Lecture 13:
Quantifying Cell Behavior

1. Cell Cultures

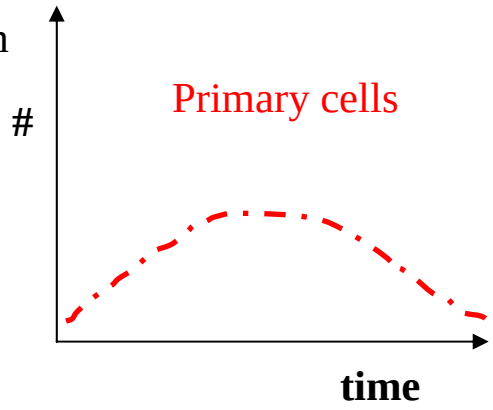
a. Why use cell cultures?

	in vivo (living organisms)	*in vitro* (glass, i.e., culture)
Pros	1. native 3D environment 2. all relevant signals present	1. simplified model systems 2. study parameters independently 3. observe as function of time
Cons	1. many variables – noisy data 2. animal rights concerns	1. unnatural 2D environment 2. may lack important signals

b. Types of cell cultures

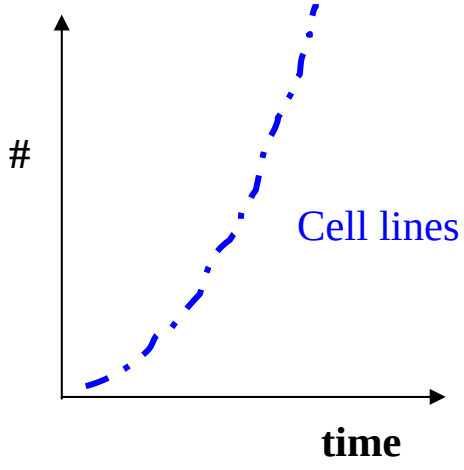
Primary cultures: directly from living organism

- ☺ reflect function of real organism
- ☹ eventually die off;
- ☹ some cell types poor dividers (nerve)



Cell lines: derived from primary cells (not clones)

- ☺ altered genes ⇒ immortality
  - isolated spontaneous mutation
  - tumor cells
  - viral oncogene transfection



2. Assays of Cell Function

- Adhesion
- Migration
- Proliferation
- Differentiation (change in phenotype, ex. blood stem cells ⇒ leukocytes)
- Secretion (protein production)

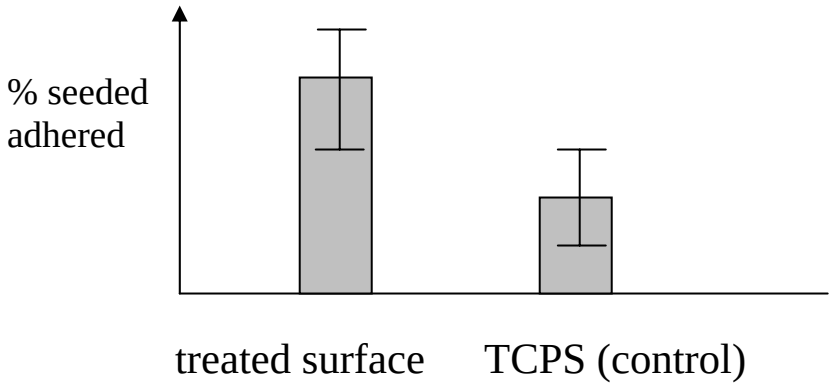
a. Cell Adhesion Assays

Importance: cell adhesion is necessary for many other cell functions—provides biophysical and biochemical stimulation

- *Sedimentation assay:*

1. Cell type seeded onto surface of interest at given density (#/area) for a specified time *in vitro*

2. Surface is gently washed, and remaining cells counted, e.g., by optical microscopy or Coulter counter (cells detached, suspended & "count" by electrical resistance change thru narrow channel)



3.For testing cell resistance, serum-containing medium is a stricter test than cells seeded in protein-free solution.

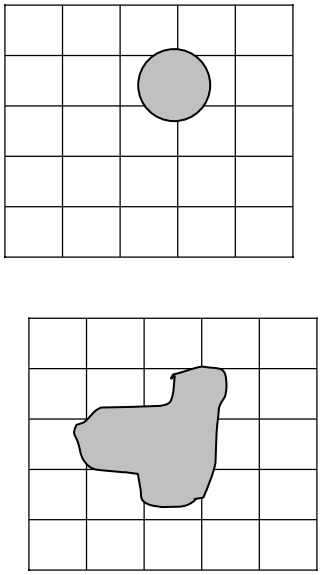
Note: plasma vs. serum
plasma—liquid portion of blood (cells removed)
serum—plasma with coagulants (e.g., FGN) removed

⇒ Sedimentation assays give no info. on strength of adhesion.

➢ *Cell Spreading Assay*

1. Cell type seeded onto surface of interest at given density (#/area) for a specified time

2. Measure projected surface area (optical microscopy)



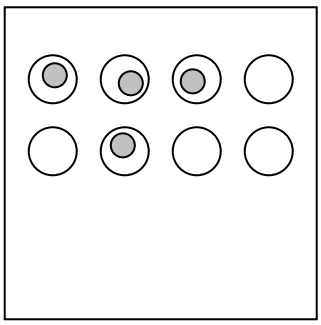
Closer in spirit, but still not a direct adhesion measurement

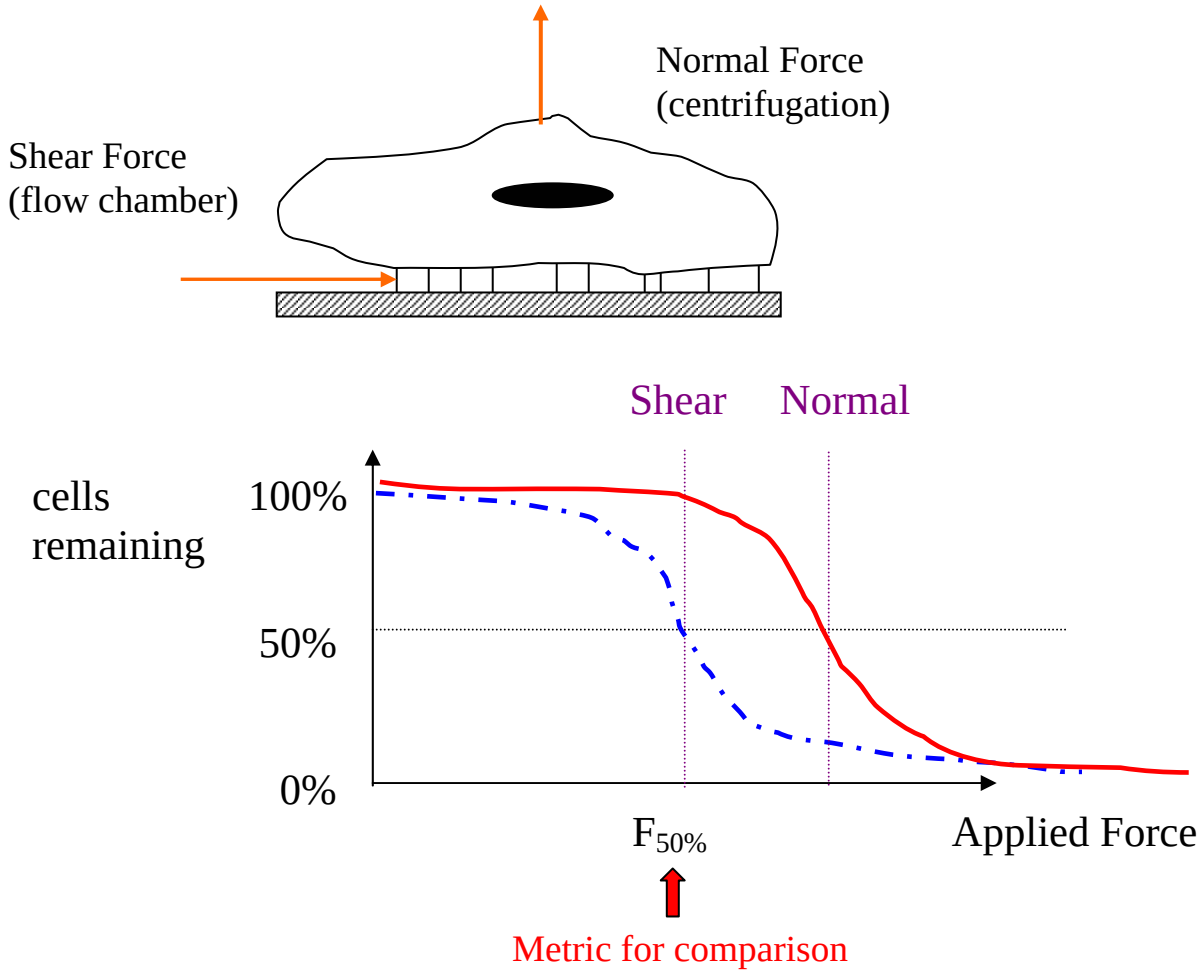
Spreading indicates focal contact formation—metabolic activity

➢ *Centrifugation assay: (normal force)*

1. Cell type seeded into 24-well plate with surface coating for a specified time

2. Plate is inverted in centrifuge and cells attached vs. applied force is measured

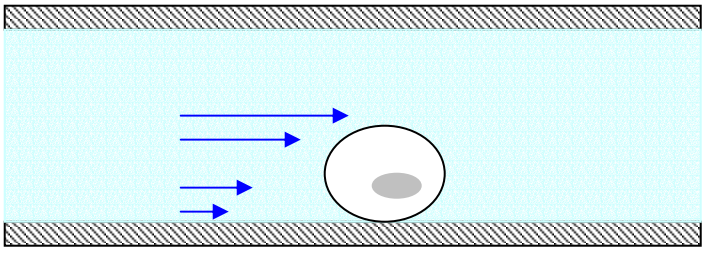




- *Flow Chamber assay: (shear force)*

1. Cells seeded into parallel plate chamber

2. Fluid flow velocity gradient results in shear force on cell-surface bonds – pop like a "seam"



b. Cell Migration Assays

Importance:

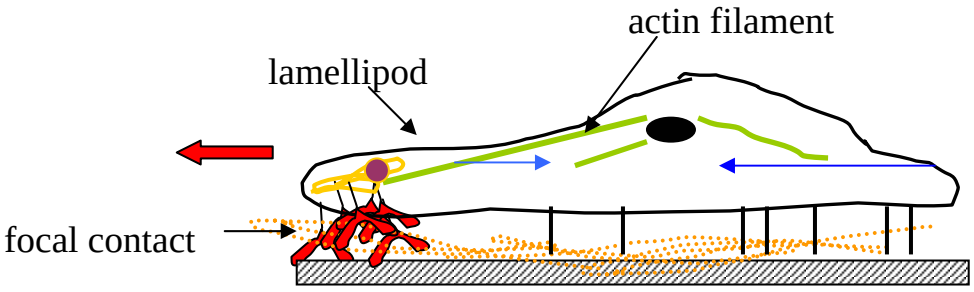
- tissue organization (embryonic)
- immune and inflammatory response (chemotaxis of white blood cells)
- angiogenesis—endothelial cell migration to form vasculature
- wound healing—fibroblast migration to form connective tissue
- tumor metastasis

How do cells migrate?

Actin polymerization in cytoskeleton ⇒ "lamellipod" extension

Contractile force generated on actin filaments

Force translated to substrate through *focal contacts*
⇒ net translation



Over short times: cell motion appears directional

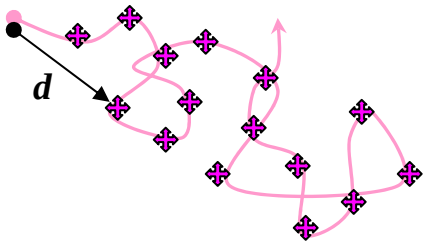
Over long times: cell motion is analogous to a diffusive process
(in absence of chemical gradients or physical barriers)

Quantification approaches:

1. individual cell measurements
2. migration of cell populations

- ***Individual Cell Measurements*:**

Time-lapse videomicroscopy tracks cell motion (beneath fluid or gel) on a surface as a function of time elapsed.



1. Measure distance traveled from starting pt. (*d*) as a function of time (*t*)

2. Fit data to model to obtain *rms speed* (S) and *persistence time* (P) (cell dependent)

P: time before memory of initial direction is lost (typically min-hrs)
S: measure of centroid displacement per time (typically 1-50 μm/h)

Persistent Random Walk Model: $\left\langle d(t)^2 \right\rangle = nS^2 \left[Pt - P^2 \left(1 - e^{-t/P}\right) \right]$

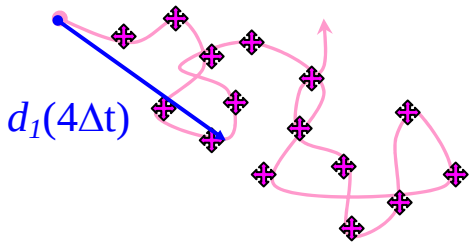
# of dimensions (2)

where $\left\langle d(t)^2 \right\rangle = \frac{1}{M} \sum_{i=1}^{M} d_i(t)^2$

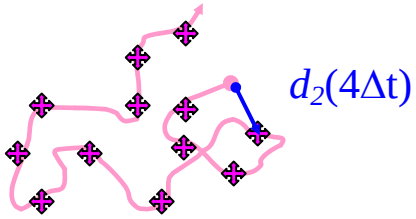
M = # of measurements

Note: $\langle \boldsymbol{d}(t) \rangle \equiv 0$, $t >> P$
Why?

M = # of cells if each time step used as a single data point



Cell 1 path

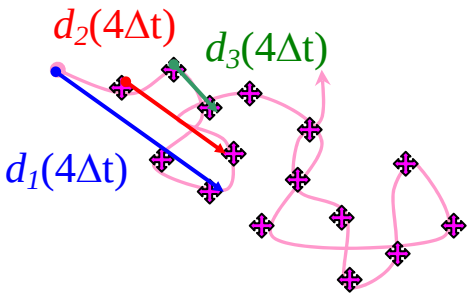


Cell 2 path

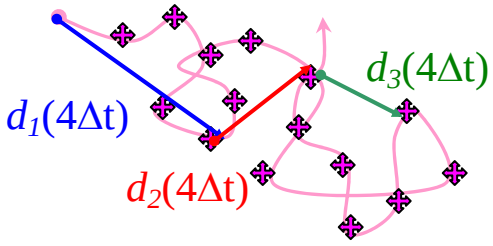
Requires measuring many cell paths for good statistics (TEDIOUS!!)

Alternately, we can use data from a *single cell:*

Strategy (i): count each pt. on its trajectory as a "starting pt." (all sampling points are equivalent)



Strategy (ii): break migration path into M = *N/j* segments of $j\Delta t$ steps (*N*=total # of sampling intervals Δt)



Fitting to the Persistent Random Walk Model

$$\left\langle d(t)^2 \right\rangle = nS^2\left[Pt - P^2\left(1-e^{-t/P}\right)\right]$$

For short times $t<P$:

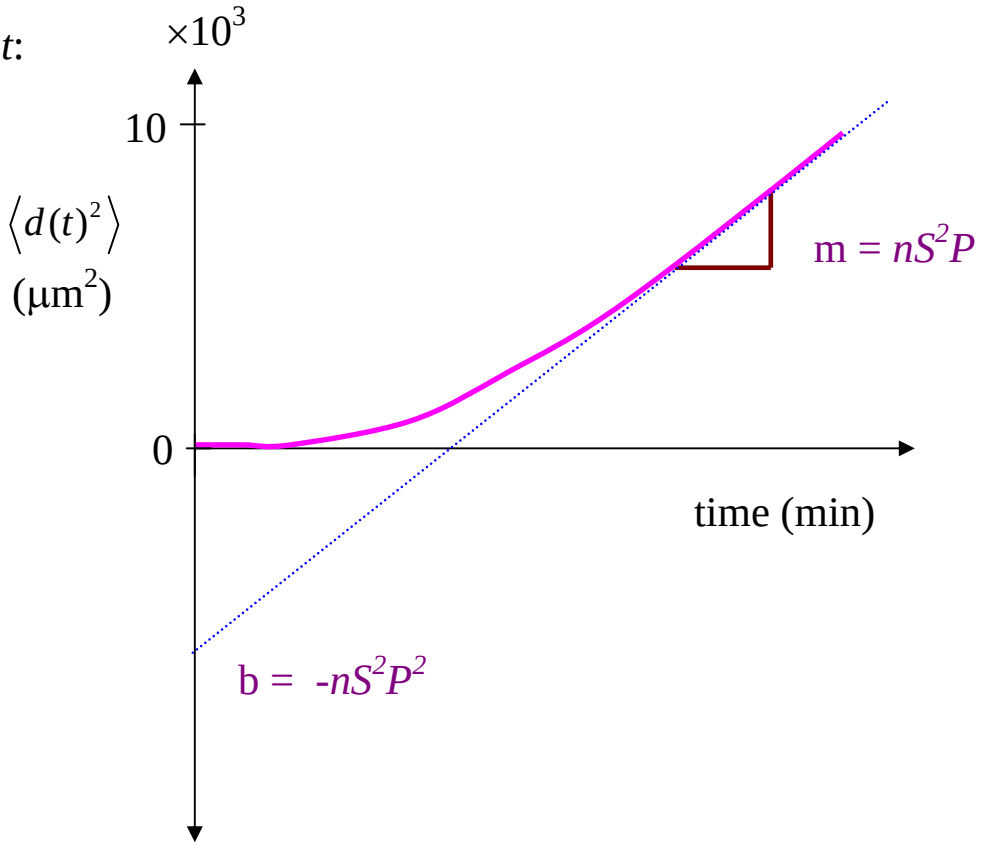
$$\exp(-ax) \approx 1 - ax + \frac{(ax)^2}{2} - \ldots$$

$$\left\langle d(t)^2 \right\rangle = S^2t^2$$

or $\left\langle d(t)^2 \right\rangle^{1/2} = St$ distance traveled = speed × time (directional motion)

For long times $t>P$: $\left\langle d(t)^2 \right\rangle = nS^2Pt - nS^2P^2$

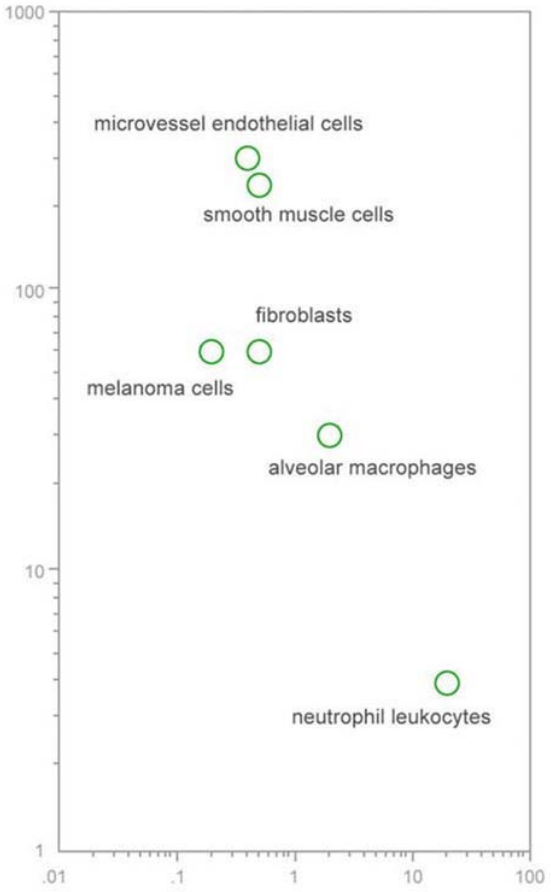
Plotting $\left\langle d(t)^2 \right\rangle$ vs. t:



slope = nS^2P
y-intercept = $-nS^2P^2$

$\Rightarrow$ *values of P & S*

Persistence time (*P*) (min)



Speed (*S*) (μm/min)

Comparison of *P* & *S* for different cell types migrating on same substrate…

How do speeds relate to event sequence in the inflammatory response?

Figure by MIT OCW.

From D.A. Lauffenburger & J.J. Linderman, *Receptors: Models for Binding, Trafficking and Signaling*, Oxford U. Press, 1993.

For $t >> P$: $\left\langle d(t)^2 \right\rangle = nS^2Pt$

or $\left\langle d(t)^2 \right\rangle^{1/2} = S\sqrt{nPt}$

distance traveled ~ time$^{1/2}$ (diffusive motion)

Analogous to the diffusion coefficient (D) for atoms and molecules, we can define a motility coefficient, μ, for cells:

$\mu = S^2P/n \quad \Rightarrow \left\langle d(t)^2 \right\rangle = n^2\mu t \quad$ or $< d^2 >^{1/2} = \sqrt{4\mu t}$ for 2d

Typical values:
μ ~ 10^{-9}-10^{-8} cm^2/sec

Chemotaxis

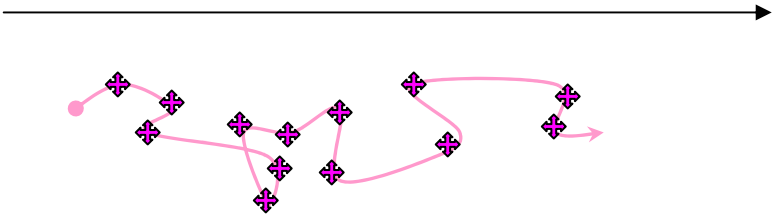
If chemotactic or haptotactic (surface-bound signaling) agent is present, mean displacement $\langle \boldsymbol{d}(t) \rangle$ is nonzero.

We can quantify the degree of chemotacticity of a migration with the *Chemotactic Index*, CI:

$$CI = \frac{\left| < \mathbf{d}(t) > \right|}{L_{path}} \left\{ 1 - \left(\frac{t}{P} \right)^{-1} \left[1 - e^{-t/P} \right] \right\}^{-1}$$

where $\langle \boldsymbol{d}(t) \rangle$ is the mean displacement up a concentration gradient, and L_{path} is the *total cell path (contour) length*.

Concentration gradient of chemotactic agent in x-direction

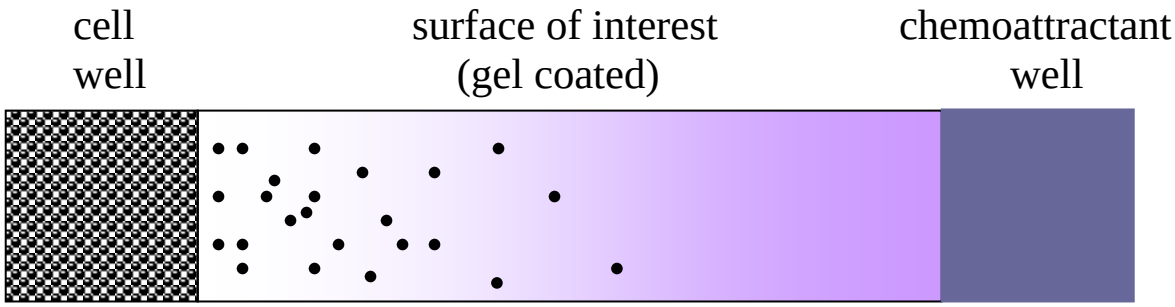


For long times ($t >> P$): $CI = \dfrac{\left| < \mathbf{d}(t) > \right|}{< L_{path}(t) >}$ $\qquad (0 < CI < 1)$

➢ *Migration of Cell Populations*

- Cells are seeded in a well (typically under a gel) from which they migrate

- cell density (*c*, cells/area or cells/volume) is measured at various distances from well at different times



Governing expression for cell density, *c* (cells/area):

$$\frac{\partial c}{\partial t} = -\frac{\partial \mathbf{J}_c}{\partial x}$$

where $\mathbf{J}_c$ is cell flux (cells/distance-time)

$$\frac{\partial c}{\partial t} = -\frac{\partial}{\partial x}\left[-\mu\frac{\partial c}{\partial x} + c\left\{-\frac{1}{2}\frac{\partial \mu}{\partial L} + \chi\right\}\frac{\partial L}{\partial x}\right]$$

L = chemotactic agent conc. (mol/liter)

χ = chemotaxis coefficient (area/time-M)

random-walk motion

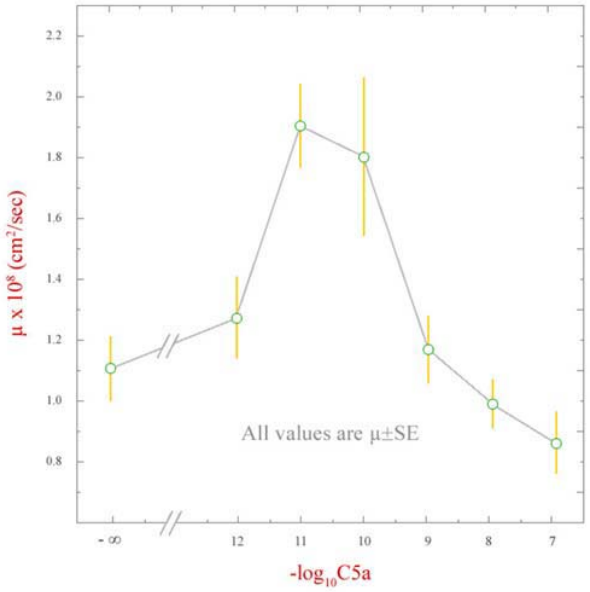
μ-dependence on chemotactic agent concentration

directional bias from chemotactic agent

χ related to single cell properties thru: $\chi = \frac{S \cdot CI}{\nabla L} - \frac{1}{n}\left(\frac{d \ln P}{dL} - \frac{d \ln S}{dL}\right)$

Cells can exhibit chemotaxis in any situation where μ exhibits concentration dependence.

Macrophage motility coefficient μ dependence on C5a concentration



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$$\frac{\partial c}{\partial t} = -\frac{\partial}{\partial x}\left[-\mu\frac{\partial c}{\partial x} + c\left\{-\frac{1}{2}\frac{\partial \mu}{\partial L} + \chi\right\}\frac{\partial L}{\partial x}\right]$$

A fit of cell data to the model requires a solving the above equation for the appropriate boundary conditions.

Example: For random cell motion with continually replenished cells:

$c(0,t) = c_o \quad t > 0$

$c(\infty,t) = 0$

$$\frac{c(x,t)}{c_o} = erfc\left(\frac{x}{\sqrt{4\mu t}}\right)$$

can determine μ from fitting c(x,t) data

obtain μ(L) for different L values