Use of Computer Enhanced Laser and Video Microscopy Techniques to Elucidate the Physical Properties of Individual Cell Surface Receptors, Channels and Adhesion Molecules

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Modeling Limitations

The following are not considered in the typical analysis of bead motion:

- Correlation between cell motion and bead motion
- Correlation between membrane motion and bead motion
- Potential clustering of beads

Algorithm Limitations

The extraction of bead position, i.e., the coordinates of the bead centroid, is crucial for the reliability of the results. However,

- The Kodak and MetaMorph algorithms may be suboptimal for bead identification and tracking,
- Bead position extraction may be difficult due to the changes in the shape and size of the image (e.g., due to membrane fluctuations).

Technical Issues

- The variances of the diffusion coefficient and the corral size increase with increasing noise level.
- Even with high quality low noise images, there appears to be problems with the camera:

  1. While a greater frame rate may be required to describe rapidly changing events, image quality suffers significantly with an increased frame rate.
  2. At high frame rates, the camera appears to cause distortion in the image by introducing different light intensities for even and odd numbered lines.

Short Term Goal

Minimize the sources of uncertainty.

Determining the Optimal Frame Rate

- When there is no noise, the variance of the estimates decreases with frame rate, asymptotically approaching zero.
- For noisy measurements, the variance of the estimates decreases with frame rate, approaching a positive constant related to the noise level.
- The variance becomes effectively constant at a certain frame rate, making it unnecessary to increase frame rate beyond this rate.
- An increased frame rate means decreased image quality, that is, an increase in the lower bound for the variance.

Therefore, there is an "optimal" frame rate where the variance becomes close enough to its lower bound. An optimal frame rate for each type of experiment will be derived. So far, the data suggest that the optimal frame rate is around 100 frames/sec.

Image Processing

The following techniques are applied to each image sequence before proceeding with the centroid finding algorithms:

- Intensity stretching: Necessary to achieve good contrast.
- Smoothing filter: The filter we use completely counters the effect of intensity difference between odd and even lines caused by the camera.

This method, when used instead of the equalization/ thresholding method used by the Kodak/MetaMorph analysis, is expected to reduce considerably the noise component due to algorithmic limitations.

Centroid Finding

A variant of the Expectation Maximization (EM) Algorithm will be used to find the centroid of the beads:

- Determine the intensity profile of the bead, $I(x, y)$.

  $$I(x, y) = h(x, y|a) + \text{uncorrelated noise term.}$$

  where a is the unknown parameter set including position and shape of the bead.

- Find the probability density function of the parameter set using the EM algorithm.
- Evaluate the centroid position for each frame.

This method is the second step in an attempt to reduce the algorithmic limitations.

Aggregated Beads

As a by-product, the algorithm provides the variance of the centroid estimate. This variance is used as a measure of the probability of the bead image corresponding to a group of beads, making it possible to identify the bead clusters and discard the related experiments.

Determining the Correlation Component

- In the case that two or more beads are observable, the correlation between the positions of two beads can be determined directly. It is also possible to calculate the effect of membrane and cell motion on bead motion.
- It is possible to extract the motion of the membrane/cell when a sufficient number of beads on the same cell are observable.

The knowledge of various correlation related components will lead to a more accurate modeling of the independent bead motion.

Motion Parameters: Traditional Approach

- Calculate mean square distance (MSD):

$$MSD(n) = \frac{1}{N-n} \sum_{i=1}^{N-n} \left[(x_{i+n} - x_i)^2 \right]$$

  n: time index,
  N: total number of images,
  x: position of the centroid.
  For confined diffusion, $MSD(n)$ is almost linear for small n and constant for large n.

- Fit a line through origin to the MSD for small n. The slope of this line is proportional to the *diffusion coefficient.*

- Fit the MSD vs. time curve to obtain the asymptotic value, which is proportional to the square of the *corral size.*

Motion Parameters: Model Assumption

This approach originates from the model assumption:

$$y(t) = aw(t)$$

where aw is the trajectory of the bead.

Motion Parameters: Proposed Model

Consider a more refined model for the process:

$$y(t) = aw(t) + be(t)$$

$w(t)$: Bead trajectory,
$e(t)$: Observation+modeling error,
a, b: Unknown parameters.

Motion Parameters: Proposed Method

Then, for confined diffusion,

1. Fit a straight line to the MSD curve for small n. The slope will be proportional to the *diffusion coefficient* and the y intercept will yield noise magnitude b.
2. Find the asymptote p of MSD. Then $p-b$ is proportional to the square of *corral size*.

Statistical analysis of experimental curves according to this model is expected to provide much better estimates. The slope estimate is unbiased and corral size estimate is more accurate.

Correlation Study

Bead 1: $x_t^{(1)} = x_{t-1}^{(1)} + \varepsilon_t^{(1)} + z_t$

Bead 2: $x_t^{(2)} = x_{t-1}^{(2)} + \varepsilon_t^{(2)} + z_t$

$E\{x_t^{(i)}\} = E\{\varepsilon_t^{(i)}\} = E\{z_t\} = 0$

$\varepsilon_t^{(i)}$ is uncorrelated white noise,
z_t is membrane and cell motion component.

$$\begin{cases} Var\{\Delta x_t^{(i)}\} = Var\{\varepsilon_t^{(i)}\} + \{z_t\} \\ \\ cov(\Delta x_t^{(1)}, \Delta x_t^{(2)}) = Var\{z_t\} \end{cases}$$