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 4-8 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.
- 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 quantification of cells on top and the bottom of the membrane.

IncuCyte ZOOM®

- Automated, Label Free Quantitation

T cell Chemotaxis to SDF-1α and CXCL11

Neutrophil Chemotaxis to IL-8, fMLP, and C5a

Specific Inhibition of T Cell Chemotaxis using CXCR3 and CXCR4 Inhibitors

 kinetic inhibition and pharmacology of AMD3100 and NBI 74330. CXCL12/DVD activated T cells were plated at a density of 5×10⁶/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.

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
- Investgate cell migration on biologically relevant surfaces
- Sustained and stable gradient over 72 hours