A New Technology for In Vitro Chemotaxis Assays

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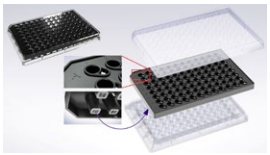
Introduction

- Chemotaxis is the movement of a cell in a direction corresponding to a gradient of increasing or decreasing concentration.
- Chemotaxis is a fundamental element of normal and pathological cell biology.
- Traditional *in vitro* methods for studying cell migration include:
  - **Scratch or Cell Exclusion Assays:** These are not measures of directed cell migration or chemotaxis. For the most part, they are a measure of "random" migration.
  - **Microfluidic Chemotaxis Assays:** Researchers can see the cells, but they suffer from small gradients across the cell, low participation rates, and low throughput.
  - **Traditional Boyden Chamber Assays:** This predominant industrial approach has good throughput (96-wells). However, the researcher can not easily visualize the process of cell migration, it requires many cells, and additional labeling or manual cell counting.

This poster describes a novel approach that combines hardware, software algorithms, and a consumable to provide a fully automated, integrated solution for studying chemotaxis using live-cell imaging.

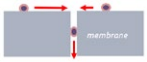
IncuCyte ClearView Cell Migration Plate

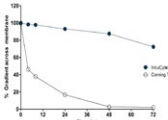


- **Visualize Chemotaxis** – The ClearView Plate incorporates an optically smooth membrane surface enabling acquisition of high-definition, phase-contrast images. Standard Boyden Chamber surfaces are not easily amenable to imaging.
- **Persistent Gradient** – The low porosity of the ClearView Plate results in a gradient that is stable for over 72 hours compared to 4 hours in traditional consumables.
- **Low Cell Density** – The combination of a long-term, persistent gradient and the interest in visualizing chemotaxis has resulted in an assay that requires significantly fewer cells compared to traditional Boyden Chamber Assays.
- **Integrin Signaling** – In the ClearView Plate, cells are required to migrate to the pores. This requires integrin interactions with the substrate that likely are not required in traditional Boyden Chamber consumables.
- **Automated Image Processing** – The unique design of the ClearView Plate facilitates quantitation of cells on top and the bottom of the membrane.

Standard Boyden Chamber

ClearView Cell Migration Plate



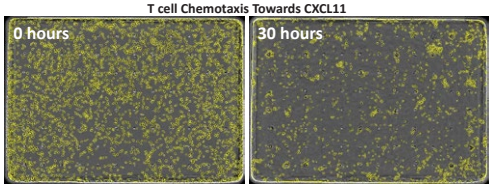


Evaluation of the gradient. A 10kD dextran (labeled with Alexa Fluor® 594) was added to the ClearView reservoir plate at a concentration of 10 µM to establish gradients over zero, 24, 48, and 72 hours. Measurements of diffusion were made by sampling the insert wells and measuring fluorescent intensity on a microplate reader. Each data point represents mean ± SEM, N=3.

IncuCyte ZOOM®

Automated, Label Free Quantitation

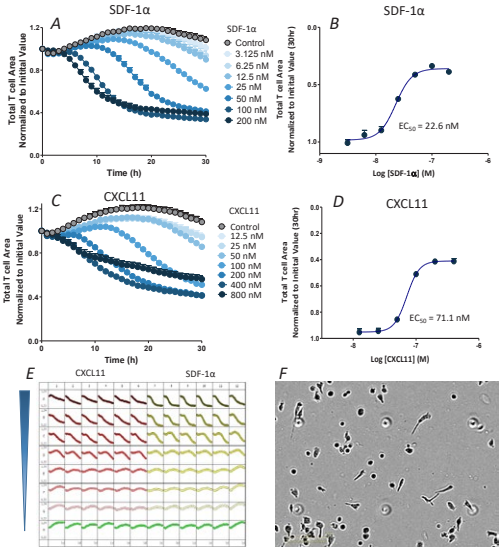
T cell Chemotaxis Towards CXCL11



The phase-contrast image is blended with image segmentation mask (yellow) created by an automated image processing algorithm.

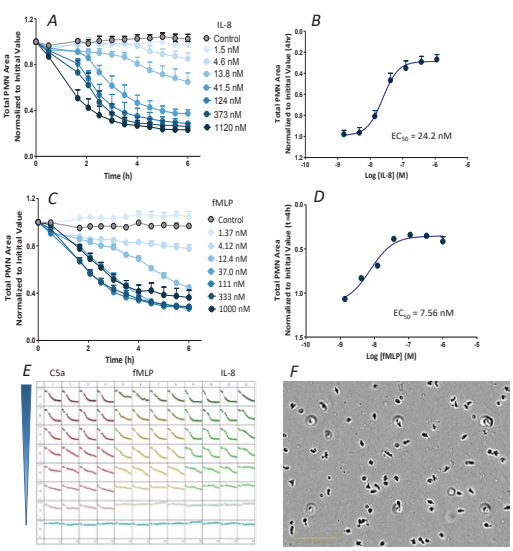
- Optically-clear surface for label-free imaging within IncuCyte ZOOM®
- Cells are added to the upper chamber and chemoattractant to lower reservoir plate.
- Chemotactic cell migration towards the pores is automatically analyzed using the IncuCyte ZOOM® instrument.

T Cell Chemotaxis to SDF-1a and CXCL11



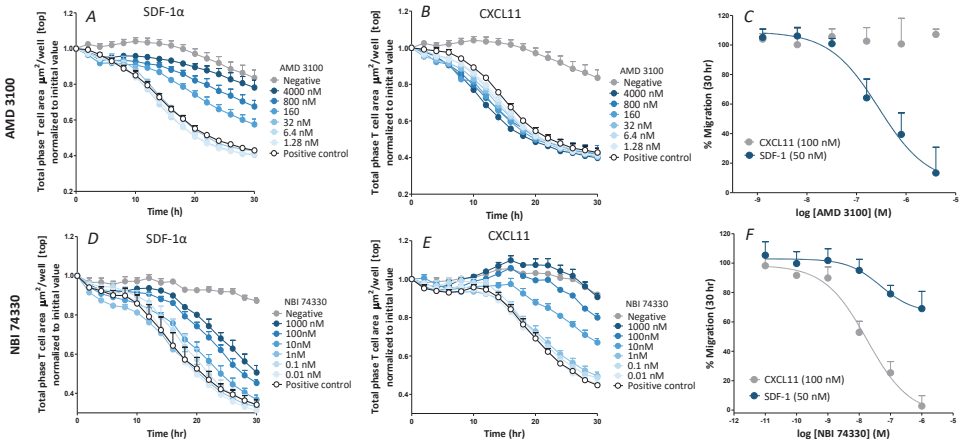
5,000 CD3/CD28 Dynabead-activated T cells were seeded in each well on an ICAM-1 coated surface. The indicated chemoattractant was added to the reservoir plate. Data were collected over a 36 hour period at 1hr intervals. **A and C**: Kinetic curve of concentration-dependent responses to SDF-1α and CXCL11. Data represent mean ± SEM; N=6. **B and D**: SDF-1α and CXCL11 agonist curve at 30 h Data represents the mean ± SEM; N=6 per condition. **E**: Each well is individually graphed in a microplate graph overview, illustrating well-to-well reproducibility. **F**: A representative image acquired at first time point of the assay from control wells (no chemoattractant). Lamellipodium/Filopodium on T cells indicates that the cell are actively interacting with the surface.

Neutrophil Chemotaxis to IL-8, fMLP, and C5a



5,000 neutrophils per well were seeded on a Matrigel + FBS coated surface. The indicated chemoattractant was added to the reservoir plate. Data were collected over a 6 hour period at 30 min intervals. **A and C**: Kinetic curve of concentration-dependent responses to IL-8 and fMLP. Data represent mean ± SEM; N=4. **B and D**: IL-8 and fMLP agonist curve at 4 h. Data represent mean ± SEM; N=4 per condition. **E**: Each well is individually graphed in a microplate graph overview, illustrating well-to-well reproducibility. **F**: Representative image acquired at first time point of the assay from control wells (no chemoattractant). Blurry cells are localized on the bottom side of the membrane.

Specific Inhibition of T Cell Chemotaxis using CXCR3 and CXCR4 Inhibitors



Kinetic inhibition and pharmacology of AMD3100 and NBI 74330. CD3/CD28 Dynabeads-activated T cells were plated at a density of 5K/well on a coated ClearView insert (Protein G + ICAM). AMD 3100 **(A and B)** or NBI 74330 **(D and E)** was added to the cells at indicated concentrations. SDF-1α **(A and D)** or CXCL11 **(B and E)** was added to the reservoir plate at 100 nM or 50 nM, respectively. Each data point represents mean ± SEM; N=3. AMD3100 inhibitor has a clear selective effect on CXCR4-mediated chemotaxis towards SDF-1α (IC50 AMD 3100 = 197 nM). No effect of AMD 3100 was found in CXCR3-meadiated chemotaxis towards CXCL11. NBI 74330 has a clear effect on CXCL11-mediated chemotaxis (IC50 NBI 74330 = 17.8 nM). NBI 74330 weakly inhibited chemotaxis towards SDF-1α.

Summary and Impact

- **Real-time visualization and automated analysis of chemotactic cell migration in a 96-well format within your incubator**
- **Measure label-free, or labelled cell migration with fixing, staining or cell scraping steps**
- **Setup and walk away – fully automated image based analysis**
- **Highly reproducible 96-well approach suitable for profiling and screening**
- **Investigate cell migration on biologically relevant surfaces**
- **Sustained and stable gradient over 72 hours**

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