Materials

* DI water (for cleaning)
* Kimwipes
* Counter
* Computer with live data sheets
* Vortex
* Two beakers
* Compound light microscope
* Samples
* Tray to hold samples
* Hemocytometer
* Glass cover slip
* Glass pipet & bulb

Protocol

1. Melt samples
2. Clean hemocytometer and slide with DI water by squirting and wiping with a kimwipe. Place 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 hemocytometer (with the cover slip on it) until liquid front completely moves across the glass slide.
5. Using 100x objective, count cells in one side of the hemocytometer.
   1. Each grid is divided into 9 large squares (Fig A), count all the symbionts in the four corner squares.
   2. Record number of cells in excel file.
   3. Rules for counting:
      1. new counters should compare your first run with someone else to ensure equivalent standards for what constitutes a cell vs. debris.
      2. Count only intact / unbroken symbionts
      3. For cells falling 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)
6. Repeat with other side of hemocytometer.
7. Clean cytometer as before with DI water. Clean glass pipet with a tube of DI water.
8. Repeat above steps twice more for 6-8 total counts.
   1. You can stop at 6 replicates if the Coefficient of variance (CV) is <15%, if not keep going until you do 8.

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Figures: Photos of the hemocytometer at A) 4x B) 10x and C) 100x magnification