Protocol for Counting Yeast Cells with a Hemacytometer

**Filling the Hemacytometer**

1. Make sure the hemacytometer and coverslip are clean. This allows the cell suspension to flow freely over the grid.
2. Place the coverslip over the hemacytometer grids.
3. Agitate the cell suspension so that it is homogenous. Withdraw a sample using a pipet.
4. Fill one side of the hemacytometer by placing the pipet tip against the indentation on the hemacytometer and allowing capillary action to draw the cell suspension over the grid. Fill so that the entire grid level is covered but do not fill the channels on either side.
5. Repeat with the other side of the hemacytometer.

**Counting Cells**

1. Focus on the hemacytometer grid using the 40x objective. You may need to find the grid using the 10x objective first. (The coverslip is too thick to use the 100x objective.)
2. Once you have focused on the grid (twenty-five squares, 0.2 mm on each side, each one divided into sixteen smaller squares), move the slide so that the center square of the twenty-five is in focus. We will refer to the twenty-five squares as “squares” and the smaller 16 squares as “subsquares.”
3. Count the cells within the center square and record the count in a table like the one shown below (Table 1). If cells span the lines that border the square, include the ones on top and on the left (not the ones on the right or the bottom lines) in your counts.
4. Count the cells in the four corner squares. Record the data.
5. Finish counting the remaining twenty squares. Record the data.
6. Repeat your counts for nine more samples, recording the data from each count.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Table for data entry from cell counts. Note the separate columns for the center square and four corners.</th>
</tr>
</thead>
</table>

7. Enter your data into a spreadsheet, using the same format and labels as Table 1.