

Evaluate Cell Migration with Corning® FluoroBlok™ Inserts on the SpectraMax® MiniMax™ Cytometer

Application Note

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Introduction

Cell migration, broadly defined as the movement of cells from one location to another, is important in diverse processes including embryonic development and wound healing. It is also a key parameter in metastasis, the spread of cancer cells to different parts of the body from their original location, which encompasses tumor cell invasion, entry into the vasculature, and distal site colonization¹. *In vitro*, cells migrate in response to chemical signals such as nutrients, and this chemoattraction can be used to study cellular migration mechanisms. Through understanding of the mechanisms of cancer cell migration, novel therapeutics can be developed to impede metastasis in cancer patients.

The proprietary Corning FluoroBlok inserts allow researchers to measure cellular migration or invasion through a polyethylene terephthalate (PET) membrane separating a suspension of cells from an underlying solution containing chemoattractant. The PET membrane blocks the transmission of light between 400 and 700 nm. Fluorescently labeled cells migrate through 8.0 μm pores in the membrane to reach the chemoattractant, and these cells are readily detected by bottom-read microplate readers or imaging systems (Figure 1). Migrating cells remain viable during the assay, enabling both endpoint and time course assays.

Raw fluorescence read from the bottom of the plate can be used to measure cell migration using the Corning FluoroBlok inserts, but there are limitations to this method. A standard curve of cells is required to standardize the RFU measurements, and variations in fluorescent cell labeling, background fluorescence, and other conditions may adversely affect data quality. By using a cellular imaging system to directly count the cells, researchers can obtain more accurate cell migration data.

Here, we demonstrate how the SpectraMax i3x Multi-Mode Microplate Reader with the SpectraMax MiniMax 300 Imaging Cytometer is used to measure cellular migration with FluoroBlok inserts by counting imaged cells.

Materials

- ▶ Corning FluoroBlok HTS 96-well Multiwell Permeable Support System with 8.0 μm High Density PET Membrane (Corning Cat. No. 351163)
- ▶ SpectraMax i3x Multi-Mode Microplate Reader (Molecular Devices Cat. No. i3x)

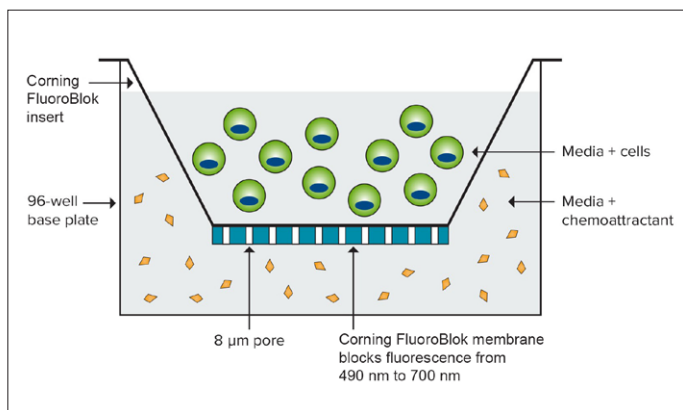


Figure 1. Corning FluoroBlok technology. Fluorescently labeled cells are seeded within Corning FluoroBlok inserts, migrate through 8 μm pores, and are detected from the bottom.

- ▶ SpectraMax MiniMax 300 Imaging Cytometer (Molecular Devices Cat. No. 5024062)
- ▶ NIH3T3 cells (ATCC Cat. No. CRL-1658)
- ▶ Minimum Essential Medium (MEM, Corning Cat. No. 10-010)
- ▶ Fetal Bovine Serum
- ▶ 0.05% Trypsin EDTA (Corning Cat. No. 25-051-CI)
- ▶ EarlyTox™ Live Cell Assay Kit (Molecular Devices Cat. No. R8342)

Methods

NIH3T3 cells were cultured in MEM with 10% FBS (complete MEM). Cells were trypsinized and resuspended in serum-free media, then stained with 4 μM Calcein AM from the EarlyTox Live Cell Assay Kit. After staining, cells were pipetted onto FluoroBlok inserts at 20K cells per well. Medium containing FBS was used as the chemoattractant for cell migration, and serum-free medium was used as a negative control.

A custom plate setting was created in SoftMax Pro Software to accommodate the dimensions of the FluoroBlok inserts. From the Plate Type window within Settings, Edit Plate was selected, and dimensions were modified according to plate dimensions supplied by Corning. The revised plate definition was saved under a new name. This custom plate setting could then be used for future FluoroBlok experiments.

The MiniMax™ cytometer was used to acquire images of NIH3T3 cells migrating through the PET membrane. Images were acquired at several time points over the course of 20 hours using the acquisition settings listed in Table 1. The reader's internal temperature was set to 37°C to maximize cell health during reads.

Migrating NIH3T3 cells were identified and quantified using SoftMax Pro Software. Discrete Object Analysis was selected as the analysis type within the Image Analysis Settings. A custom setting was created in the software by identifying cells based on their fluorescence intensity and size (Figure 2). By clicking 4 to 6 representative cells, thresholds were automatically set, and cells were identified by the software. Migrating cell numbers were graphed using the software.

Table 1. Acquisition and analysis settings.

Parameter	Setting for Cell Counts
Optical configuration	MiniMax
Read mode	Imaging
Read type	Endpoint
Wavelength settings	541 nm (green fluorescence)
Number of sites imaged	9
Image acquisition settings	541 exposure: 10 ms 541 focus adjustment: 240 µm
Image analysis settings	Analysis type: Discrete Object Wavelength for finding objects: 541

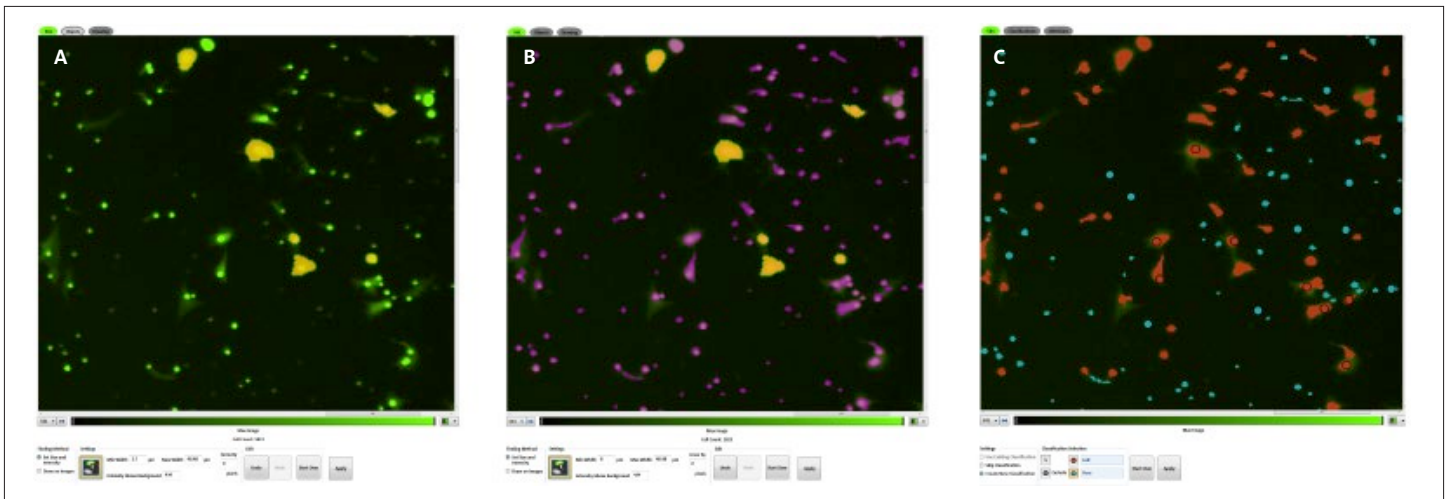


Figure 2. Object identification. NIH3T3 cells were identified by using the point and click feature in SoftMax Pro Software's acquisition editor. In Figure A, users can set fluorescent size and intensity by clicking on known cells (marked in yellow). Objects identified by the software are marked in purple (B). Using the classification tool, users can distinguish cells (red masks) from the smaller pores (blue masks) (C).

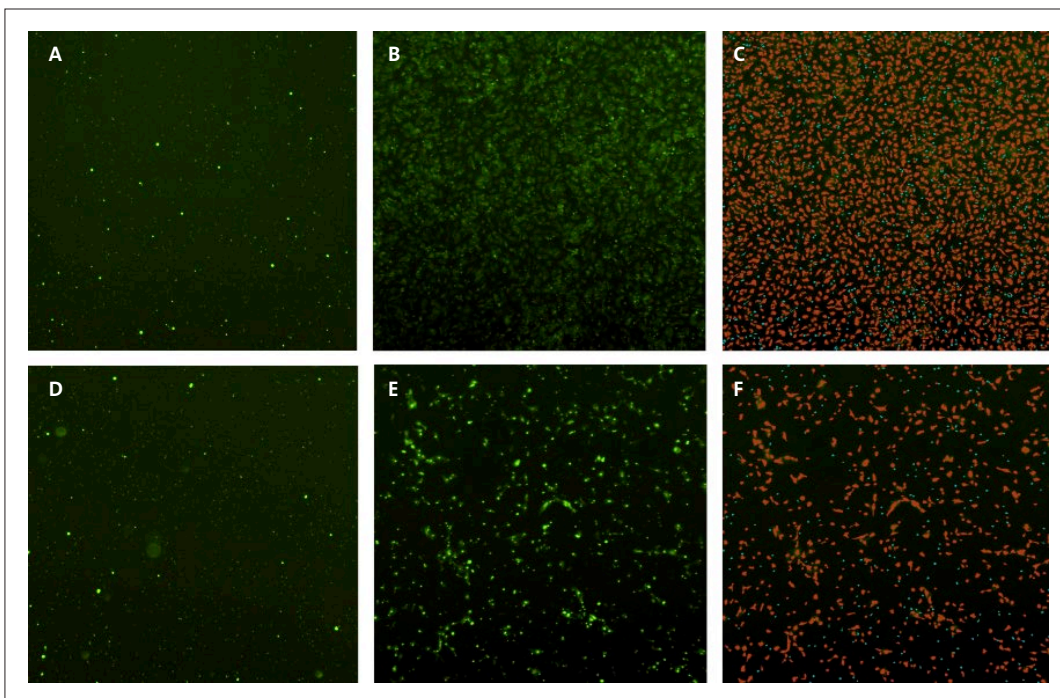


Figure 3. Tracking cell migration using the MiniMax cytometer. Images acquired 0 hours after seeding cells (A and D), 20 hours after seeding (B and E), and objects identified by the software (C and F). The red masks in C and F indicate objects classified as cells and the blue masks indicate small objects classified as pores in the Corning® FluoroBlok™ membrane. The top row shows wells containing chemoattractant, and the bottom row shows wells without chemoattractant (negative control).

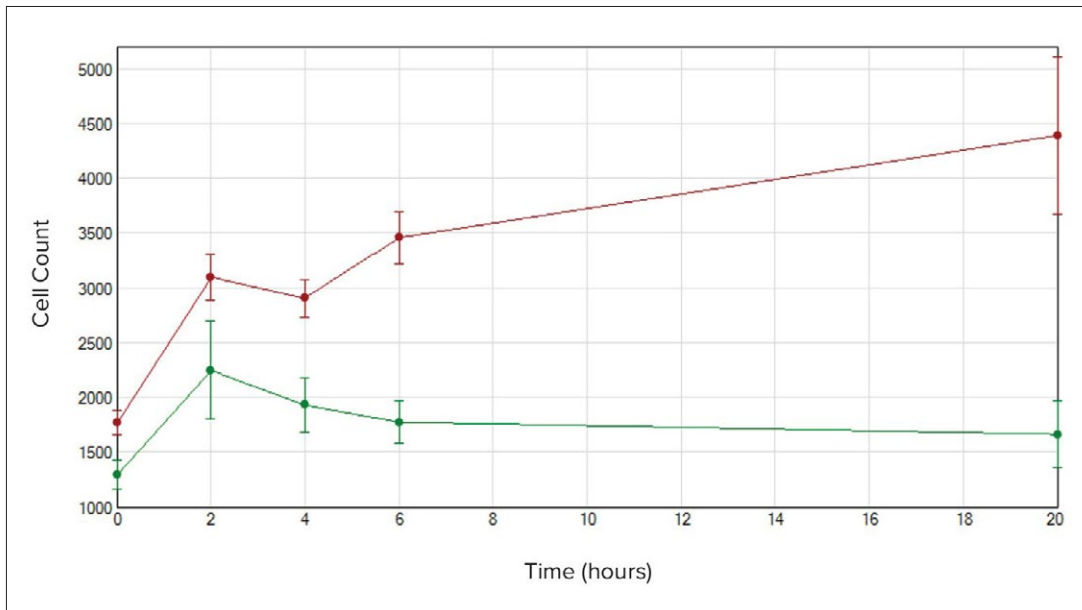


Figure 4. Cell migration cell counts. NIH3T3 cell counts were graphed using SoftMax Pro Software. Wells containing chemoattractant are shown in red, and negative control wells are shown in green. Initially both conditions show migration in the first 2 hours, but cell counts in the wells containing chemoattractant continue to increase over time.

Results

Fluorescently stained cells were identified as they migrated through the Corning® FluoroBlok™ insert using the MiniMax™ cytometer and SoftMax Pro Software (Figure 3). Although there was some migration in wells containing no chemoattractant, there was a nearly 3-fold increase in cellular migration in the presence of chemoattractant after 20 hours (Figure 4).

Conclusion

The SpectraMax® i3x reader and MiniMax cytometer can be used with Corning FluoroBlok cell culture inserts to directly count cells from high-quality images to measure cellular migration over time. SoftMax Pro Software enables users to quickly set up analysis of

cell counts via a simple point and click user interface. Image analysis provides accurate data compared to raw fluorescence, which is subject to cellular staining levels and background fluorescence from pores in the membranes. Using the image acquisition and analysis features of SoftMax Pro Software in combination with the 96-well FluoroBlok format, researchers can rapidly assess a wealth of cellular migration data to advance their studies in cancer and many other areas of biology.

Reference

1. Chambers AF, Groom AC, and MacDonald IC. Metastasis: dissemination and growth of cancer cells in metastatic sites. *Nature Reviews Cancer* 2.8 (2002):563.

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