

# The myosin II inhibitor, blebbistatin, more potently inhibits 3D cell invasion

## than 2D cell migration in a human metastatic tumor cell

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### Abstract

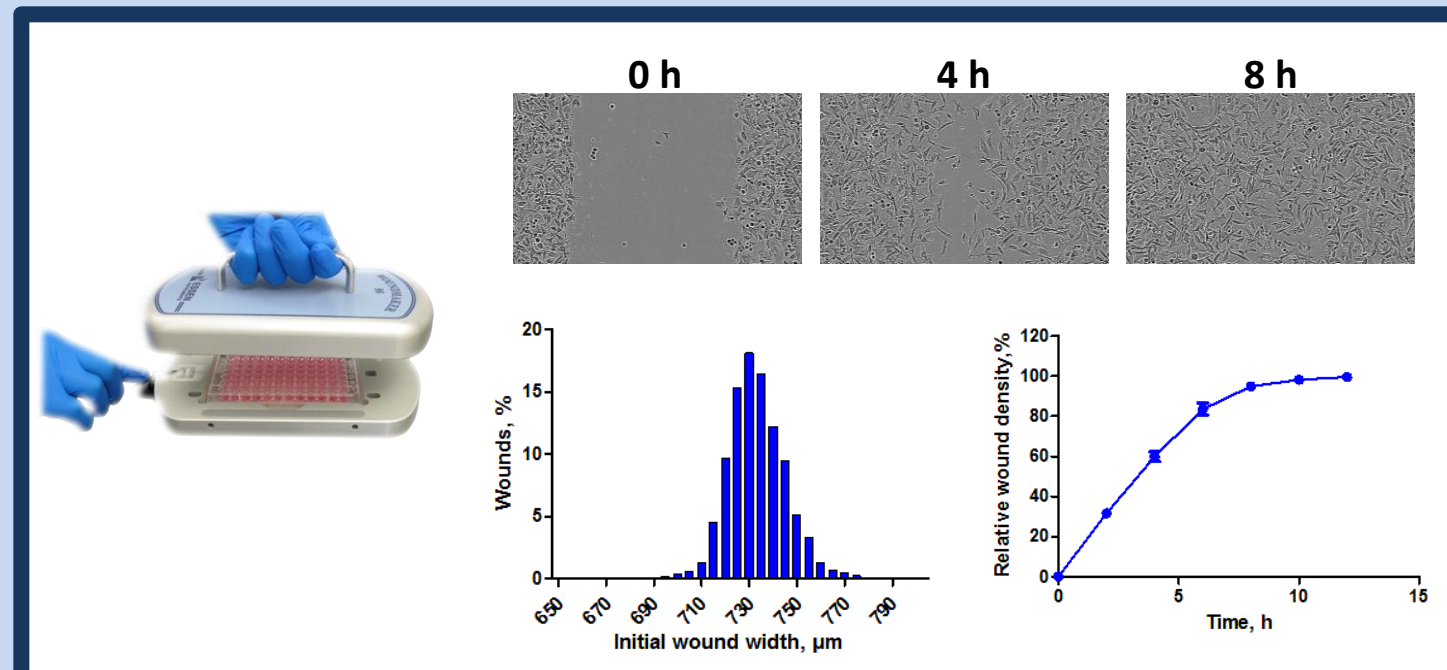
The acquired ability for tumor cells to migrate and invade through the extracellular matrix within the tumor microenvironment has long been accepted as a hallmark of metastatic potential. Numerous signal transduction pathways and a wide variety of protein classes have been implicated as important players in cell migration and/or invasion. For example, matrix-metalloproteinases (MMPs) have been implicated in the degradation of the basal lamina that is required for the movement of cells through the matrix. Likewise, the MEK/ERK pathway has been implicated in upregulated expression of MMPs in tumor cells. Furthermore, recent evidence has indicated that myosin II also plays a major role in tumor cell invasion. However, the relative contribution of myosin II in cell migration compared to cell invasion remains unclear. In this study we use the myosin II inhibitor, blebbistatin, to concurrently investigate the relative contribution of myosin II to both 2D migration and 3D invasion of human fibrosarcoma derived HT 1080 cells through collagen I and Matrigel matrices. To accomplish this, we utilized a novel assay strategy using a live-cell imaging system, the IncuCyte-FLR, to simultaneously measure migration and invasion of non-labeled cells in a 96-well plate format. Following assay initiation, images of all 96-wells were obtained every three hours until assay completion. Each image was automatically analyzed using phase contrast image based algorithms. Our results indicate that blebbistatin is approximately one order of magnitude more potent in its ability to inhibit cell invasion through both collagen I and Matrigel matrices compared to its ability to inhibit 2D cell migration (e.g. IC50 value for invasion is 5µM compared to the IC50 value for migration, >90µM). Similar, less pronounced results were also observed when human breast adenocarcinoma derived MDA-MB-231 cells were identically evaluated. These results suggest that the myosin II contractile machinery is more heavily taxed during the process of invasion compared to migration in two distinct metastatic cell types. In addition to the role of myosin II, we extended our study to investigate the relative contribution of both MMPs and the MEK/ERK pathway to cell migration and invasion using the inhibitors GM6001 and U0126, respectively. Interestingly, with both inhibitors, we observed minimal effects on cell migration, but significant inhibition of cell invasion through collagen I. In addition, whereas inhibition of myosin II had a significant, immediate effect on invasion, broad inhibition of several MMPs and inhibition of the MEK/ERK pathway did not significantly show an effect until 5, or 10 hours after assay initiation, respectively. These time points are often well past the end point of other commonly used approaches to measure cell invasion. We also found that the results of this assay were dependent both on cell density as well as matrix concentration illustrating the importance of assay optimization. Using this novel, kinetic approach, the results of this study further clarify a more significant role for myosin II in cell invasion when compared to cell migration. We also provide additional evidence that MMPs and MAPK signaling pathways are significantly involved in the process of cell invasion.

### The IncuCyte FLR



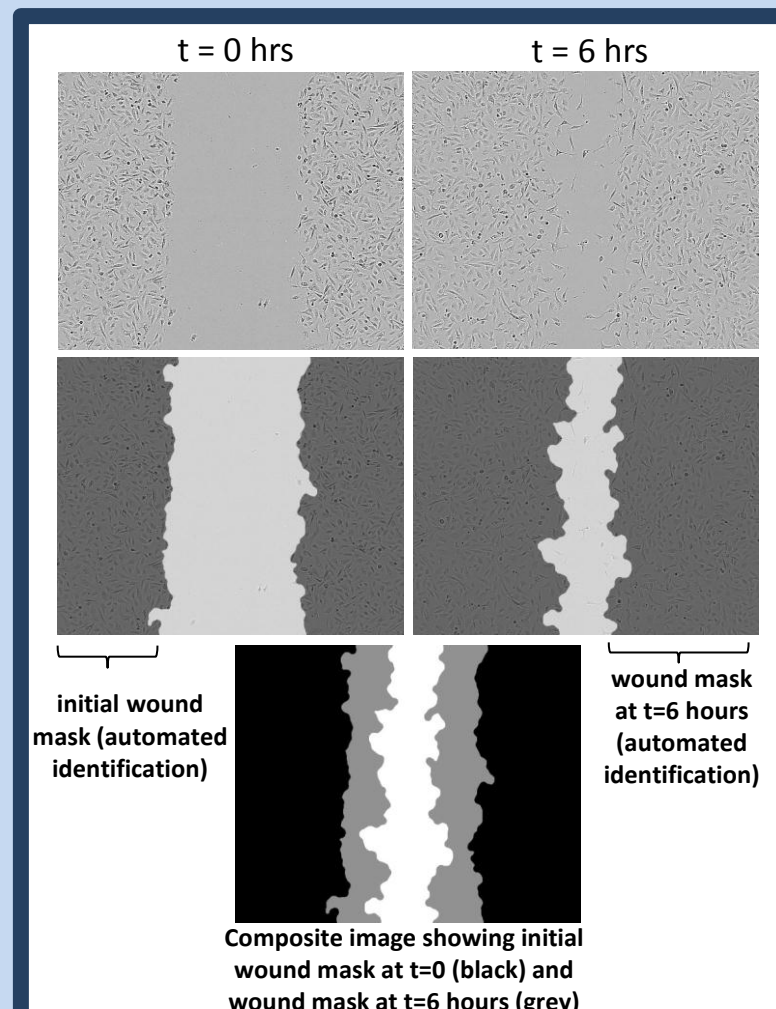
The Essen BioScience IncuCyte FLR Live-Cell Imaging System is a compact, automated microscope. The IncuCyte FLR resides inside your standard culture incubator and is used for long-term kinetic imaging in both HD-phase contrast and fluorescence (green). The IncuCyte system also comes with a robust software package that allows for automated data acquisition, image processing, and graphing capabilities.

### The 96-well WoundMaker



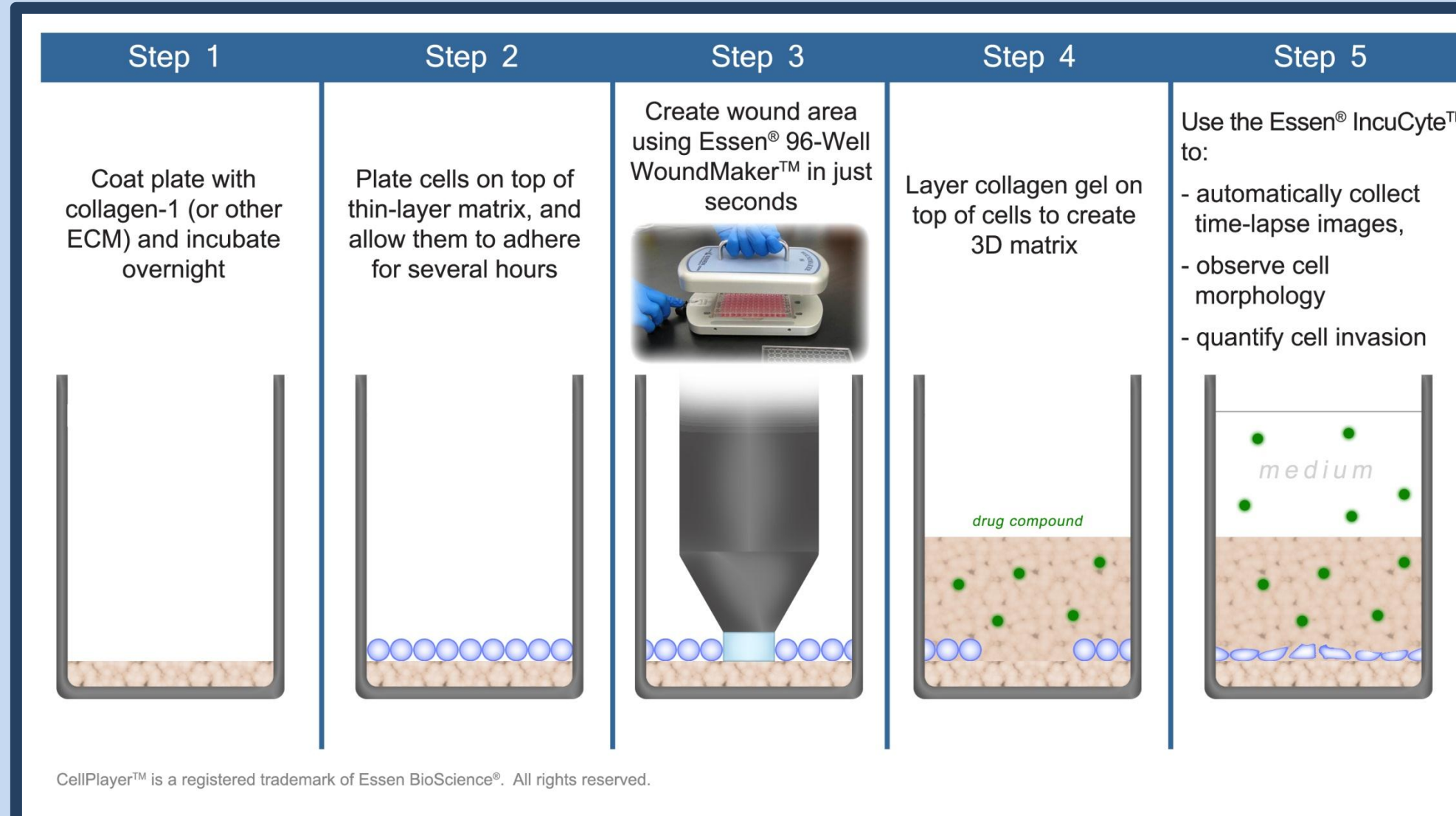
- The 96-well WoundMaker is equipped with 96 individual pins capable of precisely generating wounds in every well of a 96-well plate with one push of the button.
- Initial wound width metrics (ranging between 700 and 770 µm; CV < 3%) were automatically collected using the IncuCyte FLR basic software package.
- The custom Relative Wound Density (%) metric that analyzes both the inside of the wound and the outside cell region is used to express kinetic wound closure data.

### Automated Image Analysis

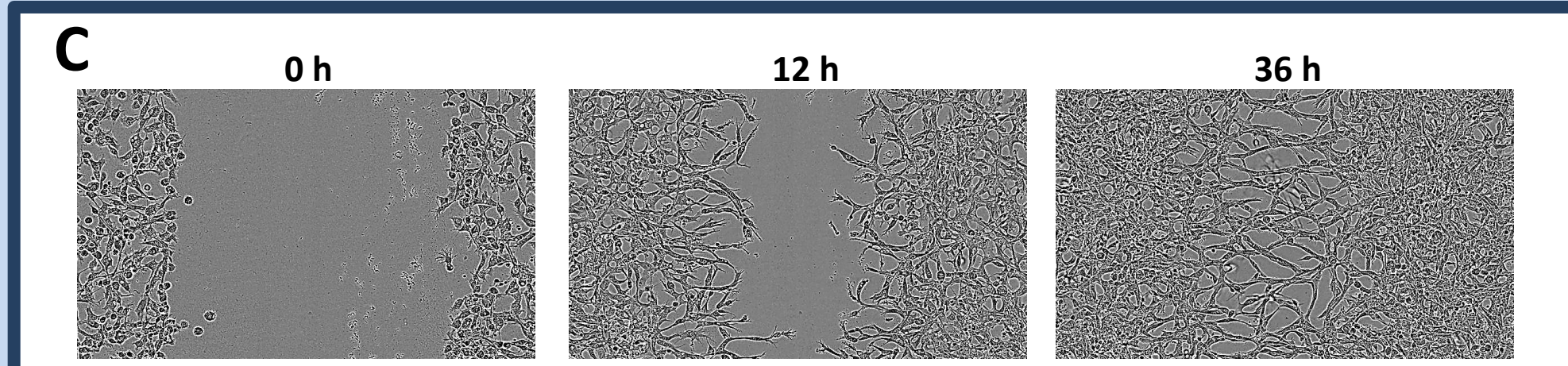
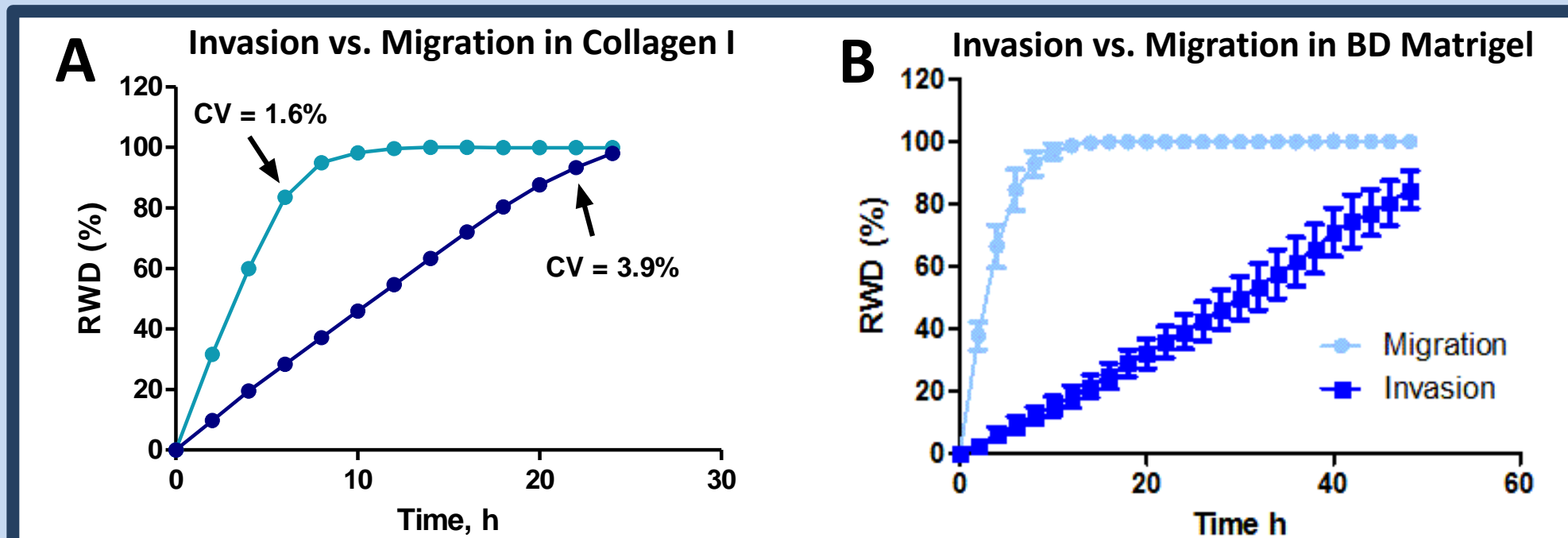


- Every HD-phase contrast image, in every well, at every time point is segmented and analyzed in real time without the use of cell labeling reagents, thereby providing instantaneous data analysis metrics.
- The novel, automated Essen BioScience Relative Wound Density (RWD) metric compares the density of both the wound region and the cell region at each time point.
- Kinetic analysis metrics are easily graphed and analyzed within the user interface.

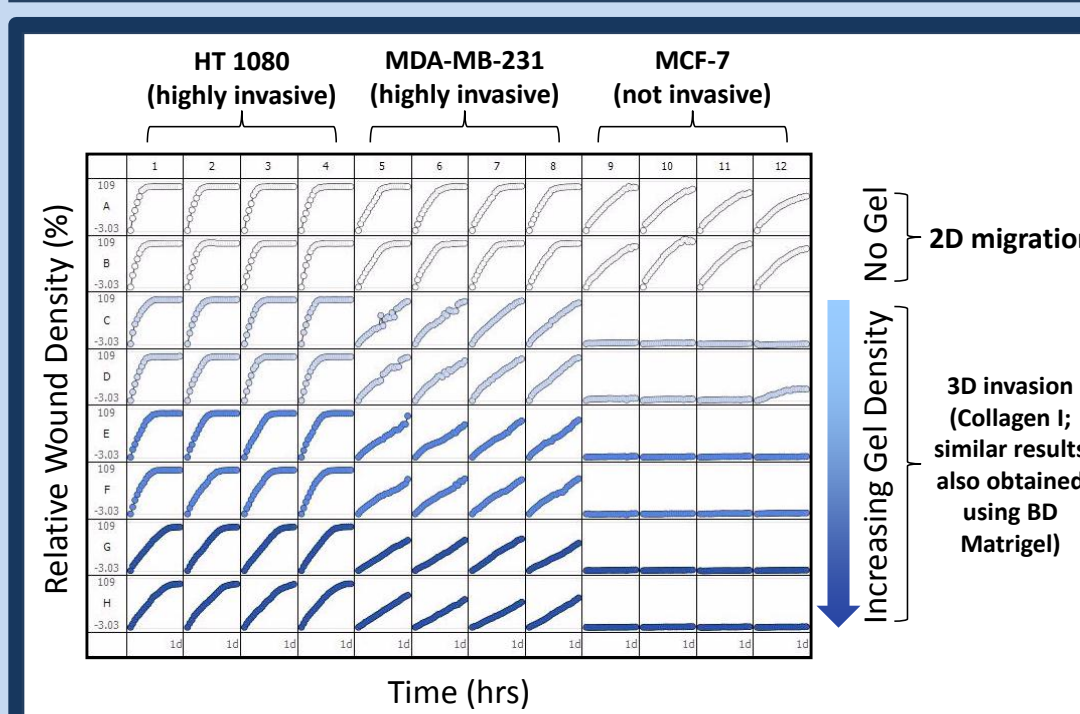
### Cell Invasion Methodology



### Results

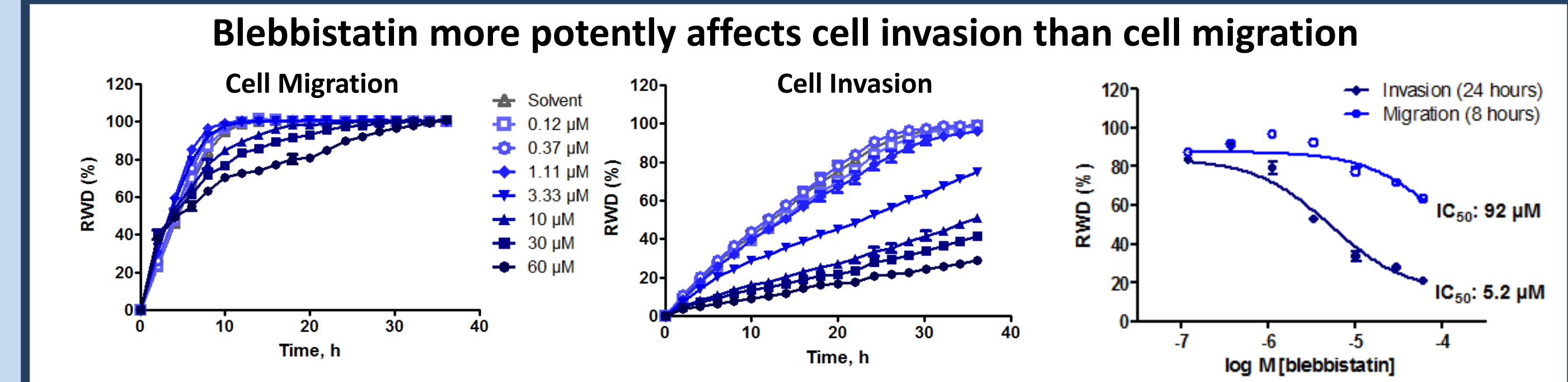


**Figure 1 (Above):** The ability of HT 1080 cells to migrate and/or invade 3mg/ml collagen I (A) or 8mg/ml BD Matrigel (B) were analyzed on the same 96-well plate (N=24 for each condition, shown as mean ±SD). CV values were calculated at the point where cells reached > 80% RWD. HT 1080 cells migrate significantly faster than they invade both Collagen I and BD Matrigel. (C) Examination of cell morphology at various time points revealed a predominantly mesenchymal invasion morphology, consistent with previous studies (Images are BD Matrigel).

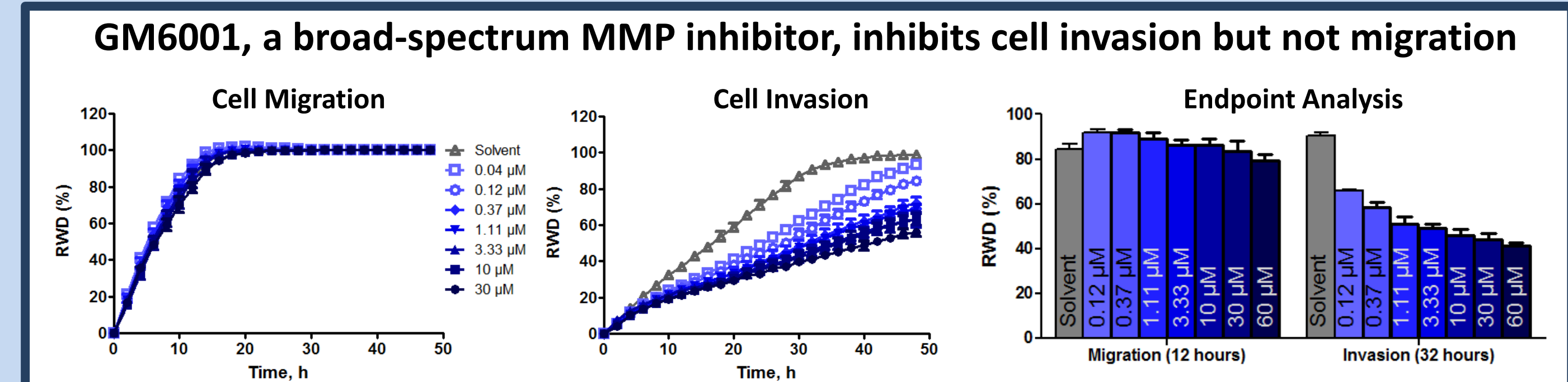


**Figure 2 (Left):** To further differentiate between invasion and migration, cells with known invasive potential were used to verify the ability to measure 3D cell invasion on the same 96-well plate. This 96-well plate micrograph illustrates that the highly invasive cell types HT 1080 and MDA-MB-231 can invade increasing densities of collagen I. In contrast, while having the ability to migrate in 2D, the non-invasive cell type MCF-7, can not invade the Collagen I ECM at any gel density tested, thus confirming the ability to differentiate between migration and invasion.

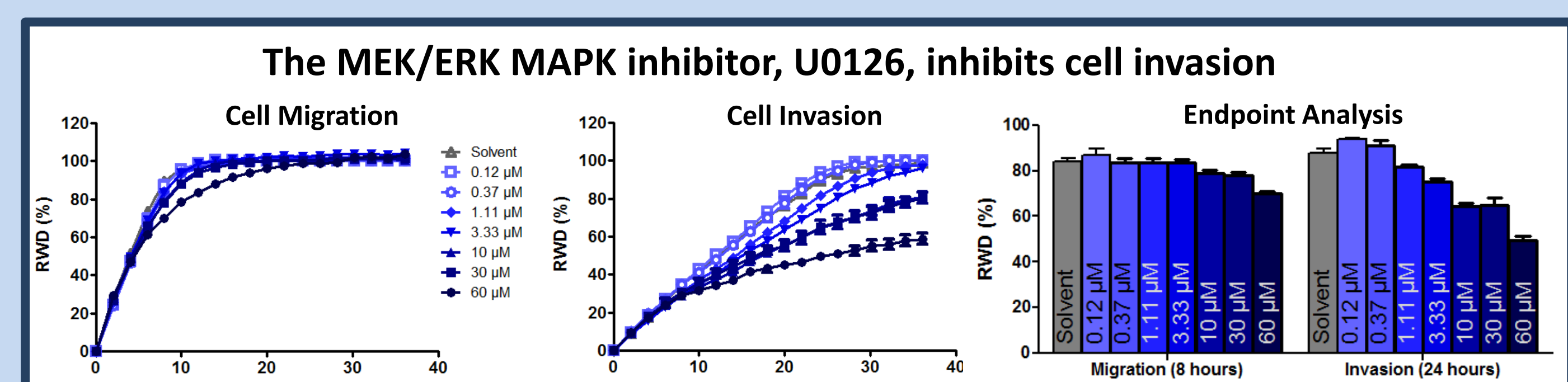
### Results



**Figure 3:** We used the myosin II inhibitor, blebbistatin, to investigate the role of myosin II in cell migration and cell invasion of HT 1080 fibrosarcoma cells through collagen I. Blebbistatin was nearly 20-times more potent in inhibiting cell invasion than cell migration. A similar increase in potency was also seen in HT 1080 cells migrating on or invading through BD Matrigel (data not shown). Data are representative of three separate experiments. Data shown as mean ± SEM (n = 3 wells).



**Figure 4:** We used GM6001 to investigate the effect of MMP inhibition in cell invasion and migration. GM6001 showed no effect on migration of HT 1080 fibrosarcoma cells at any concentration tested. However, GM6001 inhibited cell invasion through collagen I by approx. 50% at 3 µM. Unlike myosin II inhibition, inhibition of MMPs had only a marginal effect on HT 1080 invasion through BD Matrigel (data not shown). Data are representative of three separate experiments. Data shown as mean ± SEM (n = 3 wells).



**Figure 5:** We investigated the role of MAP kinase signaling in cell invasion and migration using the MEK1/2 inhibitor U0126. U0126 showed little effect on migration of HT 1080 fibrosarcoma cells under the conditions tested. The effect on cell invasion was very evident, with U0126 inhibiting invasion by approx. 50% between 30 µM and 60 µM. Data shown as mean ± SEM (n = 3 wells).

### Introduction

- Metastasis is a multistep and dynamic process governed by growth factors, hormones, and genetic and epigenetic events that alter the phenotypic characters of tumor cells.
- The basement membrane is a thin, continuous sheet of extracellular matrix that surrounds organs and represents a barrier that tumor cells must invade and migrate through in order to metastasize.
- This study demonstrates a novel, automated, label free, 96-well strategy to measure both cell migration and cell invasion in the same microplate using the IncuCyte FLR live-cell imaging system.
- This automated, flexible, kinetic, label free, quantitative strategy was then used to examine the pharmacology of the myosin II inhibitor, blebbistatin, the broad MMP inhibitor, GM6001, and the MAPK inhibitor U0126.
- Our results indicate that all three compounds were more potent inhibitors of cell invasion than cell migration.

### Conclusions

- Direct comparison of cell invasion and migration assays on the same plate, at the same time:** Investigators can measure 2D migration and 3D invasion on the same microplate. This provides the best opportunity for determining the specificity of drugs and the utility of potential drug targets.
  - Simultaneous evaluation of the effects Blebbistatin, GM6001, and U0126 on illustrate that these drugs are more potent inhibitors of invasion compared to cell migration.
- Kinetic:** The spatio-temporal, label-free format of the invasion assay allows investigators to follow both the rate and extent of cell invasion for a given set of experimental variables. This feature can be used to explore time-dependent pharmacology, assay optimization (e.g. ECM concentration, cell density), and enhance assay sensitivity.
- Automated data acquisition:** After the experiment is initiated, the data is collected automatically.
- Label-free:** The IncuCyte HD optics and software package obviates the need to label the cells.
- Integrated RWD metric:** Results are quantitative and reproducible.
- Morphological data:** HD images are acquired at every time point and can be assembled into time-lapse movies for convenient viewing.