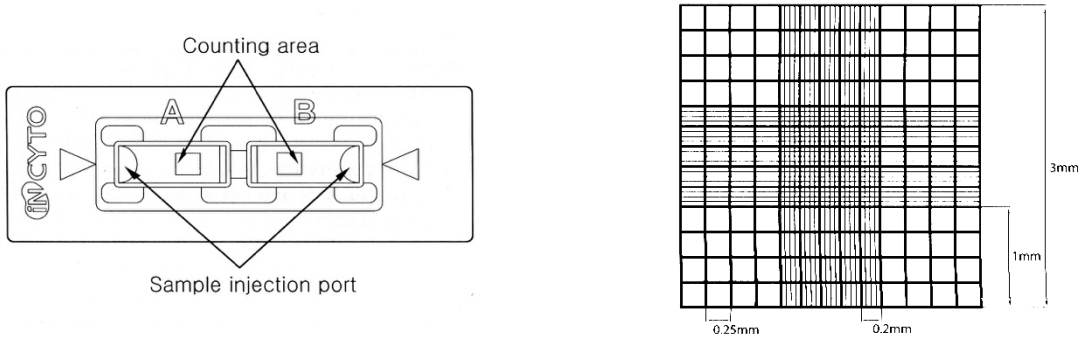
# Counting Cells with a Hemocytometer

Trypsinize cells following usual protocol. However, just before plating, after cold medium has been added to the trypsinized cells:

- mix 0.1 mL cell suspension with 0.1 mL 0.4% trypan blue. Live cells will *not* take up trypan blue. (Use a different dilution factor if cells are uncountable). Note it will be crucial to have individual, well-separated cells
- inject 10 µL to one side of the hemocytometer.
- The hemocytometer has precisely calibrated volumes that are very small, as illustrated in Figure 1. We can count how many cells there are in one of these areas and calculate cell density from that number.




- **Figure 1.** The iN Cyto disposable hemocytometer is a 1 x 3-inch microscope slide (left), but has an attached coverslip with gridlines that define the counting volume. Each slide has two **counting areas** (for two samples). When 10 µL of liquid cell suspension is introduced at the **sample injection port**, capillary action draws the culture liquid into the counting area (expanded at right). The counting area is an array of squares and rectangles. The depth of the counting area is 0.1 mm. Volumes are calibrated in $mm^3$ (length *x* width *x* depth). *(Image: http://www.incyto.com).*
- Inspect at 10x
- Count the number of live (clear) cells in:
    - ALL 9 squares of the hemocyometer if possible.
      Volume = 3mm * 3 mm * 0.1 mm = 0.9 µL
    - FOUR 1mm x 1mm squares if cells are very numerous
      Volume = 4*(1mm * 1 mm * 0.1 mm) = 0.4 µL
    - Calculate the live cell concentration (# cells/µL), for example:
        - (# live cells / 0.9 µL) for 9 squares
        - (# live cells / 0.4 µL) for 4 squares
    - OR use the calculator at *https://wahoo.cns.umass.edu/index.php/node/892*
- Correct for dilution factor
    - Multiply by 2 if you mixed cells 1:1 with trypan blue
    - Multiply by 4 if you diluted 1:3 with trypan blue (1 part cells to 3 parts of the dye)
    - Multiply by 1.33 if you had a low cell number and diluted 3:1 (3 parts cells: 1dye)

We assume the total volume in the chamber represents a random sample. This will not be a valid assumption unless the suspension consists of individual well-separated cells.

## Hemacytometer counts are subject to the following sources of error:

- Unequal cell distribution in the sample
- Improper filling of chambers (too much or too little)
- Failure to adopt a convention for counting cells in contact with the boundary lines or with each other (be consistent)
- Statistical error

For more information, see “Hemocytometer Cautions”
at *https://wahoo.cns.umass.edu/node/939*