Towards a New Generation of Chemotaxis Experiments

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Objectives:

• quantitative study of cell motion and intracellular response
• computer controlled environment and development
• characterization cell/cell variability
• application and development of imaging technology.
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CRAC:GFP in AX3 cells, 6h development

micropipette with 1 µM cAMP
id ~ 1-2 µm.

Confocal fluorescence
exc. 488 nm, emi. > 515 nm.
pinhole wide open, gain high
Olympus 40X, NA 1.15
water objective.
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• cell lights up in front - is it always translocation?
• what is the optics?
• what does it mean: pinhole is wide open?

Some facts about visualizing cells

• cells are transparent - can’t be imaged
  cells are like lenses - index of refraction changes only.
• light waves have amplitude and phase
  ~ amplitude * cos (kx-wt + phase)
• use clever techniques to change phase and visualize cells
  phase contrast, DIC, …

In a strict sense: this is not imaging!
poor man’s phase contrast

- When shallow cell is in focus it is not visible.
- When cell is out of focus the cell is visible.

=> near field diffraction pattern of the cell

Epi-fluorescence imaging

- index of refraction changes can lead to diffraction (caustics “lines at bottom of a pool”)
- cell shape changes may lead to out of focus effects (poor man’s phase contrast)
- could lead to apparent translocation signal.

To make sure have “active” marker and passive cytosolic dyes with different emission freq. (see for example talk by Tobias Meyer.)
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Confocal Microscopy:

- size of pinhole restricts depth of field
- small pinhole ==> little light
- whole region is excited in fluorescence

Better: two Photon Microscopy

- use two photon fluorescence in focus
- use infra red light (less bleaching)
- only focal region is excited
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However:

- cells are not homogeneous
- index of refraction change inside and outside may act like lenses (caustics, Talbot effect).
- three dimensional structure of cell could lead to apparent focusing of light.
  - index-match buffer
  - measure z-sections
  - use passive and active markers …

Slug (18h)

Slug in buffer: cotb:gfp marks prespore cells

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- How do we apply a chemo-attractant gradient?
- What gradients are there?

Types of Gradients

- **Purely spatial**
  - [cAMP]
  - Steady state spatial gradient

- **Purely temporal**
  - [cAMP]
  - Concentration changing with time.
Types of Gradients (cont)

Mean concentration changing with time keeping spatial gradient constant.

Spatial gradient changing with time with mean remaining constant.

Mean concentration and spatial gradient both changing with time.

Requirements at the single cell scale

- controllable time evolution
- adjustable spatial gradients
- easy change of solutions
- controlled “history”
- easy to build
- biocompatible
- ideal for optical observation
- replicable in any laboratory
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History of chemokineses and chemotaxis assays:

- 1888 Leber: describes chemotaxis
- 1917 Comandon: chemotaxis towards bacteria
- 1920s-1930s Lewis: cell shape change
- Dixon and McCutcheon: tracking of paths, chemotaxis index
- 1962 Boyden: filter assay (cellulose)
- 1970s: polycarbonate filters
- 1975 Nelson: agarose gel assay
- 1977 Zigmond: orientational assay
- 1982-83: collagen and fibrin filters
- 2002 Jeon et al: microfabricated devices
- since 1980s: computer aided tracking of cells


Boyden Filter Assay

- 120 mm
- buffer
- cellulose ester filters
- chemo-attractant

- how many cells make it through the filter
- good for identifying new chemotactic factors
- modification to count only the leading front
- many filters
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Polycarbonate Filter Assay

- filter has holes
- cells move along the top surface and through the hole
- cell adhesion can be a problem
- overlayer with monolayer of cells

Collagen or Fibrin Gels Invasion Assay

- watch migration into gel (video)
- gels can have preferred orientations
- cell adhesion may be important
- sometimes overlayer with monolayer of cells
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Fig. 3. (a) Morphology of a lymphocyte migrating through a collagen gel. (b) Lymphocytes in an aligned collagen gel. The top of the picture shows cells on the upper surface of the gel. The sharp line running diagonally from left to right is the edge of the gel. Below this line is the interior of the gel and it can be seen that most of the collagen fibres run parallel to the edge. Lymphocytes within the gel show preferential migration in the direction of this axis (Wilkinson and Lackie, 1983) (Courtesy of Dr Wendy Haston).

Polarisation Assay

Fig. 5. (a) Human blood platelets showing spherical morphology of untreated cells. (b) Human blood platelets showing round morphology after 30 min in formyl-Met-Leu-Pho (10^-7 M). From Haston and Wilkinson (1983).
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**Polarisation Assay**

Fig. 7. Correlation between polarization and invasion of collagen gels by lymphocytes responding to synovial fluids from patients with rheumatoid arthritis (○) and other forms of arthritis (other symbols). $r = 0.85$. (From Al-Mughales et al., 1996).

**Orientation Assays: Zigmond Chamber**
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Orientation Assays: Korohoda


Orientation Assays: Korohoda

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Orientation Assays: Korohoda


Orientation Assays: Korohoda

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Orientation Assays: Korohoda

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Orientation Assays: Tani


Orientation Assays: Tani

Summary so far

- temporally changing gradients
- stationary gradient possible
- cells see history
- single cell imaging possible

Micro-Pipette Assay

- difficult to reproduce quantitatively
- hard to measure response of many cells under identical conditions
- poorly defined gradient
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**Micro-Fabricated Devices**

**Promise:**
- controllable time evolution
- adjustable spatial gradients
- controlled change of solutions
- controllable “history”
- easy to build
- biocompatible
- ideal for optical observation
- replicable in any laboratory

Pyramidal Microfluidic Networks:

- arbitrary shape of gradient
- discontinuous (jumps) gradients
- multiple components
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Serpentines:
- $h = 45 \text{ m}$
- $w = 45 \text{ m}$
- $l = 9.25 \text{ mm}$

Channels:
- $h = 45 \text{ mm}$
- $w = 100 \text{ mm}$
- $l = 375 \text{ mm}$

We have:
- $B+1 = B+1$
- $B+1 = B-V + 1+V$
- $I = (B-V)(B+1) + (1+V)/(B+1)$

left $\quad$ right
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Figure 4. Neutrophil chemotaxis in heterogeneous linear IL-8 gradients. Cells and hill-type gradients were obtained by placing two linear gradient generators in parallel. At the beginning of each experiment, neutrophils were placed as a band along the side of the migration chamber that was exposed to zero IL-8. Digital images of neutrophil migration were obtained at 20-s intervals and recorded as cells were subjected to a hill-type gradient. (A) Neutrophils at the end (60 min) of exposure to a hill-type gradient. Bar, 200 μm. (B) Tracks of cells exposed to hill-type gradient. (C) Tracks of cells exposed to a hill-type gradient. (D) Snapshots of a single neutrophil (arrow) that reversed direction after it overshooted a hill-type gradient. Bar, 20 μm. (E) Migration speed of an individual cell in hill gradients as a function of channel width. Speeds were calculated from individual cell track data that were obtained every 20 s and filtered using Savitzky–Golay algorithm (Biorad, WaveMetrics, Lake Oswego, OR).

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**Optical Lithography Technique**

- **Mask**
  - Photoresist on mask
  - Chrome etch
  - Resist strip
  - Development bath
- **Wafer**
  - Spin photoresist on Si-wafer
  - Silicon wafer
- **Expose**
  - UV light
  - Etch
- **Mold**
  - PDMS photoresist wafer
  - Replica
  - Connect: glass, tubing
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Apply cAMP at opposite sides with a controlled time interval \( \Delta t \) between them.

Three Channel Flow

- \( c_{cAMP} \)
- \( v_{buffer} \)
- \( c_{cAMP} \)

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Camilla Voelz
(CU)
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Three Channel Flow

caged \( v_{cAMP} \) \hspace{1cm} v_{buffer} \hspace{1cm} caged \( v_{cAMP} \)

Water jet

Black ink

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outlet

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Three Channel Sweeper

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Three Channel Sweeper

Multiphoton imaging: Olympus 20X NA 0.5 W, $\lambda_{\text{ex}} = 780$ nm. $\Delta Z = 1\, \mu m$, 50 steps

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Three Channel Sweeper

Multiphoton imaging: Olympus 20X NA 0.5 W, $\lambda_{\text{ex}} = 780$ nm. $\Delta Z = 1\, \mu m$, 50 steps

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Single Cell Gradient Chamber

channels: 20 micron x 20 micron
5 mm long

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Single Cell Gradient Chamber

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Summary

- optics needs to be understood
- traditional chemotaxis chambers not optimal.
- microfluidic devices promise unprecedented control and repeatability

groups: Berkeley, Cornell, Harvard, MIT, UCSD,....
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THANKS!

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