COUNTING YEAST CELLS

1. Microscope set-up.
   - Carefully carry a scope from the cabinets to a table. Use two hands!
   - Plug it in and put the lowest power objective lens in viewing position.
   - Adjust the separation of the oculars to your eyes.
   - Adjust the independent focus to your eyes: with a slide to focus on, close the eye with the independent focus and focus the image for the other eye using the focus knobs on the body of the scope. Open only the eye on the ocular with the independent focus and adjust the ocular focus. (Some compound microscopes allow both eyes to be adjusted.)
   - Set the iris diaphram of the condensor to an brightness that allows you to see the cells.
   - Move the next higher objective lens in place.
2. Loading the hemacytometer.
   - Start with a clean hemacytometer cell and cover slip.
   - Swirl the culture flask to suspend the cells.
   - Suspend cells using the Vortex shaker set at *shake=3* for 15 seconds.
   - Use a P-20 micropipette and the appropriate tip we saved earlier on the paper towel to obtain a small drop of sample. Dispense the sample to one of the hemocytometer counting surfaces.
   - Cover the counting surface with a cover slip.
   - Place the hemacytometer securely on the mechanical stage and focus.
3. Counting.
   - Zero the counter.
   - Randomly choose a set of grids to count (e.g., pairs: (2,3), (2,1), (4,2)). See the figure below. The number of grids counted will depend on the density of cells. Count as many grids as necessary to have enumerated at least 100 yeast cells. If the density is low, count an entire ``large" grid. If the density if high, count an appropriate numer of small grids.
   - Record the area counted and the density of cells: number / (area x 0.1 mm (depth)). Convert to cells per liter.

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