing the capability of automatic multiple time point readouts over several days (Figure 22).

Quantification of cell migration using standard inverted microscopy techniques is a very time-consuming procedure. Tecan (www.tecan.com) offers a range of solutions to allow automated, microplate-based cell migration studies, including software support and plate definition files for common assay systems, such as BD Falcon™ FluoroBlok™, Life Technologies Transwell and Millipore InnoCyte™ assay plates. A comprehensive collaborative study with the Lyon Neuroscience Research Center, France, recently demonstrated that the Infinite® M200 PRO multimode reader, combined with FluoroBlok plates, allows easy investigation of cell migration activities. Migration quantification using this system saves time and significantly improves throughput, as well as simplifying the collection,

Figure 24

Overview of 3D Cell Migration Assay from Thermo Scientific. (A) 800nL of extracellular matrix, in yellow, is added to the input port, filling just to the opposite end of the channel through passive pumping mechanisms. (B) Cells, in pink, are then added to the large output port at the other end of the channel. (C) Cells migrate into the matrix-filled channel over the duration of the assay. Finally, cells in the indicated dashed-line box are imaged and quantified with an inverted microscope or other high content instrument



analysis and processing of data. The Infinite M200 PRO is ideally suited to cell-based studies, offering precise temperature control and – combined with the patent pending Gas Control Module (GCM™) – simultaneous regulation of CO₂ and O₂ within the measurement chamber. This powerful set-up eliminates the need for a standard CO₂ incubator, offering efficient handling and improved reproducibility for cell invasion and cell migration studies. The Infinite series of multimode readers can also be combined with Tecan's Freedom EVO® workstations to provide complete automation of cell-based studies. This comprehensive solution offers walkaway automation of cell migration assays, including automated cell seeding, plating, media exchange and incubation for a broad variety of labware types, significantly increasing throughput and streamlining your laboratory workflow (Figure 23).

Traditional cell migration assays deliver limited information and inconsistent results and offer low throughput capabilities. Two new assay plates are

available for culturing cells and performing high content analysis of cell migration during inflammation and cancer cell invasion, the Thermo Scientific (www.thermoscientific.com/cellmigration) iuvo Chemotaxis Assay Plates and iuvo Microchannel 5250 Assay Plates. These single-piece plates do not have filters or membranes and are compliant with the SLAS standard for microplates, validating them for use with automated liquid handling systems and high content analysis readers. The iuvo Chemotaxis Assay Plate can establish a stable chemical gradient to mimic inflammation and measure neutrophil cell migration. The iuvo Microchannel 5250 Assay Plate is ideal for cell invasion assays in 2D or 3D cultures in fibrillar collagen, creating a miniature model of tissue biology. Automated handling makes these plates ideal for primary screening assays. Microscopic imaging of cell migration in either plate provides quantitative data on the number of cells migrating, distance travelled and cell morphology (Figure 24).

For high content screening of the effects on cell migration, the new acumen® HCI imaging cytometer from TTP Labtech (www.ttp-labtech.com) with its large depth of field and whole well scanning capabilities, is highly compatible with bespoke labware designed for such studies. This includes both the Oris™ Cell Migration Assay (Platypus Technologies LLC) and the iuvo™ Chemotaxis assay set-up (Bellbrook labs) allowing the study of cell motility in microplate format. In a recent high content screening study of the inhibitory effect of a range of compounds on neutrophil migration, acumen HCI's simultaneous data acquisition and software analysis capabilities was shown to reduce the time taken to acquire results by four-fold compared to automated microscopy. This study demonstrated that it was possible to screen and analyse 10 plates in under an hour (approximately five minutes per plate). In addition, acumen HCI's ability to simultaneously acquire fluorescent data from multiple colours without extending the plate read time makes it possible to analyse multiple events

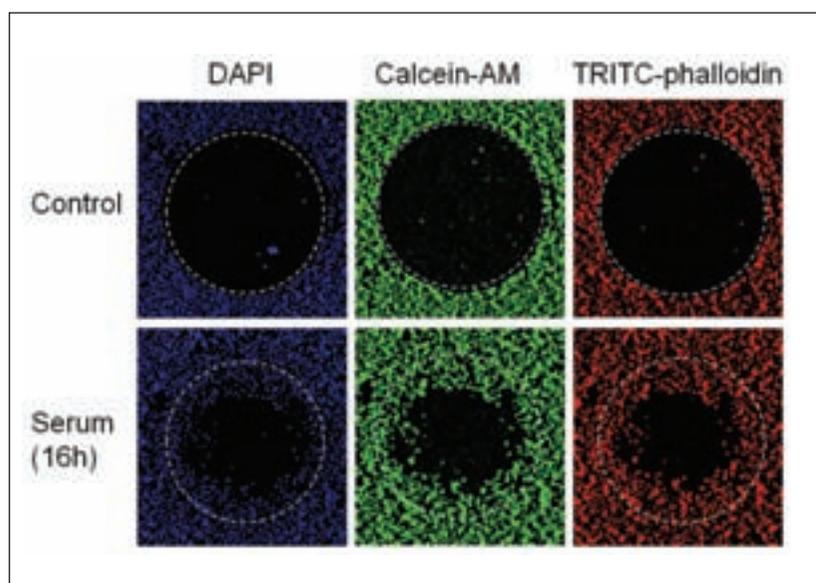


Figure 25: Widefield imaging of cell migration using the TTP Labtech's acumen® HCI. Detection of multiple colours with up to three lasers provides multiplexing capabilities with other screening targets and ensures reliable results

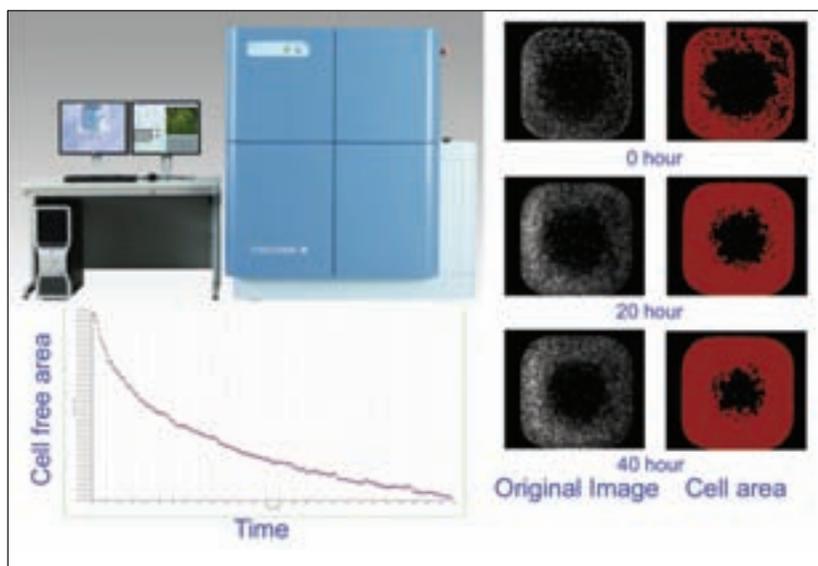


Figure 26
Yokogawa CellVoyager
CV7000 and time series
analysis of cell migration

including cell mobility thereby gaining information about potential mechanisms and efficacy of novel therapeutic compounds. The combination of acumen HCI and suitable labware are both easily integrated into an automated environment for rapid screening of novel compounds on cell motility (Figure 25).

Yokogawa (www.yokogawa.com/hca) CellVoyager CV7000 is a live-cell HCA system, which can be used for analysing cells at a single time point, but also for time-series analysis of plural time points. Shown in Figure 26 is an example of ‘Migration Assay’ which measures the migration of cells by the changes in the cell area. In this experiment 5,000 cells/well of HeLa cells expressing Azami-Green in the cytoplasm were cultured in a 384-well plate. The plates were imaged by the CV7000 system for a total of 48 hours at 10-minute intervals using a 4x objective lens. The degree of cell migration was numerically converted from the changes in the cell area. As a result, the decrease with time in the cell-free area was clearly shown in the graph. While migration assays generally analyse only the information from one time point, there is a good potential to discover new results if you can analyse multiple time points in an extended time-series. Some of the many advantages of live-cell HCA using the CellVoyager series are: 1) less cell damage thanks to the CSU, microlens-enhanced spinning Nipkow disk confocal unit; 2) whole-well imaging of a 384-well plate with the wide-view confocal unit, wide-view cameras and 4x objective lens; and 3) ultra-fast image acquisition – only four minutes to acquire a whole 384-well plate image.

Discussion

Some of the tools now offered by vendors to support the investigation of cell migration assays include novel developments and improvements in the following areas:

Membrane inserts: Of interest here is an improved fluorescence blocking version of the FluoroBlok™ insert system (BD Biosciences) and the availability of insert membranes made of polyethylene terephthalate (PET) with better defined capillary pores, in a range of pore sizes, enhanced optical clarity and excellent biocompatibility, supporting more elaborate types of invasion assays (Greiner Bio One, Corning).

Silicone stoppers/inserts: The ability to create well-defined cell-free gaps has revolutionised the set-up of reproducible cell motility assays (Cell Biolabs, ibidi, Platypus).

Higher density formats: Increasingly higher throughput is enabled through tools compatible with 96- and 384-well plate formats (BD Biosciences, Cell Biolabs, Fluxion Biosciences, Platypus, Thermo Scientific).

Bespoke labware: Specialty microplates and slides, that make use of combination of microchannels and matrices, provide tailor-made solutions for directed cell migration applications (ibidi, Thermo Scientific).

Microfluidics: Devices which incorporate microfluidic design into traditional microplate formats enable real-time, dynamic measurements of adhesion and migration in physiologically relevant microenvironments (Fluxion Biosciences).

Scale-up of migrating cells: Bulk supply of sufficient migrating cells to perform detailed biochemical and proteomic analysis is facilitated with the Cell Comb™ Scratch Assay (EMD Millipore).

Live cell compatible trackers: The availability of new photo-stable, non-toxic, well retained and bright fluorescent dyes are useful in a variety of live cell motility assay types, including co-culture (Molecular Probes).

Brightfield imaging: To overcome some of the limitations associated with fluorescent probes more imaging systems are supporting cell migration assays on unlabelled cells using brightfield mode (Brooks Automation, Molecular Devices, Solentim, Yokogawa).

Kinetic time courses: Greater insight can often be gained by analysing cell movement over an extended time-series with multiple images (or time-lapse video) (Brooks Automation, Molecular Devices, Solentim, Thermo Scientific, Yokogawa).

HCS imagers: Changes to the optics of HCS/HCI

imaging systems are facilitating simultaneous multiple colour acquisition, wider view (whole well imaging), greater compatibility with bespoke labware and faster imaging (Brooks Automation, Molecular Devices, Thermo Scientific, Yokogawa).

Automated image analysis tools: New automated image analysis tools and software (some web-based) provide fast calculation of critical parameters such as cell velocity, directionality, migration index, cell area changes, volumetric changes in the detection zone and even multi-planar invasion (Brooks Automation, ibidi, Molecular Devices, Thermo Scientific, Yokogawa).

Microplate readers: Quantitation of bulk fluorescence from migrating cells using microplate readers can be advantageous in terms of read time, throughput and simplified data processing. Plate readers have now got even more cell-friendly through improvements in temperature and gas regulation in the measurement chamber (Tecan).

In conclusion, researchers wanting to investigate cell migration are now increasingly spoilt for

choice. Many new and innovative assays tools, some of which are reported in this article, not only facilitate the wider investigation of cell migration assays but also contribute to a better understanding of the process by which cells migrate. The latter is expected to play a pivotal role in the development of novel therapeutics strategies and to improving the treatment of a wide range of conditions/diseases.

DDW

Dr John Comley is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery and the life sciences. Since its formation nine years ago, HTStec has published more than 80 market reports on enabling technologies and Dr Comley has authored more than 40 review articles in Drug Discovery World. Please contact info@htstec.com for more information about HTStec reports.

References

I Cell Migration Assay Trends 2012 Report, published by HTStec Limited, Cambridge, UK, August 2012.



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