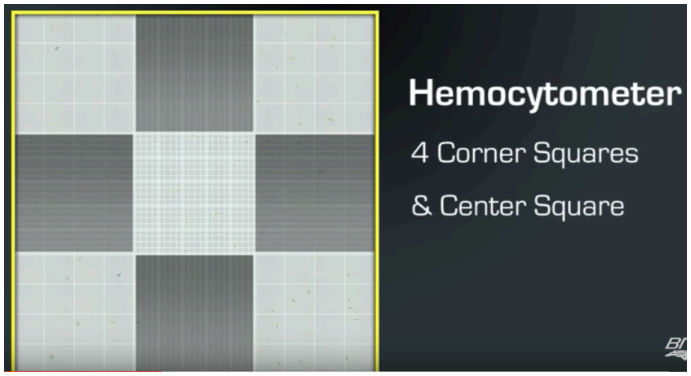
Cell Counting using a Hemocytometer
Based on YouTube video: https://www.youtube.com/watch?v=pP0xERLUhyc



Examples of when to count cells:

- **Passaging**: you may not need to count every time, but can be helpful to determine how many flasks to seed, and what volume of cell suspension you will seed into each flask
- **Freezing cells**: 1-2 million cells per cryovial
- **Experimental application**: different experimental applications will require a different number of cells or different seeding density

Supplies needed (*in addition to those required to passage/trypsinize your cells)*:

- Hemocytometer (ensure clean with 70% EtOH)
- Trypan blue
- Glass coverslip (place gently over hemocytometer)
- Small Eppendorf tube
- Optional: Gibco Cell Culture app (has helpful Cell Counter tool)

Steps (Steps 2 and beyond do not need to be done under sterile conditions):

1. Perform steps 1-9 of passaging protocol.
    a. **If counting cells with goal of freezing:**
        i. Pipette the cell suspension gently a few times in order to ensure even cell distribution.
        ii. Take **100 uL** sample of cell suspension from centrifuge tube, and add to Eppendorf tube. **Note remaining volume of main cell suspension.**
        iii. Place main suspension in centrifuge, and count cells while centrifuging main volume of cell suspension.
    b. **If counting cells during a regular passage or for experimental application**:
        i. Perform steps 10-11 of passaging protocol (centrifuge, aspirate supernatant).
        ii. Re-suspend cell pellet in 1 mL medium, or more if large pellet, but *keep track of what volume you use to re-suspend your cells.*
        iii. Take **100 uL** sample of re-suspended cells and add to Eppendorf tube.

2. Add **100 uL** of Trypan blue to Eppendorf tube and pipette up and down a few times to ensure an even suspension.
3. Gently pipette solution into each well of the hemocytometer in order to fill the area under the coverslip by capillary action.
    a. It is important not to disturb the coverslip in this process, to maintain an even distance between the glass and the hemocytometer grid. This ensures that each large square contains 10^4 mL of suspension.
4. Count the number of **dead** and **alive cells** in 5 of the large squares of the hemocytometer grid, using phase contrast microscope under ~10x power.
    a. For standardization, count those in the 4 corner squares and center square. For cells touching an edge, count the ones touching the top and left edges and exclude those touching bottom and right edges.
    b. If there are copious cells (>80 per large square), okay to count just 2 or 3 large squares.
    c. Dead cells appear dark/blue, as dye enters through cell membrane. Live cells appear more clear centrally.
5. Discard coverslip in glass/sharps container. Discard Eppendorf tube in biohazard bag. Clean hemocytometer thoroughly with 70% Ethanol.

Calculations:

1. Percentage of viable cells (# viable / total # cells)*100: ___
2. Average # of viable cells per square (# viable cells/# of large squares counted: ___
3. Dilution factor (Final volume/Volume of cells): **Always going to be 2 if adding the same volume of cells and trypan blue.**
    a. Note: final volume = volume of trypan blue + volume of cells added.
4. **Concentration** (i.e. **viable cells/mL**):
    = average # of viable cells per square * Dilution factor * 10^4
5. **Total number of viable cells:** Multiply concentration by total mL of cell suspension solution to calculate total number of viable cells in solution.
    a. If you resuspended your pellet in 1 mL of solution, total # of cells = concentration.
    b. If cell suspension volume is not 1 mL, *don't forget to multiply by total volume to get total number of cells.*