Combining cell migration and immunophenotype assessment using flow cytometry

Cancer biology applications on the BD Accuri™ C6 Plus

Features

- Assess cell migration rapidly and reliably using flow cytometry
- Streamline workflow for quantitation of migrated cells
- Combine migration with immunophenotyping to assess cancer cell marker expression in migrating cells

A new flow cytometric research application promises to make the assessment of cell migration fast, easy, and reliable, while enriching the data you can generate by multiplexing immunophenotype analysis. On the BD Accuri™ C6 Plus personal flow cytometer, which can count cells directly per unit volume, you can perform the assay right on the benchtop without the use of cell stains, counting beads, or standard curves.

Cells migrate during important physiological and pathological processes such as wound healing and tumor metastasis. Researchers use migration assays to understand the mechanisms underlying cell migration and to identify inhibitory or stimulatory molecules. In a conventional Boyden chamber assay, cells that migrate through a transwell membrane are stained and counted manually using a microscope or automatically with a plate reader. However, these conventional methods consume time, effort, and material, and do not illuminate the nature or identity of the migrated cells.

BD

Figure 1 shows the workflow for the new flow cytometric assay. Cell samples are plated in a transwell insert. Migrating cells move through the porous membrane toward chemoattractant in the receiver well and attach to the bottom of the membrane. After detachment and resuspension, the cells are directly acquired on a BD Accuri C6 Plus for volumetric count. The process is fast and consumes little sample. And unlike conventional assays, the remaining cells can be washed, stained with antibodies, and reanalyzed to assess their immunophenotype.

The unique, non-pressurized, peristaltic pump system of the BD Accuri C6 Plus accurately monitors the sample volume pulled per run, and can directly calculate sample concentrations per µL. These counts are more precise and far less tedious than manual counts of migrated cells using microscopy.

A personal flow cytometer in the lab provides many advantages for cell and cancer biology studies. When cells are ready for analysis or rare tumor samples arrive, it's crucial to have a flow cytometer at hand, ready to go.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 Plus personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. A compact design, fixed alignment, pre-optimized detector settings, and automated instrument QC result in a system that is simple to use. For walkaway convenience, the optional BD CSampler™ Plus accessory offers automated sampling from 24-tube racks or multiwell plates.

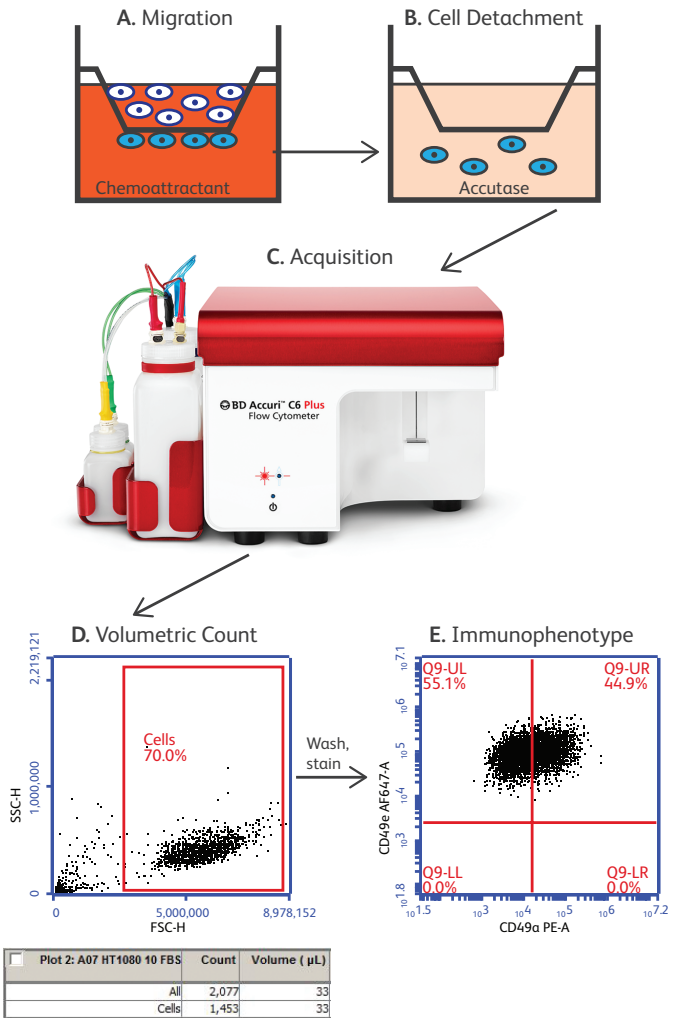

Figure 1. Cell migration assay workflow using flow cytometry
A. Cells are cultured in Corning® Transwell® inserts in serum-free medium for 6–24 hours in the presence or absence of chemoattractant in the receiver well. **B.** Cells that have migrated, which are now attached to the bottom of the inserts, are detached by 30 minutes incubation with BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527). **C.** After incubation, the whole cell suspension is collected and run directly on a BD Accuri C6 Plus for 30 seconds. **D.** BD Accuri™ C6 Plus software provides a volumetric count (number of cells/µL). E. The remaining cells are washed and stained with a cocktail of antibodies of interest, and then reanalyzed for immunophenotype.

Figure 2 shows assay validation using two cancer cell lines, HT1080 (invasive) and MCF-7 (non-invasive). In the absence of chemoattractant, neither cell type migrated to the receiver well. When FBS was added as a chemoattractant, only the HT1080 cells migrated, as expected. Migration of HT1080 was also observed in the presence of basement membrane extract, demonstrating that this approach can also be used for invasion assays (not shown).

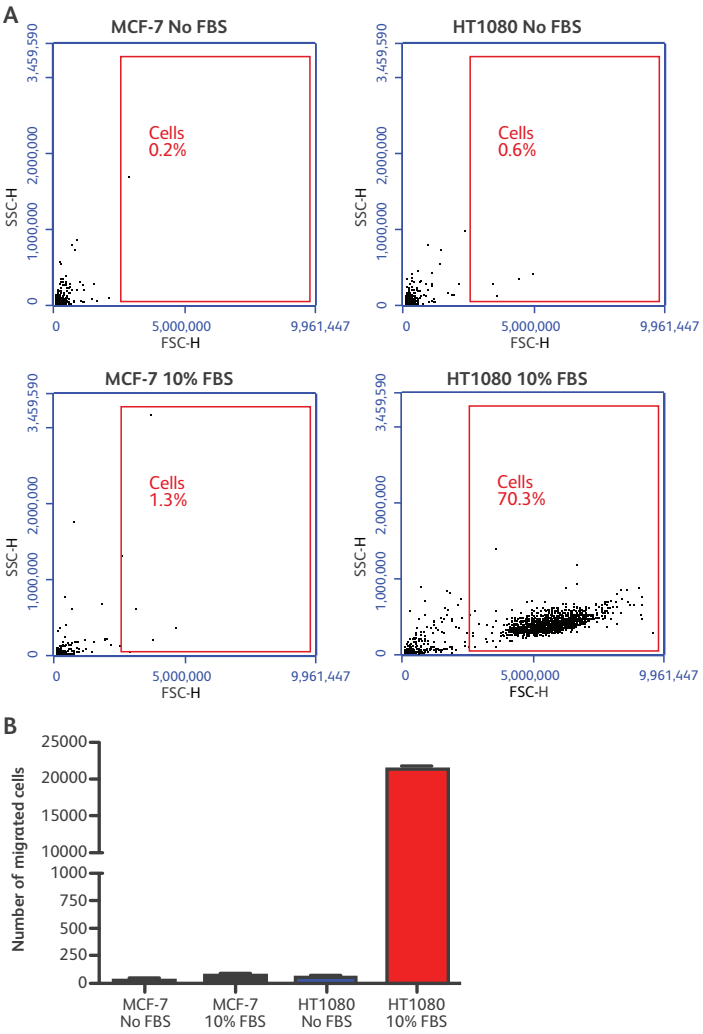

Figure 2. Cell migration assay validation
The invasive cell line HT1080 (ATCC® CCL121™) and the non-invasive cell line MCF-7 (ATCC® HTB-22™) were used as positive and negative controls, respectively, to validate the migration assay. 5 x 10^4 Cells were seeded in triplicate in 96-well plate Corning® Transwell® inserts, cultured for 6 hours in the presence or absence of FBS, and acquired on the BD Accuri C6 Plus. Results: Cells were gated and counted based on light scatter properties. **A.** As expected, in the absence of chemoattractant (No FBS, top plots), both cell lines showed minimal or no migratory activity (few events in the cells gate). In the presence of 10% FBS (bottom plots), only HT1080 cells migrated and populated the cells gate. **B.** Cell migration was quantified based on volumetric count.

Figure 3 shows the additional data that flow cytometry can provide. A549 cells were treated with TFG-β to induce epithelial-to-mesenchymal transition, a process known to increase cell motility. The treated cells were six times more likely to migrate than untreated cells. After volumetric count, the remaining cells were washed and stained with antibodies to integrins CD49e and CD49a. When analyzed on the BD Accuri C6 Plus, migrated cells previously treated with TGF-β expressed higher levels of both integrins—a finding that a conventional assay could not provide.

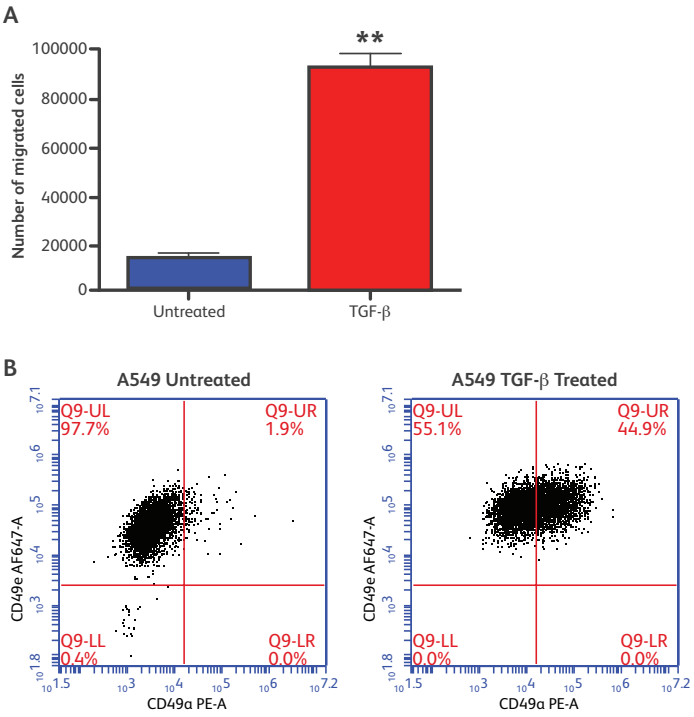

Figure 3. Combined assessment of migration and immunophenotype
A549 (ATCC® CCL-185™) cells were treated with 10 ng/mL TGF-β (R&D Systems) for 48 hours to induce epithelial-to-mesenchymal transition (EMT). Untreated cells were used as a control. Cells were then collected and 4 x 10^5 cells were seeded in triplicate in 6-well plate Corning® Transwell® inserts and cultured for 6 hours in the presence of FBS. **A.** TGF-β treatment significantly increased the migratory activity of A549 cells. **B.** Immunophenotype analysis of migrated cells revealed that A549 cells treated with TGF-β upregulated the expression of the integrins CD49e and CD49a, as measured by changes in mean fluorescence intensity (MFI) and percentage of positive cells, respectively.

Ordering information	
Description	Cat. no.
BD Accuri™ C6 Plus Flow Cytometer System	660517
BD Accuri™ C6 Plus Workstation and Software	661391
BD CSampler™ Plus Automated Sampling System (optional)	660519

Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Trademarks are the property of their respective owners.
23-18648-01

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com