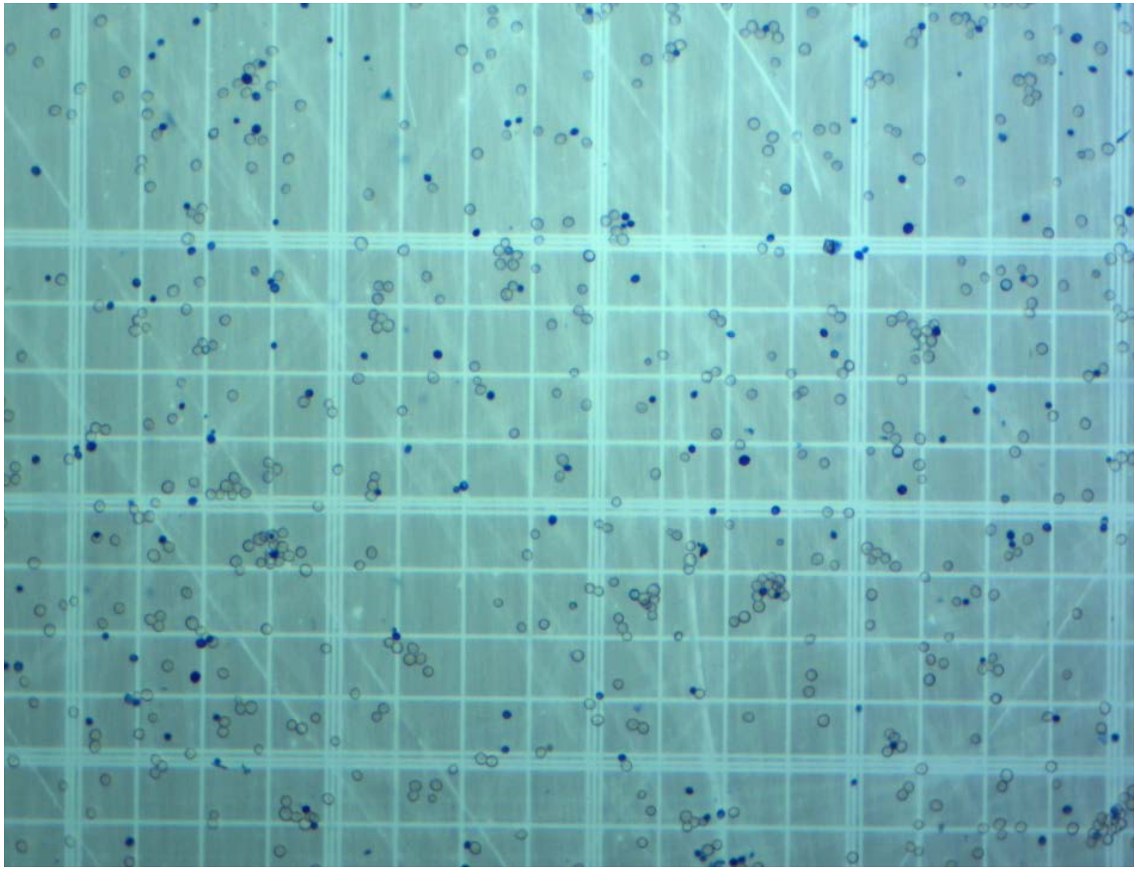
Practical Experiences with an Automated Cell Counter Using Methylene Blue in Breweries

K. Thomson, J. Carvell and A. Bhat

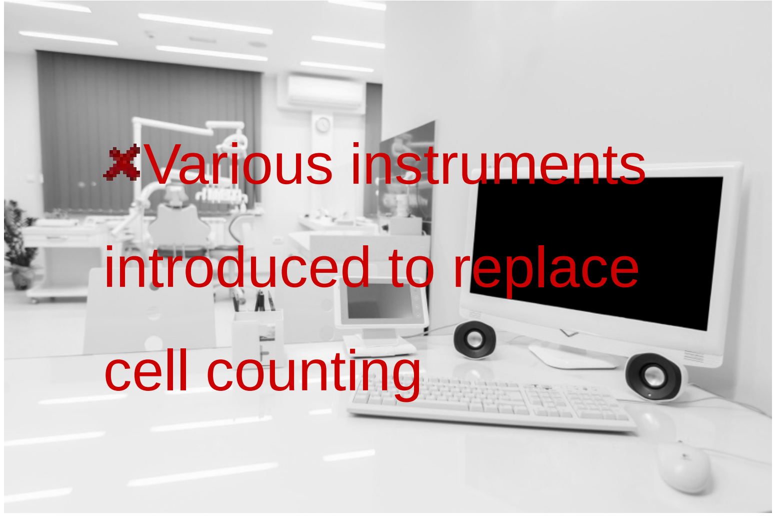
The Science of Beer

Cell Counting

- Critical step
- Number
- Viability



Current Methods



Common and Rapid Methods for Yeast Counting and Viability Estimations

- Coulter Counter (only provides a count and not viability).
- Dielectric Spectroscopy (provides viable cell count only).
- Image Analysis with a cartridge and embedded Propidium Iodide and fluorescence microscope.
- Image analysis with addition of fluorescent stain(s) to 1or2 chamber slide with single or dual channels.
- Flow Cytometry.
- Image analysis with brightfield microscope/5 chamber slide and methylene blue.

Considerations by Brewery in choosing new instrument or technology

- Ease of use and level of expertise in laboratory (e.g. Process operator up to skilled microbiologist).
- Additional factors that might influence reading (e.g. Manual focussing; Extra processing of sample).
- Safety and stability of reagents and disposal.
- Cost of instrument and reagents.
- Comparability with traditional or reference data.
- Calibration and maintenance.

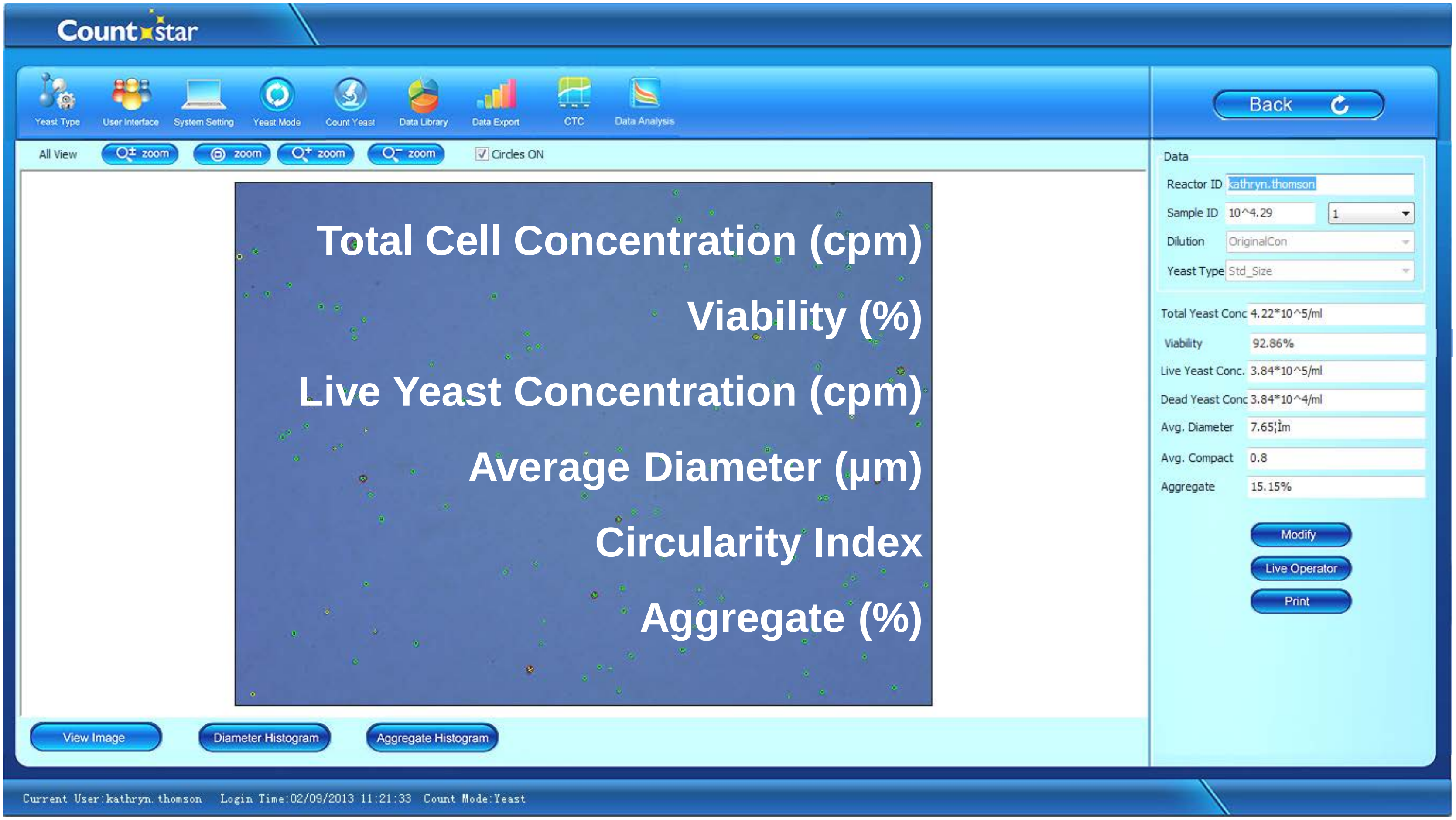
Purpose of the work

- Manual counts with Methylene Blue
  Gold Standard
- Inter-operator.
- Time consuming.
- Aber Countstar has been recently released into market.

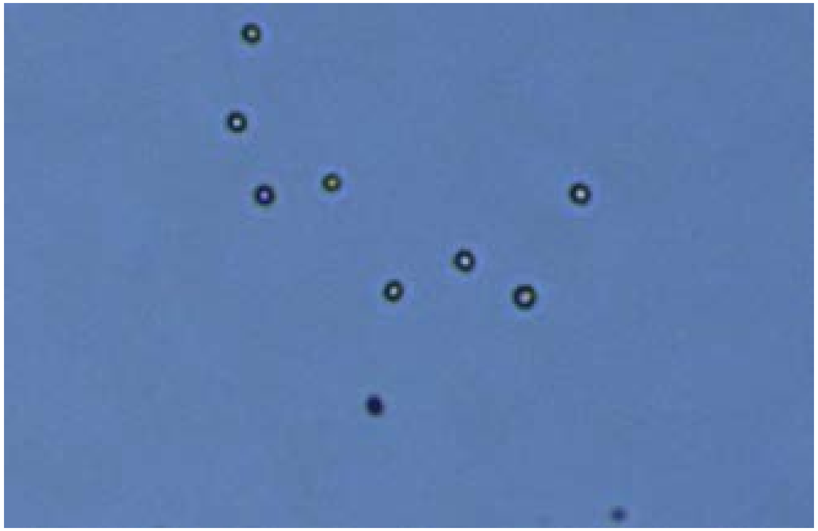
Aber Countstar

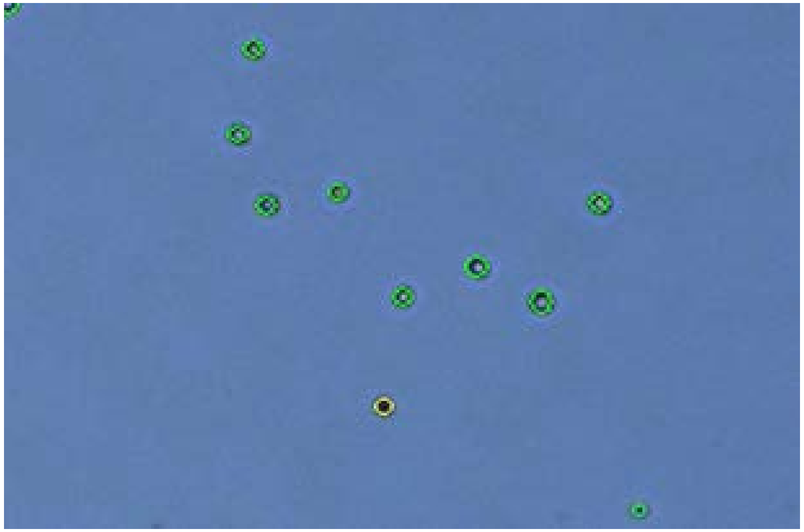
- Automated cell counter
- Methylene blue or MV
- Five chamber disposable slides
- Additional information on yeast

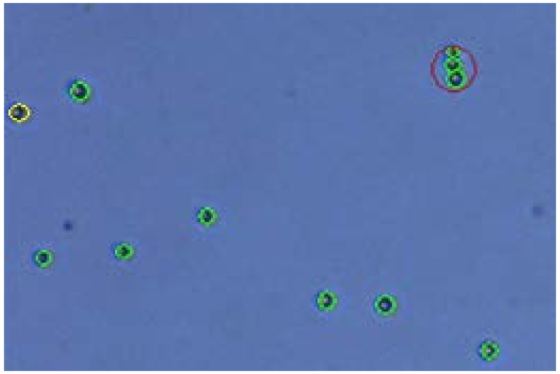
Information provided by the Countstar



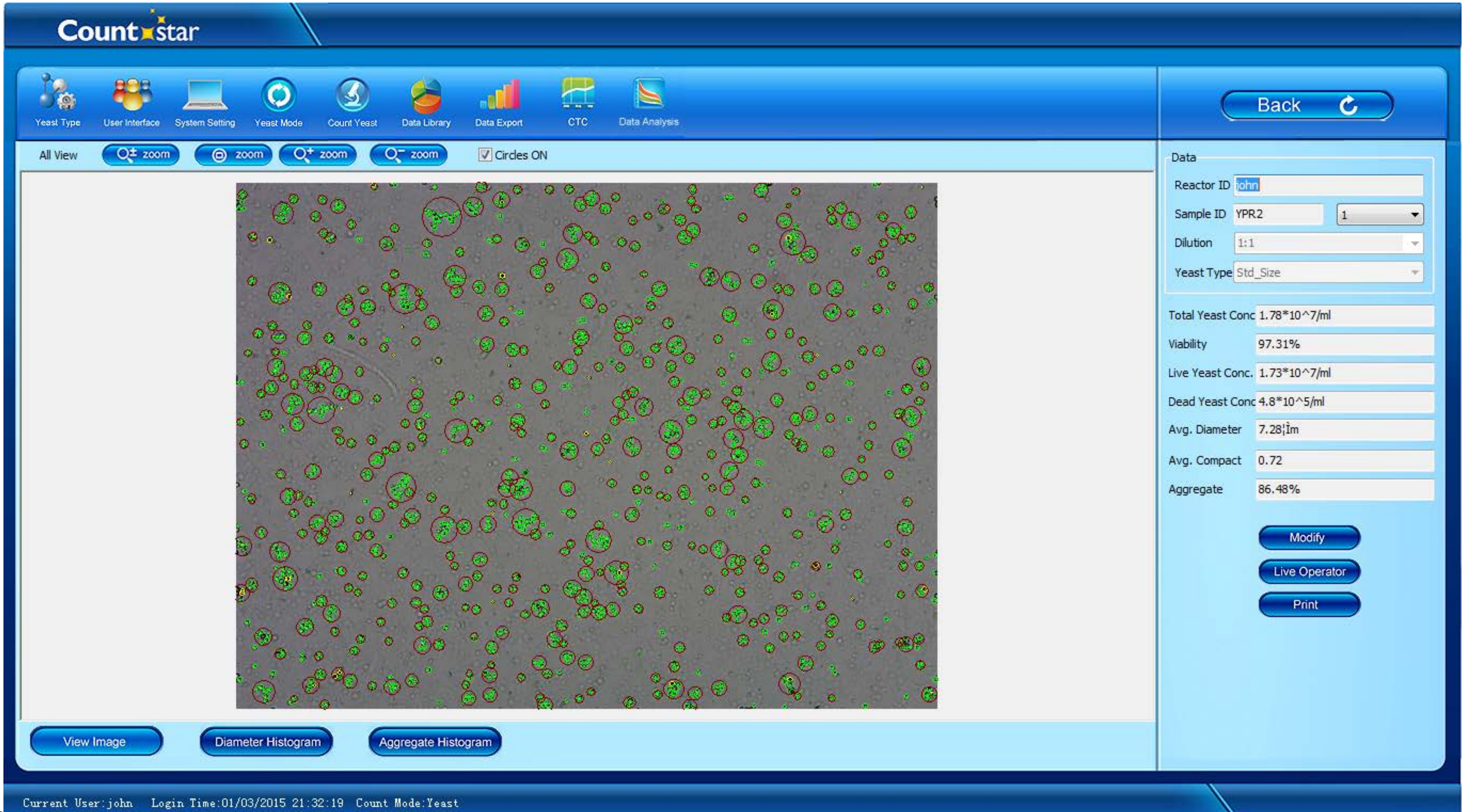
Dead vs Alive cells

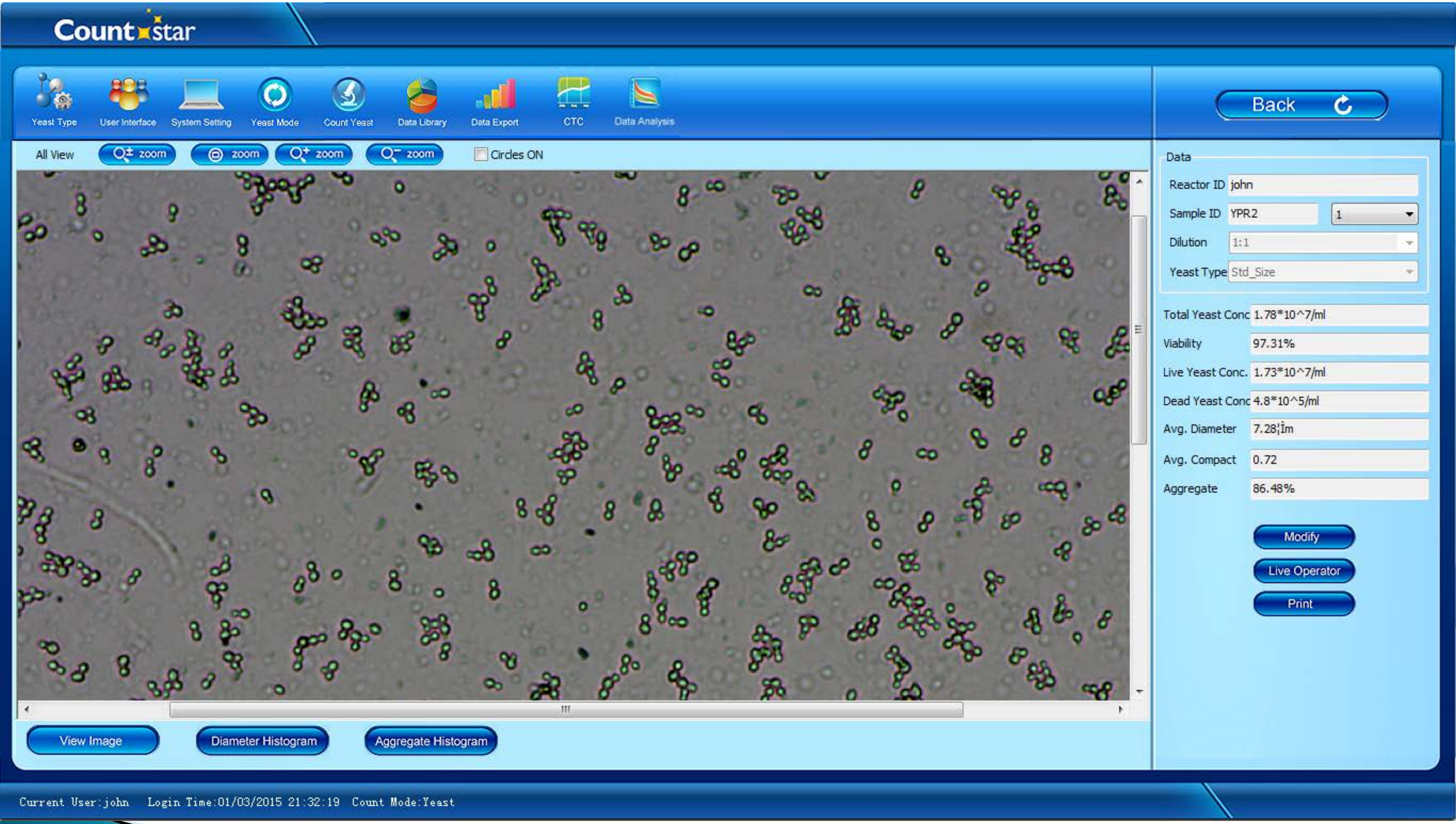


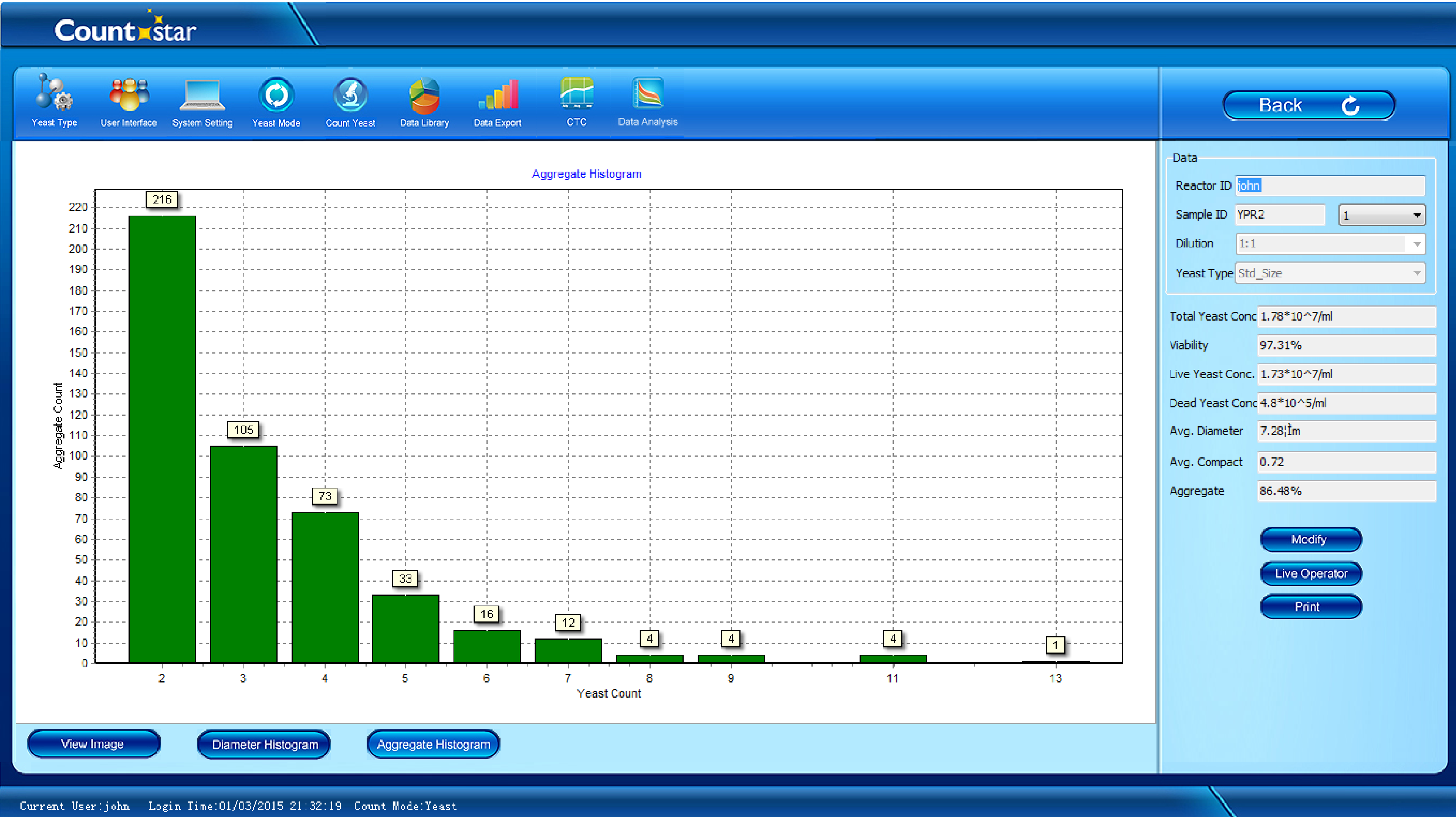




Example with propagating yeast




Countstar
Yeast Type
User Interface
System Setting
Yeast Mode
Count Yeast
Data Library
Data Export
CTC
Data Analysis
Back
All View
zoom
zoom
zoom
zoom
Circles ON
Data
Reactor ID john
Sample ID YPR2
1
Dilution 1:1
Yeast Type Std_Size
Total Yeast Conc 1.78*10^7/ml
Viability 97.31%
Live Yeast Conc. 1.73*10^7/ml
Dead Yeast Conc 4.8*10^5/ml
Avg. Diameter 7.28¦Ìm
Avg. Compact 0.72
Aggregate 86.48%
Modify
Live Operator
Print
View Image
Diameter Histogram
Aggregate Histogram
Current User:john Login Time:01/03/2015 21:32:19 Count Mode:Yeast



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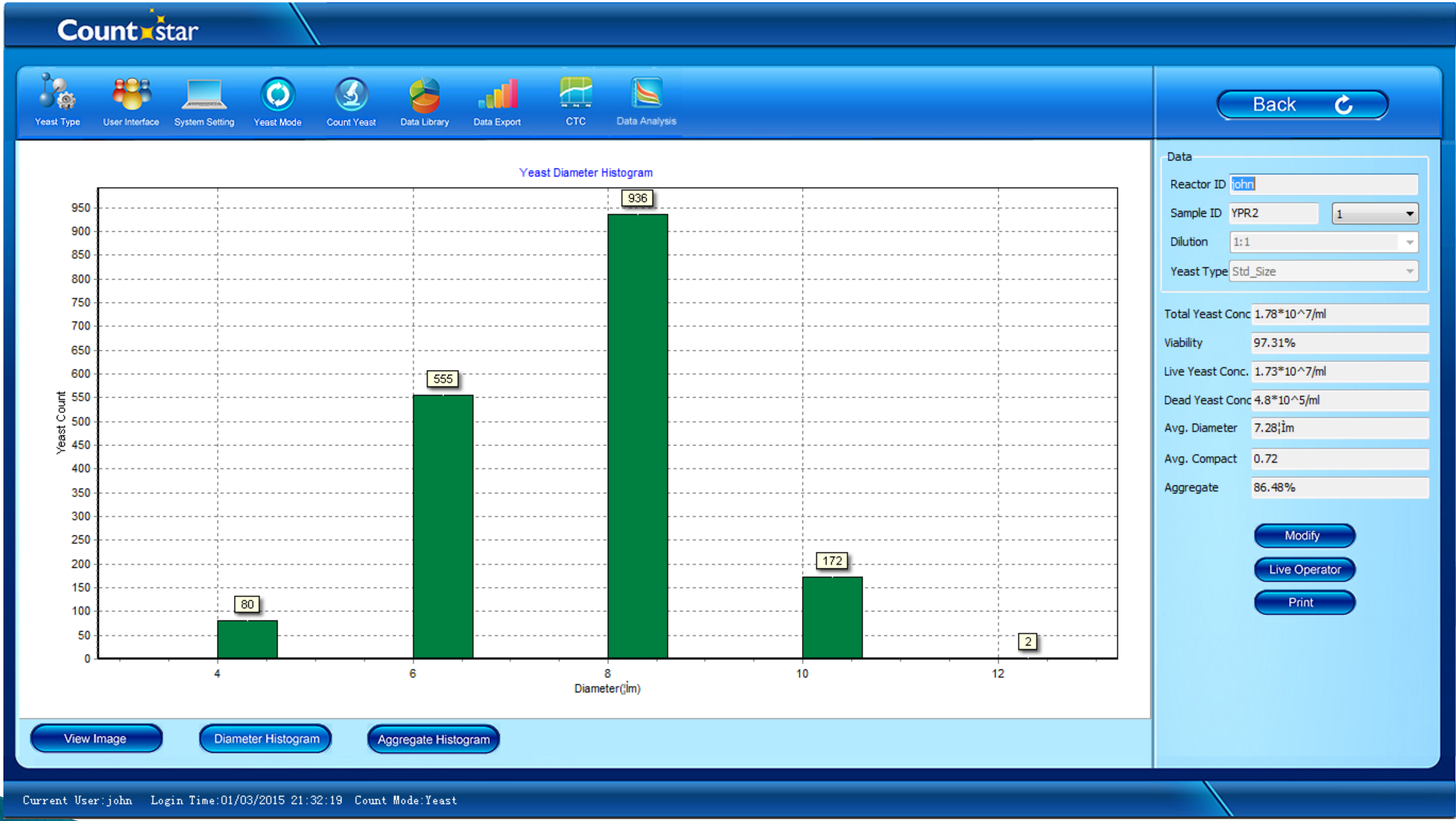

Countstar
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User Interface
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Yeast Diameter Histogram
Yeast Count
Diameter(¦Ìm)
936
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80
172
2
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Objectives of work

- To see if the gold standard stain can be retained, while automating the process.
- Investigate whether consistency, labour time and inter-operator effects were improved.
- Reveal how well an automated machine that uses methylene blue can be applied in the brewing industry.

Methods I

- Dried brewer's Yeast
  - In house testing with a range of dried brewer's yeast.
  - Samples analysed with the Aber Countstar and haemocytometer.
  - Live cell concentration (cells/ml) recorded using each method
  - Results directly compared.

Results I – Dried brewer's Yeast

Saccharomyces cerevisiae

Yeast strain name	Type	R^2 value
Nottingham	Ale yeast	0.537
BRY-97 American West Coast	Ale yeast	0.957
Belle Saison	Belgian Ale yeast	0.985
EC-118	Champagne yeast	0.919
Munich	Wheat beer yeast	0.991
Kolsch A	German Beer yeast	0.576

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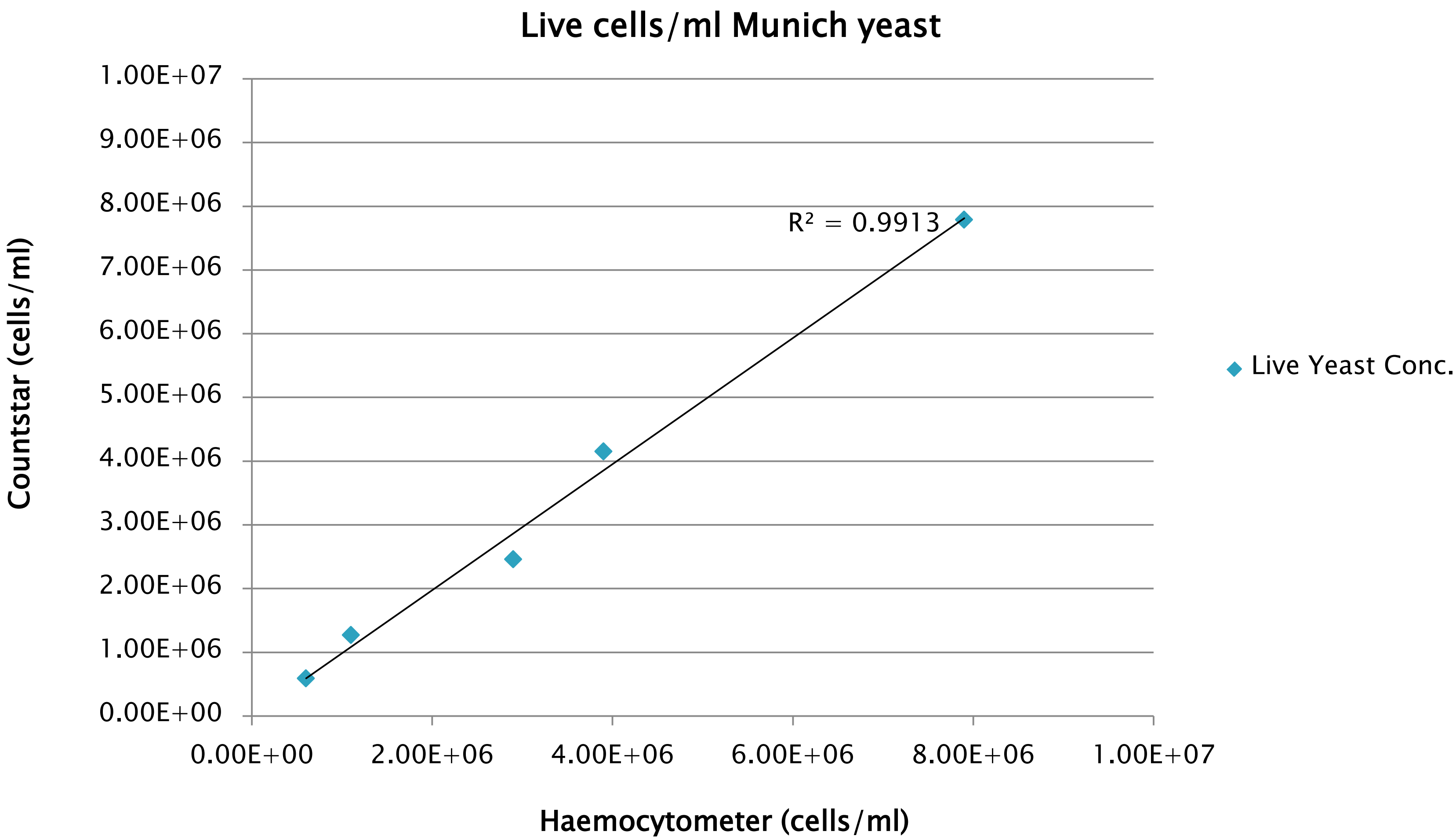

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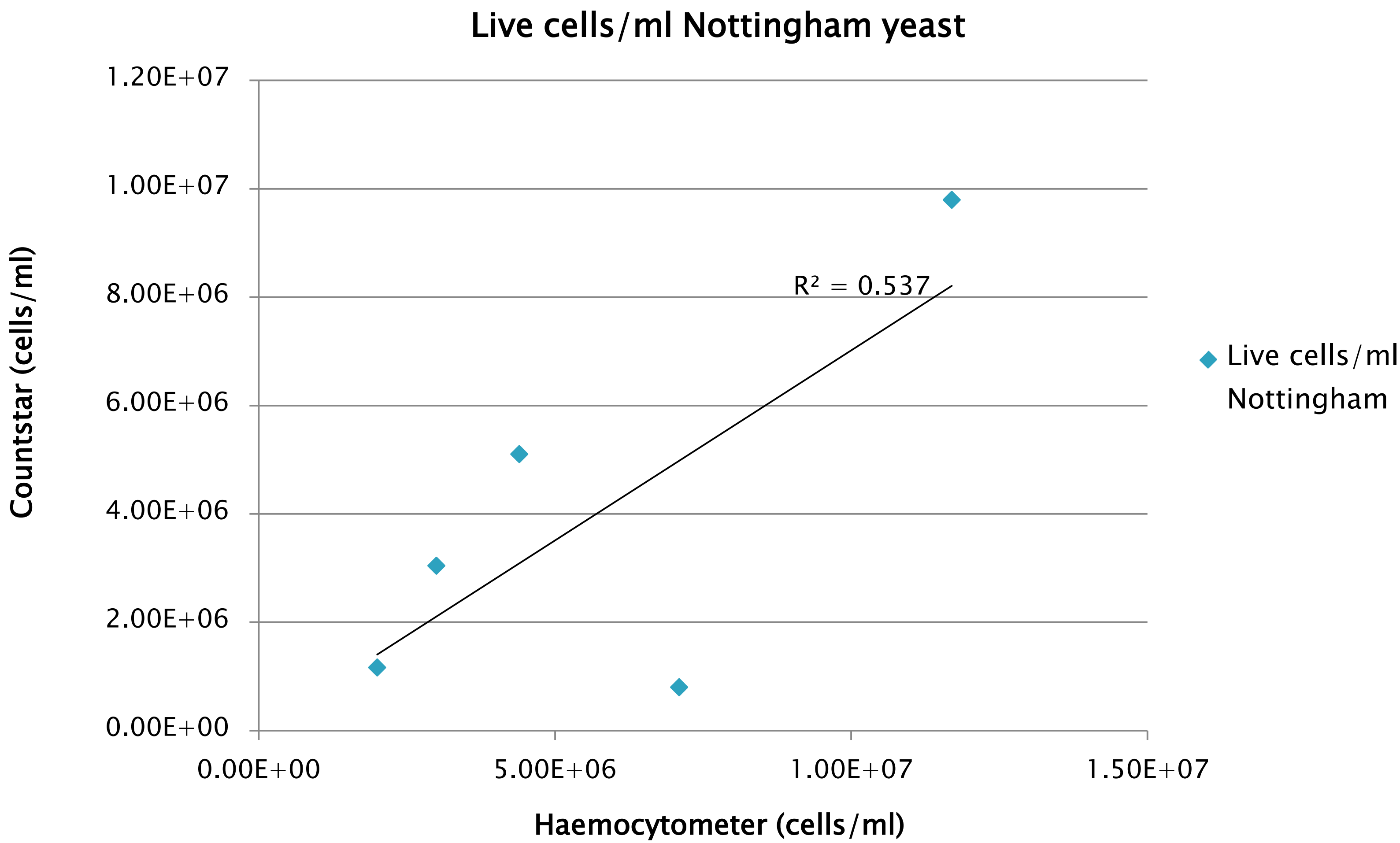

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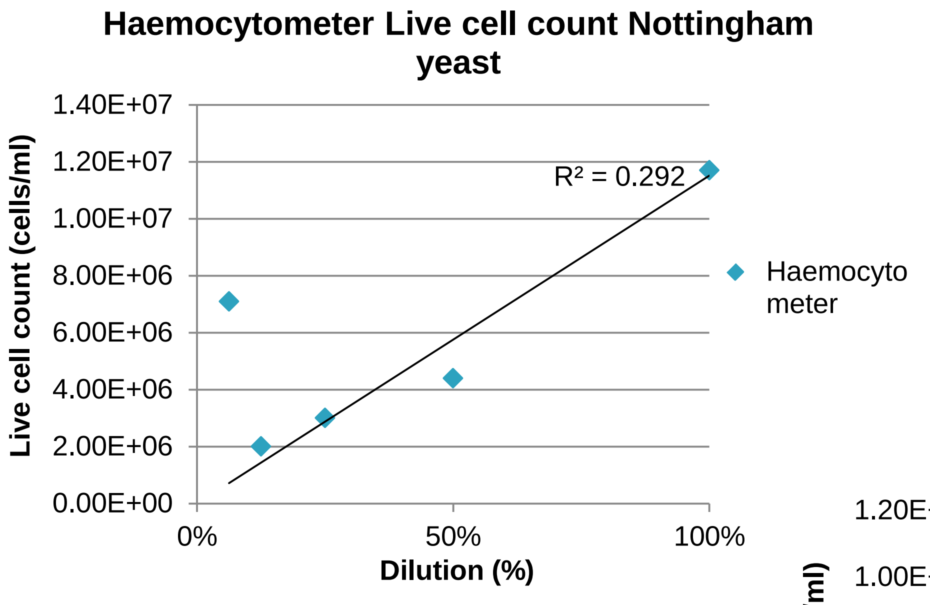
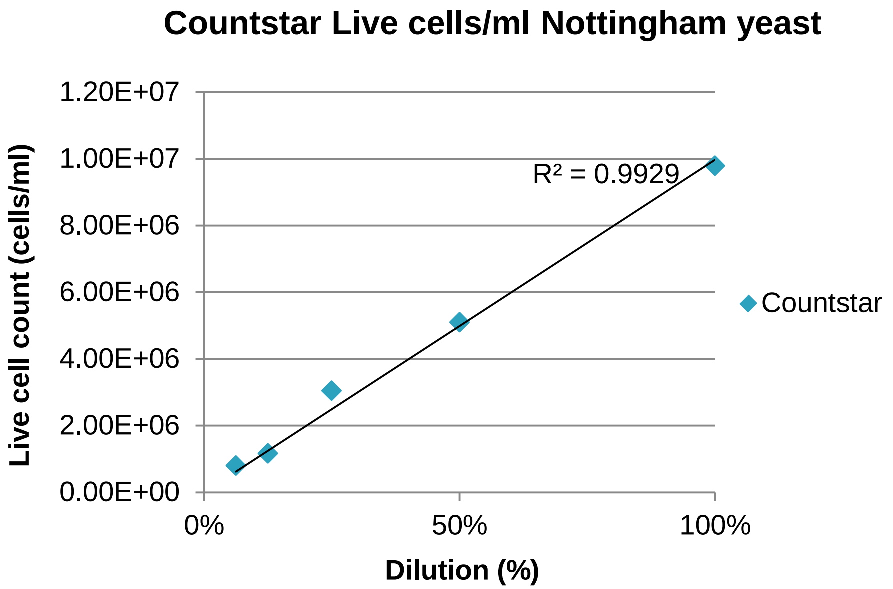
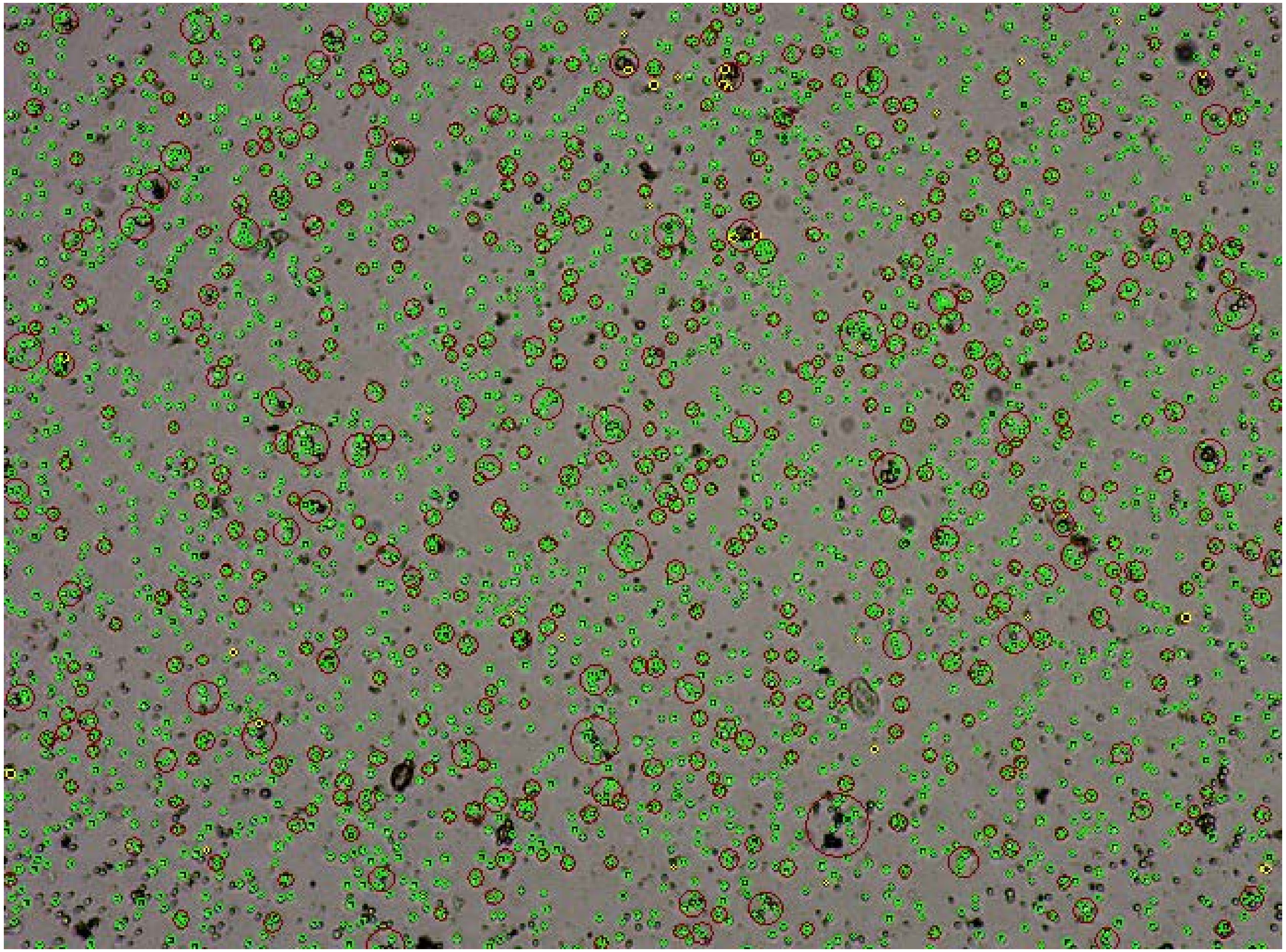




Methods II

- Wheat beer
  - Bottle conditioned
  - Analysed using haemocytometer and Countstar
  - Range of concentrations tested with 2-fold serial dilutions.
  - Live cell count using both methods assessed and compared.

Results II – Bottled Wheat Beer



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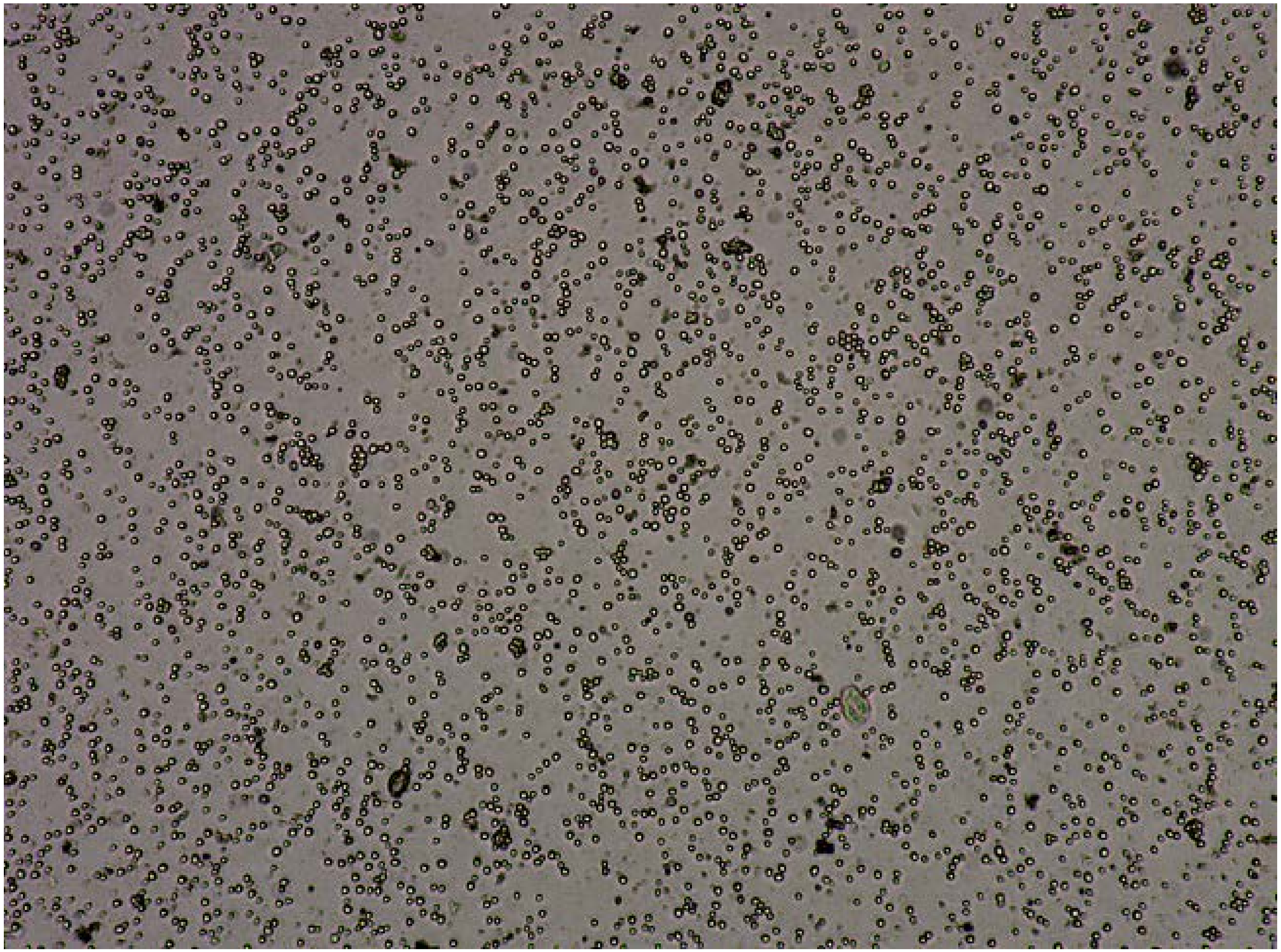

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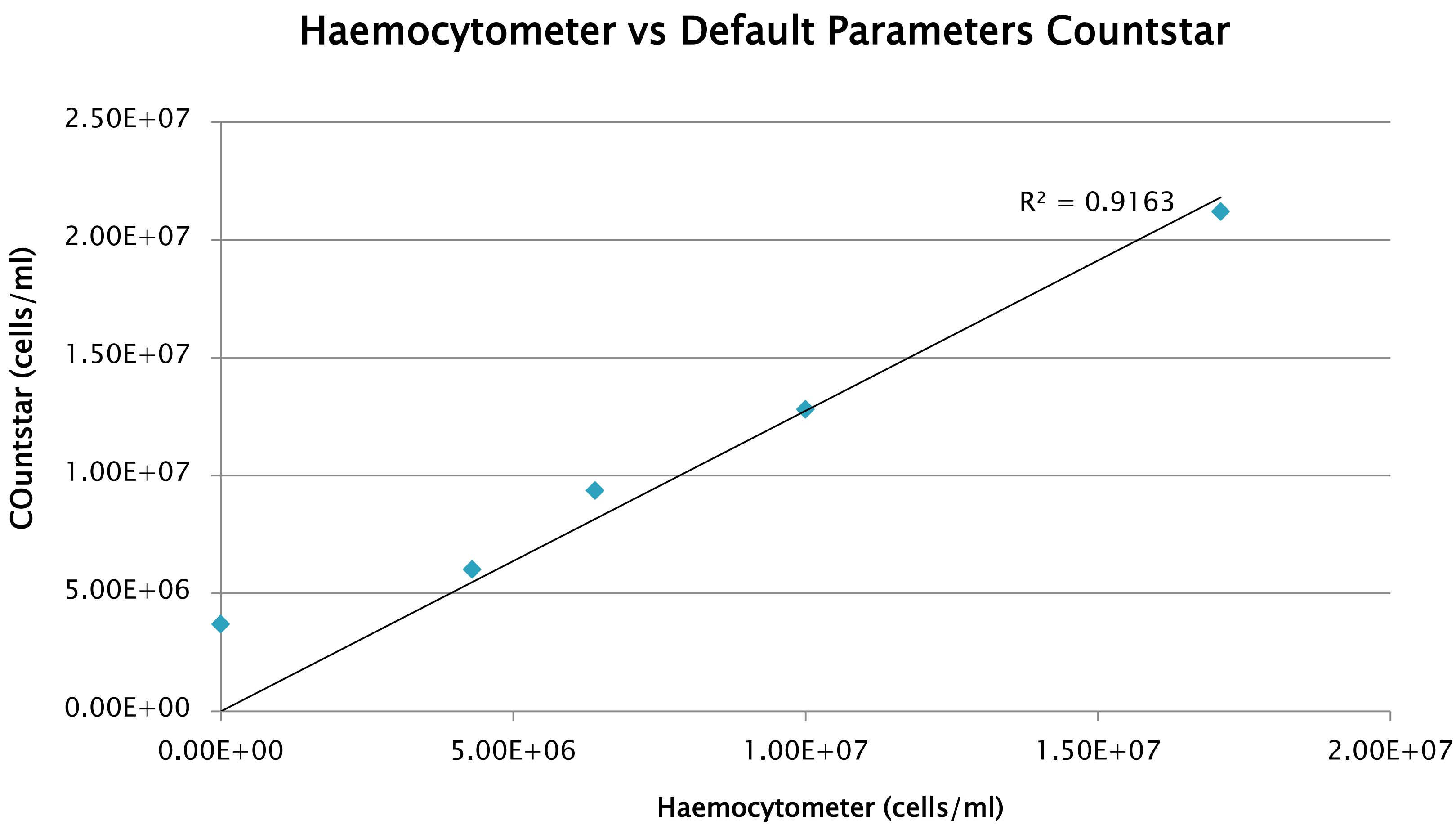

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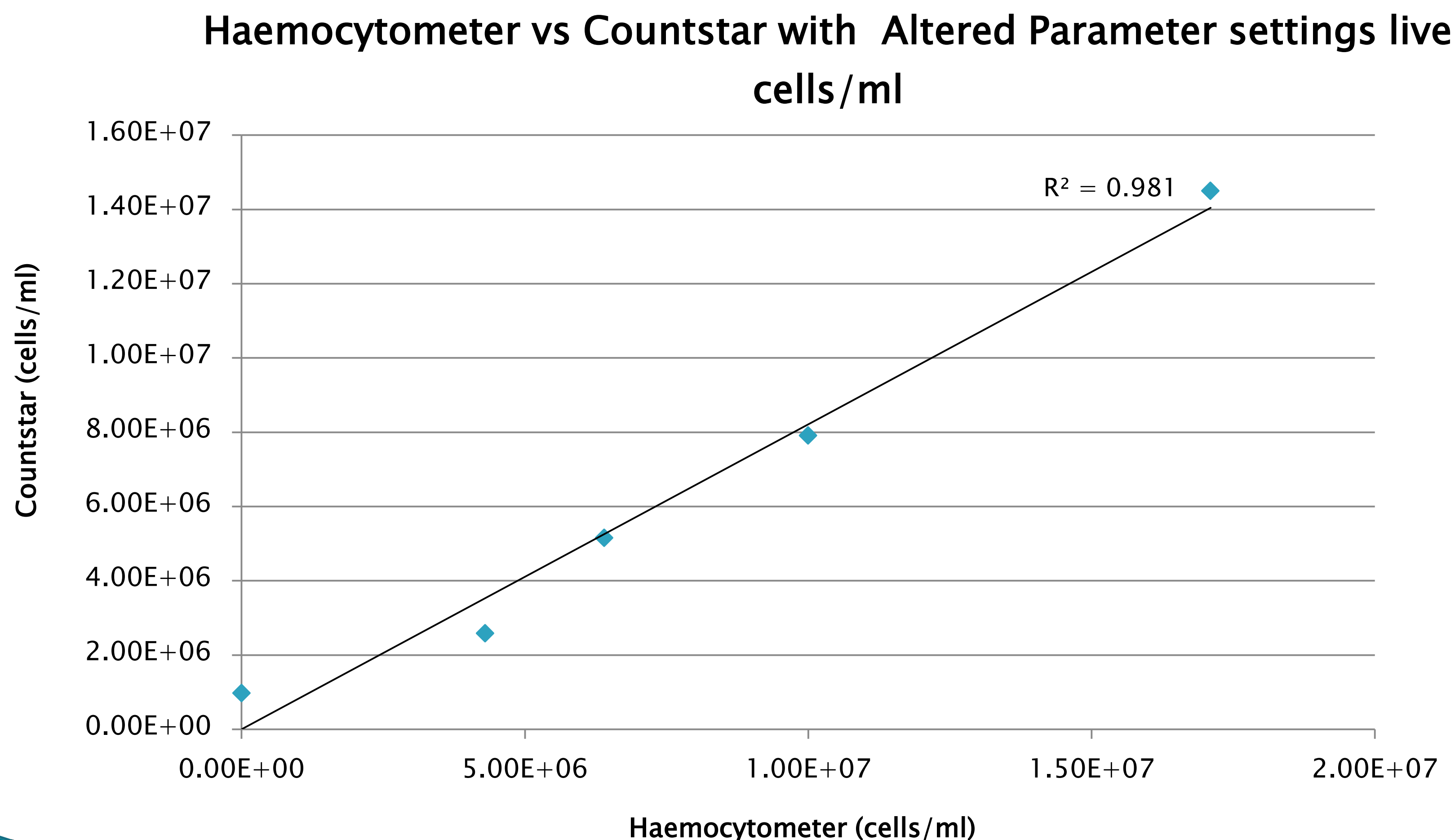
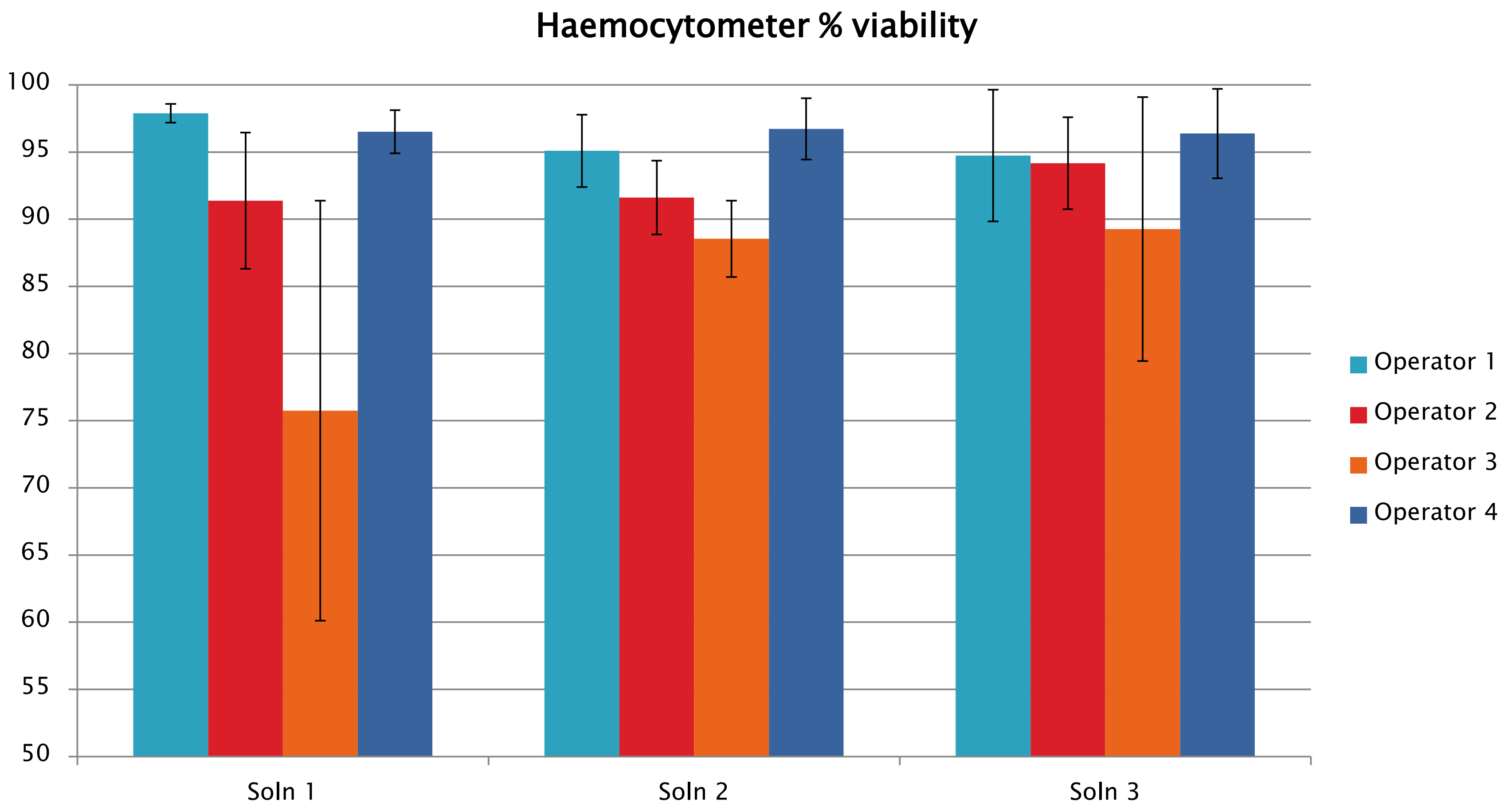


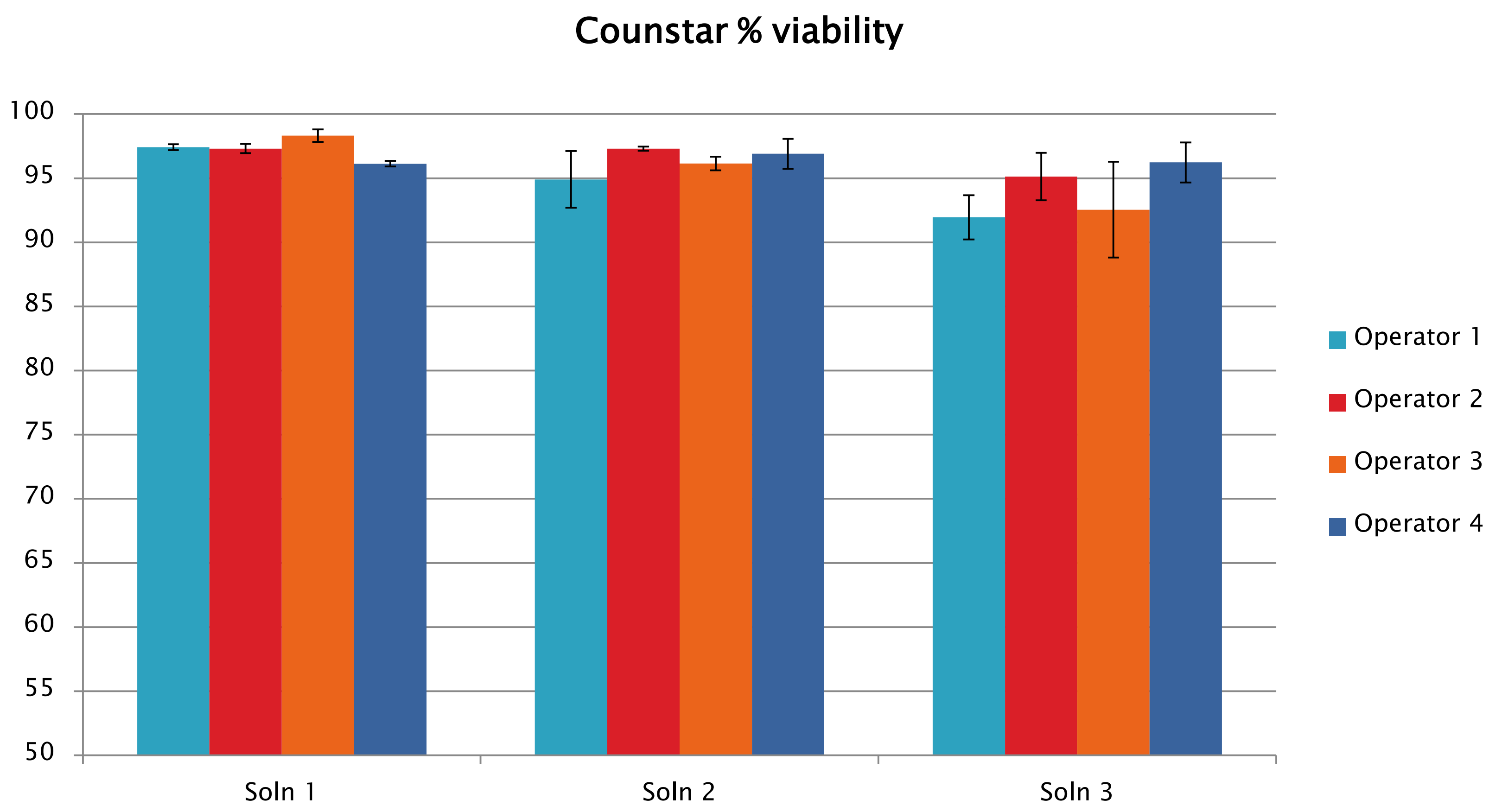
Methods III

- Inter-Operator Variability
  - Baker's yeast.
  - Three separate solutions.
  - Four operator's (different abilities) measured cell count and viability for each solution three times.
  - An average for each operator was taken for each of the three solutions.

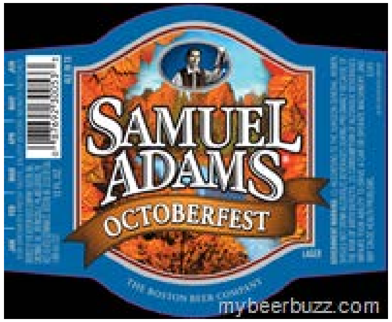
Results III – Inter-operator Variablity

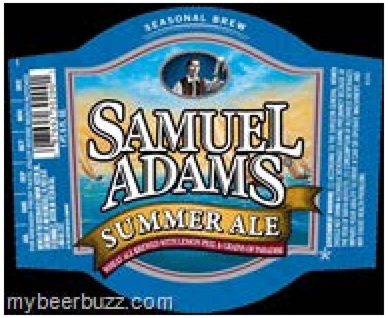


Results III –Inter-operator Variability



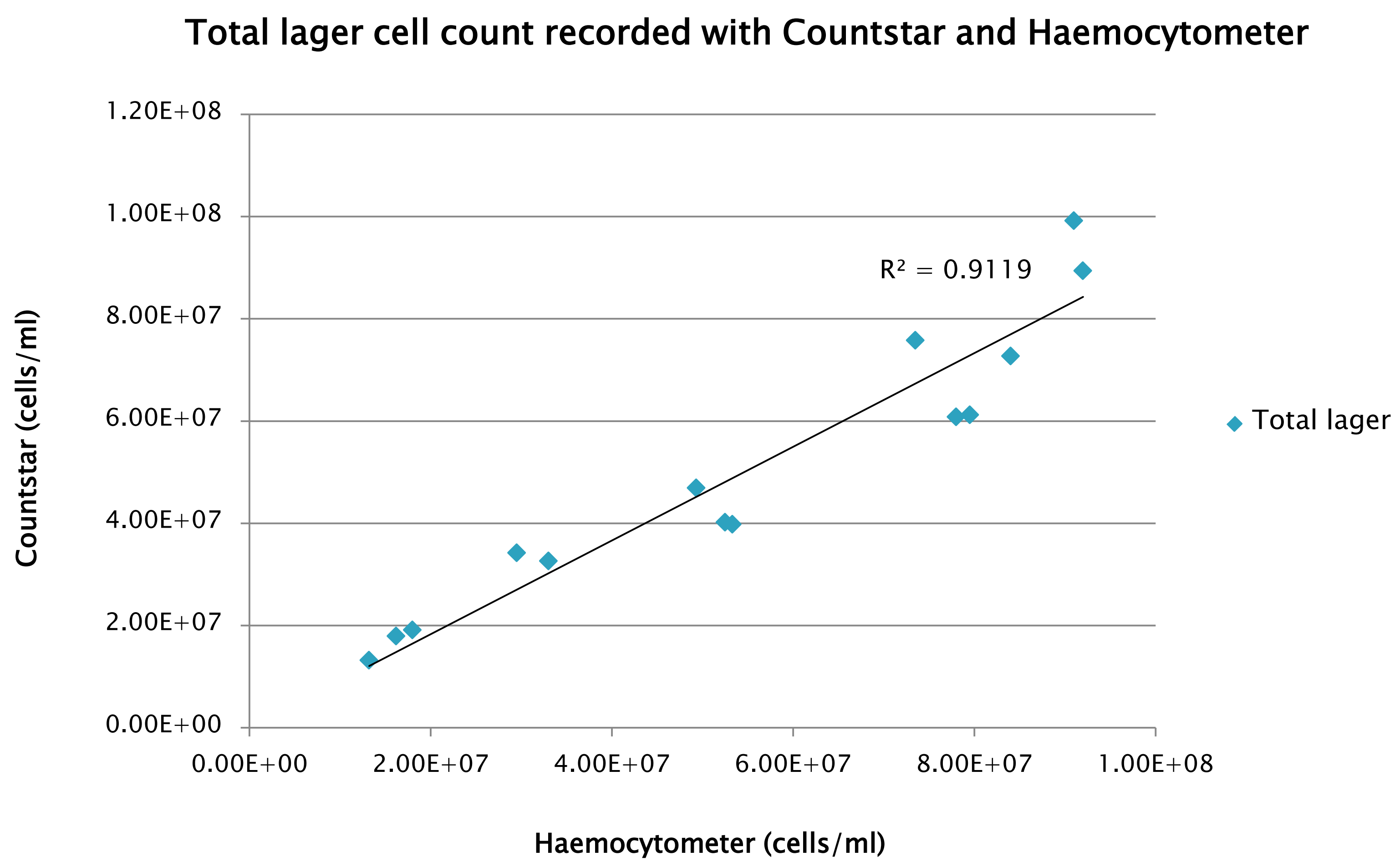
Methods IV





- Boston Beer
  - Propagation yeast
    - Total cell count most important in propagation yeast using Countstar and manually.
    - 11 samples of ale and 11 of lager yeast were analysed.
  - Harvest yeast
    - Viability% recorded.
    - 27 samples of ale and 20 samples of lager yeast.
  - Fermenter samples
    - Total cell counts recorded manual counts and Countstar.
    - 3 samples of ale and 3 of lager yeast were analysed.

Result IV –Boston Beer



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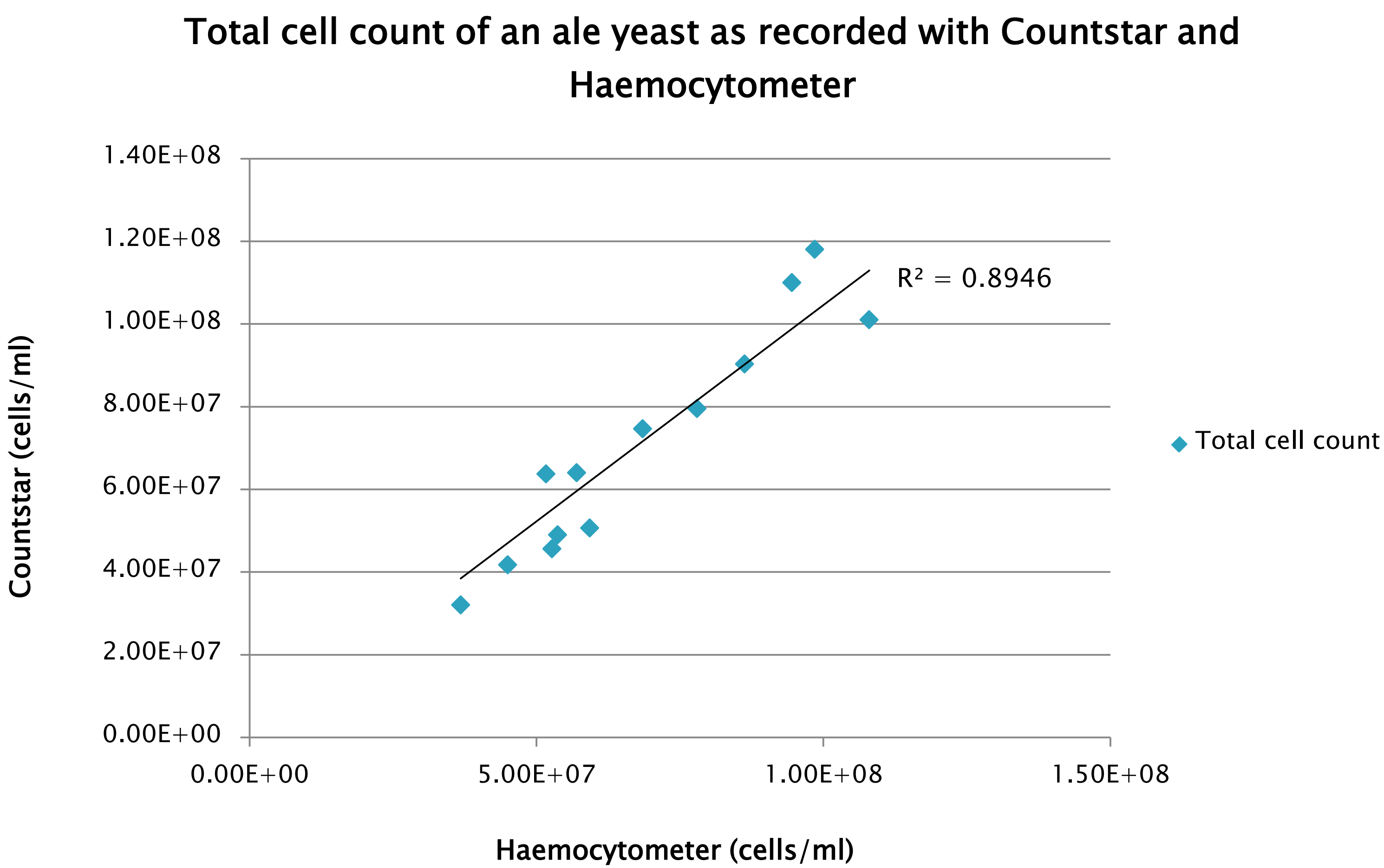

Conclusions

- The Aber Countstar can accurately perform cell counts with a range of dried brewing yeasts.
- Shown to be more reliable with some of these yeasts than haemocytometer readings.
- The Countstar is able to discount trub (Wheat beer).
- Lower deviation between repeats for one operator.
- Lower inter-operation discrepancies displayed when multiple operators analysed samples with the Countstar than when performed manually.
- Able to distinguish buds in propagation yeast.

Possible Impact to Brewing

- Save manual labour time
- Allows multiple operators to analyse samples with much lower deviation.
- Allows different multiple breweries within group to have “ the same microbiologist’s interpretation of count and viability”
- Images of yeast can be compared (across different site too) to reference images for possible cross contamination or changes in yeast morphology

Ongoing Research.

- Budding cells
- Use of technology in wine and spirits production.

Acknowledgements

- Katy Thomson, Aber Instruments
- Dr Urs Wellhoener, Boston Beer Company

Thank you for Listening.

Any Questions?