

Analysis of Cell Invasion and Motility on the EIDAQ™ 100 High Throughput Microscopy (HTM) System

A novel high throughput microscopy invasion motility assay (HTM-IMA) is described for the accurate measurement and analysis of cell motility in the presence of chemoattractant. The HTM-IMA enables the direct visualization and analysis of fluorescence in cells, without disturbing the chamber or insert environment. The assay is capable of measuring migration from a sample at multiple time-points and accurately distinguishes cells that show only partial migration or invasion from cells that show complete migration-invasion. The method employs the EIDAQ™ 100 high throughput microscopy (HTM) system and CytoShop™ analysis software from Q3DM Inc., San Diego, CA.

Background and Significance

Motility assays are an important tool for the study of cell migration and the effect of various chemoattractants. However, many analytical techniques for cell motility rely on indirect measurements that are often time and labor intensive. Furthermore, current plate readers, which rely on overall measurements of fluorescent intensity, cannot distinguish between cells that have only begun migrating and those that have completed migration. Since a certain amount of partial migration takes place in the absence of chemoattractants, the inability to accurately distinguish between partial and complete migration inflates baseline measurements and makes the accurate quantification of chemoattractant effects much more difficult. Lastly, current colorimetric and fluorimetric assays are usually restricted to measuring motility at a single time point, making it impossible to quantify the time course of migration.

Q3DM has developed a simple method for the direct measurement and analysis of migrating, or invading, cells that uses the EIDAQ™ 100 HTM system for automated fluorescence imaging and quantification.

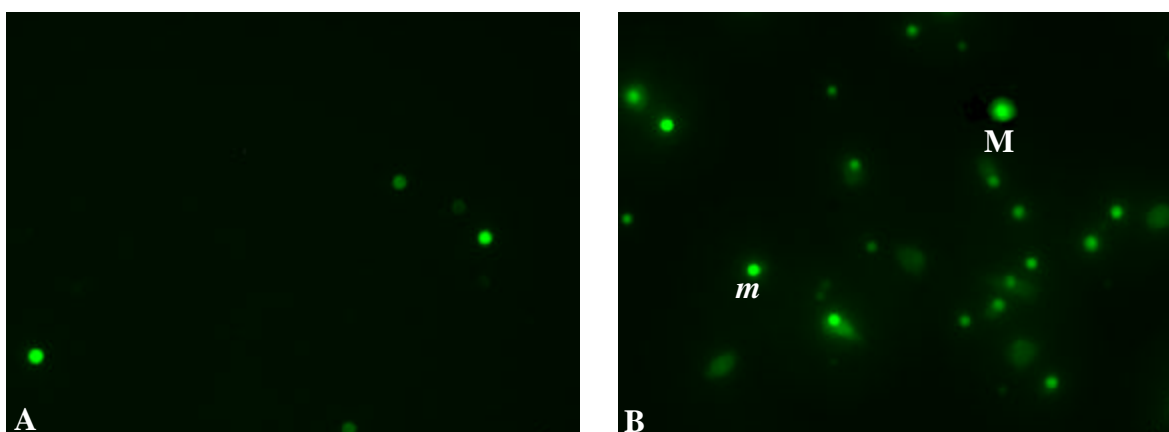


Figure 1. Effects of chemoattractant stimulation on cell motility. The EIDAQ™ 100 was used to image directional motility of a human carcinoma cell line in response to a single chemoattractant. Cells were seeded in the upper chambers of a two chamber culture system in serum-free medium, and chemoattractant was supplied in the medium below the membrane. Migration was allowed to proceed for 5 hours. **A.** Cells at 0 hours after the addition of chemoattractant. **B.** Cells 5 hours post chemoattractant. Note that there is a clear visual difference between cells that may appear to be migrating (*m*) and those that have completed migration (*M*). This difference is easily quantified with the EIDAQ™ 100 HTM system and CytoShop™ software, but cannot be distinguished by current plate readers.

Experimental Methods

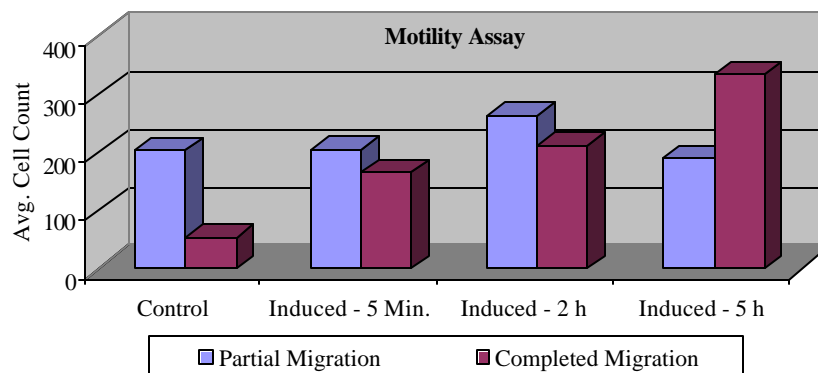
Cells were a proprietary human carcinoma cell line. All cell culture reagents were purchased from Sigma (St. Louis MO, USA). Cells were grown to near confluence and suspensions were prepared. Cells were stained with calcein, washed, and then placed in 24-Multiwell HTS FluoroBlok™ Cell Culture Inserts with 13 mm diameter, 8 µm pore poly carbonate membranes from BD Biosciences (San Jose, CA). Chemoattractant was added to the lower chamber. Migration was permitted to proceed for 5 hours, with measurements taken at t = 0, 2, 5 hrs. Q3DM's EIDAQ™ 100 HTM system was used to automatically acquire images and quantify cells which had begun and completed migration through the membrane, respectively (excitation wavelength = 485 nm, emission wavelength = 530 nm, CCD camera gain = 60). All images were analyzed with Q3DM's CytoShop™ software.

Experimental Results

The migration between chambers can be measured by directly counting the number of cells in the recipient chamber. Figure 2 provides data on a 5-hour time course study for motility in the presence of a chemoattractant vs. a control with no chemoattractant. It is important to note that a substantial amount of baseline partial migration did take place in the control as well as the stimulated cells.

Although powerful, current plate readers would not be able to distinguish partial migrations characterized by high intensity cells with small diameters (8µM) vs. cells that have completed migration, which are characterized by larger diameters and more diffuse fluorescence. The EIDAQ™ 100 HTM system allows researchers to easily distinguish between these two states (Figure 1).

Figure 2. Data was collected from a series of 24 well plates for a 5-hour time course study on motility. The control group was observed over the 5-hour time course, but did not show significant changes. A substantial amount of baseline partial migration appears to take place in the control as well as in the stimulated cells.



Conclusion

Q3DM's HTM-IMA is a highly sensitive cell-based technique for the direct visualization and measurement of cell motility and invasiveness. Its key advantages are the ability to distinguish between partially and fully migrated cells, the ability to quantify the time course of cell migration, and the possibility of measuring cell motility without disturbing the chamber or insert environment. Though the results shown above were obtained with proprietary human carcinoma cells and a single chemoattractant, the experimental conditions are generally applicable and can be easily modified to investigate other cell types and chemicals.

Q3DM is a provider of innovative products and services in high throughput microscopy (HTM) and cell-based imaging. Q3DM's proprietary and patented technologies deliver an unmatched combination of speed, accuracy and precision to quantitative imaging and analysis for use in Drug Discovery, Clinical Diagnostics, and Basic Research. Q3DM's products include the EIDAQ™ 100 HTM system, coupled with CytoShop software and services in assay development, algorithm development, automation, and high throughput screening. Q3DM's products and services bring long-needed solutions to constraints in automating high-resolution imaging and provide researchers with far greater information on complex biological processes than ever before.