**Symbiont Density Protocol**

Adapted from Putnam lab protocols by T. Lindsay

Materials

* Compound light microscope
* Symbiont cell suspension (sample in PBS)
* Haemocytometer
* Glass cover slip
* Glass pipet & bulb
* DI water (for cleaning)
* Kimwipes
* Counter

Protocol

1. Melt samples a few hours prior to starting symbiont counts.
2. Clean haemocytometer and slide with DI water by squirting and wiping with a kimwipe. Place on microscope stand, turn on microscope, and visually inspect to make sure no cells are visible on grid.
3. Vortex sample to homogenize.
4. Use a glass pipet to mix sample by pipetting up and down, then take a small amount and load one side of haemocytometer (with the cover slip on it) until liquid front completely moves across the glass slide.
5. Using 10x or higher objective, count cells in one side of the haemocytometer.
   1. Each grid is divided into 9 large squares (Fig A)
   2. Count until you reach 100 total cells or 4 squares.
   3. Counting starts in the upper left hand square, and only continues in the four corner squares.
   4. Note: counting should be standardized- compare your first run with someone else to ensure equivalent standards for what constitutes a cell vs. debris. For cells falling exactly on the border of two squares, cells on the left and top border should be considered inside of the square, while cells on the bottom and right border should be considered outside. Use the inner most lines for the borders with three lines (Fig C)
   5. Record number of squares and number of cells in excel file
6. Repeat with other side of haemocytometer, counting the same number of squares as before.
7. Clean cytometer as before with DI water. Clean glass pipet with a tube of DI water.
8. Repeat above steps twice more for 6 total counts.
9. Note: Each count should count the same number of the nine large squares. For example, if the first count for sample X requires 3 squares to reach 100 squares, 3 squares should be counted on all subsequent counts for sample X.
10. Make sure average # of cells/square is consistent
    1. Enter data into the data sheet live
    2. If the coefficient of variation of counts in a sample is more than 15%, do another 1 or 2 counts to reduce the variation
11. Multiply # of cells/square by 10^4 and the dilution factor to get cells/mL.
12. Multiply by homogenate volume to get total cells.
13. Normalize to surface area to get cells/surface area.

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Figures: Photos of the haemocytometer at A) 4x B) 10x and C) 10x magnification

References

1. Schoepf et al., 2013. Coral Energy Reserves and Calcification in a High-CO2 World at Two Temperatures. PLoS ONE 8: e75049
2. Weis Lab Protocol: <https://sites.science.oregonstate.edu/~weisv/>