Chemotaxis Assays in Microplates

Experimental procedure of chemotaxis assays

A chemotaxis assay is based on a time-course analysis of the *in vitro* migratory behaviour of fluorescently labelled cells. Labelling of the cells is ideally accomplished through the use of lipophylic dyes from the DiI/DiO/DiD/DiA series (Molecular Probes, Inc), or by transfection of Green Fluorescent Protein (GFP)-based vectors. For optimal performance of the assay, cells may be labelled *in vitro* with the above agents or tagged *in vivo* prior to explantation. In this latter case, cells may for instance be anterogradely or retrogradely labelled with the lipophylic dyes, lucifer yellow or fluorescently tagged dextrans. If the chemotaxis assay is intended to be applied to the analysis of *in vivo* generated progenies of a given cell, the progenitor cells may be labelled *in situ* through iontophoretic injection of cell impermeant cell lineage tracers such as the above mentioned fluorescent dextran-lysine complexes or similar fluorescent compounds.

Chemotaxis assay is also devised such as to permit the concomittant analysis of multiple cell types within a heterogenous cell population in which different cells are labeled with different fluorochromes. Thus, this implies that the assay procedure may include the combined *in vivo* and *in vitro* selective tagging of different cell types within the same cell population that needs to be examined. Furthermore, we have exploited the technique of molecular cell labelling with GFP-based transfection vectors in order to assure the restricted labelling *in vitro* of a given cell type. This may be accomplished by targeting the expression of the constructs through phenotype-specific regulatory elements, such as cell type specific promoters which are incorporated into the transfection vector.

Procedure and Characteristics of chemotaxis assays

Cells to be analysed are labelled *in vitro* or *in vivo* (prior to explantation) with any of the above mentioned fluorescent dyes, or may be labelled by molecular means by transient transfection with GFP-based vectors, with or without cell type-specific elements regulating their transcription. The latter is accomplished by either lipotransfection with the appropriate transfection agents (e.g. LipofectAMINE, DMRIE-C, SuperFect or DOSPER). For *invasion* assays, the specifically designed porous membranes are coated with selected, purified extracellular matrix molecules; thin layers of polymeric collagen type I or Matrigel according to standard protocols, prior to adding the labelled cells to be analysed.

For *transmigration* assays, on the other hand, various type of cell lines, including primary and immortalized endothelial, stromal or epithelial cells, or freshly isolated *ex vivo* cells, are grown on (or better beneath; see later) the ”membranes” to reach confluency. Labelled cells to be examined are then plated on top of this cell monolayer and fluorescent readings are performed at different time-intervals defined by the operator.
Thus, these may need to be pre-established empirically for each given cell type and/or experimental condition. At each of these time points, fluorescence is detected from top and bottom. Ideally *Bottom* fluorescence corresponds to the total number of cells plated on the lower side of the membrane”, i.e. located at their starting point. *Top* fluorescence corresponds to the total number of cells that have crossed the membrane”, i.e. are located at their end point. This procedure is more successful and more sensitive as the original reversed one as the performance of top reading is much better than the bottom reading performance of fluorometers. As the number of plated cells (starting point) is much higher than the number of migrated ones the procedure of having the star point beneath the membrane is preferred.

The currently commercially available ”Transwells™” are made of transparent plastic and contain a transparent membrane, which allows for the emitted fluorescent light to interfere between bottom and top of the Transwell™. Therefore, to be able for a physical separation of the top and bottom fluorescent signals, a special plate was devised in *black* plastic, into which a special fluorescent shielding membrane has been inserted. Plates of that type are available through Polyfiltronics (inserts are fully black, membrane is red) and similar products are available from Becton Dickinson (transparent inserts, blue membrane) and Corning Costar.

Mithras LB 940 and Twinkle LB 970

The BERTHOLD TECHNOLOGIES microplate instruments Mithras LB 940 and Twinkle LB 970 can be efficiently used with these assays as both can be equipped with fluorescence bottom reading optics.

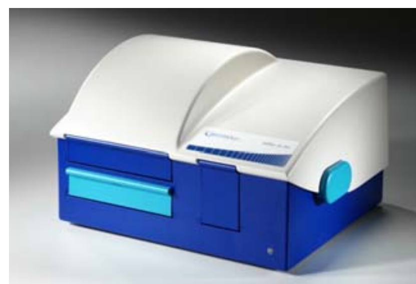

Figure 1:
Mithras LB 940 Multimode Reader Twinkle LB 970 Fluorometer