Probing the Not-So-Black Box Using a Homemade Flow Chamber

Michael DeSantis
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So what does the signal look like?

- Dictyostelium interacts with neighboring cells by emitting periodic waves of cAMP resulting in chemotaxis, or rectified motion towards the source.
- Experimentally we attempt to reproduce these symmetric waves.
- Goal: To further our understanding of dictyostelium's gradient sensing mechanism by varying the frequency and duration of repeated chemical signals.

Flow Chamber

- Two ports permitting injection of buffer (DB) or cAMP into cell chamber via an electronically controlled actuator; a third port, located at rear, is connected to a three-way luer stopcock valve and syringe pump for generating flow.
- Developed for use with high-resolution fluorescence microscopy.

Experimental Setup

Characterization of the System

- Fluorescein has a comparable diffusion coefficient, D, to cAMP.
- Time-measurements of the fluorescein intensity, averaged over a small rectangular region at different pulse lengths.

Theoretical predictions

- Solution to Fick's second equation describing a three-dimensional Gaussian distribution with flow.
- We have numerically integrated C(r,t).
- We can predict the shape of the wave as a function of the pulse duration.

\[
C(r,t) = \frac{N}{(4\pi D t)^{3/2}} e^{-r^2 / (4Dt) / 2D}.
\]
Transition from high to low velocity regimes

- We can also predict the shape of the wave as a function of the flow rate.

Experiment matches theory!

- For generally laminar flow with parabolic velocity profiles transverse to the flow, Taylor dispersion plays a significant effect.
- Manual fits to the data for a single free parameter, $v$.

\[
D_{\text{long}} / D = (1/210)P_v^2
\]

where $P_v = \nu h / D$

Matlab image analysis

- Upon exposure to cAMP, the CRAC-GFP protein accumulates preferentially at the leading side of the cell.
- To quantify the degree as to how well a cell responds to a pulse of chemoeattractant, these intensities are measured about its periphery.
- Matlab GUI reads in a directory of images.

CRAC-GFP Intensity Measurements

- Intensity as a function of the angle, binned every 10°, counter-clockwise starting along the positive x-axis.

Summary

- We have made a device to produce spatio-temporal pulses of cAMP.
- Using this device, we will allow us to change the amplitude and the frequency of the cAMP waves.
- We have characterized the pulses using fluorescein and fluorescent beads.
- We have preliminary results showing cells respond to pulses of cAMP.
- Use of 60x objective optimized for fluorescence microscopy will improve image resolution.

Possible Sources of Error

- Yeast (S. cerevisiae)
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- 3:30pm snacks at the colloquia

*“Piled Higher and Deeper” by Jorge Cham [http://www.phdcomics.com/]