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

- Visualized 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 up to 72 hours compared to 4-8 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.
- Integrid Signalling: 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 quantification of cells on top and the bottom of the membrane.

IncuCyte ZOOM®

- Automated, Label Free Quantitation

T cell Chemotaxis Towards CXCL11

- 5,000 CD8 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: CXCL11 (100 nM). D and E: CXCL11 (100 nM) and AMD-1300 (50 nM). 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 is actively interacting with the surface.

Specific inhibition of T cell chemotaxis using CXCR3 and CXCR4 inhibitors

- Kinetic inhibition and pharmacology of AMD-1300 and NBI-74330. CD8 T cells were seeded at a density of 5x10^4/well on a coated ClearView insert (protein G + ICAM). AMD-3100 (A and B) or NBI-74330 (D and E) was added to the reservoir plate at 100 nM or 50 nM, respectively. Each data point represents mean ± SEM, N=3. AMD-3100 inhibitor has a clear selective effect on CXCR4-mediated chemotaxis towards SDF-1α (K50 AMD-3100 = 197 nM). No effect of AMD-3100 was found in CXCR3-mediated chemotaxis towards CXCL11. NBI-74330 has a clear effect on CXCL11-mediated chemotaxis (K50 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