Towards a New Generation of Chemotaxis Experiments

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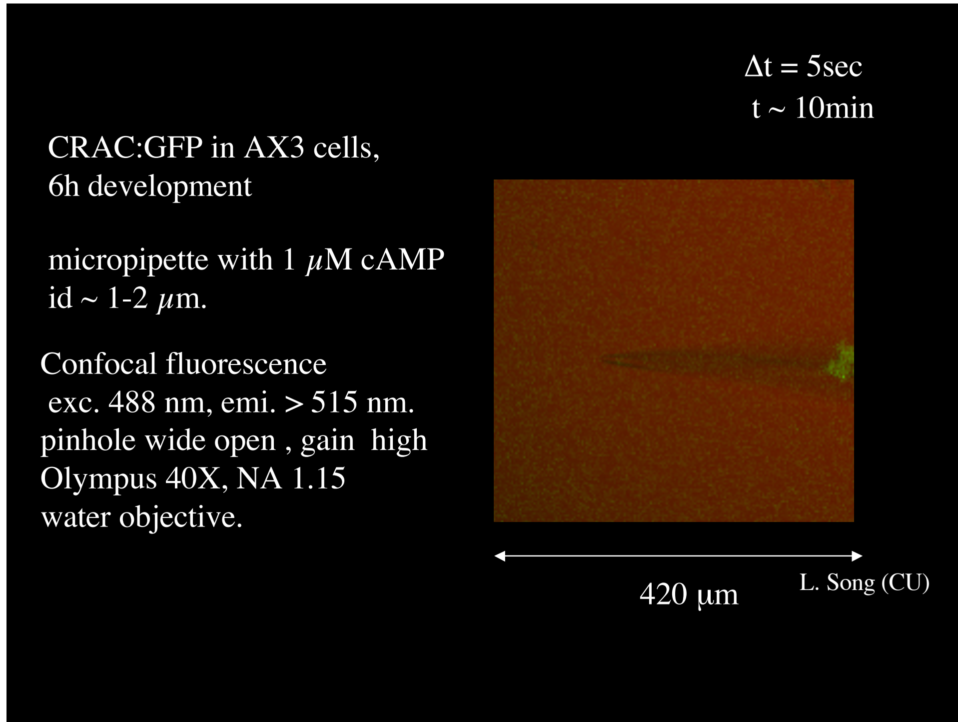
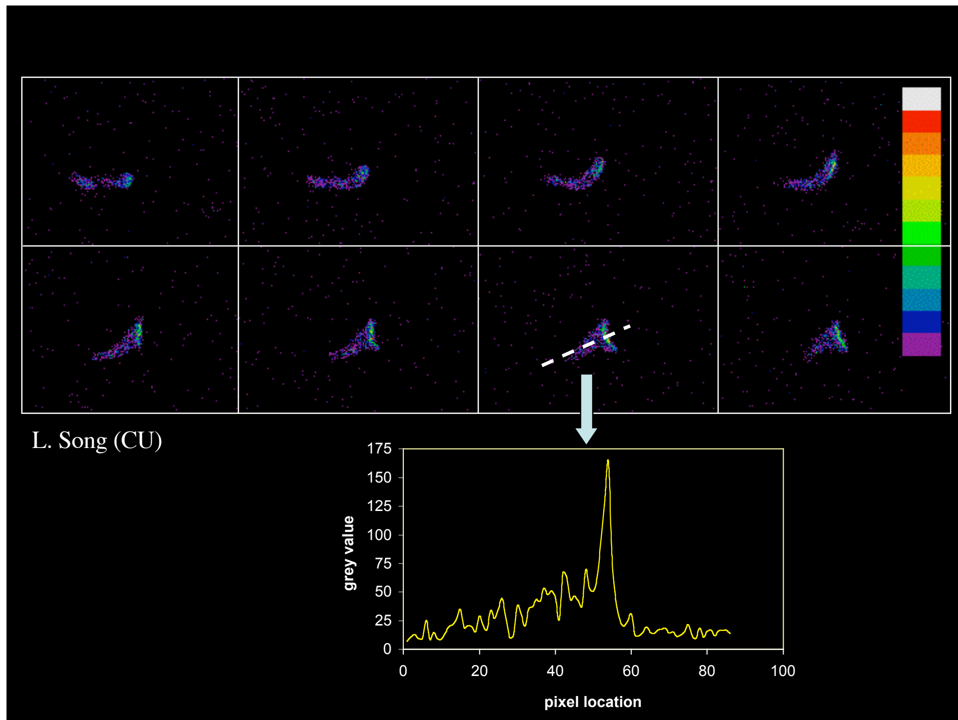
Objectives:

- quantitative study of cell motion and intracellular response
- computer controlled environment and development
- characterization cell/cell variability
- application and development of imaging technology.


Δt = 5sec
t ~ 10min
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.
420 μm
L. Song (CU)


L. Song (CU)
grey value
pixel location
0
25
50
75
100
125
150
175
20
40
60
80
100

- 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

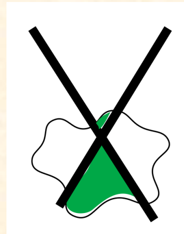
Epi-fluorescence imaging

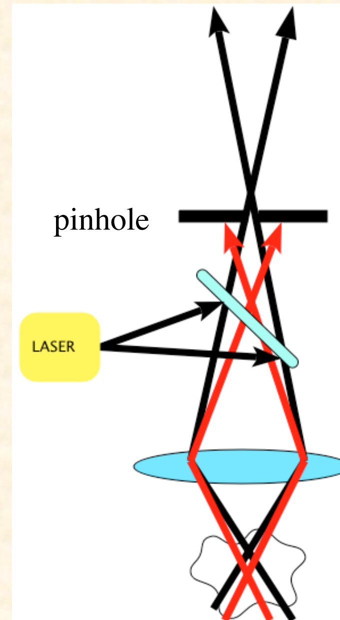
- index of refraction changes can lead to diffraction (caustics "lines at bottom of a pool")
- cell shape changes may lead to out of focus effects (poor man's phase contrast)
- could lead to apparent translocation signal.

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

Confocal Microscopy:

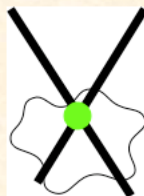
- size of pinhole restricts depth of field
- small pinhole ==> little light
- whole region is excited in fluorescence

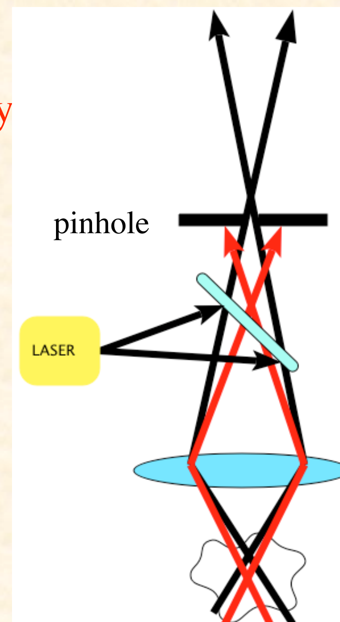




Better: two Photon Microscopy

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



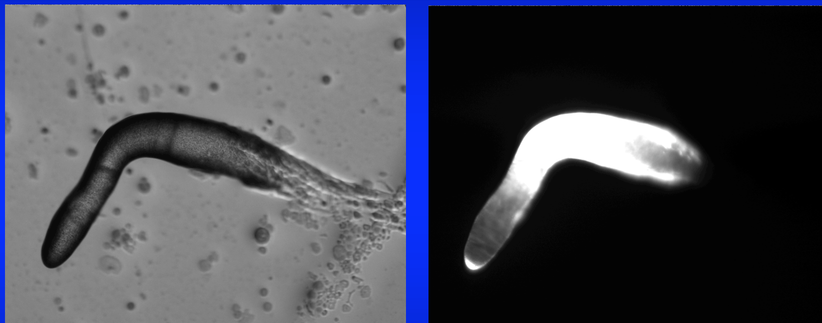


However:

- 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)



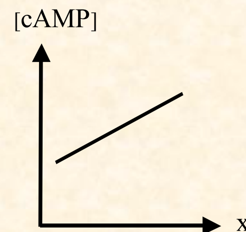
I. Rafols (CU)

Slug in buffer: cotb:gfp marks prespore cells

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

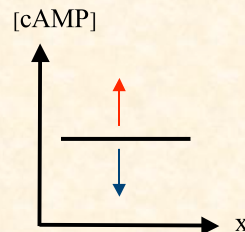
Types of Gradients

Purely spatial



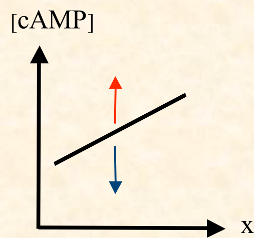
Steady state
spatial gradient

Purely temporal

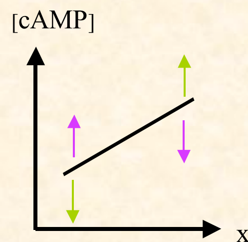


concentration
changing with time.

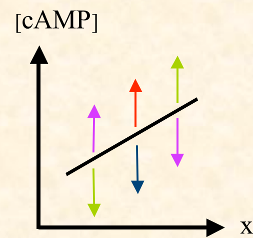
Types of Gradients (cont)



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

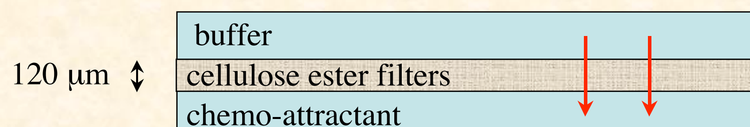
History of chemokineses and chemotaxis assays:

1888 Leber:	describes chemotaxis
1917 Comandon:	chemotaxis towards bacteria
1920s-1930s Lewis: Dixon and McCutcheon:	cell shape change tracking of paths, chemotaxis index
1962 Boyden: 1970s:	filter assay (cellulose) polycarbonate filters
1975 Nelson:	agarose gel assay
1977 Zigmond:	orientational assay
1981 Gerisch and Keller:	micropipette studies of single cells.
1982-83:	collagen and fibrin filters
2002 Jeon et al:	microfabricated devices
since 1980s:	computer aided tracking of cells

(P.C. Wilkinson, J. Immun. Methods 216 (1998) 139-153)

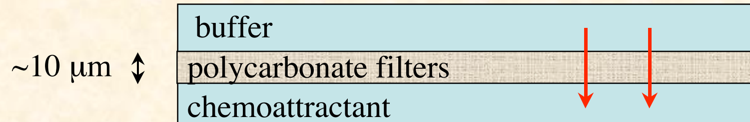
Boyden Filter Assay

120 μm ↕



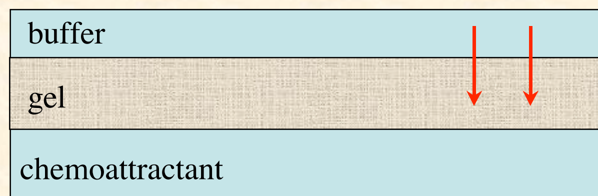
- how many cells make it through the filter
- good for identifying new chemotactic factors
- modification to count only the leading front
- many filters

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

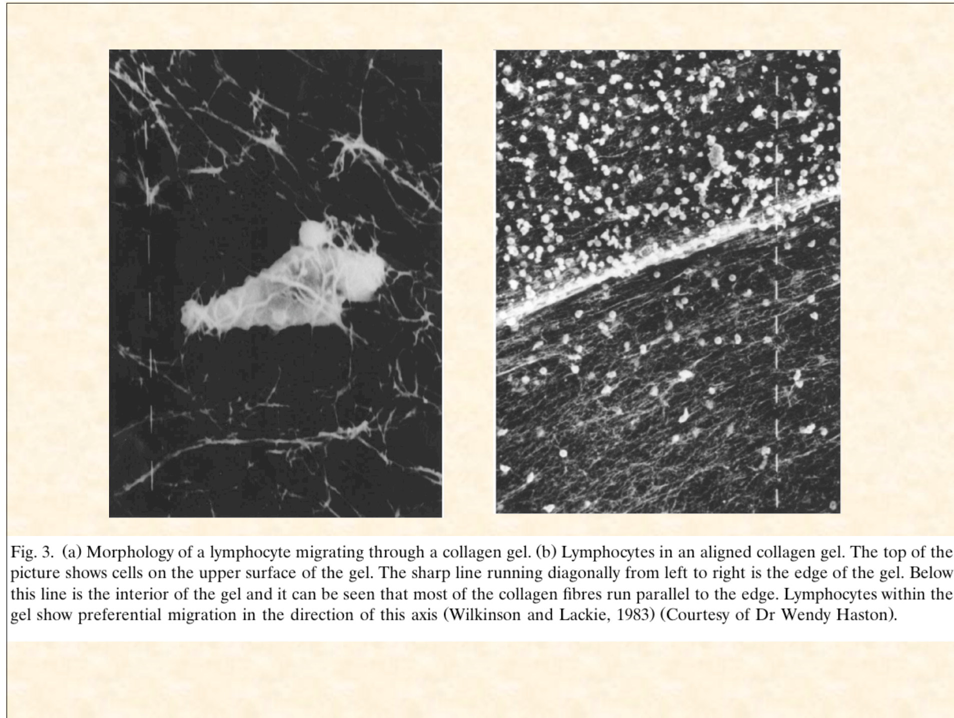


Fig. 3. (a) Morphology of a lymphocyte migrating through a collagen gel. (b) Lymphocytes in an aligned collagen gel. The top of the picture shows cells on the upper surface of the gel. The sharp line running diagonally from left to right is the edge of the gel. Below this line is the interior of the 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

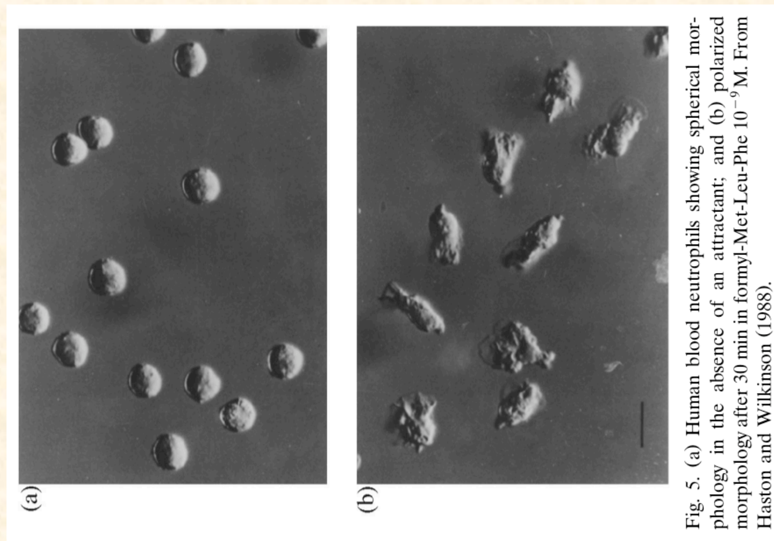


Fig. 5. (a) Human blood neutrophils showing spherical morphology in the absence of an attractant; and (b) polarized morphology after 30 min in formyl-Met-Leu-Phe 10^{-9}M. From Haston and Wilkinson (1988).

Polarisation Assay

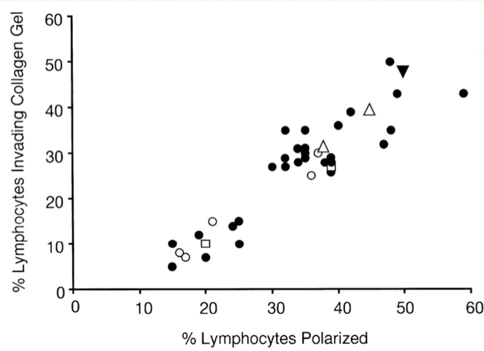
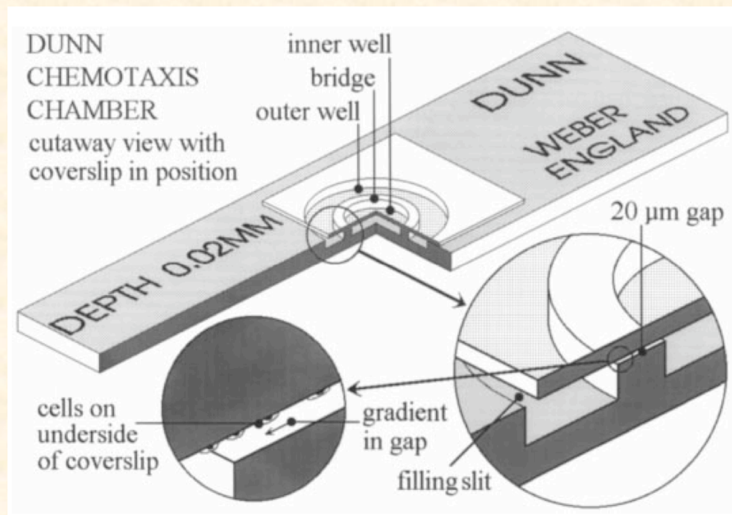
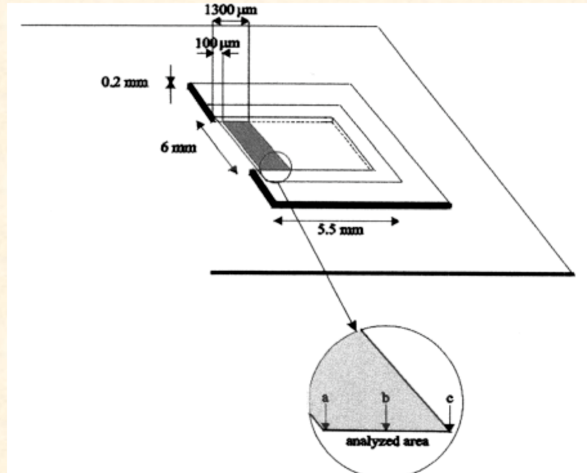


Fig. 7. Correlation between polarization and invasion of collagen gels by lymphocytes responding to synovial fluids from patients with rheumatoid arthritis (○) and other forms of arthritis (other symbols). $r = 0.85$. (From Al-Mughales et al., 1996).

Orientation Assays: Zigmond Chamber

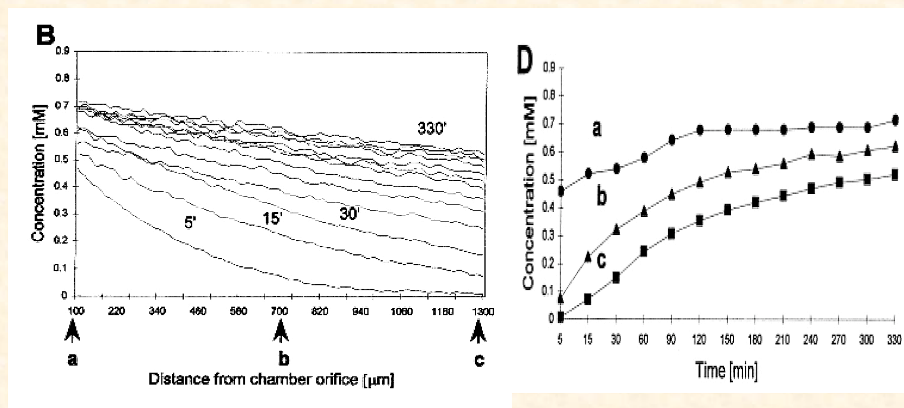


Orientation Assays: Korohoda



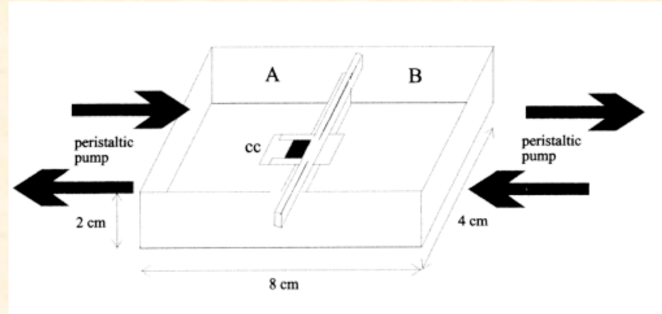
W. Krohoda et al., Cell Mot. Cytoskel. **53** (2002) 1-25

Orientation Assays: Korohoda



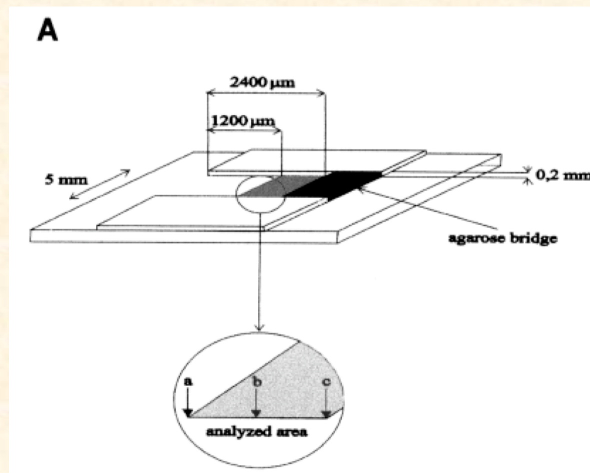
W. Krohoda et al., Cell Mot. Cytoskel. **53** (2002) 1-25

Orientation Assays: Korohoda



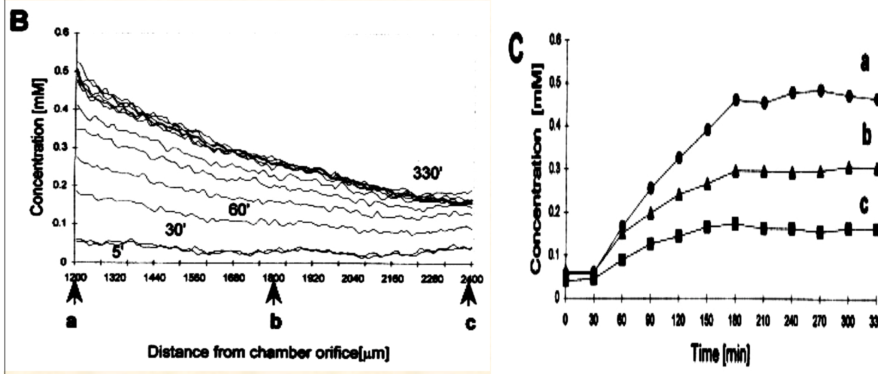
W. Krohoda et al., Cell Mot. Cytoskel. **53** (2002) 1-25

Orientation Assays: Korohoda



W. Krohoda et al., Cell Mot. Cytoskel. **53** (2002) 1-25

Orientation Assays: Korohoda



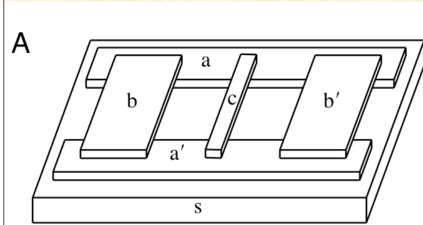
W. Krohoda et al., Cell Mot. Cytoskel. **53** (2002) 1-25

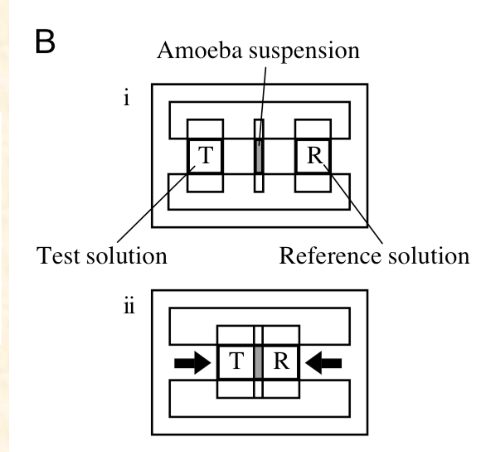
Orientation Assays: Korohoda

Parameters (± SEM)	cAMP (+Ca^{2+},+Mg^{2+}, +cAMP, starved) Control	Gradient	Gradient
Total length of cell trajectory (μm)	431.2 ± 7.9	535.5 ± 8.7*	454.2 ± 11.3^{NS}
Average speed of cell movement (μm/min)[a]	21.6 ± 0.4	26.7 ± 0.5*	22.7 ± 0.6^{NS}
Total length of cell displacement (μm)	70.8 ± 7.9	357.7 ± 11.9*	121.1 ± 10^{NS}
Average rate of cell displacement (μm/min)[b]	3.5 ± 0.4	17.9 ± 0.6*	6.0 ± 0.5^{NS}
Coefficient of movement efficiency (CME)[c]	0.16 ± 0.01	0.66 ± 0.01*	0.3 ± 0.01^{NS}
Average directional cosine ($\Sigma_n \cos \beta/n$)[d]	−0.02 ± 0.01	−0.60 ± 0.01*	0.01 ± 0.01^{NS}
Average directional cosine ($\Sigma_n \cos \gamma/n$)[e]	−0.13 ± 0.01	−0.82 ± 0.01*	−0.03 ± 0.01*
McCutcheon index[f]	−0.01 ± 0.01	−0.65 ± 0.01*	0.01 ± 0.01^{NS}
Levy parameter[g]	1.1	1.7	1.3
	spatio-temporal		spatial

W. Krohoda et al., Cell Mot. Cytoskel. **53** (2002) 1-25

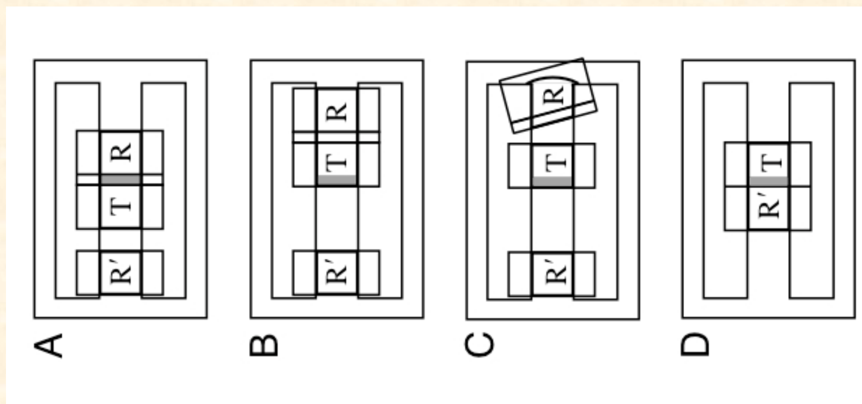
Orientation Assays: Tani





Tani & Naitoh J. Exp. Bio. **202** (1999) 1-12

Orientation Assays: Tani

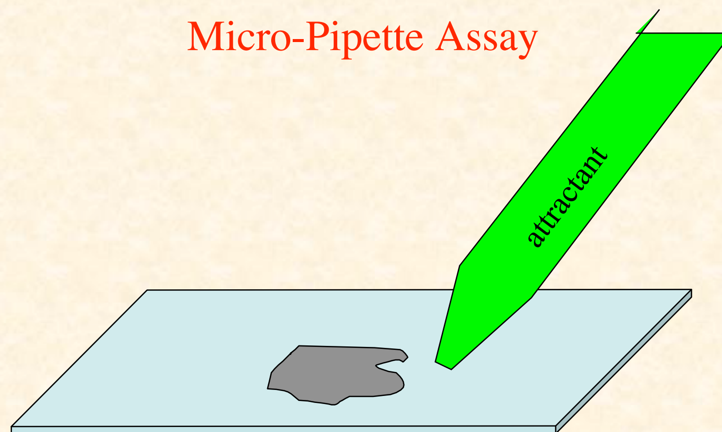


Tani & Naitoh J. Exp. Bio. **202** (1999) 1-12

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

Micro-Fabricated Devices

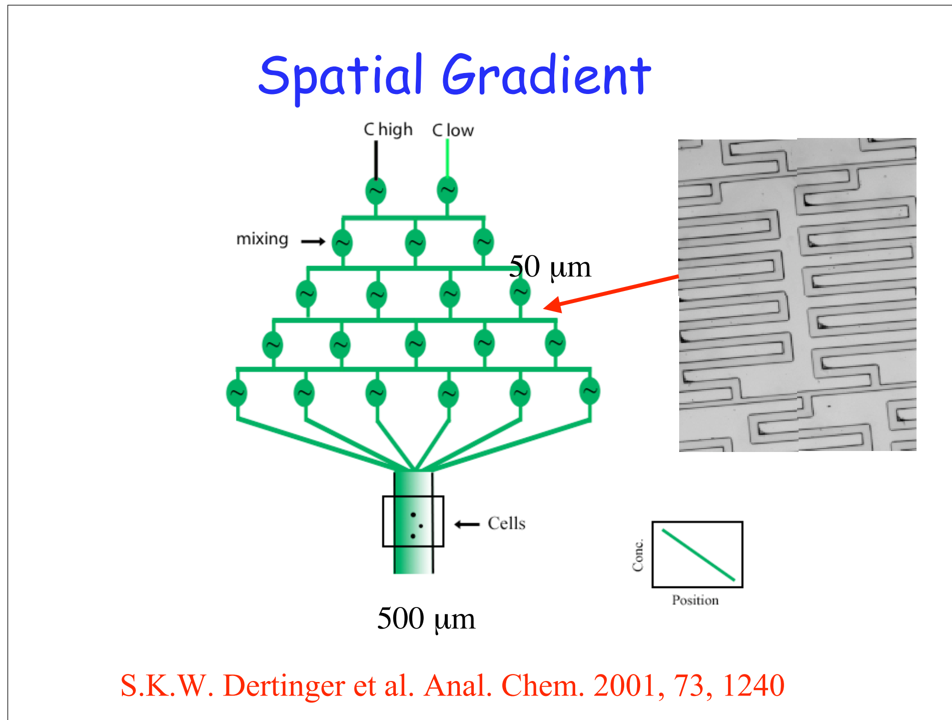
Promise:

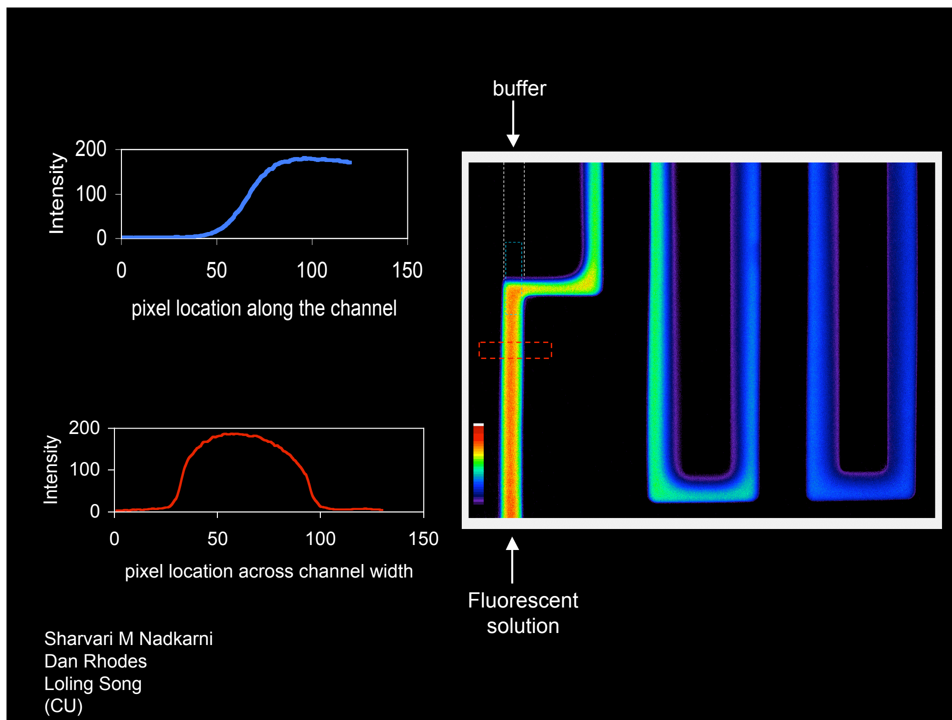
- controllable time evolution
- adjustable spatial gradients
- controlled change of solutions
- controllable "history"
- easy to build
- biocompatible
- ideal for optical observation
- replicable in any laboratory

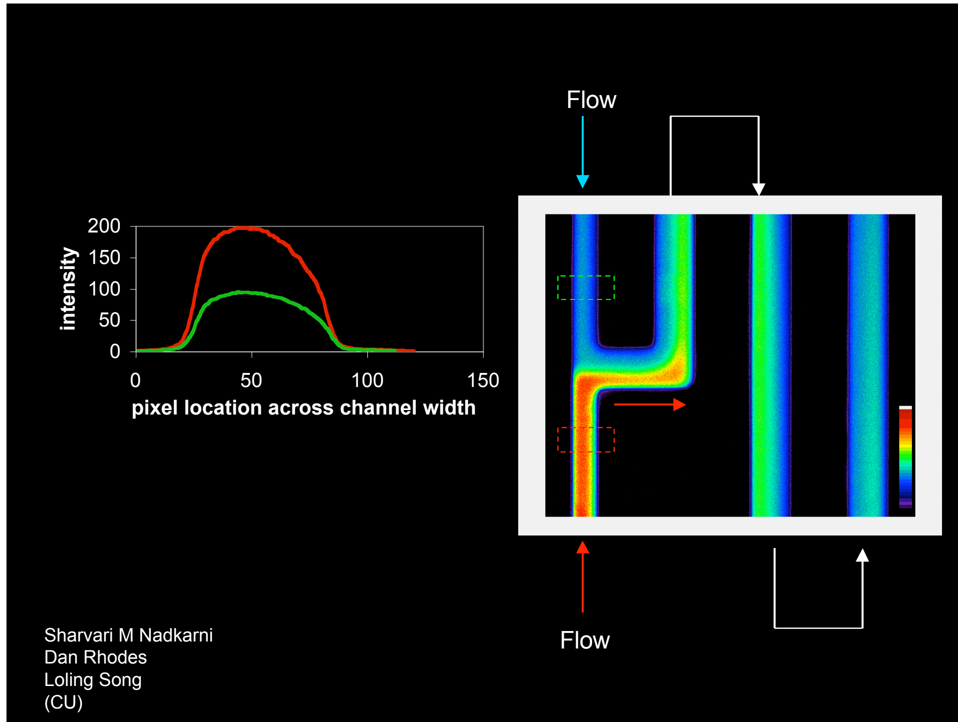
Pyramidal Microfluidic Networks:

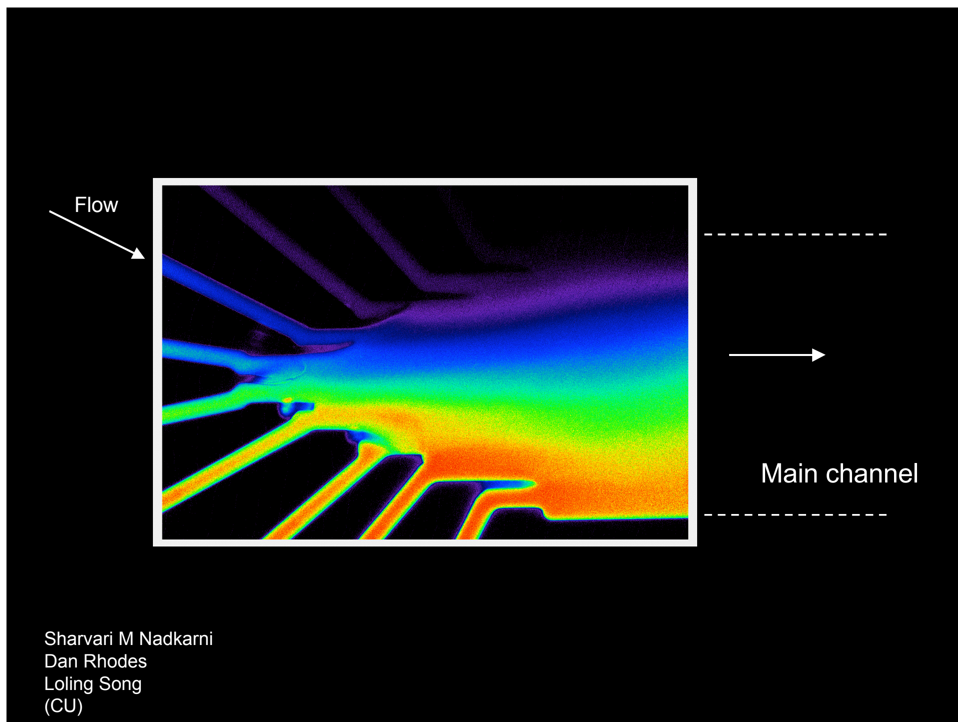
S.K.W. Dertinger et al. Anal. Chem. 2001, 73, 1240

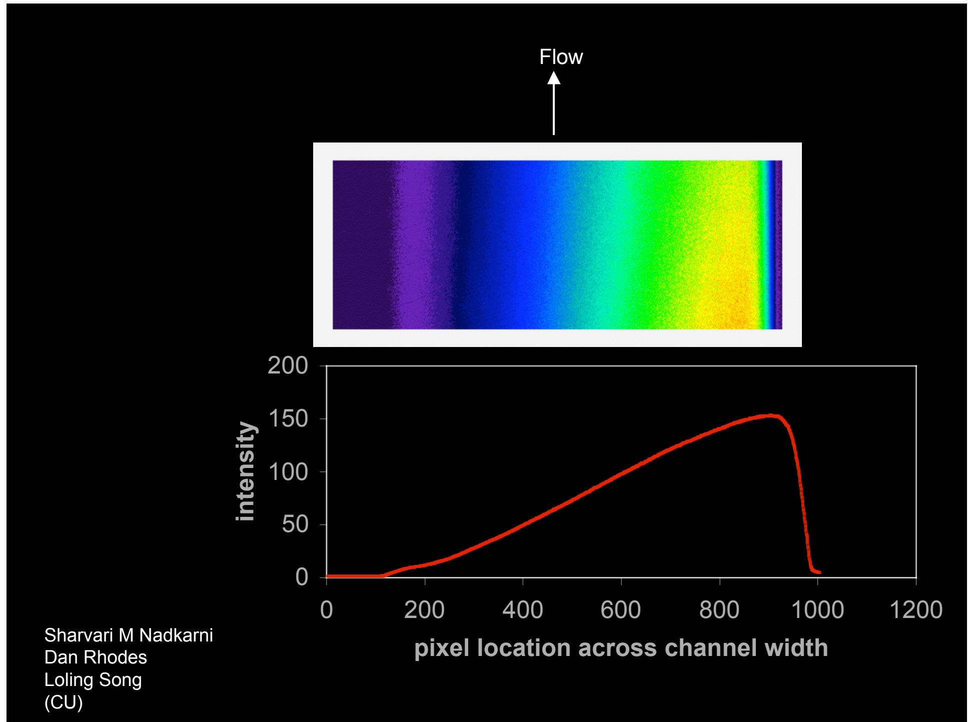
- arbitrary shape of gradient
- discontinuous (jumps) gradients
- multiple components

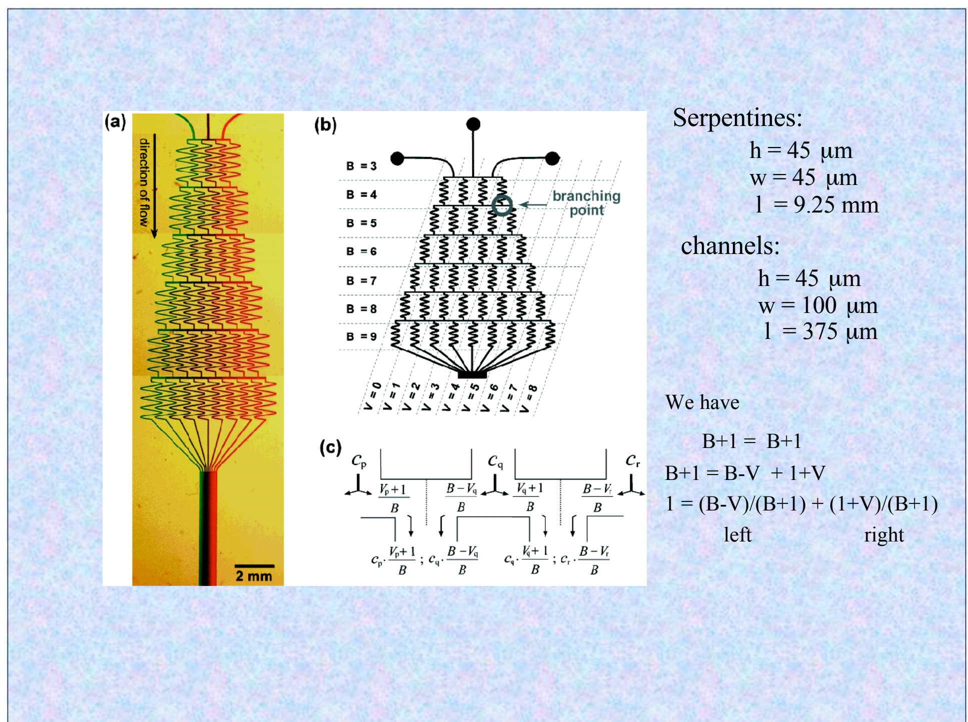

Spatial Gradient
C high
C low
mixing
50 μm
Cells
Conc.
Position
500 μm
S.K.W. Dertinger et al. Anal. Chem. 2001, 73, 1240

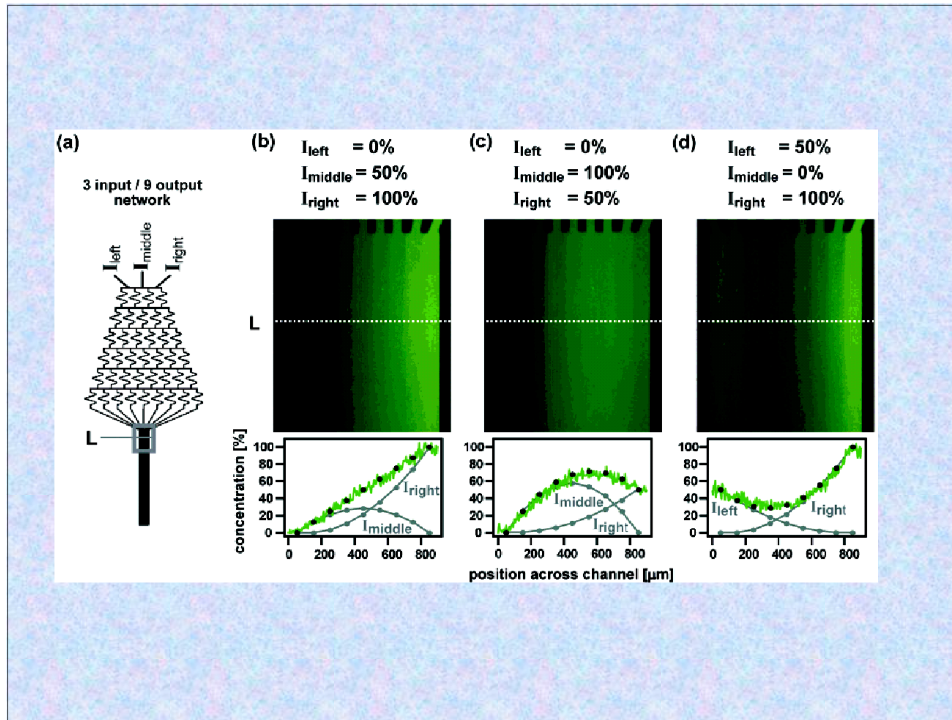

buffer
Intensity
200
100
0
0
50
100
150
pixel location along the channel
Intensity
200
100
0
0
50
100
150
pixel location across channel width
Fluorescent solution
Sharvari M Nadkarni
Dan Rhodes
Loling Song
(CU)


Flow
200
150
100
50
0
intensity
0
50
100
150
pixel location across channel width
Flow
Sharvari M Nadkarni
Dan Rhodes
Loling Song
(CU)


Flow
Main channel
Sharvari M Nadkarni
Dan Rhodes
Loling Song
(CU)


Flow
intensity
200
150
100
50
0
0
200
400
600
800
1000
1200
pixel location across channel width
Sharvari M Nadkarni
Dan Rhodes
Loling Song
(CU)


(a)
direction of flow
2 mm
(b)
B = 3
B = 4
B = 5
B = 6
B = 7
B = 8
B = 9
branching point
V = 0
V = 1
V = 2
V = 3
V = 4
V = 5
V = 6
V = 7
V = 8
(c)
Serpentines:
h = 45 μm
w = 45 μm
l = 9.25 mm
channels:
h = 45 μm
w = 100 μm
l = 375 μm
We have
B+1 = B+1
B+1 = B-V + 1+V
1 = (B-V)/(B+1) + (1+V)/(B+1)
left
right



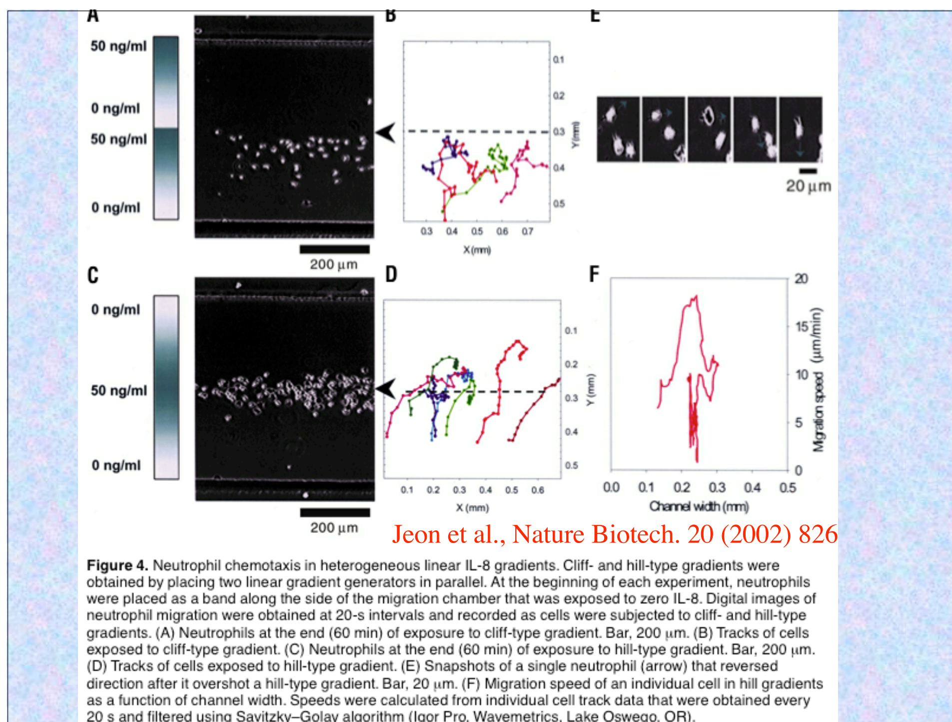
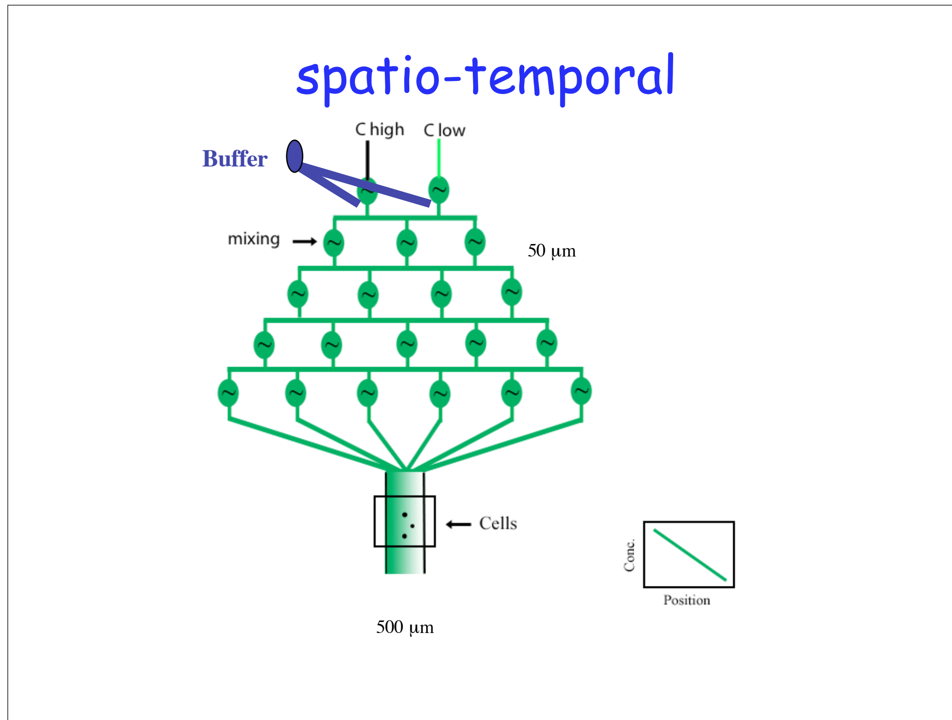
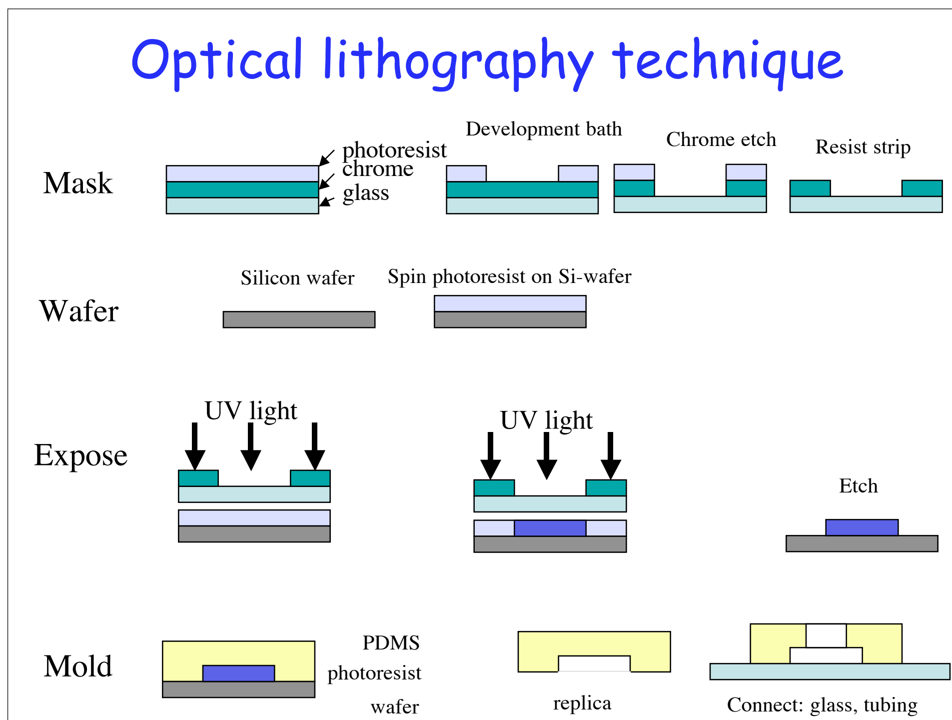
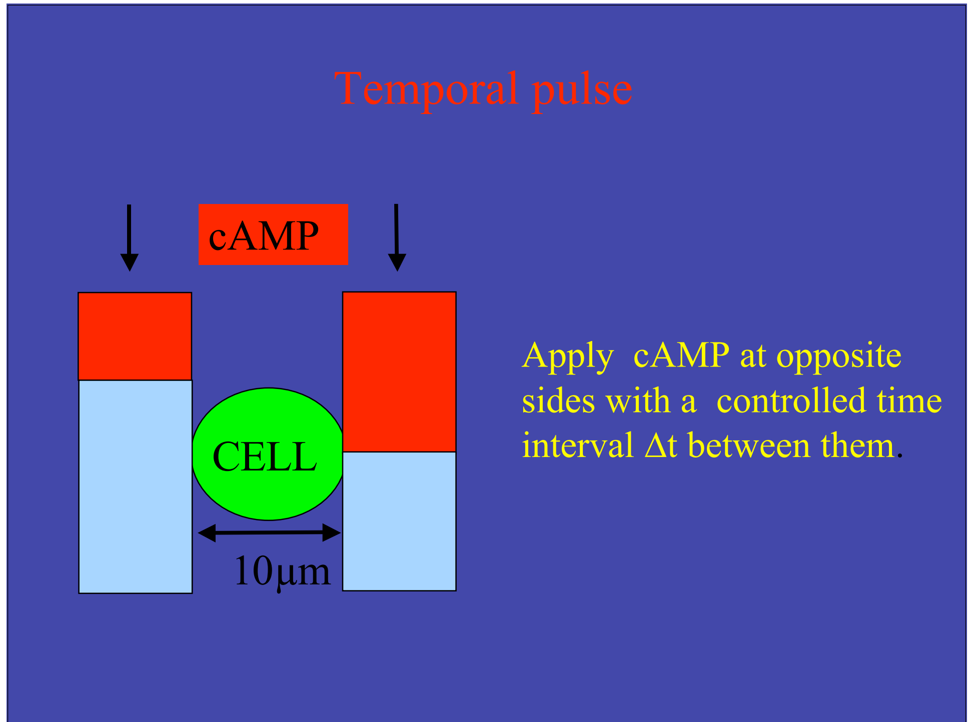
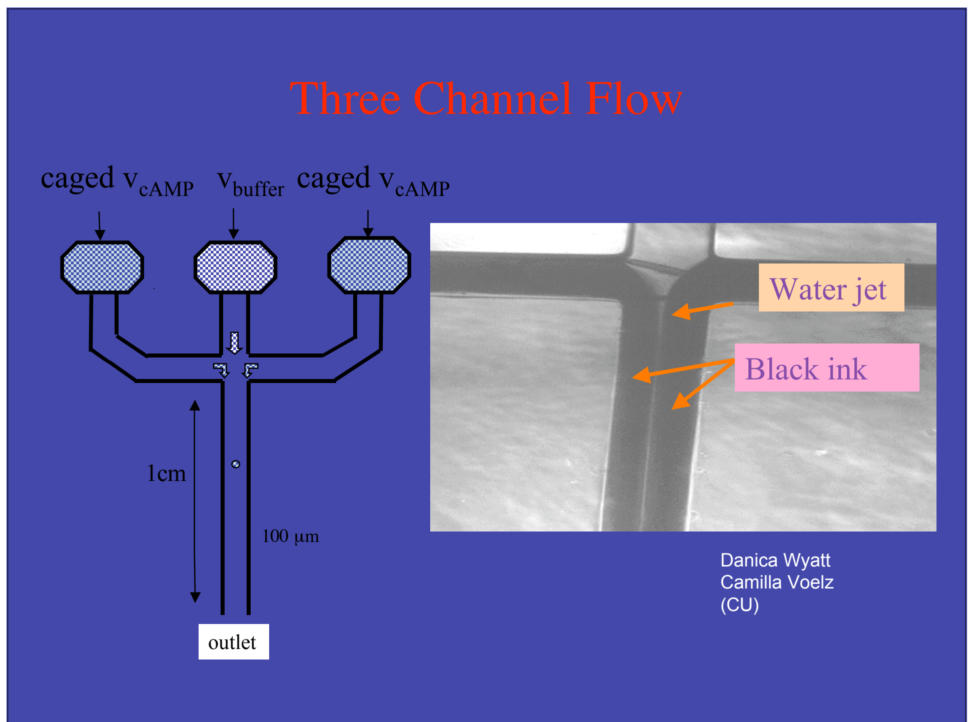


Figure 4. Neutrophil chemotaxis in heterogeneous linear IL-8 gradients. Cliff- and hill-type gradients were obtained by placing two linear gradient generators in parallel. At the beginning of each experiment, neutrophils were placed as a band along the side of the migration chamber that was exposed to zero IL-8. Digital images of neutrophil migration were obtained at 20-s intervals and recorded as cells were subjected to cliff- and hill-type gradients. (A) Neutrophils at the end (60 min) of exposure to cliff-type gradient. Bar, 200 µm. (B) Tracks of cells exposed to cliff-type gradient. (C) Neutrophils at the end (60 min) of exposure to hill-type gradient. Bar, 200 µm. (D) Tracks of cells exposed to hill-type gradient. (E) Snapshots of a single neutrophil (arrow) that reversed direction after it overshot a hill-type gradient. Bar, 20 µm. (F) 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 (Igor Pro, Wavemetrics, Lake Oswego, OR).


spatio-temporal
Buffer
C high
C low
mixing
50 μm
Cells
Conc.
Position
500 μm


Optical lithography technique
Mask
photoresist
chrome
glass
Development bath
Chrome etch
Resist strip
Wafer
Silicon wafer
Spin photoresist on Si-wafer
Expose
UV light
UV light
Etch
Mold
PDMS
photoresist
wafer
replica
Connect: glass, tubing


Temporal pulse
cAMP
CELL
10µm
Apply cAMP at opposite sides with a controlled time interval Δt between them.


Three Channel Flow
caged v cAMP
v buffer
caged v cAMP
1cm
100 µm
outlet
Water jet
Black ink
Danica Wyatt
Camilla Voelz
(CU)

Three Channel Flow

caged v_{cAMP} v_{buffer} caged v_{cAMP}

1cm

100 µm

outlet

Water jet

Black ink

Danica Wyatt
Camilla Voelz
(CU)

Three Channel Flow

caged cAMP buffer caged cAMP

1cm

100 µm

outlet

Water jet

Black ink

Danica Wyatt
Camilla Voelz
(CU)

Three Channel Sweeper

V_{buffer} V_{cAMP} V_{buffer}

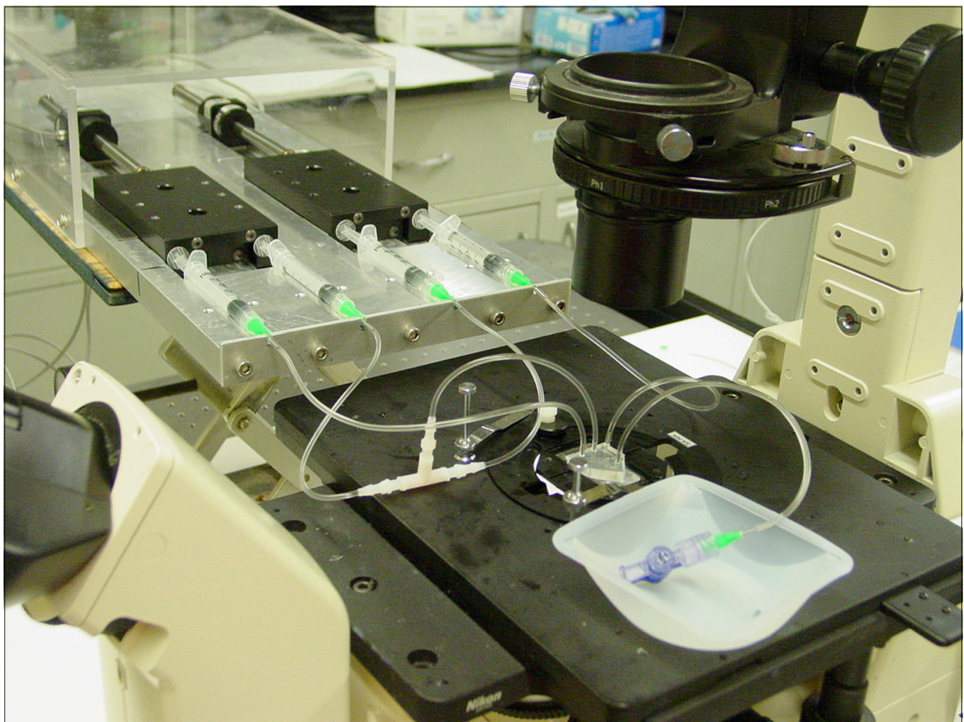
1cm

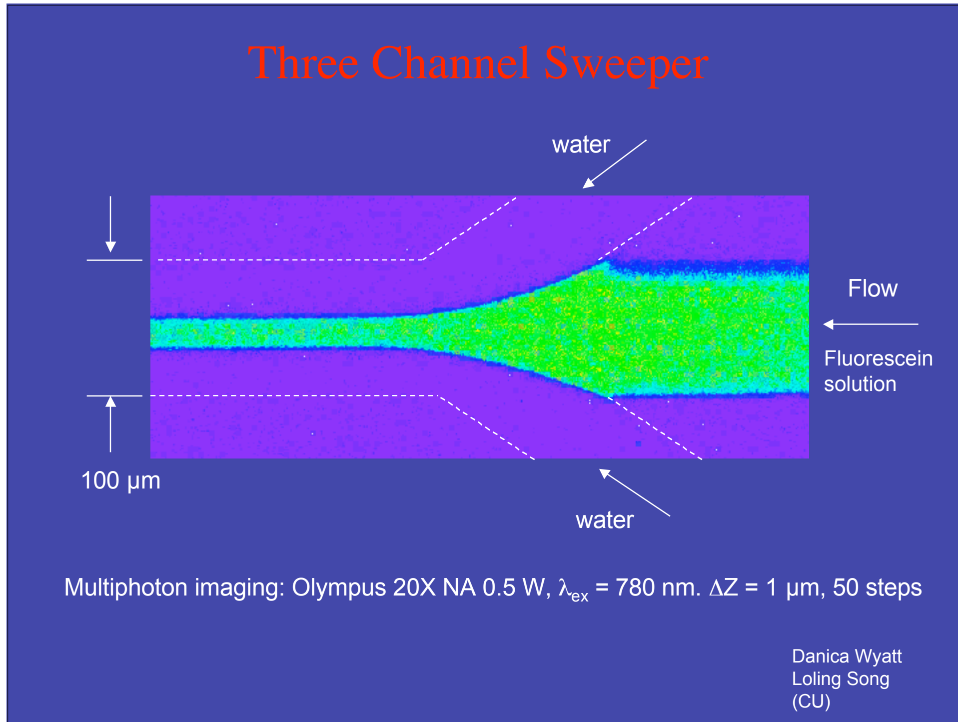
100 μm

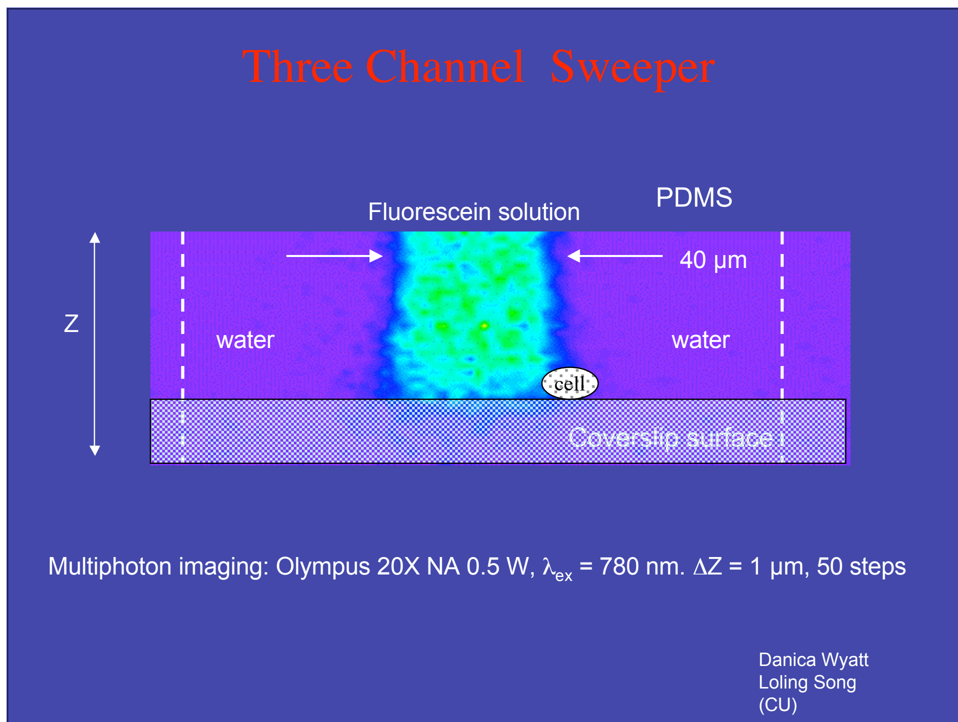
outlet

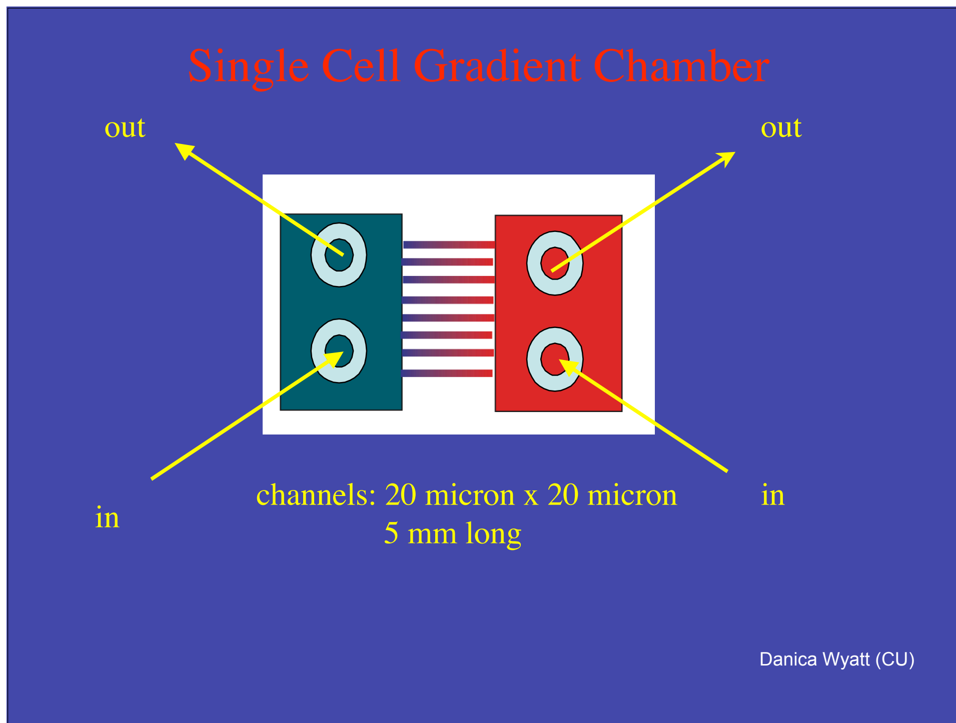
4mm/sec

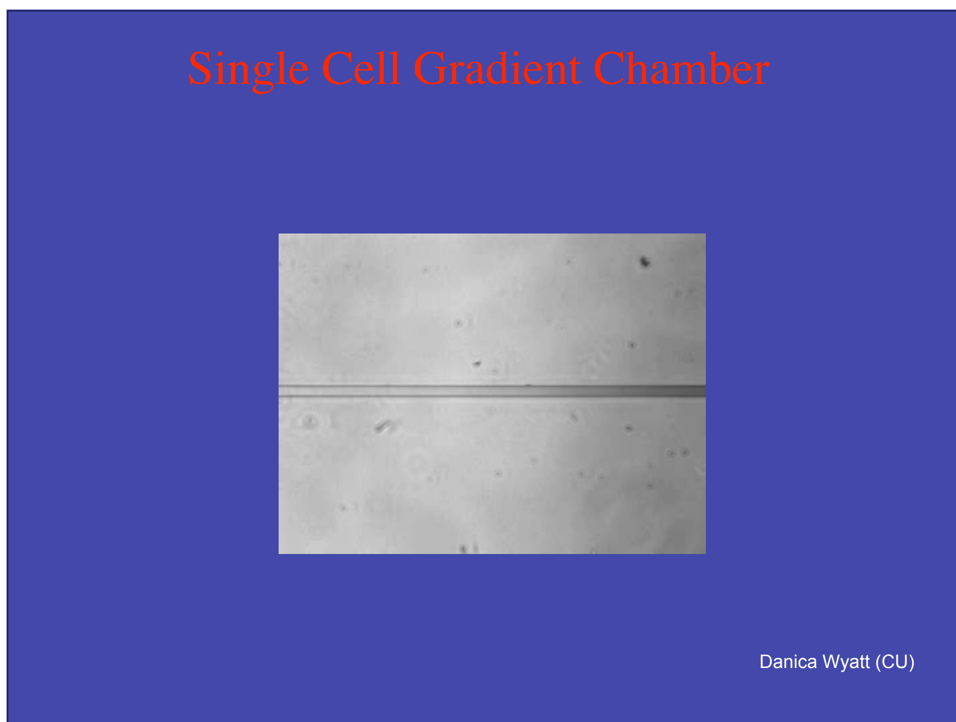
Danica Wyatt
Camilla Voelz
(CU)




Three Channel Sweeper
water
Flow
Fluorescein solution
100 µm
water
Multiphoton imaging: Olympus 20X NA 0.5 W, λex = 780 nm. ΔZ = 1 µm, 50 steps
Danica Wyatt
Loling Song
(CU)


Three Channel Sweeper
Fluorescein solution
PDMS
40 µm
Z
water
water
cell
Coverslip surface
Multiphoton imaging: Olympus 20X NA 0.5 W, λex = 780 nm. ΔZ = 1 µm, 50 steps
Danica Wyatt
Loling Song
(CU)


Single Cell Gradient Chamber
out
out
in
channels: 20 micron x 20 micron
5 mm long
in
Danica Wyatt (CU)


Single Cell Gradient Chamber
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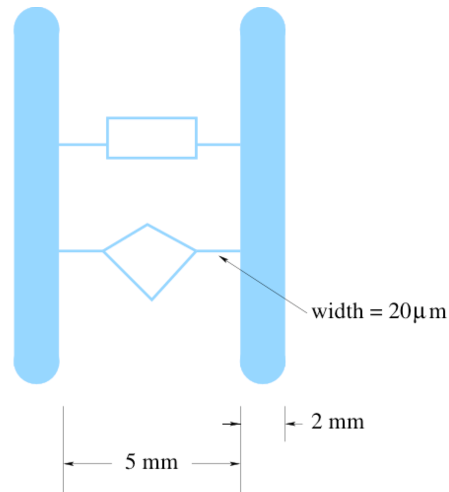
Single Cell Gradient Chambers …



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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,….

THANKS!
Sharvari Nadkarni
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