Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Material

Established and Novel Methods of Interrogating Two-Dimensional Cell Migration

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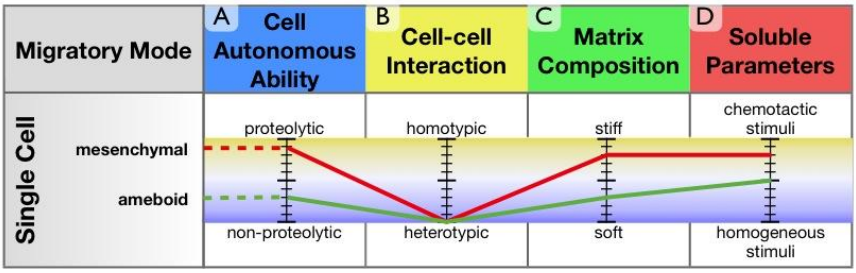
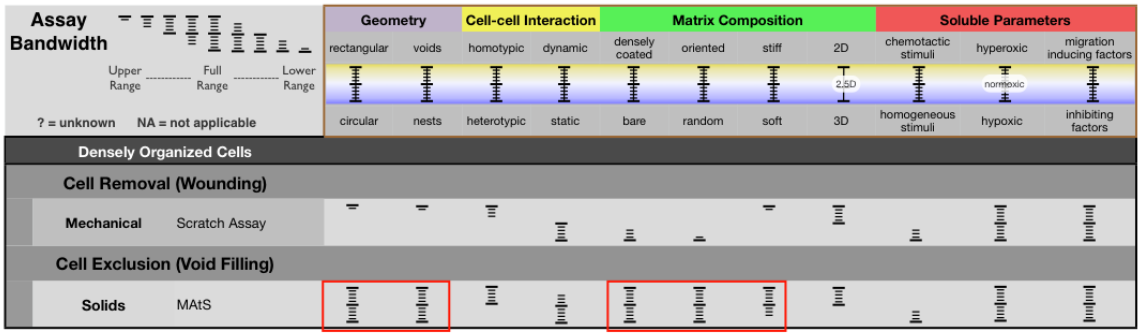


Supplemental Figure S1. The multi-scale tuning model. A multi-scale model presents multiple interdependent parameters classified under four distinct categories (cell autonomous ability, cell-cell interaction, matrix composition and soluble parameters). Individual migratory parameters are displayed in a tuning model in which the tuner ( ) represents the continuous range between two opposing properties for the same parameter. The magnitude of any parameter influences its impact on the mode and means of migration as well as the influence of related parameters. This example demonstrates the utility of the tuning model: (A) Proteolytic modification of the microenvironment promotes mesenchymal migration while non-proteolytic properties perpetuate ameboid movement. (B) Heterotypic interactions with adjacent cells facilitate the movement of individual cells while homotypic interactions generally suppress single cell movement and are associated with collective movement. (C) Stiff substrates promote movement in a mesenchymal fashion while soft substrates facilitate ameboid movement. (D) Mesenchymal motility benefits greatly from a chemotactic stimulus while ameboid movement is supported by both chemotactic and homogeneous stimuli.



Supplemental Figure S2. Assay bandwidth. In Table I a panel of assays is represented with the respective ability to assay across different geometries, and assess the contributions of cell-cell interactions, matrix composition, and soluble stimuli. The tuning model introduced in Figure 1 is used to represent the continuous range between two opposing properties for each parameter. The ability of each assay to assess a given parameter is represented as a tuner where the visible range represents the bandwidth associated with that assay. In this example the scratch assay and the MAtS assay are shown. The display of tuners for each assay readily reveal the limited range of the scratch assay: Scratch assays provide only rectangular voids which allow for analysis of migration of cells experiencing homotypic interactions under relatively static conditions. The matrix underlying the cells is damaged and largely removed by the act of scratching. Consequently, the matrix composition during this migration is “bare” and mostly “random”. This assay also requires either glass or plastic as a culture surface thereby limiting the analysis of migration of “stiff” substrates. Like most assays described herein, the scratch assay is limited to 2D arrangements of cells responding to a homogeneous stimulus of soluble factors. In contrast, the MAtS assay uses a non-destructive stencil to exclude cells rather than remove them. Consequently this assay distinguishes itself from the scratch assay primarily by the geometries that are possible and the ability to evaluate the contribution of the underlying matrix (outlined in red).

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Supplemental Table SI: Terms and definitions

Term	**Definition** (in relation to morphology and migration.)
Ameboid	Cells that exhibit an asymmetric morphology with poorly discernible leading front. These cells are generally poorly adherent to the matrix and other cells. Their migration is exclusively as isolated, individual cells.
Cell autonomous ability	The intrinsic ability of a cell to move independent of external stimuli.
Cell-cell interaction	Direct contact between adjacent cells
Densely Organized	Refers to the migration of a group of cells engaged in continuous-to-intermittent cell-cell contact.
Epithelial	Cells that exhibit a broad and flat morphology often seen as cuboidal when cells are confluent. These cell migrate with a broad leading front. These cells are typified by strong cell-cell adhesions and exhibit collective migration in sheets and strands.
Matrix composition	The composition of the extracellular matrix and substrate onto which the cells are adherent.
Mesenchymal	Cells that exhibit an elongated fibroblast-like appearance with a discernible leading front and trailing back. These cells possess weaker cell-cell adhesions and do not exhibit the collective migration than might be seen in epithelial cells.
Migration	Refers to the movement adherent cells use to mobilize themselves.
Nest	Refers to the formation of a cell population with defined dimensions in order to monitor migration from this “nest” onto adjacent cell-free surface.
Single Cell	Refers to the migration of an individual, isolated cell free of cell-cell adhesions.
Soluble Parameters	The condition and composition of the soluble environment surrounding the cell. This includes the traditional growth factors and their presentation (e.g. chemotactic gradient vs homogeneous availability) as well as gases (oxygen) and nutrients (glucose).
Void	Refers to an area on the culture surface that is left devoid of cells for the purpose of of creating an empty space into which adjacent cells can migrate.