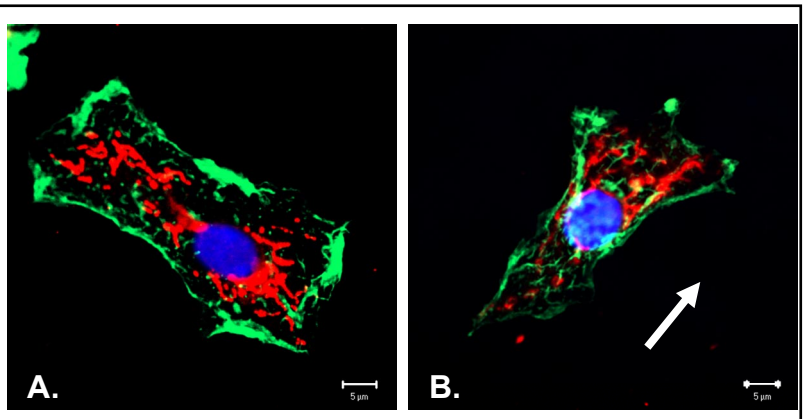
Lights! Plate Reader! Action!: Fluorescence-based cell migration assays

Cell migration is a multistep process that requires the orchestrated signaling and movement of hundreds of molecules that sense extracellular cues, reorganize the cytoskeleton, redistribute internal organelles and recycle lipid compartments in order to achieve motility. Our understanding of the cellular events regulating cell migration can, in part, be attributed to the pioneering efforts of Dr. Stephen Boyden at the Australian National University, who created a novel assay for the analysis of cell migration and cell invasion in 1962.[1]

The basic principle of the "Boyden Chamber" is to culture cells in the upper reservoir of a two-part chamber, which is separated from the bottom by a filter membrane of defined porosity, and allow them to migrate through the filter toward a chemoattractant placed in the lower reservoir. After a given period of time, migrated cells on the lower part of the membrane are fixed, stained and counted. For cell invasion assays, the same system is used only the filter is coated with an extracellular matrix material that the cells must enzymatically degrade in order to migrate through the pores.

Several companies now offer the latest rendition of the Boyden Chamber that use fluorescence-based technologies to quantify cell migration and invasion. Although little has changed with the basic properties of the assay, investigators can now fluorescently label cells using Calcein AM or Cell Tracker™ Green (both available from Invitrogen) prior to plating, or they can be fixed and labeled with Hoechst 33342 (nuclear stain) or a fluorescence-conjugated phalloidin (F-actin). After migration is completed, cells on the bottom of the membrane can be disassociated and analyzed with a fluorometer. The amount of fluorescence observed is taken to be proportional to the number of migrated cells. As an alternative, BD Biosciences has developed 24-well and 96-well culture inserts with a specialized porous filter (FluoroBlok™) that permits use of a fluorescence plate reader for specific analysis of migrated cells without cell dissociation and without interference of non-migrated cells in the upper part of the insert.



Phenotype of a migrating cell: Adherent mononuclear cells stained for nuclei (blue), mitochondria (red) and actin (green). In panel A, the non-migrating cell has a non-polar distribution of mitochondria and actin. In panel B, the migrating cell appears polarized with a protruding lamellipodium at the front of the cell, reorganization of mitochondria to the leading edge and retraction of the tail end of the cell.

A variant of migration analysis called "wound healing" has also been adapted to analyze fluorescently labeled cells in order to better quantify cell migration. The Oris™ Cell Migration Assay developed by Platypus Technologies also uses a 96-well format with small inserts in each well that can be lifted off after fluorescently labeled cells have adhered, creating a cell-free migration zone. After a defined period of time, cell migration can be quantified by applying a "migration mask" on the bottom of the 96-well plate that only allows fluorescence from migrating cells (within the migration zone of each well) to be detected by the plate reader.

Considerable progress has been made in understanding the molecular mechanisms involved in cell migration since the advent of the Boyden chamber in 1962. It is interesting to note that cell migration was only a side interest in the career of Dr. Boyden. To read an interview with Dr. Boyden, visit http://www.science.org.au/scientists/interviews/b/sb.html .

References:

1. Boyden, S. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med. 1962 Mar 1;115:453-66.