Shape Determination of Attached Vesicles

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Abstract

The procedure to determine the shapes of a thermally fluctuating attached phospholipid vesicle is described. Large number of digitized video images are segmented and the vesicle outlines are determined using a contour following algorithm.

1 Introduction

Phospholipid vesicles have been intensively studied as a model for biological membranes. They form spontaneously in water when a thin film of amphiphilic phospholipid molecules closes upon itself due to the hydrophobic effect. As the phospholipid membrane is virtually an incompressible two dimensional liquid its shape is predominantly controlled by the bending rigidity [1].

The bending moduli of phospholipid membranes have been experimentally determined by various methods. One of them is to digitize a large number of microscope images of a fluctuating vesicle. By the Fourier analysis of the vesicle cross-sections the mean square amplitudes of normal-mode displacements are determined as a function of wave numbers [2, 3, 4, 5]. From these data the membrane bending modulus is calculated by the least square fitting to the appropriate theoretical model, the choice of which remains crucial for the whole procedure.

It was thus of great importance to statistically analyze the shape fluctuations of phospholipid vesicles. For this purpose a large number of video images must be digitized and automatically processed. The procedure to do this was developed and successfully implemented.

2 Experimental Procedure

The phospholipid vesicles were prepared in sucrose solution by the modified method of electro-formation [6]. The solution containing vesicles was placed into a flow chamber positioned under the inverted microscope. The flow velocity could be hidrostatically controlled. At first no flow wa applied, allowing the vesicles to settle down and to attach to the bottom glass plate of the flow chamber. Then the flow velocity was stepwise increased. Although the vesicles were observed to deform in the flow, they nevertheless remained attached to the glass by a long thin lipid tube (tether). Its length was controlled by the flow velocity.

The vesicles were observed by an inverted optical microscope (Olympus IMT-2) using phase contrast technique. Video camera (Cohu 6500 with electronic shutter option) attached to the microscope served to record the images. The frame grabber (Data Translation DT2851) in a personal computer was used to digitize the images at the rate about one per second [7]. This yielded a series of 512x512 pictures with 256 gray levels that were stored on the computer disk and later archived on CDs.

3 Image Processing

Image analysis was performed on an Pentium II computer operating under Linux. It received the data from the image acquisition computer by ftp via local network. Images were processed in series of about 1000 images. These represented time sequences of shape fluctuations at a fixed tether length. For each series the starting region of interest was determined in such a way that it encompassed only the observed vesicle. As the vesicle slowly moved on the screen during the measurement, the region of interest was shifted together with the determined center of the vesicle. All processing was done only over the region of interest.

3.1 Image Thresholding

Average value of the pixels is calculated. As the contrast is usually poor the image is normalized inside the narrow interval (e.g. ± 6) about the average. This means that the values outside the chosen interval

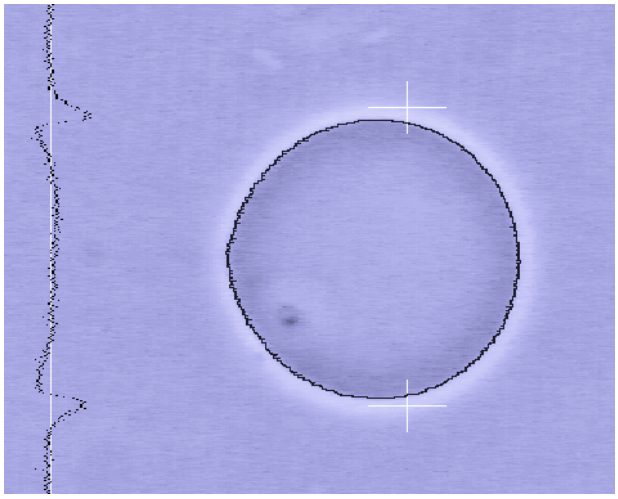

Figure 1: Determination of the contour of a vesicle.

are dropped and the ones inside are stretched to the full interval (0 to 255). This facilitates the following numerical manipulation of the picture. If necessary the image is then slightly smoothed.

To determine the vesicle outline the image is binarized, where the choice of the thresholding value is somewhat arbitrary. Fig.1 shows a typical image. At its left side the intensity profile across the vesicle is plotted where the white line in the graph represents the image average value. The two crosses mark the line for which the profile was calculated. Since the vesicle was observed by the phase contrast technique its contour is dark with a white halo around it. From the intensity profile we notice that the image average could be used as the threshold to discriminate the vesicle outline.

But sometimes the background was evidently not the same over the picture. In these cases the local averages were calculated for each point by considering sufficiently large regions around it.

3.2 Determination of the Vesicle Outline

From the binary image it is rather straightforward to determine the outline of the vesicle. We use an modified maze solving algorithm. A starting point is determined as the top brightest point on the line across the vesicle. This is the position of the top cross in fig.1. From this point we continue down until a black point is encountered. Now we can follow the contour to the left. We follow the white points in such a way that in each step we start to investigate points from the right to the left of our path. In this way we finally come to the starting point where procedure stops. This procedure encounters problems if the contour is not connected. In this case the algorithm walks into the vesicle and out again resulting in an unusually long path. Smoothing the image usually corrects for these problems, since it eliminates the isolated channels to the vesicle interior. The other problem present small dark particles that are not attached to the vesicle. If one of them happen to be between the staring point and the vesicle, the algorithm of course determines its contour, which is very short. A slight shift of the starting point normally corrects for these errors.

This procedure yields the coordinates of the vesicle contour. They may still contain some thin threads. These are removed and the center of the contour is calculated. All the contour coordinates are then calculated with respect to the origin in the center.

4 Discussion

The described method of shape analysis proved to be efficient if not very fast. It was used to determine the contours of at least 40 series of shape fluctuations. The results are used to investigate the dependence of the membrane tension as the function of the tether length - the work which is now in progress.

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