SORCERER

Chemotaxis Assay Image Analysis

Introduction

Application of IA to Cellular Migration assays.
The quantification of eukaryotic cellular migration has been greatly aided by Image Analysis technology.

Cellular migration may be assessed by a variety of techniques, many of which require enumerating cells that have migrated to a specified location or measuring the distance that subsets of cells have moved.

Image Analysis of data obtained from these assays has provided an efficient and relatively unbiased means of quantitating large volumes of migration data. Eukaryotic cells whose migration has been studied in vitro with Image Analysis include: Neutrophils, Monocytes, Lymphocytes, Endothelial cells, Retinal pigmented epithelial cells, Macrophages, and Macrophage-like cell lines.

One of the most popular methods for studying cell migration in response to stimulatory agents, involves the movement of cells through pore-containing membrane filters. In this assay, the membrane filter is situated in a chamber between two wells, one of the wells containing the stimulatory agent, and the other containing a suspension of the cells. Cells that are activated to migrate in response to the stimulatory agents migrate from the cell-surface side of the membrane, through the pores, and into or through the filter. The filters are then fixed and stained, and the responding cells are enumerated.

The in vitro conditions for migration assays vary and depend upon the cell type that is studied. In membrane filter assays, two commonly used filter types are Polycarbonate and Cellulose nitrate.

Polycarbonate membranes, which are only 10 um in thickness, are substantially thinner than cellulose nitrate membranes, which are 100-150 um in thickness

Cells that migrate through polycarbonate filters have a relatively short distance to move in order to pass through the filter, and all of the responding cells are found in one optical plane of the filter.

In contrast, cells that migrate into cellulose nitrate membranes do not move uniformly through the filter to the opposing surface, and consequently, cells are found in numerous optical planes throughout the filter.

Quantification of the total number of responding cells in cellulose nitrate filters requires summation of cells that are counted in multiple optical planes throughout the filter.

Image Analysis Hardware and Software for the enumeration of cell migration in each of these membrane filter types has been developed by Optomax.

System Overview

The Optomax SORCERER Image Analysis unit consists of a PC, High Resolution Video Camera, Image Analysis Measurement Hardware and Software.

When coupled to a light microscope, the camera produces a video image of fixed, stained cells on a membrane filter, as seen by the microscope.

Digitizing and processing this video image, the system automatically counts cells that have migrated through thin polycarbonate membranes, or partially through thick cellulose nitrate membranes.

Spreadsheet analysis of the cell count information then generates a data table and graph showing cell migration in response to stimulatory agents.

Program operation

The Image Analysis unit is programmed to detect stained cells in the video image, based on optical contrast of the cells against the filter background.

Detected cells present in the field of view are scanned and counted in approximately one second. Controlling software allows cells to be rapidly counted over multiple microscope fields, and over a number of replicate filters. In the case of thick membrane filters, cell counts are also made at a number of pre-determined depths in the filter.

The **Thin membrane program** measures Area occupied by the cells that have migrated through the filter. This technique is used in preference to a direct cell count that could be inaccurate due to frequent cell clumping encountered on the membrane. The generally accepted measurement of total cell Area divided by a previously established Mean single cell Area is utilized, and results in 'equivalent cell count ' data.

The **Thick membrane program** measures direct cell count at different depths in the membrane. Here cell clumping is normally not a problem, the migrating cells being well separated at different optical planes throughout the membrane.

Sorcerer Chemotaxis Assay Analyzer - Hardware & Software Specifications

Image Analyzer	
Video Input	625 line, 50 Hz CCIR CCD camera
Image Resolution	768 x 576 square pixel array
Detection	Matrix or Binary type
Binary Image Operators	*Remove* function digitally eliminates pore images in Polycarbonate membranes
Measurement Parameters	Cell Area or Count for Chemotaxis. Perimeter, Fiber length, Fiber width, Longest Dimension, Axial Ratio, Circularity and Spherical diameter data is also available.
Measurement Speed	Up to 1000 cells / second
Minimum cell size.	1 um
Data generated	Cell Area, Count, Size Distribution and Summary statistics.
Spreadsheet	Measurements are imported to Microsoft Excel spreadsheet for data processing, graphics display and reporting.
Computer	Minimum 733 MHz Pentium III, 128 MB RAM, 10GB / 1.44 MB drives, SVGA 1024 x 768 graphics, Mouse, Microsoft Excel, Windows 98 / NT4 / 2000 / XP.
Microscope	Transmitted Bright field illumination with, 5x, 10x, 20x, 50x biological objectives and optical coupler for video camera.

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Image Analysis Products for Science & Industry

9 Ash Street, P.O. Box 840, Hollis, NH 03049, USA.

Tel: (603) 465 3385 FAX: (603) 465 2291 E-mail:optomax@msn.com