ASBC THE SCIENCE OF BEER

Yeast Cell Counting, Viability, and Vitality

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Introduction

- Why is this measurement important?
- Impact of yeast health/pitch rate on fermentation
  - Tank dwell time
    - Fermentation speed
    - Fermentation consistency
  - Under-attenuation
    - Out-of-spec ABV
  - Slow or stalled diacetyl/acetolactate metabolism

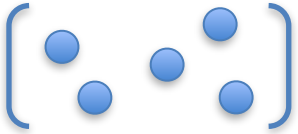
Introduction

- Yeast health impact on flavor profile
  - Off-flavor control
    - Too high cell count
      - Ethyl butyrate, ethyl hexanoate
    - Too low cell count
      - Isoamyl acetate, acetaldehyde, diacetyl
    - Too long fermentation time
      - Autolysis, ethanethiol/mercaptan

Introduction

- What are characteristics of healthy yeast?
  - Capable of cellular proliferation
  - Capable of cellular growth
  - Strong resting metabolism
  - Strong membrane integrity
- What we're going to cover
  - Cell count, viability, and vitality via hemocytomter and microscope
    - Citrate methylene blue
    - Alkaline methylene violet

Definitions

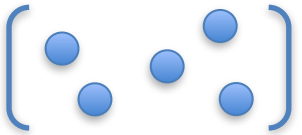


- Cell count
  - Concentration of yeast cells in a volume of liquid
  - Typically expressed in $x10^6$ (million cells per mL)

- Viability
  - % yeast cells that are capable of reproduction
    - ultimately determined by colony culture
  - Viability can be correlated with cell stain tests

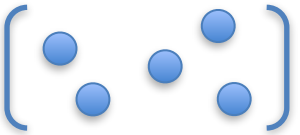
- Vitality
  - A measure of the health or vigor of living cells
    - ultimately determined by fermentation performance
  - Vitality can be correlated with cell stain tests

How to measure cell count



- Dye is not necessary
- Cell count = concentration
  - Count of cells in a set volume
- *An accurate cell count is all about sample prep*
  - Inconsistent results are rarely due to counting errors
- Hemocytometer
- Dilution calculations must match procedure
- ASBC Method of Analysis “Yeast-4”

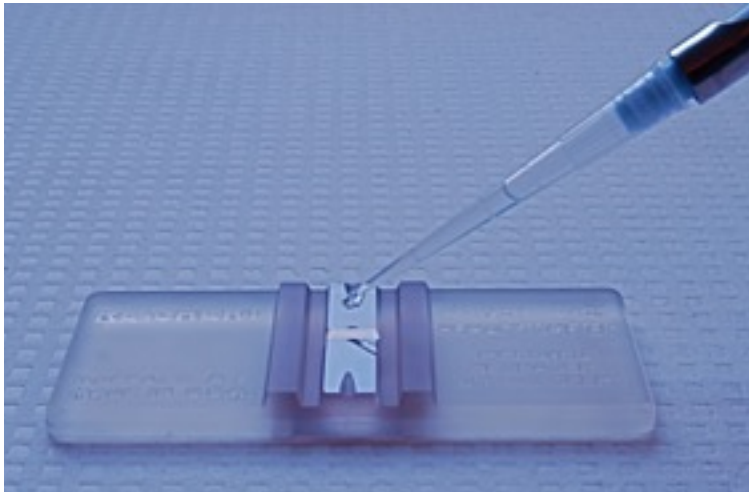
How to measure cell count

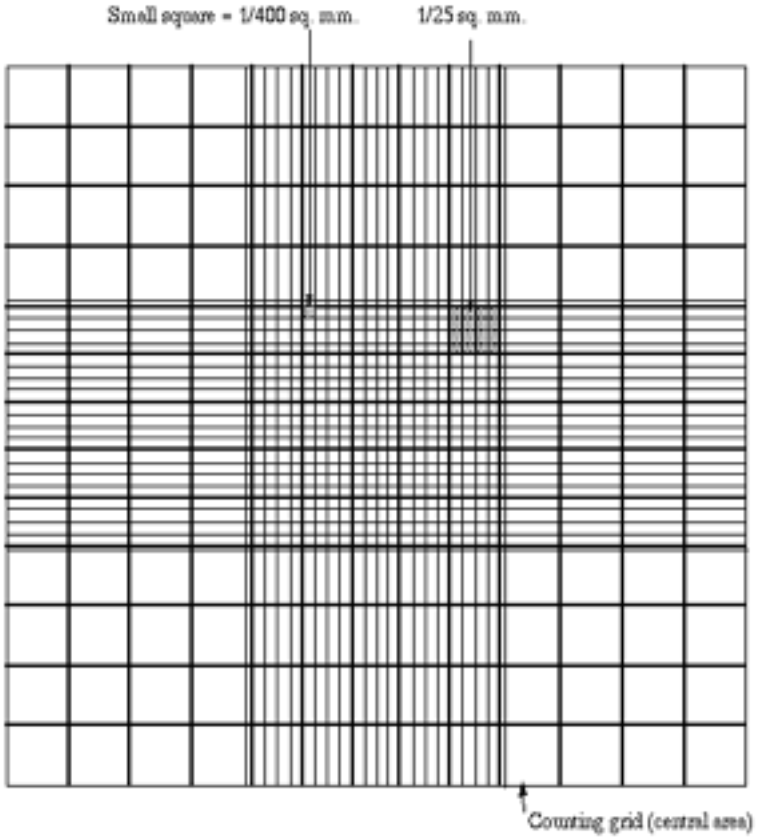


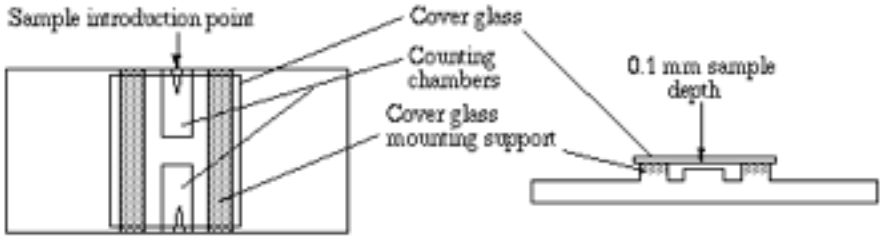
- All about sample prep!
- Degas the sample; bubbles displace volume
- Yeast settles very quickly, especially in a dilution
  - Homogenize it well at every step of the process
- Flocculent yeast clumps should be separated
  - Common deflocculents:
    - 0.5M EDTA
    - 0.5% sulfuric acid
    - NaOH or HCl also works
    - *depending on your dye, this may alter its function slightly*

How to measure cell count

- Hemocytometer
  - Set volume per square
  - Measures concentration

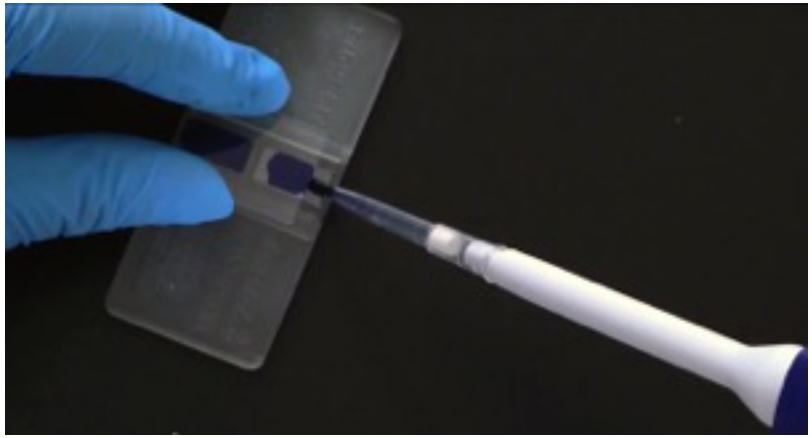




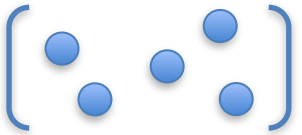


How to measure cell count

- Loading the Hemocytometer
  - Take up a small volume in a fine-tipped pipette
  - Dry the outside of the tip
  - Expel a few drops before loading your sample
  - Capillary action will draw the sample into the grid

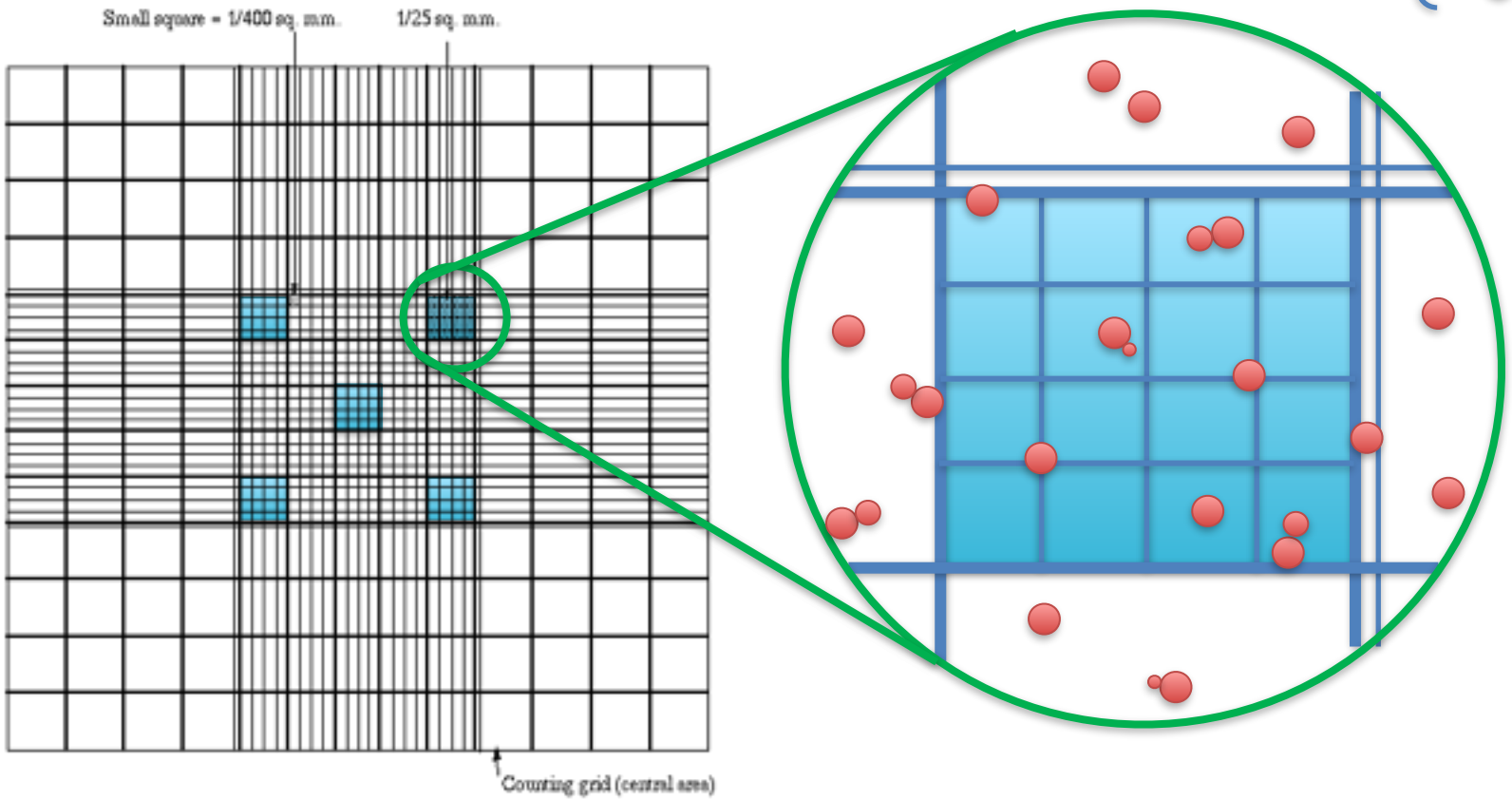


How to measure cell count



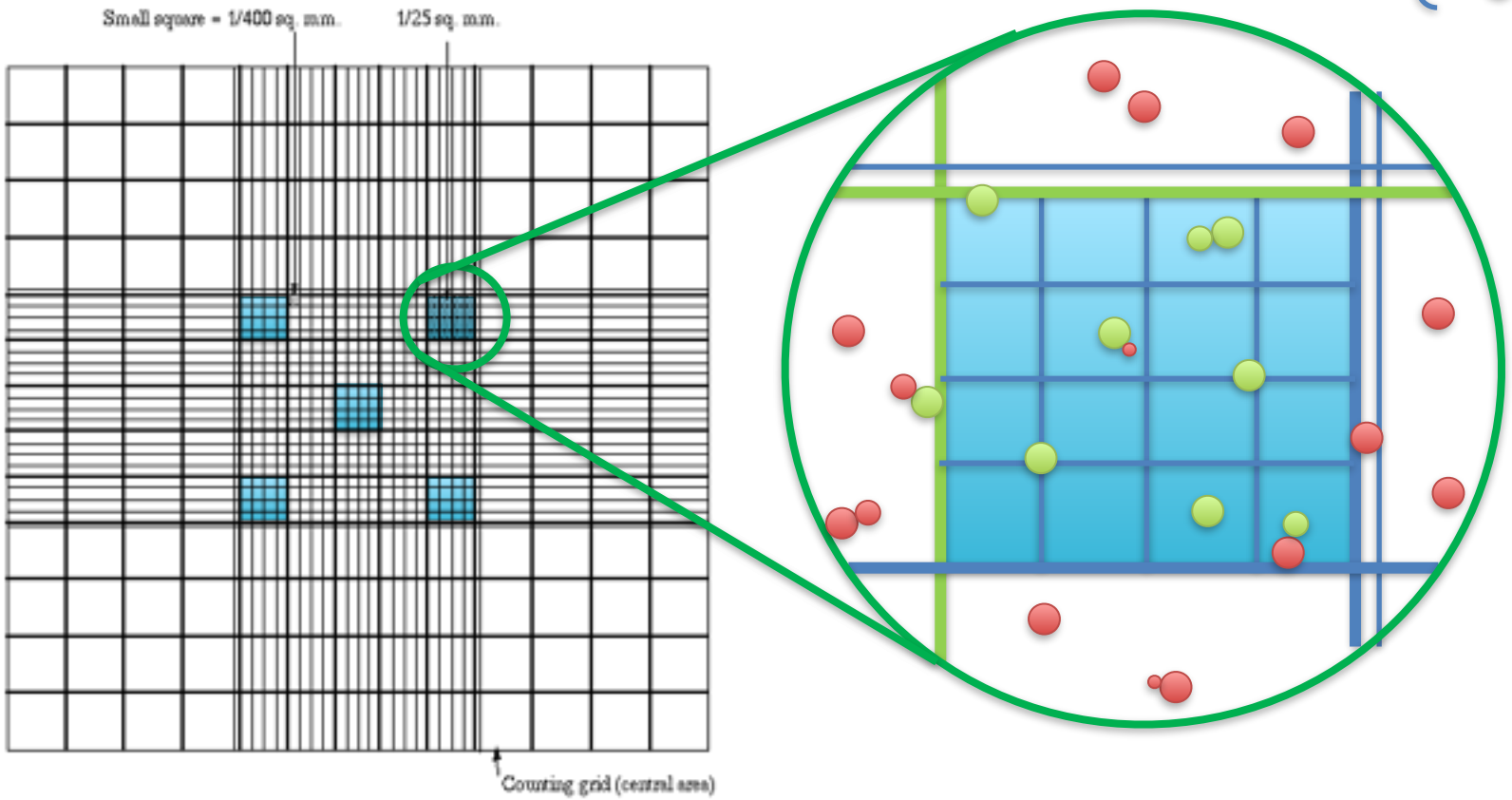
- ASBC Yeast-4 describes two counting methods
  - You may count all cells on one entire side
    - 25 counting areas
  - You may count all cells in 10 counting areas
    - 5 counting areas on both sides and average the result
  - We will cover in-depth this second method referenced here, since it is faster

How to measure cell count



- Budding cells:
  - Count if >50% parent's size
- Cells touching the outer lines:
  - Only count on 2 perpendicular sides

How to measure cell count



- Budding cells:
  - Count if >50% parent's size
- Cells touching the outer lines:
  - Only count on 2 perpendicular sides

How to measure cell count

- Dilution calculations must match procedure
  - Keep careful track of your dilution
  - Dilution factor is a ratio of total volume to sample volume
    - (10mL total volume):(1mL sample volume) = (10:1) = dilution factor of 10
    - 10mL total volume = 1mL sample volume + 9mL diluent
  - Count 5 squares on both sides of the hemocytometer
  - Most hemocytometers give results in $x10^4$ but yours may vary
    - Need to move the decimal over 2 spaces to get $x10^6$

$$(\text{count} \div 2) \times 5 \times \text{dilution factor} \div 100 = \text{cell count (million cells per mL)}$$

Average both sides

Compensate for sample dilution

Extrapolate to all 25 squares

Moving the decimal: $x10^4$ to $x10^6$

How to measure cell count

- Dilution calculations must match procedure
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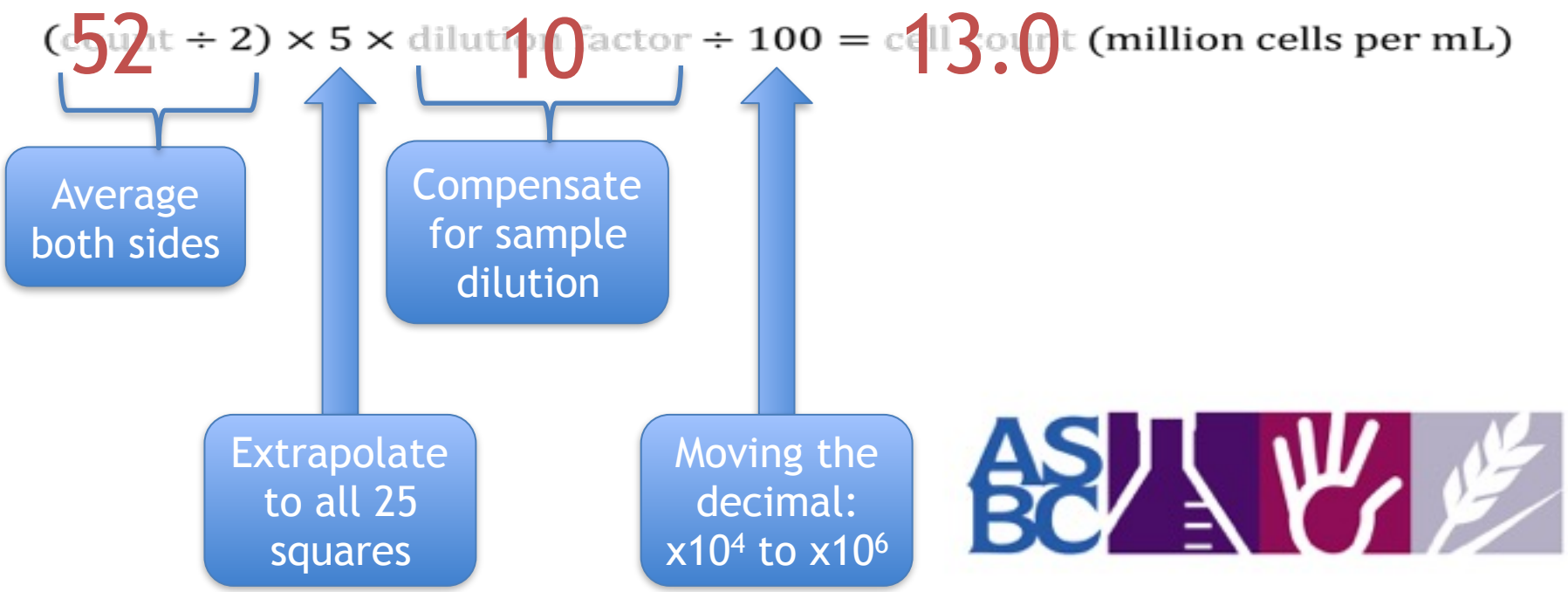
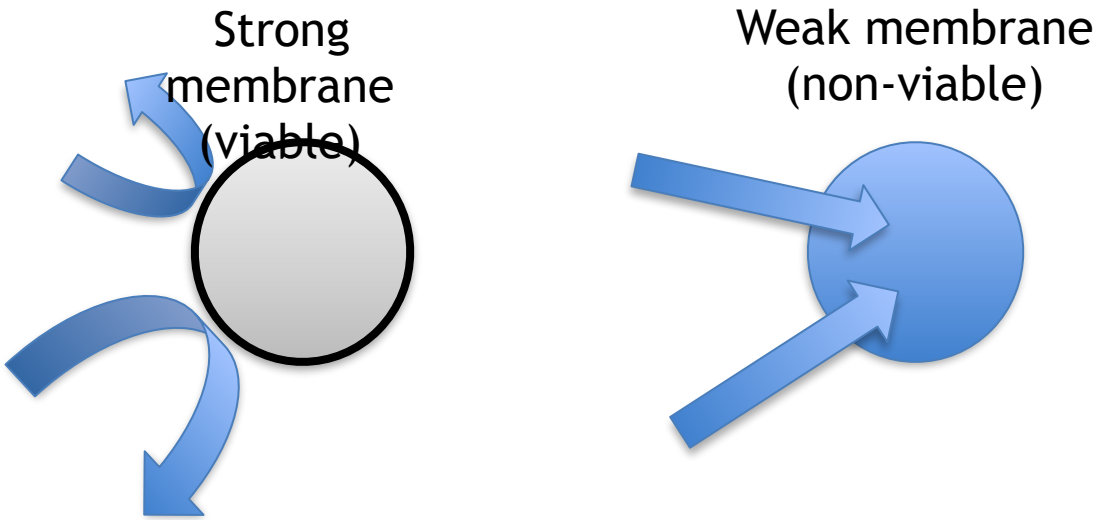


How to measure viability

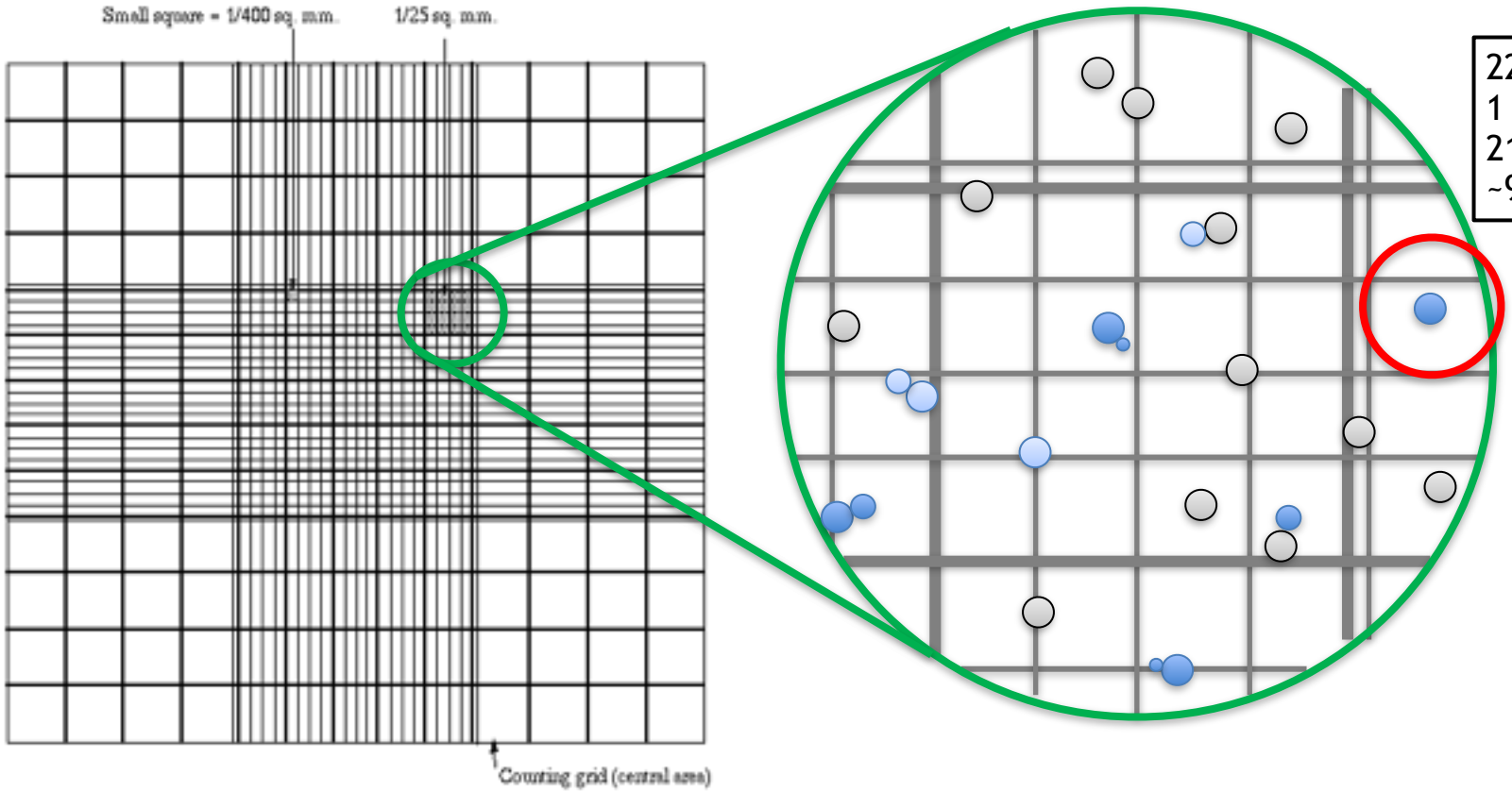
- Viability is ultimately determined by cell culture
- Cell culture is correlated with a dye exclusion assay
  - Based on membrane potential
  - ASBC Method of Analysis “Yeast-3”



How to measure viability

- Industry standard viability dye: methylene blue
  - Intact membranes slow the uptake of methylene blue
  - Methylene blue is reduced in the cytoplasm
    - Cells w. weak membranes uptake dye too fast to reduce
  - Strong correlation w/ high range viability (90%-100%)
    - Tends to over-estimate viability below 90%
    - At 75% and below, results are too inaccurate to be relied on
  - Viable cells remain clear
  - Non-viable cells stain blue
  - Mix your sample 1:1 with 0.1% dye
  - Count at least 100 cells total and note how many of those were stained blue

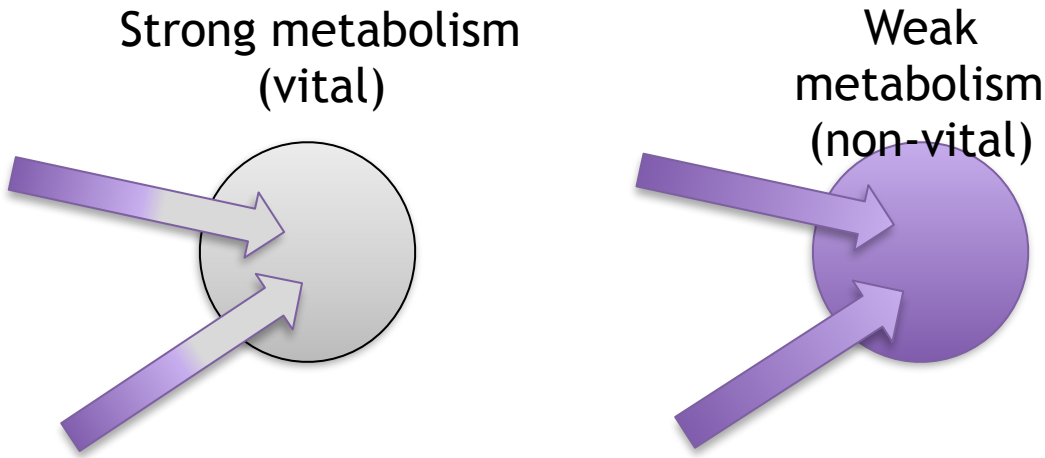
How to measure viability



- Solitary, darkly-stained cells are non-viable
- Budding cells:
  - Count budding cells as viable
- Lightly stained cells:
  - Count lightly stained cells as viable

How to measure vitality

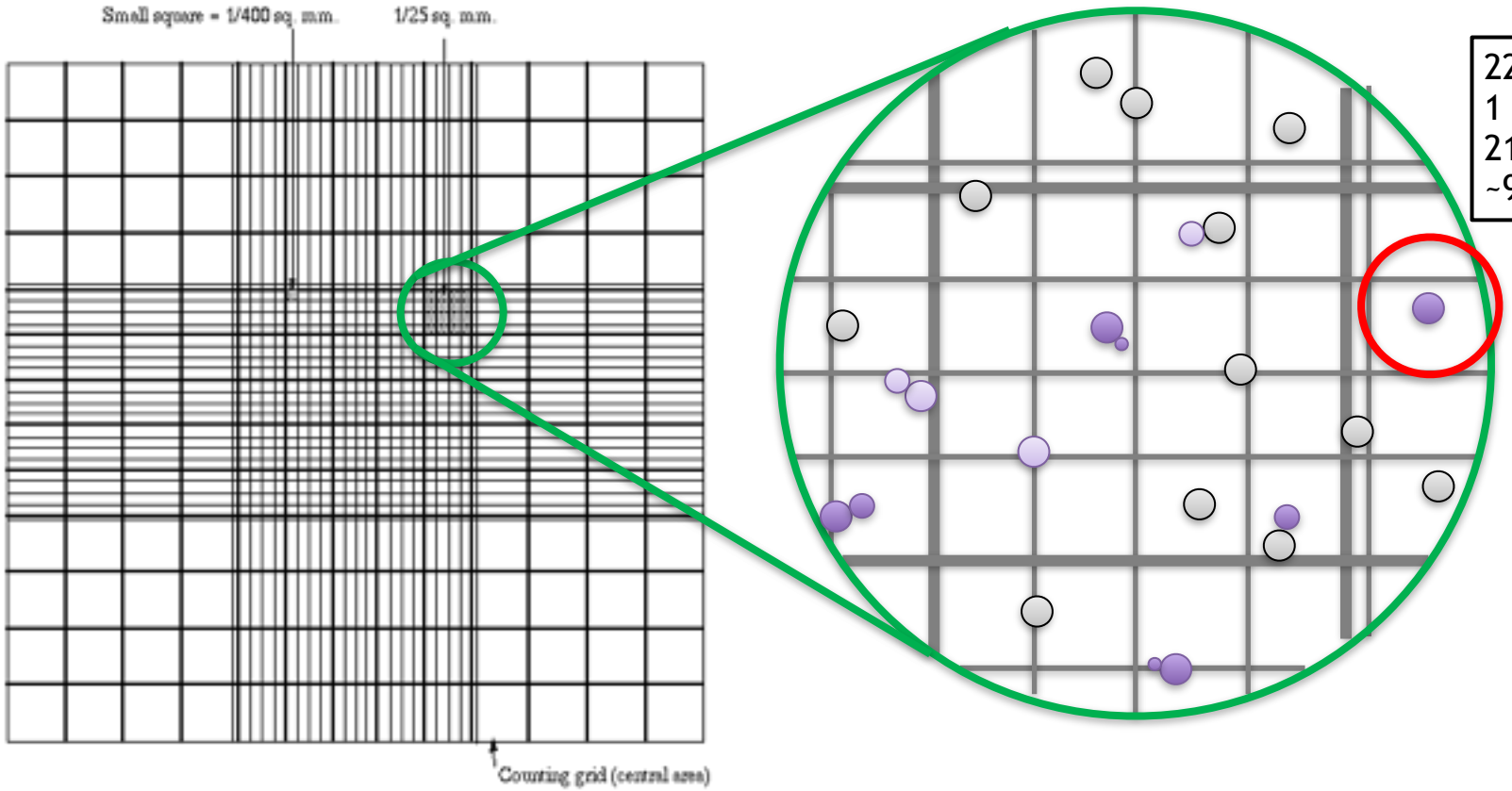
- Vitality is ultimately determined by fermentation performance
- Yeast health is correlated with a metabolic dye reduction assay
  - Based on intracellular reducing power



How to measure vitality

- Good vitality dye: alkaline methylene violet
  - Alkalinity lowers membrane potential, letting more dye into cells with stressed membranes (helps mitigate false positive viability)
  - Only strong metabolisms will reduce the dye
    - Vital cells remain clear
    - Non-vital cells stain violet
  - Mix your sample 1:1 with 0.1% dye
  - Count at least 100 cells total and note how many of those are stained violet

How to measure vitality



- Solitary, darkly-stained cells are non-vital
- Budding cells:
  - Count budding cells as vital
- Lightly stained cells:
  - Count lightly stained cells as vital

Pro's and Con's

Pro's

- Quick and effective in high viability/vitality situations
- Easy to set a go/no-go threshold
  - Ex: Find alternate yeast source <85% viability

Con's

- No good way to quickly and accurately measure all aspects of yeast health, these tests are no exception
- Color gradient "gray areas" can leave a lot to individual's judgement

References

- Boulton, C. & Quain, D. Brewing yeast and fermentation. Blackwell Science Ltd. Oxford, UK. 2001.
- Smart, K.A., Chambers, K.M., Lambert, I. & Jenkins, C. (1999) Use of methylene violet staining procedures to determine yeast viability and vitality. Journal of the American Society of Brewing Chemists, 57, 18-23.
- Priest, F. & Campbell, I. Brewing Microbiology, 3rd ed. International Center for Brewing and Distilling, Heriot-Watt University. Kulwer Academic/Plenum publishers, New York, NY 2003.

Thank you for your kind attention!

- Questions?