Getting The Most Out Of Your Hemocytometer

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March 28, 2013

Introduction

- Brewers have access to the same set of ingredients.
- How you combine them and control the process is what makes your beer unique.
- Monitoring yeast health and pitching rates is one more point of control to fine tune your process.

Getting Started

- The technology is simple and relatively inexpensive
- A hemocytometer: $15-30
- A microscope: $50-200
  - 400x objective
- 1 ml transfer pipet
- A scale that can measure grams accurately or a 100 ml volumetric flask
- A Pasteur pipette or a fine tipped glass pipet
- A hand held counter
- Pipette Pump
- Methyene Blue Stain

What is a Hemocytometer?

- A slide that you can look at under a microscope
- Chamber has a known volume of 0.0001 ml^3

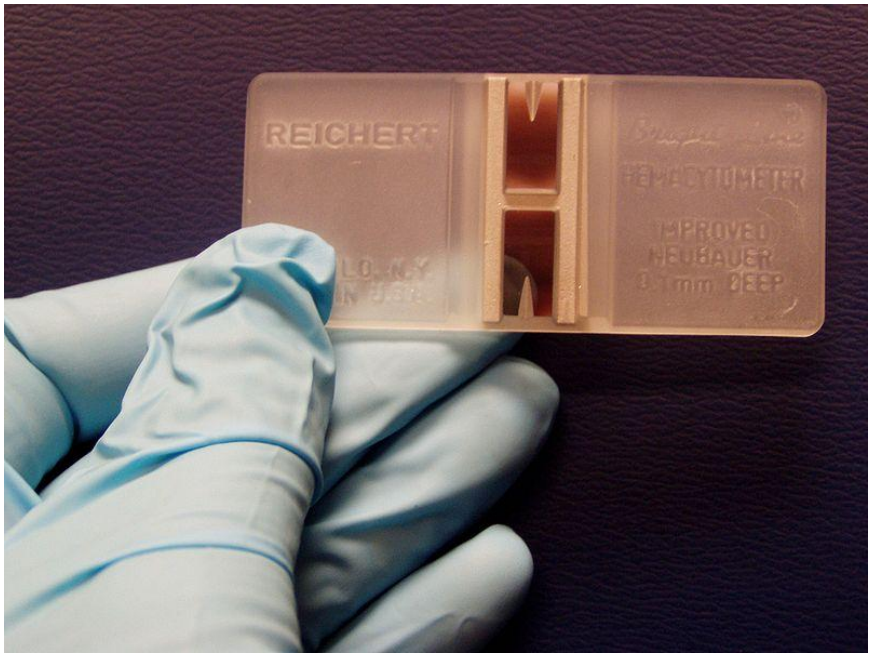
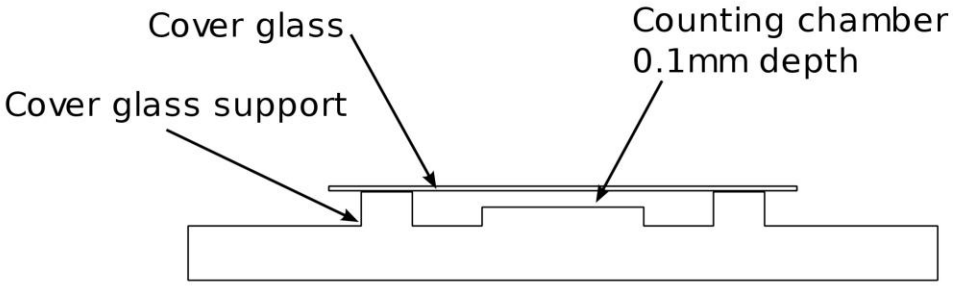
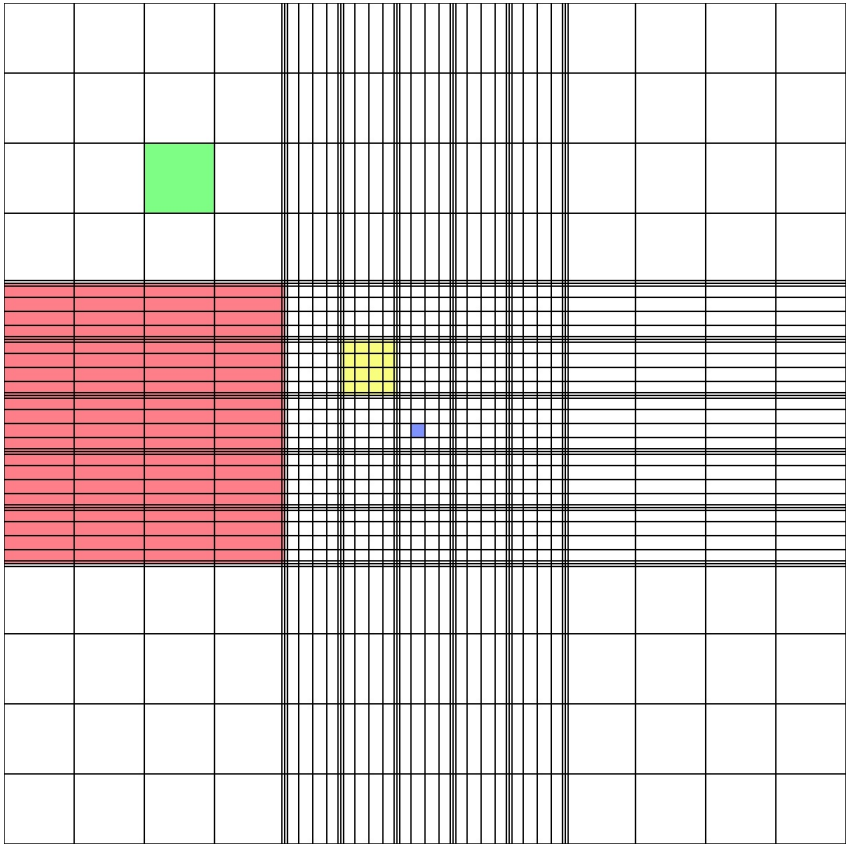


Photo by Jeffery M. Vinocur



What is a Hemocytometer?

- Laser etched in the surface are gridlines to aid in counting cells
- Cell counting occurs in the matrix where the triple lines converge



Make it Easy

- If the equipment is not accessible and ready to use you and your brewers won't count cells regularly.
  - Not everyone has a lab.
  - Or an office.
  - Or a desk.
- Build and maintain a kit.
  - Use a tray, tool box to store everything.
- Develop or borrow a spreadsheet to calculate pitching quantities.

Cell Counting Procedures

- Sampling of yeast slurry
  - Yeast should be stored in a vessel that make mixing and sampling easy.
  - Sampling needs to be done in a sanitary manner.
- Sample Size
  - Will you pitch by weight or by volume?
  - 1 ml if pitching by volume or 1 g if pitching by weight.

Cell Counting Procedures

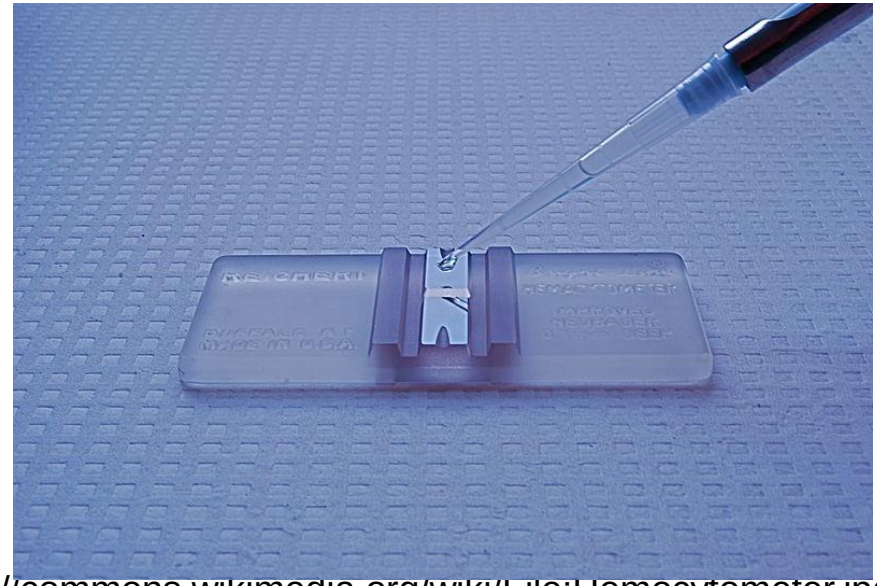
- Create a dilution
  - 1:100 is target using 1 part yeast slurry and 99 parts distilled water
  - 1 gram of slurry to 99 grams of water
  - 1 ml of slurry to 99 ml of water
  - Include the methylene blue as part of the dilution to determine viability

Cell Counting Procedures

- A word about Viability
  - Viabilities below 95% may not be accurate
  - The test only demonstrates the yeasts ability to metabolize the methylene blue stain
  - We are interested in the yeasts ability to ferment wort
  - The test measures viability but what we are really interested in is *vitality*

Cell Counting Procedures

- Load the hemocytometer
  - Make sure the hemocytometer is clean and dry
  - Place a lens on the hemocytometer
  - Take the Pastuer pipette and fill with dilution
  - Create a droplet at the tip of the pipette
  - Touch the droplet to the edge of the slide and the and the sample will wick into the chamber



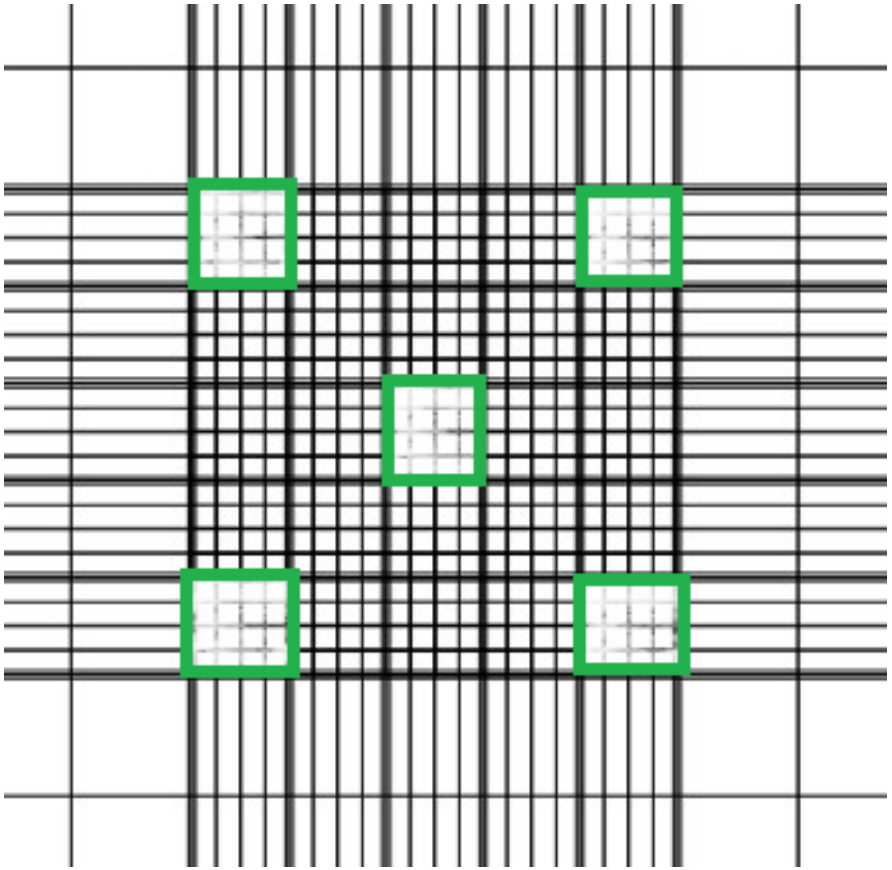
http://commons.wikimedia.org/wiki/File:Hemocytometer.jpg

Cell Counting Procedures

- Counting Cells
  - No need to count every cell in the chamber
  - The grid is 5 x 5 of the large squares
  - Use a hand held counter.
  - Budding cells are counted as one cell unless the bud is at least one half the size of the mother.

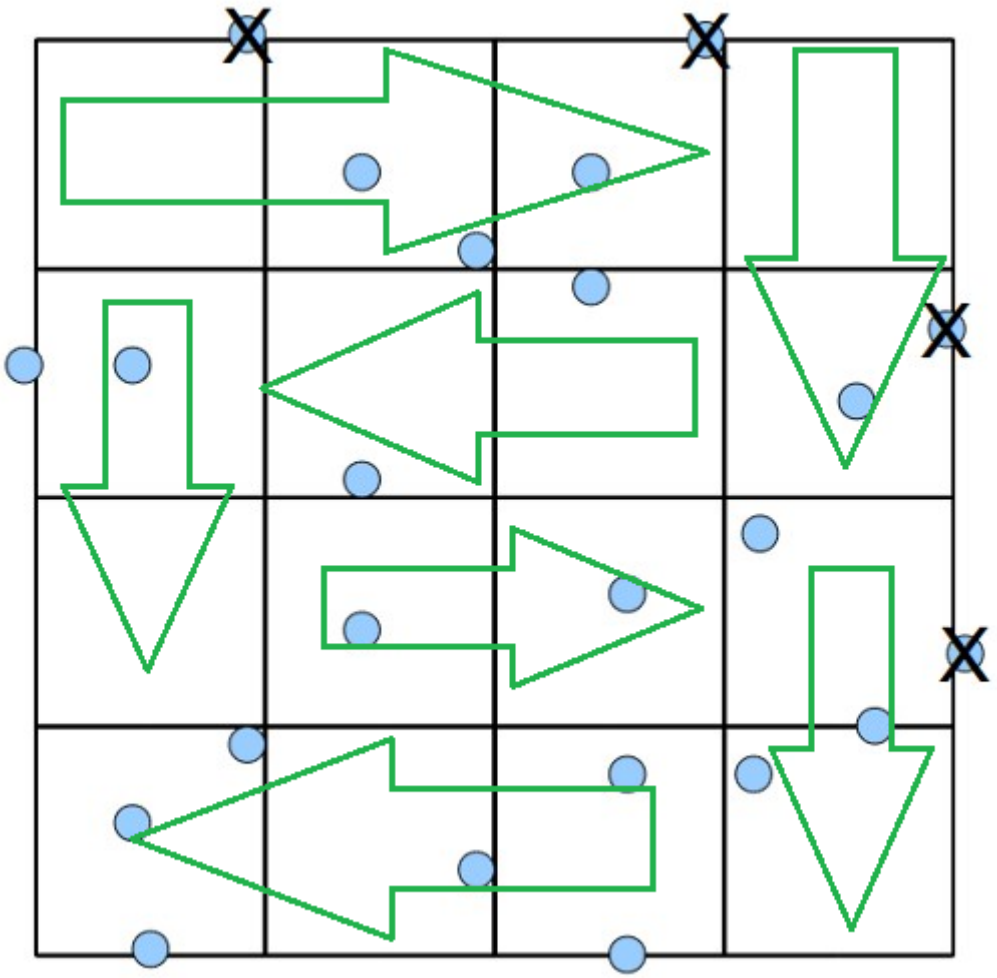
Cell Counting Procedures

- Counting Cells
  - Count 5 of the 25 squares in the 5 x 5 grid.



Cell Counting Procedures

- Move methodically through the grid
- If the cell touches to top or right line of the grid do not count.
- If the cell touches the bottom or left line of the grid count it.



Determining Pitching Rate

- Traditional pitching rates
  - 1 million cells per ml per degree plato for ale
  - 2 million cells per ml per degree plato for lager

$$°Plato\ wort \times \frac{1 \times 10^6\ viable\ cells/ml_{wort}}{1\ °Plato} = viable\ cells/ml_{wort}$$

$$bbl_{wort} \times \frac{117.35\ L_{wort}}{1\ bbl_{wort}} \times \frac{1{,}000\ ml_{wort}}{1\ L_{wort}} x\ \frac{vialble\ cells}{ml_{wort}} = Total\ cells\ needed$$

Cell Counting Procedures

- The Calculation:

$$\frac{(viable\ cell\ count)(5)(dilution)}{(chamber\ volume)} = \frac{yeast\ cells}{ml\ or\ g_{slurry}}$$

- *Viable* cells are what we are interested in
- The number 5 in the equation takes our sample of 5 squares up to 25 squares to represent the whole grid
- The dilution factor in this example would be 100 because we did a 100:1 dilution.

Determining Pitching Rate

$$\frac{Total\ Cells\ Needed}{yeast\ cells/ml_{slurry}} = Volume\ of\ yeast\ slurry\ required\ (in\ ml)$$

$$\frac{Total\ Cells\ Needed}{yeast\ cells/g_{slurry}} = Volume\ of\ yeast\ slurry\ required\ (in\ g)$$

- Convert to gallons or pounds for easier use:

$$ml_{slurry} \times \frac{1\ gal_{slurry}}{3785\ ml_{slurry}} = gal_{slurry}$$

$$g_{slurry} \times \frac{1\ lb_{slurry}}{453.592\ g_{slurry}} = lbs_{slurry}$$

Calculation Example

Yeast requirements for brewing 15 barrels of 13 plato wort of ale

13 million cells per ml would be target pitching rate

$$15\ bbl_{wort} \times \frac{117.35\ L_{wort}}{1\ bbl_{wort}} \times \frac{1{,}000\ ml_{wort}}{1\ L_{wort}}\ x\ \frac{1.3\ x\ 10^{7}}{ml_{wort}} = 2.288\ x\ 10^{13}\ cells\ needed$$

$$\frac{(viable\ cell\ count)(5)(dilution)}{(chamber\ volume)} = \frac{(98\ cells)(5)(100)}{(.0001\ ml)} = 4.9\ x\ 10^{8} cells/ml_{slurry}$$

$$\frac{Total\ Cells\ Needed}{yeast\ cells/ml_{slurry}} = \frac{2.288\ x\ 10^{13} cells}{4.9\ x\ 10^{8} yeast\ cells/ml_{slurry}} = 4.67\ x\ 10^{4}\ ml_{slurry}$$

Calculation Example

- Convert to gallons or pounds for easier use:

$$4.67\ x\ 10^4\ ml_{slurry} \times \frac{1\ gal_{slurry}}{3785\ ml_{slurry}} = 12.34\ gal_{slurry}$$

$$4.67\ x\ 10^4 g_{slurry} \times \frac{1\ lb_{slurry}}{453.592\ g_{slurry}} = 102.96\ lbs_{slurry}$$

Determining Pitching Rate

- Non traditional pitching rates
  - Some fermentation flavors from expressive yeasts can be manipulated by intentional under or over pitching
  - Not all strains can perform well with under pitching, you still need to achieve attenuation
  - Rates of 750,000 cells per ml per degree plato in German Style Hefeweizen and Belgian styles have worked well balancing flavor and performance

Determining Pitching Rate

- High Gravity Beers
  - Over pitching high gravity beers typically leads to better attenuation rates
  - Can lead to lower flocculation and an abundance of yeast in the fermenter
- Beyond fermentation flavors
  - Yeast cells absorb isomerized alpha acids
  - Pitch rate effects finished IBU levels in beer
  - Consistent pitching rates will result in more consistent bitterness.

Beyond Primary Fermentation

- Determining the best time to filter beer
  - Establish a *measureable* target for pre-filtration clarity
- Establishing consistent yeast levels in unfiltered beer.
  - Consistent haze and yeast count in beer package with yeast across several operators

Beyond Primary Fermentation

- Determining bottle or can conditioning pitching rates
  - Evaluate unfiltered beer prior to package
  - Determine fresh yeast dosing rates
    - To much yeast in the bottle leads to off flavors and shorter shelf life
    - Too little viable yeast can result in a failure to re-ferment in the bottle.
  - 500,000 cells/ml is sufficient if only a portion of final co2 is gained from bottle conditioning.
  - Over 1 million cells/ml can result in excessive yeast in the bottle.

Conclusion

- You monitor your mash temp, calculate IBUs and weigh out your grain already
- All are points of control that impact the final flavor of your beer
- Using a hemocytometer gives the brewer one more point of control to fine tune flavor and ultimately achieve a more consistent product.

Thank You

Steve Parkes – American Brewers Guild and Drop In Brewing Company

Ray Romero – Brewers Supply Group

The Brewers Association

QUESTIONS?

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