Rapid yeast viability detection method in complex brewing samples using Cellometer X2 Image Cytometry

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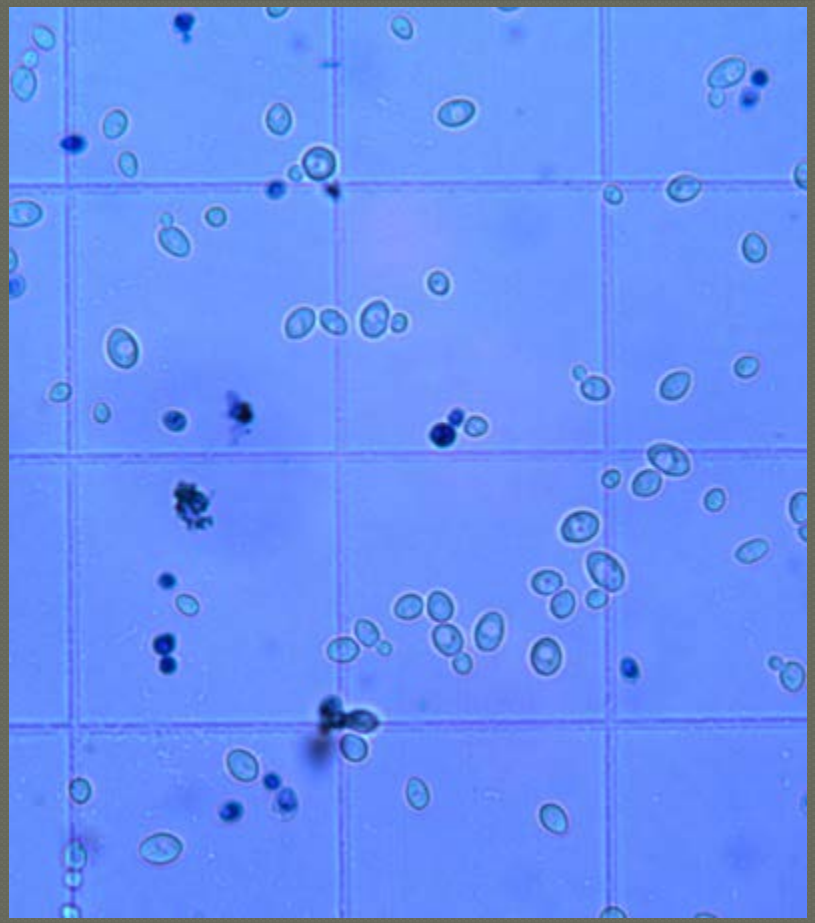
Presentation Outline

- Importance of yeast viability for fermentation
  - Difference between simple and complex samples
- Yeast viability detection methods
  - Traditional methods for yeast viability
  - Cellometer X2 image cytometry method
- Developing a viability detection method for yeast in complex media
  - Testing multiple fluorescent viability stains
  - AOPI yeast viability detection method
- Validation of yeast viability detection method
  - Testing yeast in corn mash, corn stover, and sugarcane
- Summary

Importance of yeast viability for fermentation



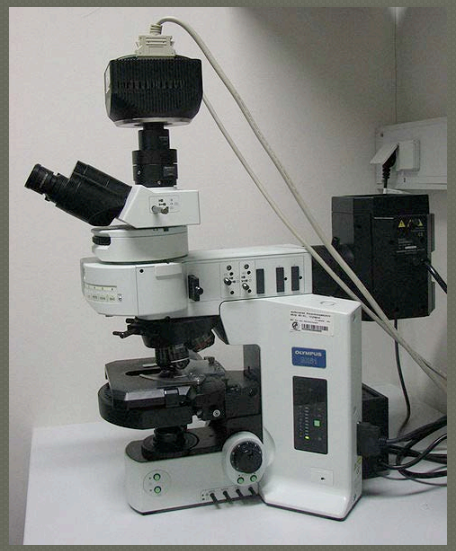
https://eurekabrewing.wordpress.com

- The ability to measure yeast viability allowed brewers to better control fermentation process
- The liveliness of the yeast can affect the fermentation time as well as the quality of the beer products

Yeast in complex brewing sample

- The common yeast sample collected from fermentation tanks is mostly clean
- However, some specialty beer requires other materials such as herbs, spices, seeds, roots, and fruits
- These materials often generate high amount of nonspecific particles in the yeast sample, which make it difficult for manual and automated cell counting

Traditional methods for yeast viability



Manual counting method

- Traditional method
- Manually count methylene blue stained yeast cells
- Prone to human-error
- Time-consuming

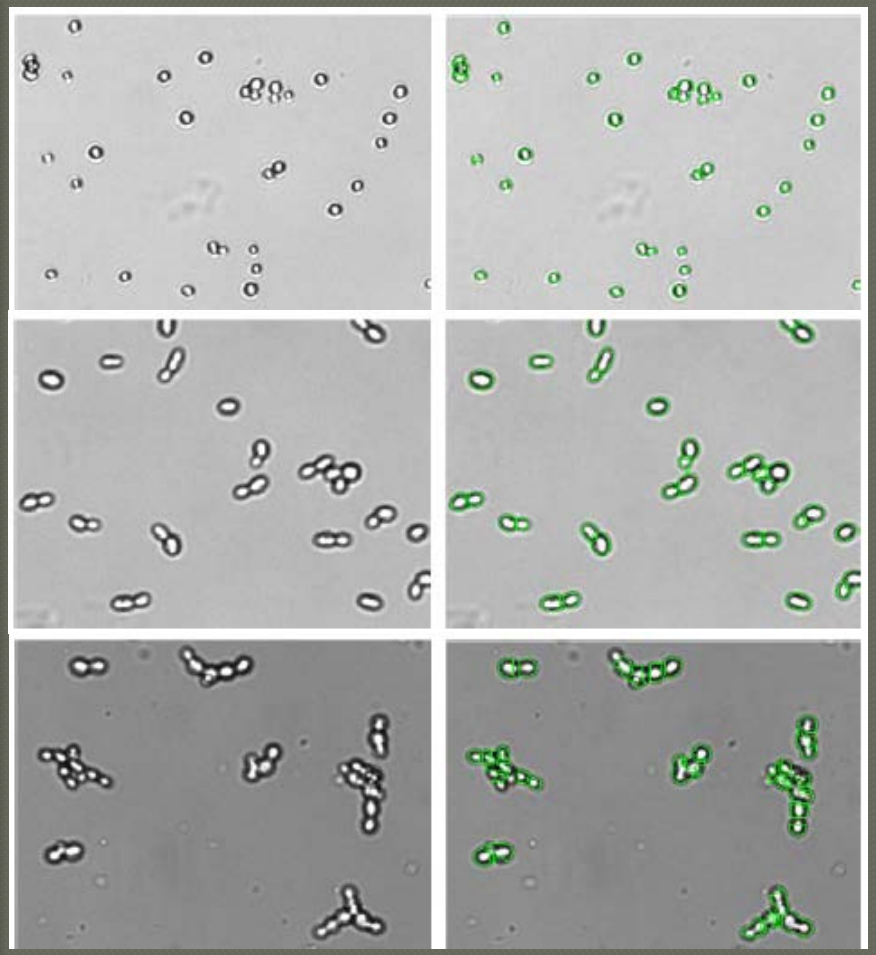
Fluorescence Microscopy

- Ability to study fluorescence
- Image-based observation
- Lacks automation
- Qualitative analysis

Flow Cytometry

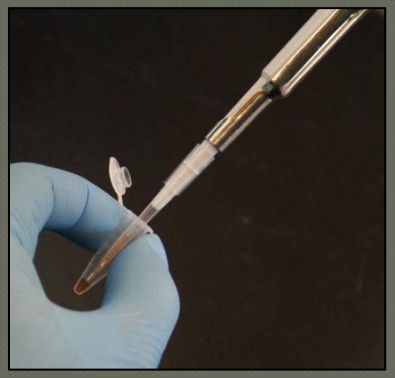
- Provides automation
- Improves statistical analysis
- Considerable maintenance
- Lack of imaging capability

Cellometer X2 image cytometry method

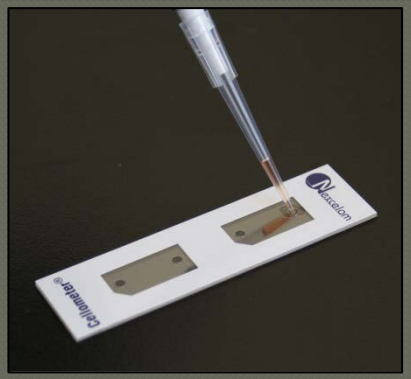


- Cellometer X2 is an image cytometry system
- Captures bright-field and fluorescent images
- Automatically count the yeast in images to generate concentration and viability

How to do an image cytometric analysis protocol?

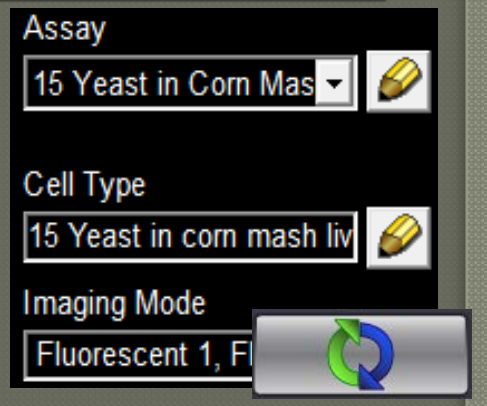


1. Stain sample

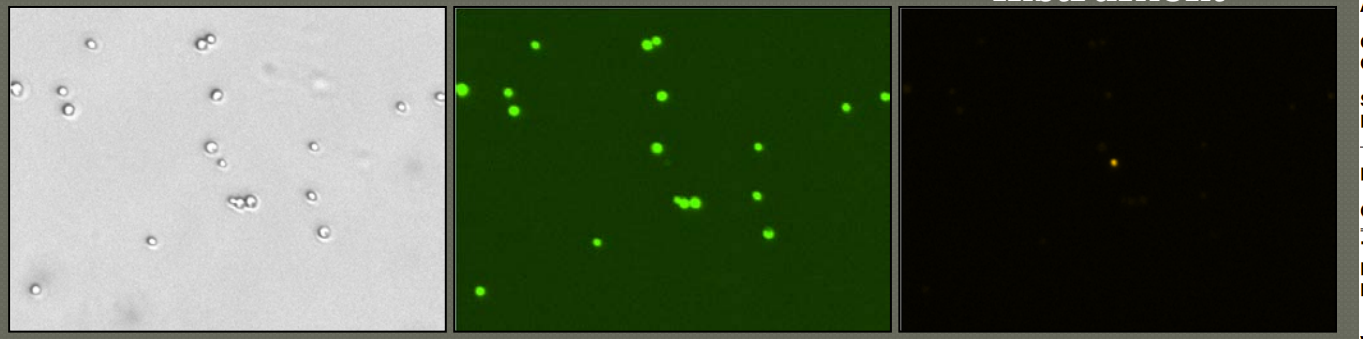


2. Pipette 20uL into counting chamber

3. Insert chamber into instrument



4. Select assay and click count



5. Bright field and fluorescent images are acquired and analyzed

Assay: 15 Yeast in Corn Mash Viability

Cell Type F1: 15 Yeast in corn mash live
Cell Type F2: 15 Yeast in Corn Mash dead

Sample ID: sample 2-2_20100422_152120-2
Dilution: 1.00

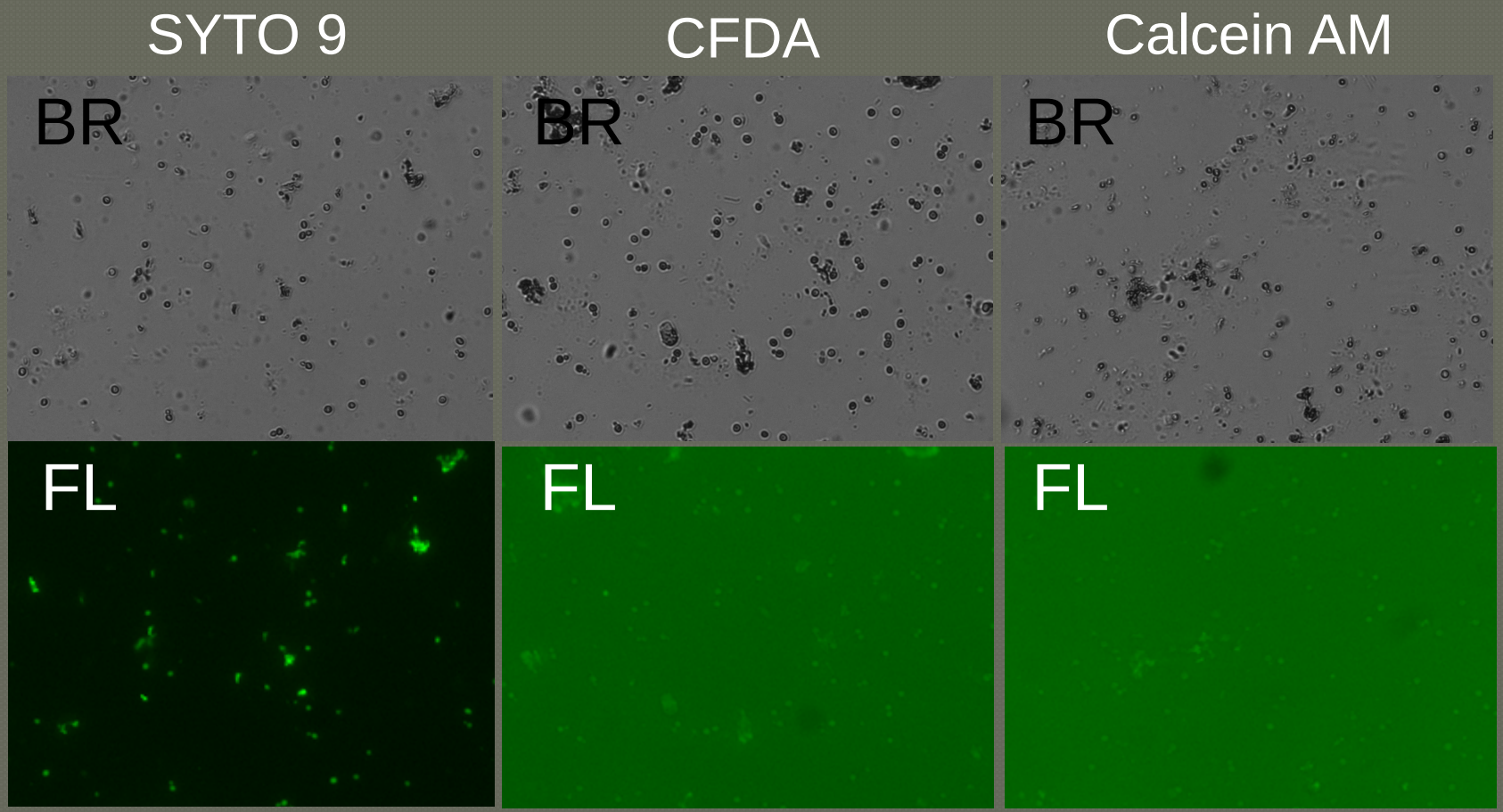
Results:

Count	Concentration
Total: 713	4.01x10^6 cells/mL
Live: 466	2.63x10^6 cells/mL
Dead: 247	1.39x10^6 cells/mL

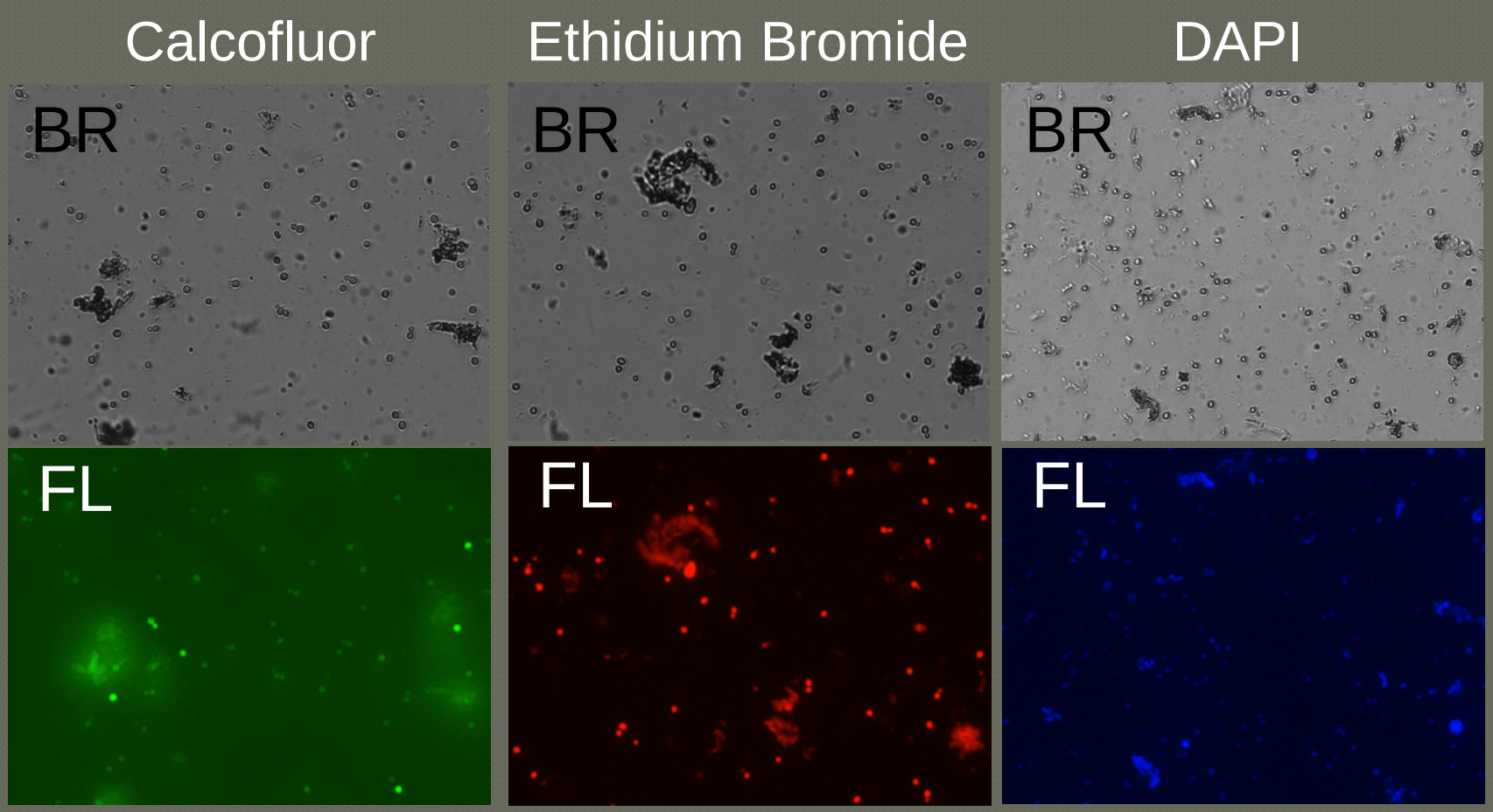
Viability: 65.4%

6. Cell count / viability are calculated automatically

Fluorescent viability dyes can stain nonspecific particles in the sample



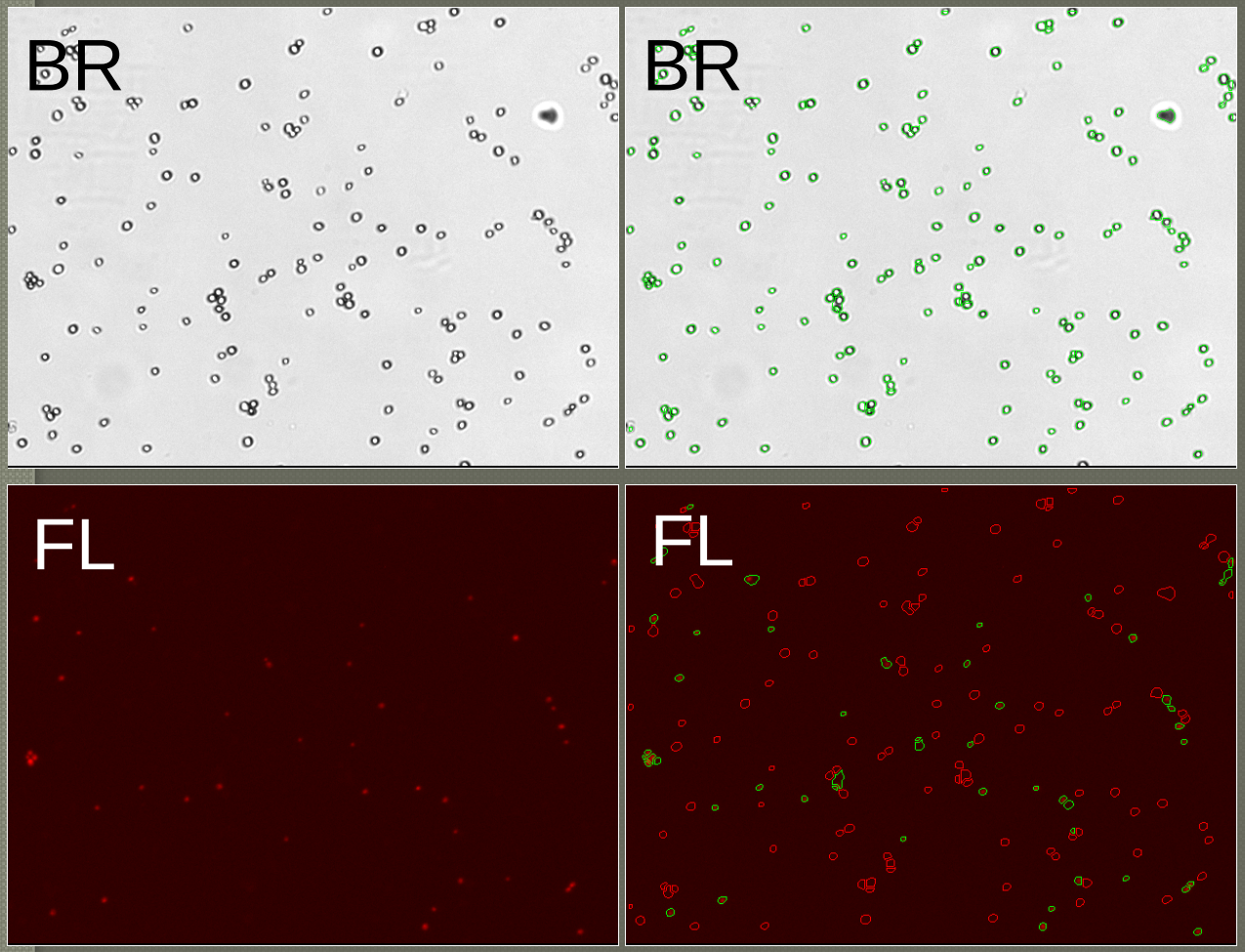
Fluorescent viability dyes can stain nonspecific particles in the sample



Measure yeast viability in clean sample using PI

Images

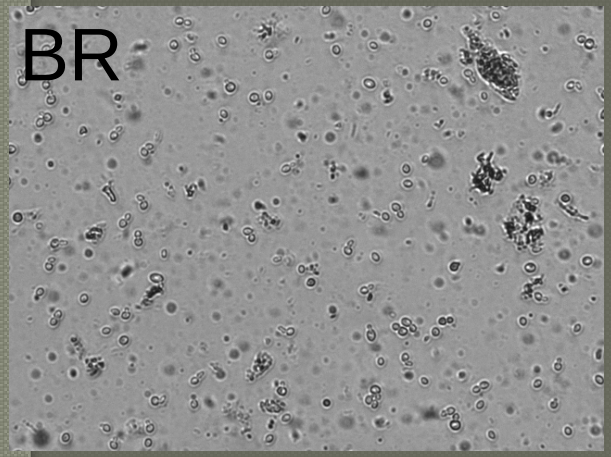
Counted

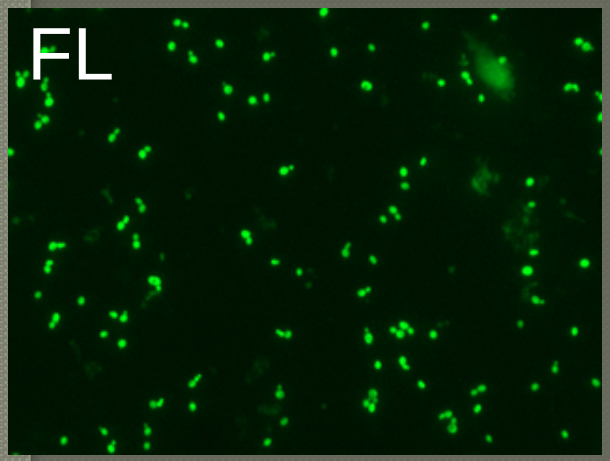


- If the yeast sample is clean, viability can be measured by
  - Total cells in bright-field
  - Dead cells with PI fluorescence

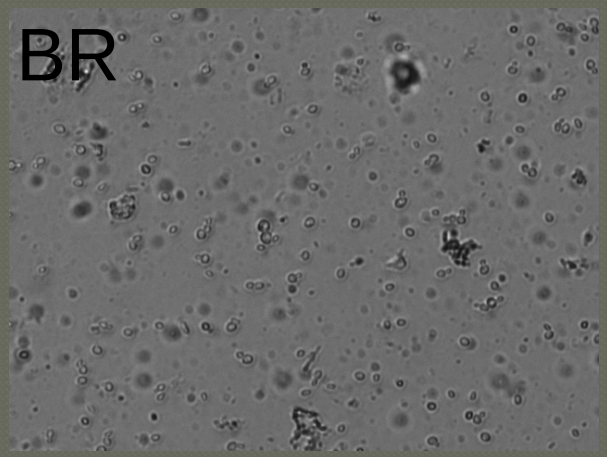
Fluorescent viability detection using AOPI

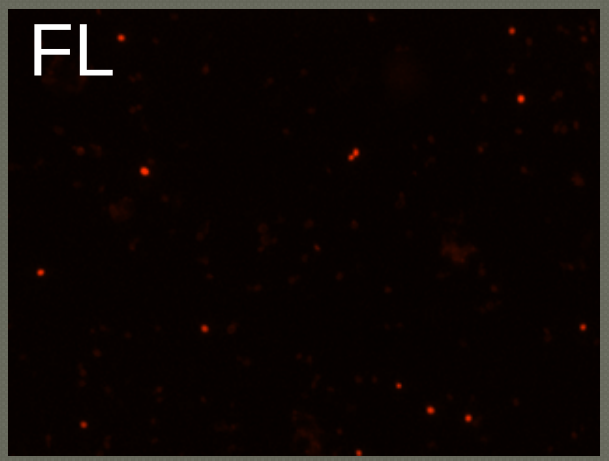
Acridine Orange (AO)





Propidium Iodide (PI)





- AOPI is used in combination with a yeast dilution buffer developed in Nexcelom
- This allowed clear identification of live and dead yeast particles in the sample

Dual AOPI fluorescence for measuring viability of yeast in complex sample

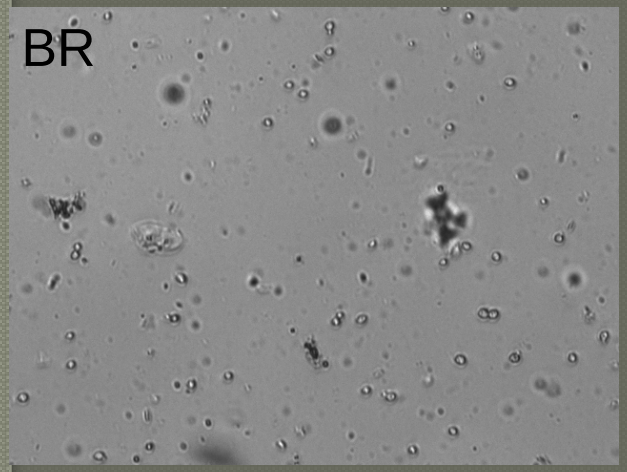
- Yeast samples that are clean can be stained with only propidium iodide for dead cells
  - Viability is calculated by measuring total cells in bright-field and dead cells in fluorescence
- Yeast in complex samples will require 2 fluorescent stains for live and dead cells, which can eliminate the debris and nonspecific particles
  - Viability is calculated by measuring total live and dead cells in fluorescence

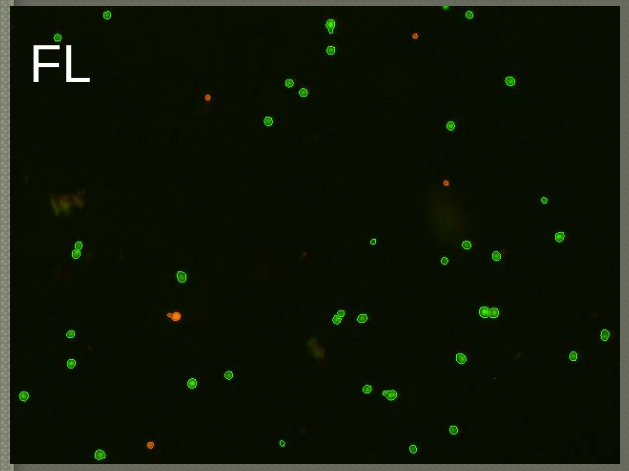
Validation of yeast viability detection method

- In order to validate the AOPI method for yeast viability in complex samples, we showed the specific staining of live and dead yeast in fermentation with
  - Corn mash
    - Measure concentration, viability, and compared to manual counting
  - Examples of corn stover and sugarcane

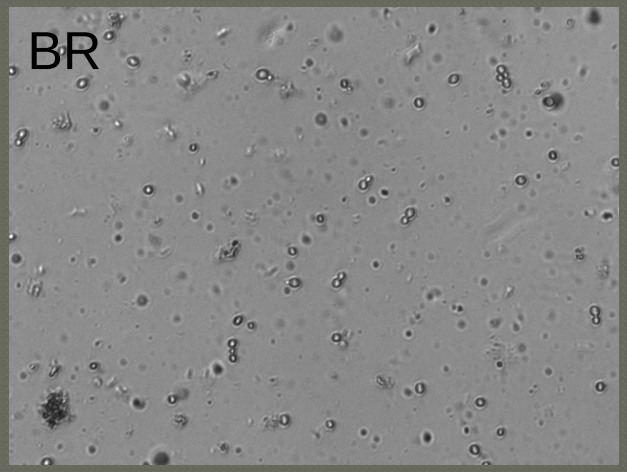
Fermentation with corn mash

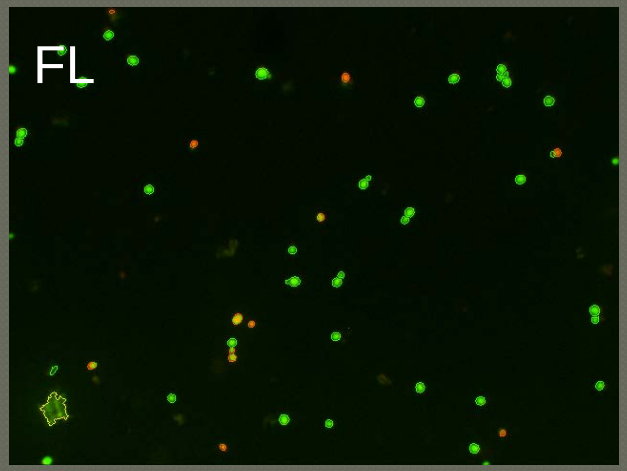
2.65 hours



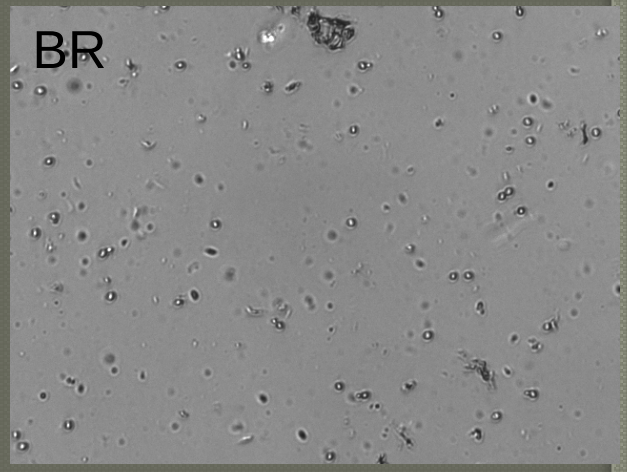


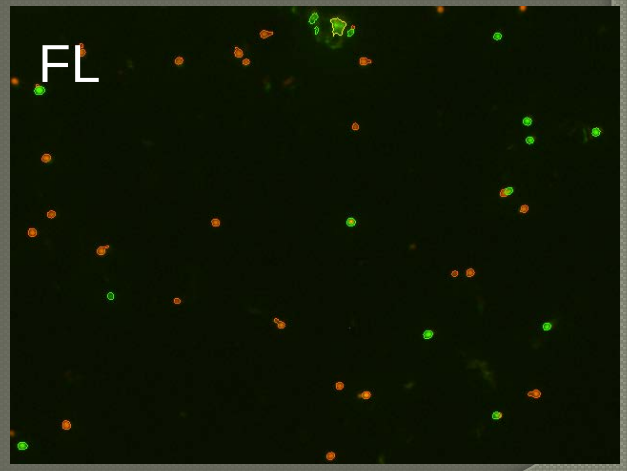
25 hours





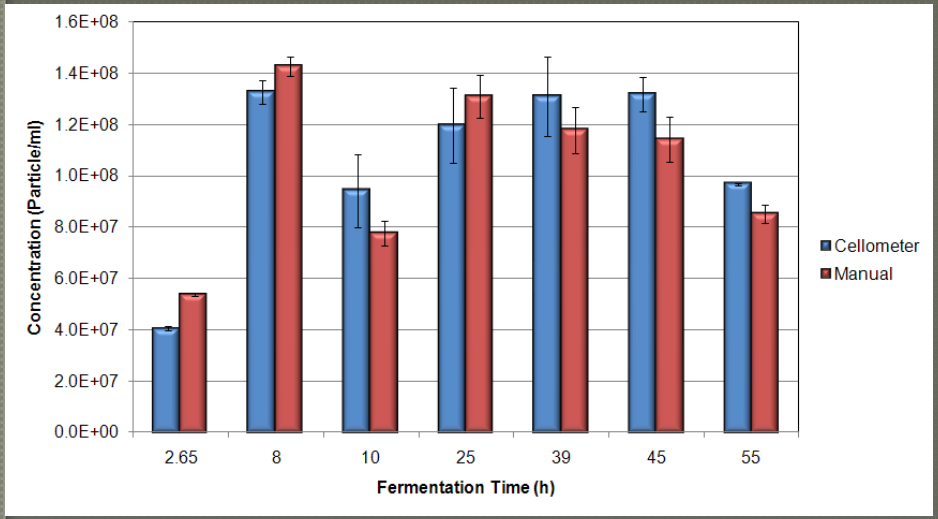
55 hours



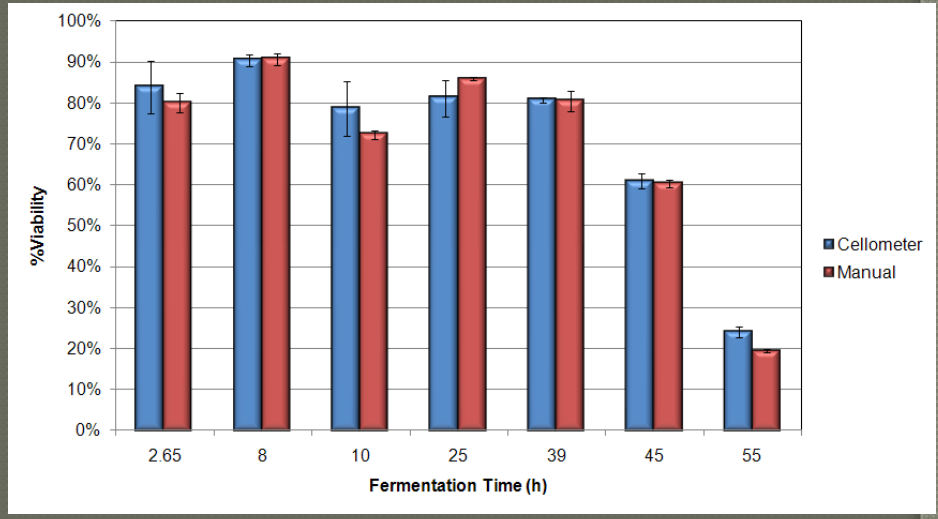


Corn mash concentration and viability results

Concentration

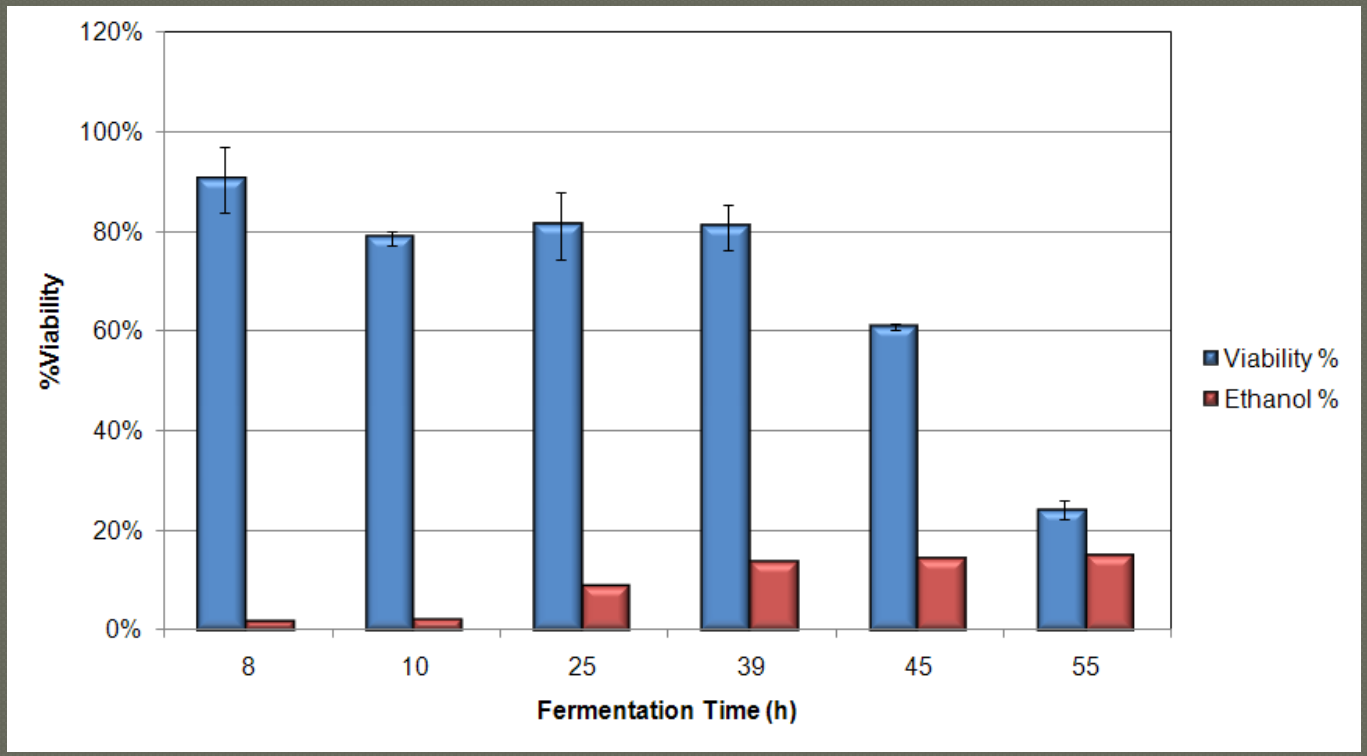


Viability



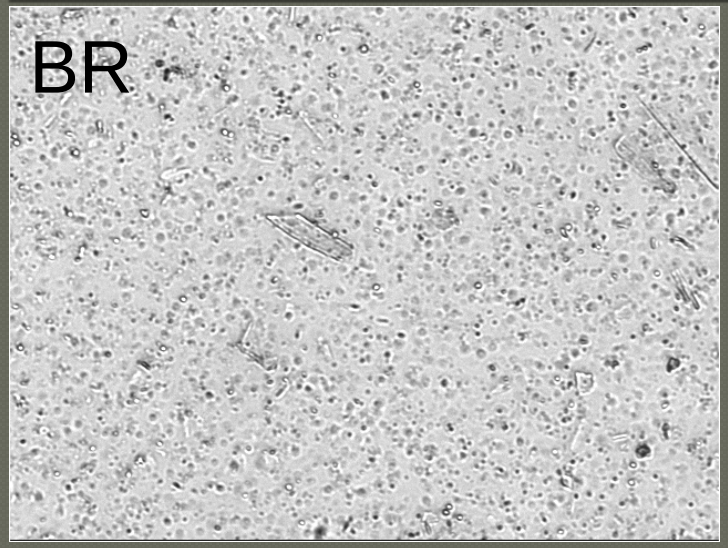
- Manual and automated counting showed comparable concentration and viability results
- By using AOPI method, viability can be monitored throughout fermentation, where by 55 hours, the viability significantly decreased

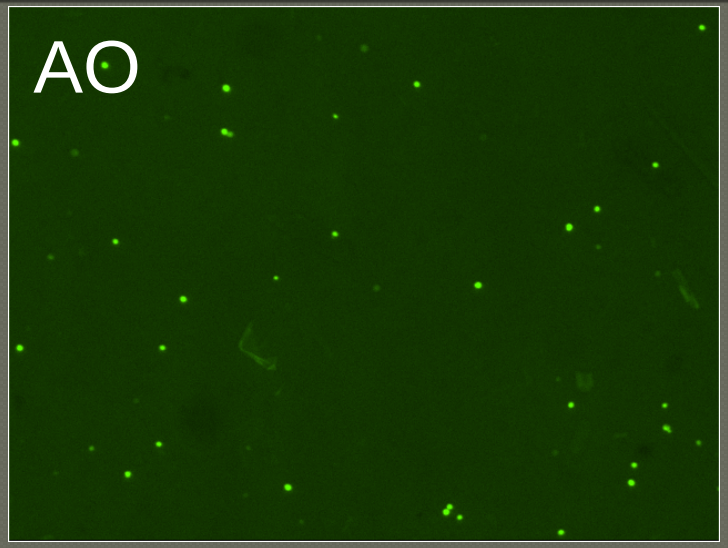
Correlation to ethanol %

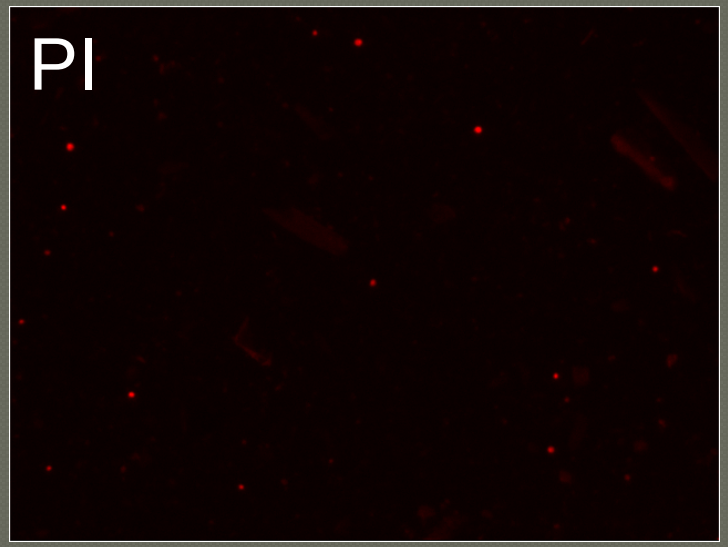


- As the viability decreased, the ethanol % increased to over 10%, which induced yeast cell death over time

Example: Yeast in corn stover

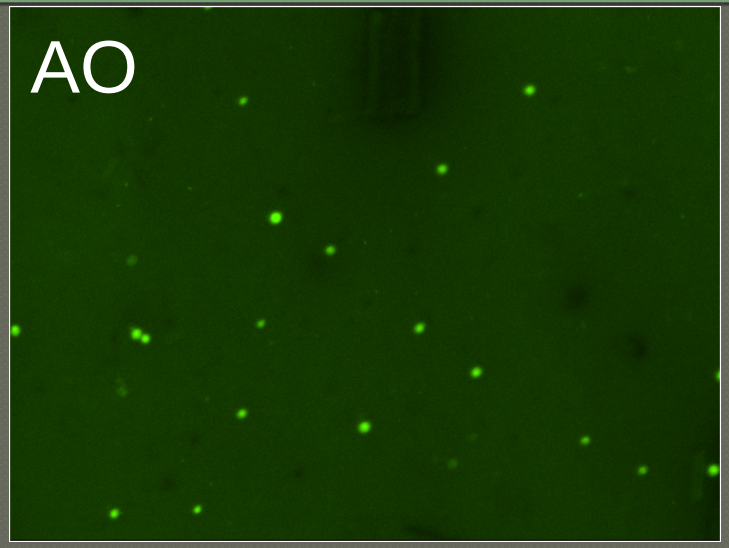


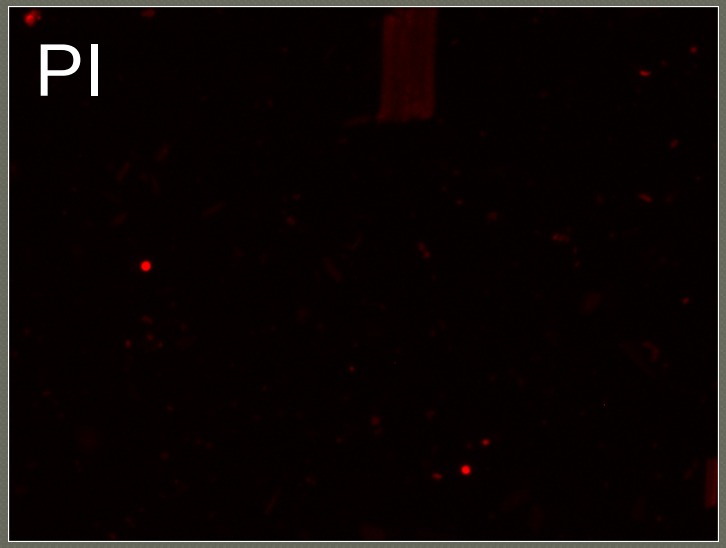




- Adding corn stover to fermentation increased the debris and nonspecific particles in the sample
- AOPI was able to specifically stain the live/dead yeasts

Example: Yeast in sugarcane



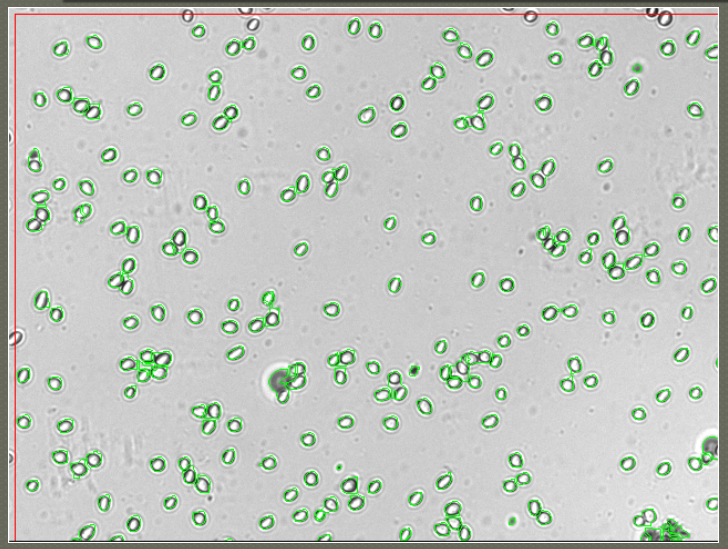


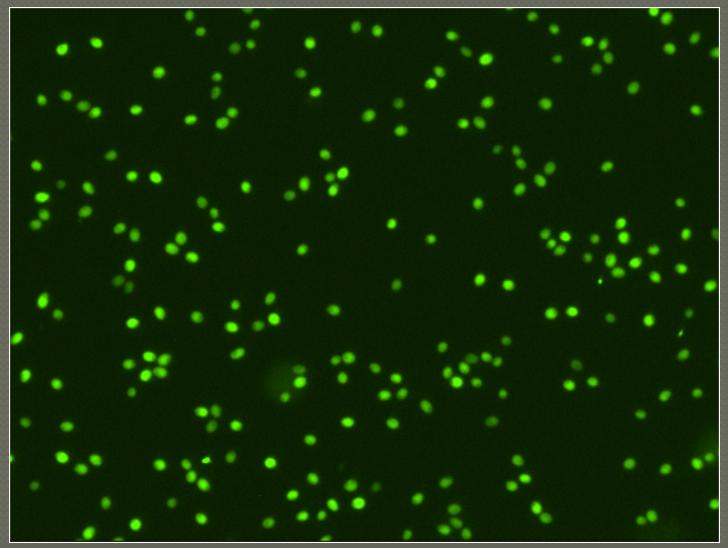
- Sugarcane has large debris and nonspecific particles in the sample
- AOPI was also able to specifically stain the live/dead yeasts

Summary

1. Yeast viability is highly important for an optimal beer fermentation
2. Manual and automated counting can be used for yeast viability analysis of clean sample
3. Complex samples with materials added increase difficulty in counting
4. By using AOPI, live and dead yeast can be specifically counted
5. This allows brewers to effectively measure viability from simple to complex yeast samples
6. Other than viability, yeast vitality is also an important parameter to measure for fermentation

Yeast vitality assay by Cellometer X2





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Assay: Yeast + CFDA

Cell Type F1: Yeast

Sample ID: F9 Joes Vitality 1_20130814_130900-1
Dilution Factor: 4.00

Results: Count Data

Count	Concentration	Mean Diameter
BR1: 3579	8.22x10^7 cells/mL	7.7 micron
FL1: 3479	7.93x10^7 cells/mL	6.4 micron

Formula: F1 / BR * 100%
96.5%

FL1 Mean Intensity:
56.6 Counts

Results: Image Data

Raw Brightfield Image BR1: A, Frame 1

Fluorescence Image F1: A, Frame 1

- Cellometer X2 can also be used to measure yeast vitality by using CFDA-AM

Any Questions?

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